REVIEW ARTICLE

Biomarkers for diagnosis and prediction of therapy responses in allergic diseases and asthma

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Abstract

Modern health care requires a proactive and individualized response to diseases, combining precision diagnosis and personalized treatment. Accordingly, the approach to patients with allergic diseases encompasses novel developments in the area of personalized medicine, disease phenotyping and endotyping, and the development and application of reliable biomarkers. A detailed clinical history and physical examination

followed by the detection of IgE immunoreactivity against specific allergens still represents the state of the art. However, nowadays, further emphasis focuses on the optimization of diagnostic and therapeutic standards and a large number of studies have been investigating the biomarkers of allergic diseases, including asthma, atopic dermatitis, allergic rhinitis, food allergy, urticaria and anaphylaxis. Various biomarkers have been developed by omics technologies, some of which lead to a better classification of distinct phenotypes or endotypes. The introduction of biologicals to clinical practice increases the need for biomarkers for patient selection, prediction of outcomes and monitoring, to allow for an adequate choice of the duration of these costly and long-lasting therapies. Escalating healthcare costs together with questions about the efficacy of the current management of allergic diseases require further development of a biomarker-driven approach. Here, we review biomarkers in diagnosis and treatment of asthma, atopic dermatitis, allergic rhinitis, viral infections, chronic rhinosinusitis, food allergy, drug hypersensitivity and allergen immunotherapy with a special emphasis on specific IgE, the microbiome and the epithelial barrier. In addition, EAACI guidelines on biologicals are discussed within the perspective of biomarkers.

KEYWORDS

allergen immunotherapy, allergic rhinitis, asthma phenotypes and endotypes, biomarkers, food allergy

1 | INTRODUCTION

Allergic diseases represent a group of conditions caused by hypersensitivity of the immune system to allergens present in the environment.[1] These diseases include food allergy, asthma, atopic dermatitis (AD), allergic rhinitis (AR), conjunctivitis and chronic rhinosinusitis with or without nasal polyposis (CRSwNP).[2–4] The 100-year-old personalized allergen-specific management of allergic diseases has been a particular advantage in our specialty contributing to the early awareness of personalized approaches and precision medicine. The use of multiple omics, big data and systems biology has demonstrated a profound complexity and dynamic variability and enabled the discovery of novel biomarkers.[5]

Generally, a biomarker is a measurable indicator of the presence and severity of diseases or their response to a treatment with clear cutoff points. Regarding the prediction, diagnosis or monitoring of diseases, biomarkers are gaining importance in clinical practice as they provide an objective and measurable way to characterize a disease. However, it is challenging to identify convincing biomarkers as the genetic and regulatory networks for individual patients differ significantly. Biomarkers represent measurable indicators linking an underlying pathway to a phenotype or endotype of a disease.[6–8] Regrettably, current biomarkers are not precise in selecting the specific endotype that will respond to a targeted treatment. A good example is the observation that blood eosinophilia predicts therapeutic responses to all currently available or future-targeted interventions in severe asthma (i.e, anti-IL-5, IL-4/IL-13, CRTH2 antagonists).[8,9] Precision medicine in allergic diseases demands accurate diagnoses,[10] which mostly rely on

Respiratory viral infections may exacerbate chronic airway inflammatory diseases, including allergic inflammation through both Type 2 (e.g, IL-25, IL-33 and TSLP) and non-Type 2 (e.g, IFN types I and III, RIP3, OSM, MCIDAS) mechanisms.

the combination of the clinical history and respective gold standards, which are all subject to the operator, observer and interpretation variability.[11,12] Some of the approaches are time-consuming, and in vivo challenges may result in severe side-effects and, in rare cases, even death. Therefore, the discovery, validation and clinical applicability of molecular biomarkers become increasingly important.[13]

The cellular, biochemical or molecular changes in allergic patients which are measurable in blood, sputum or nasal secretions can be considered as biomarkers.[14] These biomarkers are used for disease diagnosis, selection of targeted therapy, disease monitoring and prediction of prognosis.[15] Except for the well-known biomarkers (e.g, IgE, blood or sputum eosinophilia, fractional exhaled nitric oxide [FeNO]),[16–18] research focusing on pro-inflammatory mediators, genes, the epithelial barrier and microbiomes is now emerging, which highlights more potential biomarkers for allergic diseases.[19,20] Some of the biomarkers showing a strong ability to identify disease endotypes or phenotypes may also act as therapeutic targets.[21,22] This article reviews the biomarkers identified to date and potential targeted therapies in allergy. In addition, it briefly reviews the biomarkers included in EAACI guidelines.

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2 | BIOMARKERS IN ASTHMA

In the past decades, it has been increasingly recognized that asthma is a highly heterogeneous disorder with different underlying mechanisms and pathways translating into variable responses to standard treatment across the different subsets or clinical phenotypes.[23,24] Unbiased approaches and cluster analyses identified four major clinical phenotypes: (a) early-onset allergic asthma, (b) early-onset allergic moderate-to-severe asthma, (c) late-onset nonallergic eosinophilic asthma and (d) late-onset nonallergic noneosinophilic asthma.[25] The late-onset subsets tend to present as more severe or more difficult to treat than early-onset asthma. To promote an adequate treatment strategy, asthma can be subdivided into Type 2 (high) and non-Type 2 (or Type 2 low) endotypes based on their underlying inflammatory pathways.[26] As part of a more general syndrome often including nasal polyps with or without NSAID-Exacerbated Respiratory Disease (NERD),[27,28] Type 2 asthma currently comprises the best defined asthma subset(s) in terms of underlying immunopathology, corresponding biomarkers[8] and targeted treatment options with biologicals and small molecules.[13,29,30]

In parallel with the available (targeted) treatment options, biomarkers have been validated along the corresponding inflammatory pathways aimed for pheno-/endotyping and to guide treatment for Type 2 asthma.[8] Clinically applicable point-of-care biomarkers include blood eosinophils, or whenever feasible, sputum eosinophil counts, serum-specific IgE and FeNO.[8,31] Although overlapping in Type 2 biomarkers may occur within patients, all biomarkers represent different aspects of the Type 2 inflammatory pathways with IgE associating with allergy, while FeNO is linked to the IL-13 pathway and epithelium-derived inflammation.[8] Based on these point-of-care biomarkers in combination with clinical characteristics (age of onset, comorbidities, exacerbations, need for maintenance systemic corticosteroids) and physiological parameters (lung function, airway hyperresponsiveness, etc), current guidelines have now adapted algorithms which can help to predict a response to (targeted) treatments and/or can be used to monitor the subsequent treatment response.[23,32,33] In this context, some confounders have been recognized for the existing point-of-care biomarkers, *i.e*, for FeNO mainly related to ICS use, smoking, dietary nitrate intake, virus infections and bronchoconstriction, while for blood eosinophils circadian variation, parasites and systemic corticosteroids were found to be the most common perturbing factors.[23,34] In parallel with FeNO, oxidative stress can also be caused by an excess of reactive oxygen and nitrogen species. Many direct or indirect markers of oxidative stress such as bromotyrosine, malondialdehyde, isoprostane, thiobarbituric acid, glutathione disulfide have been detected in urine, plasma, sputum and BAL fluids of patients with asthma, and the level of these markers correlated with the clinical output and severity of the disease.[35–37] A noninvasive way of analysis, exhaled breath condensate collection, has allowed direct measurements of pH changes, H_2O_2 and the measurement of several indirect by-products of oxidation like 8-isoprostane and ethane.[38,39] Currently, the presence of high levels of urinary bromotyrosine is a promising noninvasive

biomarker of oxidative stress for clinical use in asthma patients. In this context, a clinically relevant issue has been raised, *i.e*, whether "true" non-Type 2 (noneosinophilic) asthma really exists among patients with severe asthma, given the fact that high-dose inhaled and oral corticosteroids may potentially mask preexisting Type 2 inflammation interfering with its biomarkers, especially blood eosinophils and FeNO.[40,41] Currently ongoing corticosteroid-tapering studies (RASP-UK) in patients with non-Type 2 severe asthma should answer this question. Alternatively, airway neutrophilia ("neutrophilic asthma") may often reflect (subclinical) airway infection.[41–43]

In contrast, for non-Type 2 asthma which is by default defined as asthma without Type 2 biomarkers, underlying pathways and, hence, clinically applicable biomarkers and targeted treatment options are still largely under exploration.[41,44] Apart from most patients with mild clinically stable asthma,[26,45] clinical phenotypes frequently associated with non-Type 2 asthma include very late-onset asthma (women), obesity-associated asthma, smoking-associated neutrophilic asthma and paucigranulocytic asthma. Although generally based on increased sputum neutrophils or absence of normal levels of (sputum) eosinophils and neutrophils (paucigranulocytic) with normal levels of other Type 2 markers, the diagnosis of non-Type 2 asthma is difficult to establish as often based on cross-sectional data potentially affected by confounders including respiratory infections or anti-inflammatory therapies.[41] In the absence of targeted biologicals, in non-Type 2 asthma treatable traits should be targeted,[46–48] e.g, obesity, smoking habits, psychological aspects, neutrophilia as a potential indicator of respiratory infection and airway narrowing or airway hyperresponsiveness as an indicator of ASM dysfunction, while corticosteroids may not be effective and should be tapered off (Figure 1).[23]

In conclusion, despite substantial progress in our understanding, applicable biomarkers and targeted treatment options for Type 2 asthma, further characterization of molecular pathways by omics technologies,[49–51] sophisticated imaging[52] and innovative anatomical approaches[53] should help to further unravel the complexity of asthma and to define reliable (composite) biomarkers and therapeutic strategies for patients nonresponsive to currently available (targeted) treatment options including non-Type 2 asthma.

3 | BIOMARKERS LINKED TO MICROBIOME AND ASTHMA

An enormous variety of microbes colonize mucosal body surfaces, and these microbes are organized within complex community structures, utilizing nutrients from other microbes, host secretions and the diet. Modern lifestyles, medications and social interactions have fundamentally altered and disrupted the human microbiome metacommunity and, as a consequence, risk of immune-mediated diseases such as allergy and asthma.[54,55] The mechanisms that mediate host–microbe communication are highly sophisticated and are being intensely investigated by many research groups across the world. However, there is accumulating
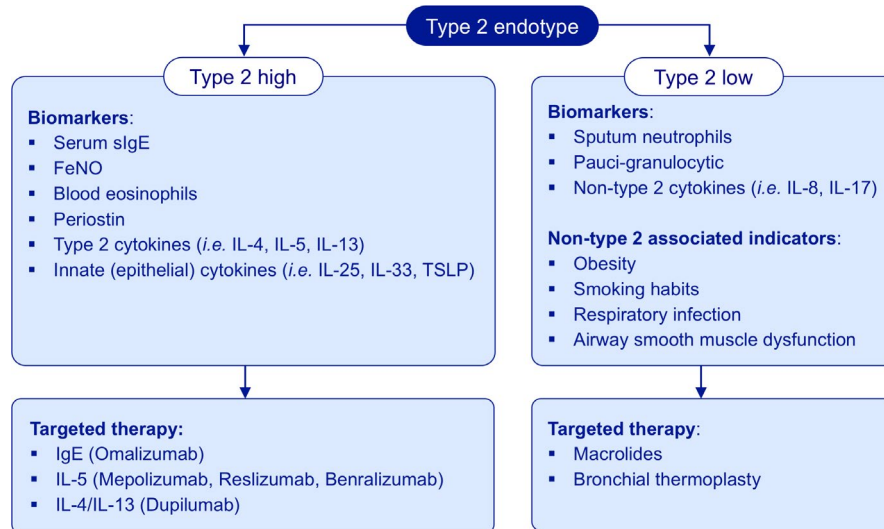
FIGURE 1 Treatment based on molecular biomarkers for endotypes in asthma. Asthma can be subdivided into Type 2 (high) and non-type 2 (or type 2 low) endotypes based on their underlying inflammatory pathways. For Type 2 high asthma, potential biomarkers could be serum-specific IgE (sIgE), fractional exhaled nitric oxide (FeNO) and blood or sputum eosinophils, and in some more specialized centers periostin. Moreover, Type 2 cytokines (IL-4, IL-5 and IL-13) and innate (epithelial) cytokines (IL-25, IL-33 and TSLP) can also be important biomarkers. The options to treat with biologicals emphasizing biomarkers of Type 2 high endotype have entered the market: IgE (omalizumab), IL-5 (mepolizumab, reslizumab, benralizumab) and IL-4/IL-13 (dupilumab). In contrast, the diagnosis of Type 2 low asthma is difficult to establish as generally based on increased sputum neutrophils or paucigranulocytic with normal levels of other Type 2 markers, and non-Type 2 cytokines (IL-8 or IL-17). There are still some associated indicators including obesity, smoking habits and psychological aspects. Therefore, therapeutic strategies for patients with Type 2 low asthma could be macrolides and bronchial thermoplasty
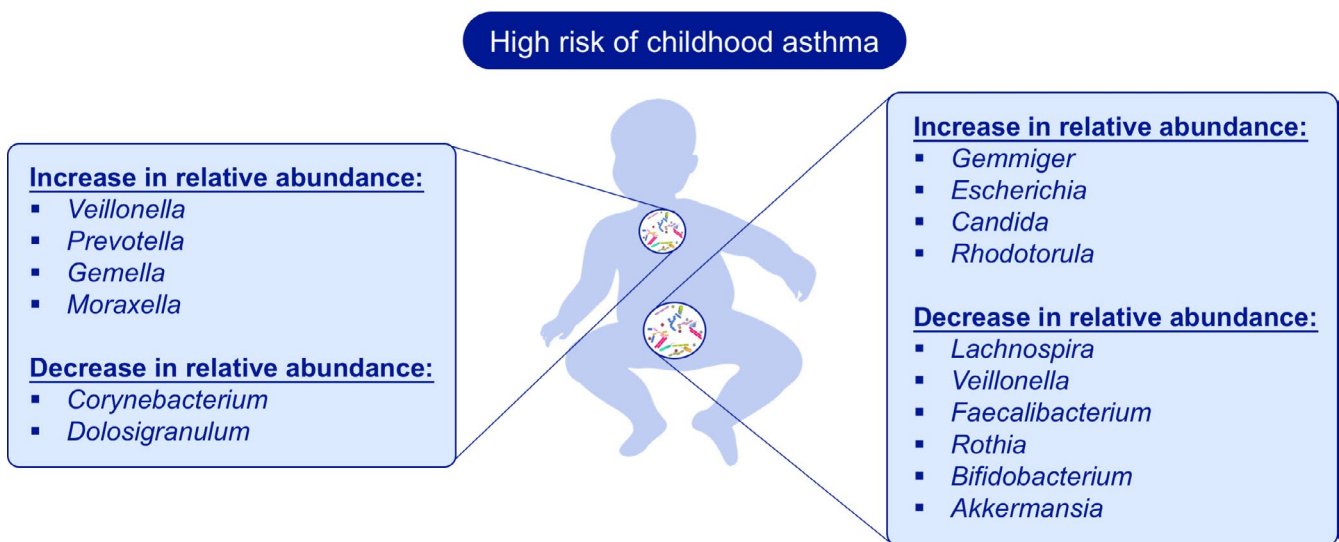
FIGURE 2 Microbiome Biomarkers in Asthma. Alterations in the gut and airway microbiota during childhood have been associated with asthma risk. The higher relative abundance of *Veillonella* and *Prevotella* and a switch from a *Corynebacterium* and *Dolosigranulum* cluster to a *Moraxella* cluster in the upper-airways were associated with a higher risk of severe asthma exacerbation in children with asthma. The lower relative abundance of genera including *Lachnospira*, *Veillonella*, *Faecalibacterium*, *Rothia*, *Bifidobacterium* and *Akkermansia* in the gut during early life has been associated with the development of asthma. The increases in relative abundance of *Gemmiger*, *Escherichia*, *Candida* and *Rhodotorula* within the gut were also associated with the subsequent development of asthma

evidence that microbiome composition and metabolic activities within the gut and the airways can influence asthma pathogenesis.[56–58] Here, we summarize some of the key recent findings that identify specific microbes or associated metabolites that may be useful as biomarkers to predict asthma risk, asthma severity or guide existing or novel therapies.

Alterations in the gut microbiota within the first year of life have been associated with asthma risk in multiple birth cohort studies.[19]

The lower relative abundance of genera including *Lachnospira*, *Veillonella*, *Faecalibacterium*, *Rothia*, *Bifidobacterium* and *Akkermansia* in the gut during early life has been associated with the development of asthma.[59,60] While fewer studies have examined preschool children (2-4 years of age), a recent study in this age group demonstrated that certain bacterial genera within the gut were still associated with wheezing (*Collinsella* and *Dorea*) or subsequent development of asthma (*Gemmiger* and *Escherichia*).[61] In addition to the gut microbiota, studies are also showing changes in the microbial populations of the airways. Microbial diversity and the relative abundances of *Veillonella* and *Prevotella* in the airways at age 1 month were associated with asthma by age 6 years.[62] Interestingly, higher relative abundance of these bacteria was associated with reduced TNF-α and IL-1β and increased CCL2 and CCL17 within the airways. A switch from a *Corynebacterium* and *Dolosigranulum* cluster in the upper airways to a *Moraxella* cluster was associated with a higher risk of severe asthma exacerbation in children with asthma.[63] In adults, increased relative abundance of the phylum *Proteobacteria* (including *Haemophilus*, *Comamonadaceae*, *Sphingomonadaceae*, *Nitrosomonadaceae*, *Oxalobacteraceae* and *Pseudomonadaceae*) is often associated with asthma or with worse asthma control.[64] Microbial changes within the gut, upper and lower airways of adult asthma patients are magnified in obese asthma patients and in those with severe disease.[65] Bronchoalveolar lavage levels of IL-5 and eosinophils correlated with a variety of microbes within the airways. Of note, severe asthma negatively correlated with fecal *Akkermansia* levels and oral administration of *Akkermansia* to murine models significantly reduced airway hyper-reactivity and airway inflammation (Figure 2).

In addition to microbiota composition, microbial metabolites may also be useful biomarkers in asthma. The fecal metabolome of children at increased risk of asthma contained increased levels of pro-inflammatory metabolites, among which 12, 13 DiHOME was able to induce IL-4 production in CD4+T cells and decreased the abundance of Tregs.[60] High levels of short-chain fatty acids (SCFAs), such as butyrate and propionate, at 1 year of age were associated with reduced risk of atopic sensitization and asthma by school age.[66] Multiple immune modulatory effects have been described in murine models for SCFAs, which include the promotion of Treg development and the inhibition of pulmonary ILC2 functions and subsequent development of airway hyper-reactivity.[67] In adults, an increased abundance of histamine-secreting bacteria was observed within the gut of patients with asthma, while disease severity correlated with high levels of the histamine-secreting microbe *Morganella morganii*.[68] Murine models have demonstrated that bacterial-derived histamine within the gut can influence inflammatory responses within the lungs.[69]

In the future, the application of recent advances in metagenomic sequencing technologies and bioinformatics will likely lead to the identification of novel functional traits and metabolites within the gut and airway microbiome of asthma patients.[70] In addition, future asthma studies should include the microbiome as potential biomarkers that predict or associate with responses to biologics, as already observed for *Faecalibacterium*, *Bifidobacterium* and *Akkermansia* that associate with immunotherapy responses in certain groups of cancer patients.[71]

4 | SKIN BACTERIAL MICROBIOME AS CLINICAL BIOMARKER IN ATOPIC ECZEMA

Diagnosis of atopic eczema (AE) severity is still today a semi-quantitative clinical score based on subjective information from the patients together with a doctor's subjective estimation of the severity of skin lesions and patient's history of itching and sleep loss.[72,73] In the era of targeted therapy, and thus more complex therapy management requirements, more objective criteria are urgently needed. The serum thymus and activation-regulated chemokine (TARC) level has been reported as the most reliable biomarker for disease severity with strong pooled correlation coefficients with AD. Additional biomarkers that could prove useful but require additional research include serum cutaneous T-cell-attracting chemokine (CTACK), sE-selectin, macrophage-derived chemokine (MDC), lactate dehydrogenase (LDH) and IL-18.[74] A diagnostic biomarker to distinguish between the different subgroups of AE is still needed. AE, likewise, lacks a prognostic biomarker: AE[75] affects 30% of children but only 5% of adults—thus, the question remains who keeps the disease, who emerges from it and who embarks on the full career of an atopic individual. Skin microbiome dysbiosis, measured either as microbiome diversity or more reliably as abundance of *S aureus*, was shown to correlate with both the AE clinical score and the expression of skin barrier molecules.[76] It is still a matter of scientific debate whether the relative frequency of various bacteria (e.g, *S aureus* frequency as obtained from 16S-based NGS) is an adequate biomarker or rather the absolute microbial load (e.g, as obtained from qPCR) is better. Furthermore, is it enough to quantify the DNA abundance from nonstandardized amounts of skin samples, or rather is the absolute microbial load of standardized skin samples needed?

S aureus is important for AE pathogenesis even though it is still a matter of debate whether overgrowth of *S aureus* is a cause or a result of barrier disruption.[77] Thus, microbiome analysis, at least on the species level, but ideally on the strain level, would enable us to identify personalized biomarkers. This highlights a methodological drawback, as currently tools for annotation on species level are not reliable. Furthermore, the current methods for skin microbiome measurement are not standardized; testing the same material in different laboratories is prone to give different results. For skin microbiome to be used clinically as a biomarker, standardized methodology needs to be developed and validated so it can be reliably used across different laboratories.[78] Combinatory biomarkers between skin microbiome and biomarkers of Type 2 immunity would also be of great potential.[79] Recently, biofilm propensity of *S aureus* skin isolates as a cause and possible target has become more and more of a central issue.[80] Thus, resolving the enigma of skin–microbe interaction as a function of skin homeostasis has to take more players into the sight.[81]
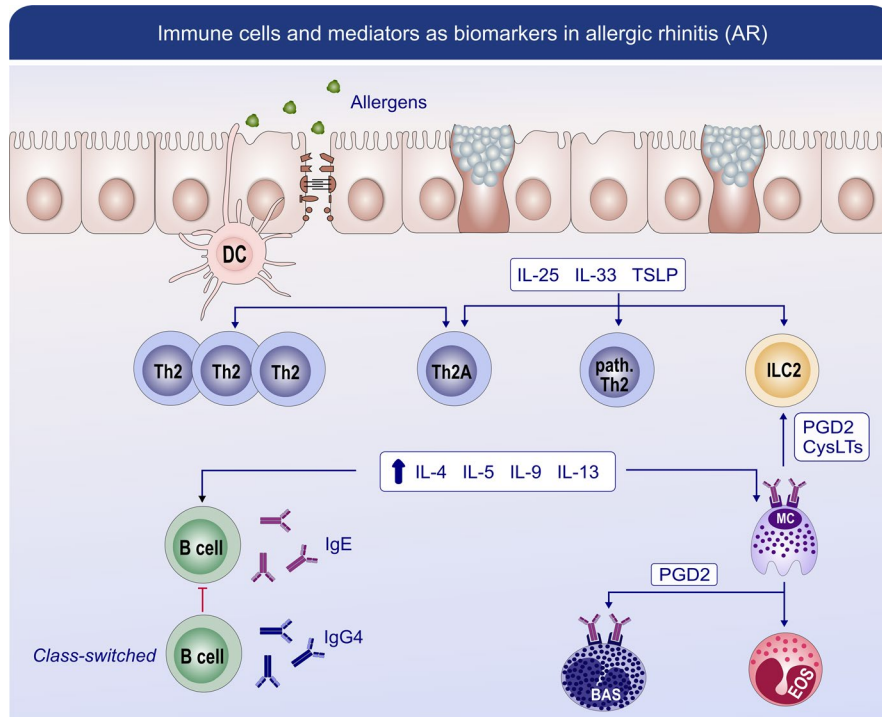
FIGURE 3 Immune cells and mediators as biomarkers in allergic rhinitis (AR). AR is associated with abnormalities in epithelial barrier function which is caused by exposure to exogenous proteases from allergens bacteria and viruses. These changes in epithelial barrier could contribute to the allergen absorption and disruption of epithelial tight junction. Activated dendritic cells (DCs) present allergen peptides to naive T cells and drive them to differentiate into Th2 cells and also allergen-specific Th2A cells. Damaged epithelial cells release a high level of alarmin (TSLP, IL-25, and IL-33), which activate the group 2 innate lymphoid cells (ILC2s) as well as pathogenic memory T helper (Th) 2 cells. All these cells produce large amounts of proinflammatory mediators including IL-4, IL-5, IL-9, and IL-13. Besides, IL-4 and IL-13 are involved in IgE class switch in B cells. IgE binding to mast cells can trigger the release of mast cell-associated mediators, such as prostaglandin D2 and leukotrienes, which could also activate the function of ILC2. PGD2 signaling could be a promising biomarker, as it can also activate eosinophils and basophils. Moreover, CD203c expression on basophils exhibits a time-of-day-dependent variation, which could partly be responsible for temporal symptomatic variations in AR. IgG4 increased during allergen immunotherapy (AIT) is purported to be a blocking antibody by competing for allergen binding with IgE bound to Fcε receptors on mast cells and basophils. cysLT, leukotrienes; PGD2, prostaglandin D2

5 | BIOMARKERS IN DIAGNOSIS OF ALLERGIC RHINITIS

With deeper insights into mechanisms of AR, novel biomarkers have recently been identified in its diagnosis. Furthermore, several immune cells and mediators, genes and metabolites have been studied to explore their potential utilization in diagnosis of AR.

In conclusion, skin bacterial microbiome shows great potential to be used as a clinically important biomarker for atopic eczema. To reach this aim, we need to perform prospective clinical trials and large longitudinal registries that include skin microbiome testing. Furthermore, it is critical to advance standardized and foremost quantitative methodologies for skin bacterial microbiome analysis. New technologies, such as single-molecule real-time (SMRT) sequencing , need to be further developed and tested in order to improve skin microbiome analysis with higher accuracy and/or longer sequencing length. Collaboration between large academic consortia and pharmaceutical companies is essential for such endeavors.

5.1 | Immune cells and mediators

Several potential immune cells (granulocytes, lymphocytes, etc) and mediators might serve as diagnostic biomarkers of AR.[14,82] Izuhara and colleagues have reported that induction and increased expression of periostin reflect Type 2 inflammation and remodeling and could be regarded as an emerging biomarker for allergic diseases.[83] One study has demonstrated that allergen-induced surface CD203c expression on basophils exhibits a time-of-day-dependent variation, and allergen-specific basophil reactivity shows daily variations depending on circadian clock activity in basophils, which could partly be responsible for temporal symptomatic variations in AR.[84] One recent study has suggested that circulating group 2 innate lymphoid cells (ILC2s) may play an important role in the pathology of AR, particularly as increased levels of ILC2s correlated with symptom scores and IL-13 levels in house dust mite (HDM)-sensitized AR patients,[85] and these cells produce large amounts of proinflammatory mediators in response to Th2 cytokines.[86,87] Indeed, a more recent study by Tojima and colleagues found that prostaglandin D2 (PGD2) and cysteinyl leukotriene (cysLTs) might induce ILC2s to produce Th2

cytokines such as IL-5 and IL-13.[88] Similarly, ST2-expressing pathogenic memory T helper (Th) 2 cells, producing substantial amounts of IL-33-induced IL-5 and IL-13, have been shown to be linked to sensitization and the onset and progression of AR (Figure 3).[89]

5.2 | Genes

Epigenetic modifications, particularly DNA methylation and micro-RNAs (miRNAs), might have the potential to identify AR patients. One recent study has demonstrated changes in DNA methylation of tryptase gamma 1 (*TPSG1*), schlafen (*SLFN12*) and mucin 4 (*MUC4*) genes, following controlled allergen challenge, and suggested that baseline epigenetic status may act as a potential biomarker for AR symptom severity.[90] Another recent study has indicated that the nasal epigenome associated with asthma, FeNO and IgE may serve as a sensitive biomarker of asthma, allergy and airway inflammation in children.[91] Other studies have reported that subsets of circulating miRNAs are solely expressed in the blood of patients with AR and asthmatics and may therefore be used as noninvasive biomarkers for diagnosis and characterization of these diseases.[92,93]

5.3 | Metabolites

Metabolites have also been proposed as biomarkers for AR. A very recent study of serum metabolomics has demonstrated that at least nine metabolites (13(S)-HPODE, bilirubin, leukotriene D4, hypoxanthine, L-stercobilinogen, N-succinyl-L-diaminopimelic acid, chlorophyll b, 15-hydroxyeicosatetraenoic acid and urate) were significantly altered in the serum of AR patients and therefore may provide a better understanding of the metabolic pathways involved in the etiology of AR.[94] Additionally, a decreased serum lactoferrin level has been reported to be associated with the phenotype of *Dermatophagoides pteronyssinus* (Der p 1)-sensitive AR and, in combination with serum Der p 1-specific IgE levels, may serve as a serologic biomarker for early detection of AR.[95]

5.4 | Clinical biomarkers of allergic rhinitis

Clinically, rhinitis phenotypes include nonallergic rhinitis (NAR), AR, local allergic rhinitis (LAR) with a localized allergic response but no systemic atopy. In some cases, basic SPT and an sIgE test are not efficient to discriminate between these phenotypes. Recently, a retrospective study was conducted to investigate the safety and reproducibility of the nasal allergen challenge (NAC) carried out over 12 years in Spain. It was shown that 99.97% NACs were well tolerated without delayed, local severe or systemic adverse events in allergic patients for both children and adults. Moreover, there were no significant differences in three consecutive NAC with a single allergen per session (NAC-S) proving the reproducibility of NACs.[96] Regarding the monitoring of the NAC, it was shown that the

%Vol2-6 cm by acoustic rhinometry (AcRh) displayed an optimal discriminative power for AR patients from both NAR and HC subjects.[97]

Although the nasal provocation test (NPT) is considered a key tool to diagnose LAR, it requires well-trained personnel and is time-consuming. In this regard, the basophil activation test (BAT) should be helpful for supporting the diagnosis of LAR. BAT shows 50%-66.6% sensitivity and 90%-100% specificity of LAR, which is more sensitive than an sIgE test and less time-consuming than NACs as an in vitro test.[98-100] There is a new AR phenotype named dual allergic rhinitis (DAR), where patients show SPT positivity to seasonal allergens only, but suffer from perennial symptoms and react to both perennial and seasonal allergens. For these patients, BAT displays 100% positivity with seasonal allergens and 60% positivity with perennial allergens.[101]

Overall, NAC can act as a gold standard in distinguishing the AR phenotype. And BAT could also be useful as an in vitro tool for LAR/DAR diagnosis in the daily practice.

5.5 | Biomarkers in therapy of allergic rhinitis

Currently, optional therapeutic measures for AR involve patient education, environmental control, pharmacotherapy, allergen immunotherapy (AIT) and surgery.[102,103] Traditional medications include nasal corticosteroids, antihistamines, mast cell stabilizers, decongestants, etc MP29-02, a combination of nasal corticosteroid and antihistamine, is a novel topical medication which has proved to be effective in reducing nasal hyperreactivity and nasal mediators such as substance P, in patients with AR.[104] As ILC2s have been shown to produce significant amounts of proinflammatory mediators in response to epithelium-derived cytokines[86,87] and PGD2 and cysLTs[88] in AR patients, agents targeting the ILC2s and the mediators activating these cells have become targets for therapy. Rittchen and Heinemann have recently reviewed the central role of hematopoietic PGD2 synthase in allergic inflammation and indicated that PGD2 signaling might be a promising therapeutic target for AR, as PGD2 can activate Th2 cells, eosinophils and basophils.[105] Indeed, a randomized controlled phase II clinical trial has recently demonstrated that ONO-4053, a novel prostaglandin D receptor 1 antagonist, was more effective than pranlukast, a leukotriene receptor antagonist, in treating patients with seasonal AR.[106] Most recently, emerging studies have focused on biologics for treating allergic diseases; especially severe, uncontrolled asthma and AD, as well as AR.[107,108] To date, a high number of specific biologics targeting markers of Th1/2/17 inflammation have been introduced; with more underdevelopment.[107,109] In particular, targeting IgE by omalizumab, a recombinant humanized anti-IgE antibody, has been shown to significantly improve symptoms in patients with inadequately controlled AR.[110] Furthermore, combining omalizumab with subcutaneous immune therapy (SCIT) in patients with SAR and comorbid seasonal allergic asthma has been shown to lead to greater clinical improvements in AR and lung function than SCIT alone.[111] Similarly, dupilumab, a biologic which targets IL-4Rα to block the activity of both IL-4 and IL-13,

has been shown to provide nasal symptom relief in patients with uncontrolled asthma and comorbid AR.[112]

6 | BIOMARKERS OF VIRAL INFECTIONS IN EXACERBATION OF ALLERGIC RHINITIS AND ASTHMA

Over the past decade, our understanding of immunological mechanisms underlying allergic diseases such as AR has substantially increased through the discovery of T helper (Th) subsets and their importance in allergic inflammation. Emerging data now provide new insights on the Type 2 immune response that is an immune response to allergens and involves Th2 cells, Type 2 B cells, ILC2s, Type 2 macrophages, a small fraction of IL-4-secreting NK cells, IL-4-secreting NK-T cells, basophils, eosinophils and mast cells.[113] At the same time, it has also been established that viral infection synergizes with allergic inflammation causing more severe exacerbations and symptoms compared to both conditions alone.[114,115] There are increasing evidences that most respiratory viral infections could trigger or exacerbate chronic Type 2 inflammatory responses via excessive release of chemokines and cytokines into the airways.[116–118] While much of these studies focus on lower airway inflammatory diseases instead of AR, insights from these studies can be applied to ongoing studies of virus-induced AR exacerbations and the search for its associated markers.

Like other chronic airway inflammatory diseases, AR patients also suffer from altered responses and potentially increased susceptibility toward viral infection.[119–121] This is similarly due to the reduced Type 3 interferon response, which is crucial against incoming viral infection in the upper airway.[119–121] Hence, markers for virus-induced AR exacerbation may have a significant overlap with findings from other inflammatory airway diseases. Proinflammatory cytokines such as TNF-α, IL-4, IL-5, IL-13, RANTES, Eotaxin, TSLP, IL-25 and IL-33 are usually expressed at higher concentrations in chronically inflamed airways, some of which are also found in AR.[113,122,123] These cytokines can be further triggered directly or indirectly by virus-induced IFNs, cytokines and chemokines. Infections such as RSV can even further shunt antiviral responses toward a more Type 2-centric response.[124–128] In addition, the discovery of ILC2s, a group of lymphoid cells, further emphasized the role of epithelial alarmins IL-25, IL-33 and TSLP in viral-induced exacerbation.[129] During viral infection, these three cytokines were secreted in response to epithelial injury.[130–132] It has been reported that IL-5, IL-13 and IL-33 levels were elevated in both the BAL and nasal fluid of asthmatics after RV16 infection compared to healthy subjects. The nasal IL-33 level was significantly and positively correlated with the total lower respiratory symptom score. Moreover, IL-33 secreted by RV-infected BECs directly induced IL-5 and IL-13 production by human blood ILC2s.[133] Together, it indicated that activated ILC2s in the upper and lower airways could cooperate to aggravate a Type 2 inflammation resulting in acute viral exacerbation. However, there are higher ILC2 levels in the blood of allergic asthmatics compared to patients with allergic rhinitis and even higher levels in patients with combined

asthma and AR.[87,134] Furthermore, ILC2s from allergic asthmatics were more responsive to IL-33 and IL-2 treatment compared to patients with allergic rhinitis.[134] These differences may cause the diverse severity of allergic airway diseases (Figure 4).

In addition, respiratory viral infections may also exacerbate chronic airway inflammatory diseases, including allergic inflammation through other non-Type 2 mechanisms, in which other markers can also be used as an indicator of these exacerbations. Viral infections can lead to the destruction of epithelial barrier and disruption of mucociliary function due in part to cell death in the virus-infected epithelium. Hence, markers for cell death (e.g, RIP3) and mucociliary dysfunction (e.g, MCIDAS) constitute part of the viral exacerbation repertoire.[124] Viral infection also causes increase in factors such as OSM and ANGPTL4 which disrupts tight junctions leading to increased allergen invasion and their contact with immune cells in the sub-epithelium region, thereby exacerbating allergic symptoms.[135,136] In addition, miRNAs are increasingly implicated in the mis-regulation of inflammatory responses and several of them are found to be dysregulated in an inflamed airway. For example, expressional changes of miRNAs such as miR-21 may coincide with viral infection responses and hence linked to virus-induced exacerbations.[137] Finally, an emerging field of bioenergetics and mitochondrial function may also contribute to the mechanism of virus-induced exacerbation in AR. Oxidative stress and mitochondrial dysfunction from viral infection may induce increased inflammation, and thus ROS and its associated markers may potentially serve as key markers for viral exacerbation.[138,139]

7 | BIOMARKERS IN CHRONIC RHINOSINUSITIS

CRS can be divided into different pheno- and endotypes. The mostly used phenotype is the division into CRS with and without nasal polyps (CRSwNP and CRSsNP), although many other pheno- and endotypes are known.[28,140] However, recently, the options to treat with biologicals have put more emphasis on markers of Th2 disease irrespective of the presence of nasal polyps. The first Type 2 targeting biologic anti-IL4Rα (Dupilumab) has entered the market for CRSwNP patients, and others like anti-IgE, anti-IL5 and anti-IL5Rα may follow shortly.[141–143] Cluster analysis of CRS has shown that CRSsNP and CRSwNP are not dichotomous but instead have overlapping inflammatory signatures with Type 2 inflammation as the predominant endotype mainly in CRSwNP but also CRSsNP, especially in western parts of the world. Interestingly, some patients with CRS express a mixture of two or more inflammatory endotypes.[22,144,145] The recently published EPOS2020 proposes a new clinical classification based on the disease being localized (often unilateral) or diffuse (always bilateral). Both these groups can be further divided into Th2 or non-Th2 disease.[4]

In the very near future, it may be possible to offer personalized medicine for CRS patients where treatment is based on molecular
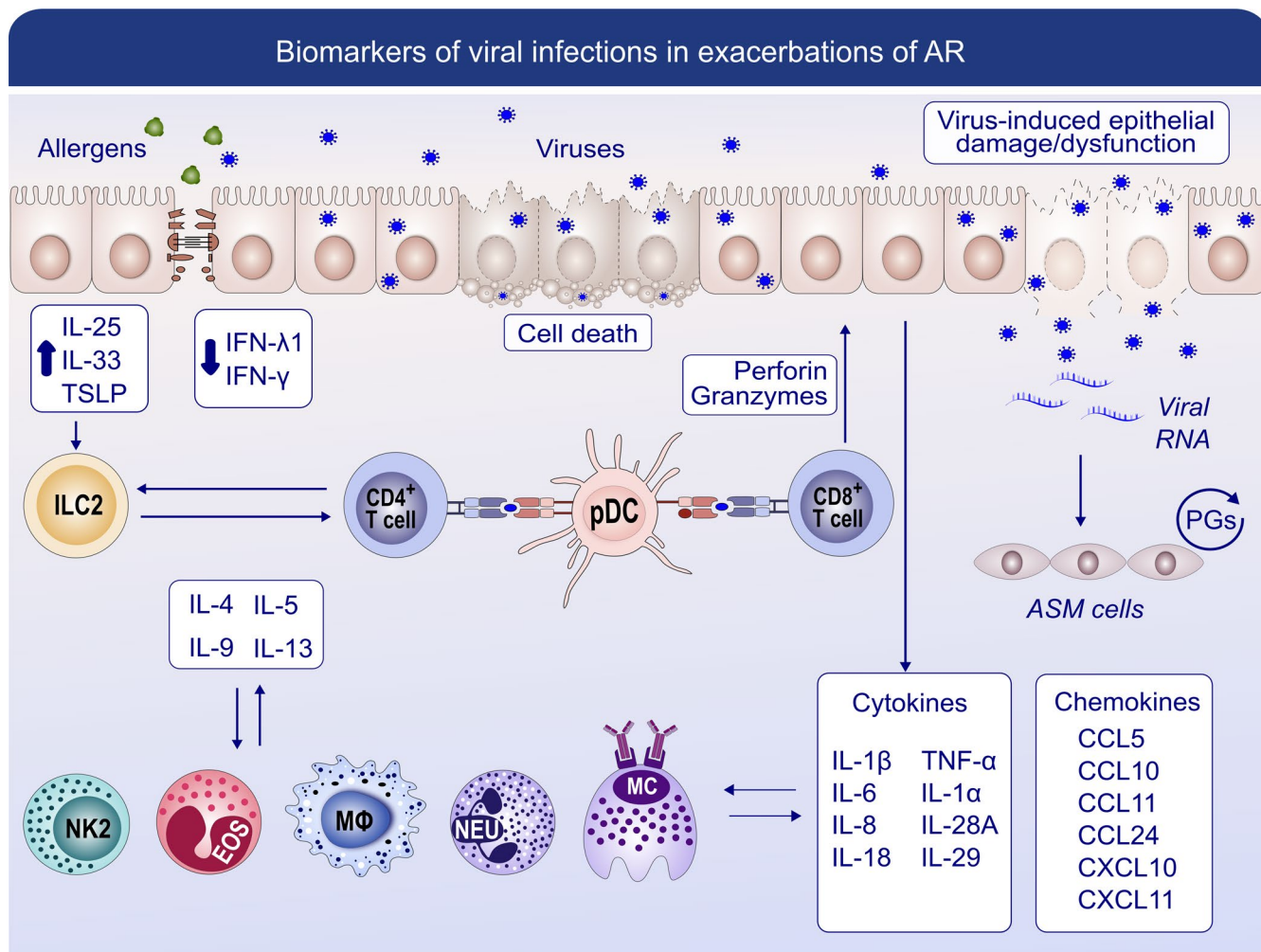
FIGURE 4 Biomarkers of viral infections in the exacerbation of AR. After the epithelial cells are infected with viruses, the replicating virus can cause cell lysis and direct damage to the epithelium, which causes deficiency in the production of antiviral interferon (IFN)-β and IFN-λ1. Together with the allergen-induced cytokines IL-25, IL-33 and TSLP, ILC2s are activated and produce more Type 2 cytokines. Subepithelial plasmacytoid dendritic cells (pDCs) recognize virus antigens and present them to CD4+ T cells and CD8+ T cells through MHC class II or I and drive them toward a more Type 2 centric response. Excessive release of chemokines and cytokines can be triggered by infections such as respiratory–syncytial virus (RSV). Together with Type 2 cytokines, they could further promote the function of Type 2 macrophages, a small fraction of IL-4-secreting NK cells, IL-4-secreting NK-T cells, neutrophils, eosinophils and mast cells and augment Type 2 responses in chronically inflamed airways. With the production of perforin and granzymes, CD8+ T cells can show cytotoxicity to virus-infected epithelial cells and induce apoptosis. The viral RNA is released and detected by airway smooth muscle cells and stimulates the production of prostaglandins (PGs) in an autocrine manner

biomarkers for the endotype or subendotype activated in an individual patient.[27,146] The major challenge is to find reliable biomarkers that define Th2 inflammation and predict reaction to treatment. Ideally, these biomarkers should be supported by a body of evidence clarifying the biological significance, be quantifiable in a cost-efficient way and be easily measurable, preferably in blood or nasal secretion.[14] Potential biomarkers could be eosinophils, neutrophils,[147,148] IgE,[149] Th2 cytokines,[150] innate (epithelial) cytokines,[123,149,151] but also phenotypical phenomena like smell loss,[152] asthma and response to systemic corticosteroids.[146] Contrary to FeNO in asthma, nasal NO has not been shown to be helpful to identify the T2 endotype because the main source of production of nasal NO is the sinuses that are closed off when CRS occurs.[153] The main biomarkers used at the moment to define Th2 disease are eosinophils, IgE levels and in some more specialized centers periostin and/or IL-5. There is quite some evidence showing that tissue and blood eosinophils are a reasonable surrogate marker for Th2 disease and that blood eosinophils are a reasonable biomarker to predict eosinophilic CRS with or without nasal polyps.[145] On the other hand, a lack of tissue eosinophilia, lower serum eosinophilia and absence of tissue squamous metaplasia may predict a CRS phenotype suitable for a trial of long-term macrolide therapy when surgery and topical therapy have failed.[154] Unfortunately, recent large studies with monoclonal antibodies directed to Type 2 endotypes have not found reliable biomarkers to predict response to treatment.[141,142,155–157] As in asthma,[8] we need large, maybe real-life studies to find better predictors to identify responders to biological treatments. For now, our treatment decisions still heavily rely on phenotypical characteristics such as smell loss, asthma and response to surgery and systemic corticosteroids.[4,27]

8 | BIOMARKERS IN FOOD ALLERGY

Apart from clinical determinants of food allergy and the respective gold standard, the oral food challenge, biomarkers represent an area of extensive research. In food allergy, the focus is on genetic risk factors, allergen-specific and nonspecific humoral and cellular biomarkers.[158–160]

Although genetic markers for food allergy are not yet at the level of clinical relevance, genes linked to HLA-genes, and more importantly to epithelial integrity and consequently reduced barrier function like filaggrin, SPINK5[161] and SERPINB7, are linked to eczema development and also food allergy.[162–168] Given the importance of the exposome in allergy development, epigenetics may even play a more important role. Promising results in the context of peanut allergy await replication in larger cohorts.[169,170] Regulation at another level has been linked to the microRNA 193a-5p. It is involved in the posttranscriptional regulation of IL-4 and downregulated in PBMCs from milk allergic children.[171] Due to the importance of barrier (dys) function in atopic diseases,[172–174] measurement of skin integrity may be a very important tool to identify high-risk populations. Electrical impedance spectroscopy, successfully tested in rodents, may be capable of assessing this biomarker also in humans.[175]

Allergen extract-based testing methodologies like skin prick test (SPT) and/or specific IgE (sIgE) tend to be less efficient for the diagnosis of food allergy. Thus, more specific approaches focusing on specific allergens (see section on allergens) and epitope-specific antibody response patterns are explored.[176] Diversity of IgE binding to linear epitopes correlated with the severity of peanut and milk allergy,[177–179] and IgG4 and IgE antibody binding to specific milk epitopes was stronger and more diverse in children who do not outgrow their milk allergy.[178] By measuring IgE and IgG4 responses with bead-immobilized milk epitopes and applying machine learning approaches, nonreactivity to baked milk could be predicted twice as successful as by conventional approaches.[180–182] The soluble high-affinity IgE receptor (FcεRI) may also act as a biomarker for IgE-mediated pathologies in a less allergen-independent way.[183]

Although allergen-specific T-cells are extremely rare, they display a pronounced Th2 type in allergic individuals.[184,185] A subset of allergen-specific memory Th2 cells called T_H2a cells, which are $CD45^+CD27^-CD45RB^-CRTH2^+CD161^+CD49^+$, has been discovered. They are almost exclusively found in allergic individuals, secrete IL-5 and IL-9, and within that group, the percentage of Type 2 cytokine double, triple, or quadruple positive cells is higher compared to Th2 cells. Moreover, mRNA expression of IL-25, IL-33 and TSLP receptors is higher.[186]

Our understanding of B-cell regulation has significantly evolved over the last few years.[187] Evidence is pointing toward an extreme rarity of IgE memory B-cells in peripheral blood of allergic individuals, which may be absent in nonpeanut allergic individuals.[188] New therapeutic and diagnostic options opened up from data on allergen-specific monoclonal antibodies that were generated via single-cell sorting of allergen-specific memory B-cells.[189,190] B-cell IgE antibody mutational maturation has been associated with barrier dysfunction.[191] Recently, the co-emergence of short-lived IgE plasmablasts and IgG memory B-cells early in grass AIT in the absence of memory IgE + B-cells has

been reported.[192,193] Both subsets shared clonotypes supporting the existence of pools of specific B-cell subsets, e.g, from IgG1-positive allergen-specific B-cells upon switch factors and stimulation as demonstrated in mice.[192,194] Yet many questions on the emergence of IgE-producing cells and their regulation have to be answered, and new biomarkers in this context will arise.

Functional tests that simulate allergen exposure in vitro like the basophil activation test (BAT) offer the possibility to assess allergen-induced IgE cross-linking. The BAT suggests adding significant diagnostic value to IgE-based test methods.[195–197] Promising results on the usage of passive sensitization strategies, mast cell lines[198] or precursors[199] have been reported.

There is still a significant need to develop biomarkers to diagnose and predict anaphylaxis to prevent near fatalities and fatalities.[200,201] Beyond tryptase, which can be a very good marker in the emergency setting when baseline values exist,[82,202] predictors of life-threatening reactions which can be measured on a routine basis or in multicenter trials are still missing.

There is the hope that the expanding array of novel mechanistic and diagnostic biomarkers provide the toolkit to develop algorithms or machine learning approaches to diagnose food allergy and predict treatment outcomes (Figure 5).

9 | sIGE AS BIOMARKER IN DIAGNOSIS OF FOOD ALLERGY

Accurate diagnoses are essential for the management of food allergy.[159] They depend on a detailed clinical history, objective markers of sensitization and double-blind placebo-controlled food challenges (DBPCFC).[203] These are time-consuming and require specialized medical facilities, and side-effects may occur. Consequently, molecular allergy diagnosis aims to reveal significant associations between sIgE and clinical phenotypes.

9.1 | Peanut

A retrospective study of 205 peanut-challenged Danish patients found the best correlation between sIgE and clinical thresholds for the 2S albumin Ara h 2.[204] A diagnostic model for peanut allergy predicted the food challenge outcome with 100% accuracy in 59% versus Ara h 2 in 50% of 100 Danish peanut-allergic patients.[205] Co-sensitization to Ara h 2 and 6 was associated with severe allergy in peanut-challenged Finnish patients.[206] A French study of 48 peanut-allergic children found that Ara h 2 sIgE titers could predict the risk of anaphylaxis.[207]

9.2 | Soy

The cupins Gly m 5 and 6 were suggested as markers for severe allergic reactions in 30 soy-allergic European patients.[208] A
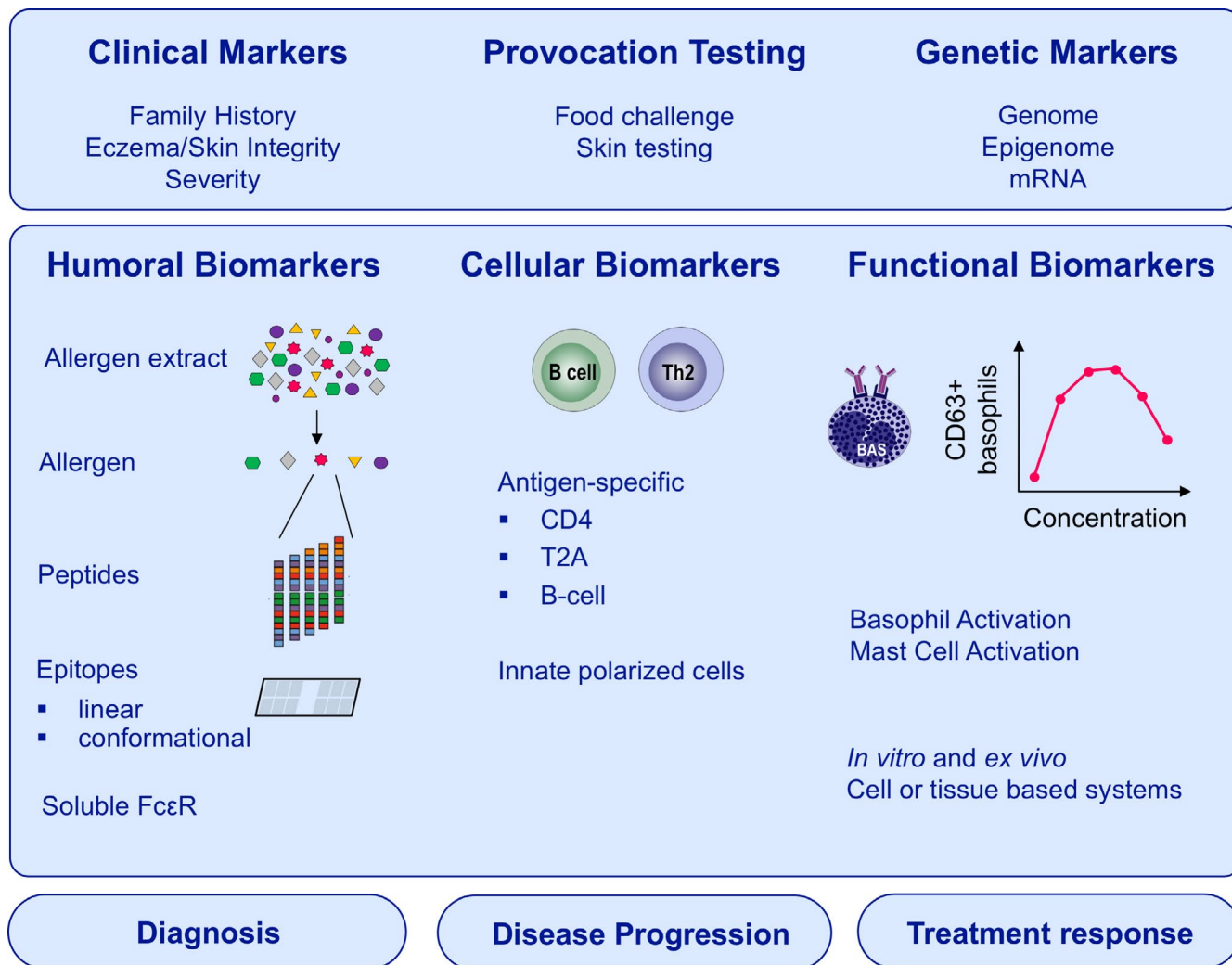
FIGURE 5 Biomarkers in food allergy diagnosis and treatment outcomes prediction. Conventional clinical approaches to diagnose food allergy include family history, skin integrity and the oral food challenge. Nowadays, expanded approaches focusing on genetic risk factors, allergen-specific and nonspecific humoral and cellular biomarkers were explored. Genome, epigenome and mRNA linked to epithelial integrity and barrier (dys)function are linked to the development of food allergy. The measurement of IgE and IgG4 binding to linear or conformational epitopes could be more powerful to diagnose food allergy than conventional approaches. The soluble high-affinity IgE receptor (FcεRI) may also act as a biomarker for IgE-mediated pathologies in a less allergen-independent way. Moreover, allergen-specific Th2A cells and memory B cells have been discovered as new cellular biomarkers. Functional tests that simulate allergen exposure in vitro or ex vivo like the basophil activation test (BAT) and mast cell activation test (MAT) offer the possibility to assess allergen-induced IgE cross-linking

high diagnostic value of the 2S albumin Gly m 8 was reported in soybean-sensitized Japanese children with and without symptoms.[209] Gly m 8 had an AUC = 0.75 for soy allergy, while the values for Gly m 5 and 6 were 0.69 and 0.64, respectively. In a study on soy allergy diagnostics, Gly m 8 had the highest AUC (0.79), comparable to skin prick test (0.76) and sIgE to soy extract (0.77).[210] In this study, the cupins Gly m 5 and 6 were related to mild symptoms.

9.3 | Hazelnut

sIgE to the cupin Cor a 9 and the 2S albumin Cor a 14 was strongly associated with clinical symptoms in 161 Dutch hazelnut-sensitized

patients.[211] sIgE to Cor a 9 and 14 allowed correct diagnosis of 90% of severely hazelnut-allergic Belgian children.[212] In 423 hazelnut-allergic patients, Cor a 9 and 14 were associated with severe symptoms (AUC = 0.70).[213] A model combining clinical symptoms and sIgE to Cor a 14 and walnut increased the AUC to 0.91. In a prospective multicenter study of 90 peanut- and 44 hazelnut-allergic German children, a 90% probability for a positive food challenge was calculated for Ara h 2-specific IgE at 14.4 kU/L and for Cor a 14-specific IgE at 47.8 kU/L.[214]

9.4 | Walnut

sIgE to the 2S albumin Jug r 1, the cupin Jug r 2 and the nsLTP1 Jug r 3 (AUC = 0.79, 0.70, 0.62, resp.) predicted anaphylaxis in 45

TABLE 1 Specific IgE to these allergens is associated with severe symptoms

Allergen source	2S albumin	Cupin *vicilin type **legumin type	nsLTP1	Ovomucoid	Tropomyosin	Caseins	References
Peanut	Ara h 2 Ara h 6	Ara h 1*					204–207,218
Soy	Gly m 8	Gly m 5* Gly m 6**					208–210
Hazelnut	Cora 14	Cor a 9**					211–214,218
Walnut	Jug r 1	Jug r 2* Jug r 4**	Jug r 3				215,216,218
Cashew	Ana o 3	Ana o 1* Ana o 2**					219–221
Egg				Gal d 1			222
Shrimp					Lit v 4		223
Cow's milk						Bos d 8	225

walnut-allergic children.[215] In 91 walnut-allergic subjects from Switzerland, Germany and Spain, severe reactions correlated with higher sIgE levels to Jug r 1 and the cupin Jug r 4.[216] sIgE to Jug r 1 (AUC = 0.79) from 32 walnut-allergic Korean children better discriminated clinical walnut allergy from tolerance than sIgE to walnut extract (AUC = 0.56).[217] In 34 peanut-, hazelnut- or walnut-allergic Italian children, sIgE to Ara h 1 and Ara h 2, Cor a 9 and particularly Cor a 14 or Jug r 1, 2 and 3 was associated with anaphylaxis.[218]

9.5 | Cashew

In 63 cashew-allergic Greek children, sIgE to the 2S albumin Ana o 3 (AUC = 0.97) performed better than extracts for predicting cashew allergy.[219] A markedly greater risk of a positive food challenge was observed for higher levels of sIgE to the cupins Ana o 1 and 2, and to Ana o 3 in 173 Dutch children with suspected cashew nut allergy.[220] Ana o 3 discriminated between allergic and tolerant children better than extract-specific IgE with an AUC = 0.94 versus 0.78. A 95% probability for a positive cashew challenge was estimated for Ana o 3-sIgE at 2.0 kU/L.[221]

9.6 | Egg and shrimp

sIgE to the ovomucoid Gal d 1 correlated with an increased risk of persistent egg allergy.[222] Sensitization to tropomyosin and sarcoplasmic calcium-binding protein was associated with clinical reactivity in 58 shrimp-allergic patients.[223]

9.7 | Cow's milk

Caseins (Bos d 8), the major protein fraction of cow's milk (80%), comprise four different proteins, αS1-casein (Bos d 9, 32%), αS2-casein

(Bos d 10, 10%), β-casein (Bos d 11, 28%) and κ-casein (Bos d 12, 10%). α-lactalbumins (Bos d 4) and β-lactoglobulins (Bos d 5) make up the whey proteins in cow's milk.[224] An Italian study including 79 children found that Bos d 8 could differentiate children at risk for cow's milk anaphylaxis (AUC = 0.718) compared to Bos d 4 (AUC = 0.491) and Bos d 5 (AUC = 0.634). The levels of Bos d 8 sIgE reflected the severity of the milk allergy.[225] Additionally, low or undetectable levels of Bos d 8-sIgE indicated tolerance to baked milk products.[226]

In conclusion, severe reactions to legume seeds and tree nuts are predominantly caused by sensitization to storage proteins rather than by pollen-related allergens such as Bet v 1 or profilin homologues, or nsLTPs (Table 1).

However, biomarkers for food allergy are also affected by geographical variations and can be age-related. In Mediterranean pediatric patients, Ara h 6 and Ara h 2 are the best predictors of peanut allergy with the prevalence of 64% and 63%, respectively.[227] In birch-endemic regions, preschool and school-aged children with systemic reactions to hazelnut are mostly sensitized to Cor a 9. However, adults in these regions are highly sensitized to Cor a 1.04. Therefore, it is important to take regional and age variations into account when working on sIgE for food allergy.[228]

10 | BIOMARKERS IN DRUG HYPERSENSITIVITY

Drug hypersensitivity reactions include those mediated by a specific immunological mechanism and those nonspecific immune mediated (Figure 6).[229]

10.1 | Immunologically mediated specific reactions

These reactions are classified into immediate reactions (IR) and non-immediate reactions (NIRs) depending on whether they occur within

1-6 hours or later after the drug intake. The first group is mostly associated with an IgE-mediated mechanism and the latter with a T-cell-dependent type.[229,230]

Skin tests (STs) are useful biomarkers for IRs to beta-lactam (BL).[14,229–232] However, their sensitivity based on the classical antigenic determinants has decreased over the last decades [233] possibly due to the changing patterns of consumption (e.g, amoxicillin/clavulanic acid is replacing penicillin).[231] Interestingly, one antigenic determinant recognized by most patients with confirmed reactions to clavulanic acid has been recently identified.[234] Therefore, amoxicillin and any suspected BL must be included when performing STs.[230,233,235,236] For other drugs and for NIRs, the value of STs is very limited.[14,230,231,237]

Regarding in vitro tests, during the acute phase of the reaction, serum tryptase is the most valuable biomarker for confirming mast cell activation in IR.[229] The expression of granzyme B and granulysin in blood cells may be useful for detecting lymphocyte activation in severe NIR.[238] At the resolution phase, immunoassays are used in IRs to determine sIgE, although the sensitivity is lower than for STs[229,239,240] and only commercially available for limited drugs. For BLs, it shows a variable sensitivity (0%-50%),[229,230,239] with the possibility of inducing false-positive results when testing for Penicillin V.[241] The value of BAT in IR has been proven for BL and quinolones, giving a sensitivity up to 55%[242,243] and 83%,[237,244] respectively. The sensitivity of both sIgE and BAT correlates with severity reaction,[239] decreases with time,[243,245] and depends on the activation basophil marker assessed, e.g, the detection of CD203c increases BAT sensitivity although remaining low (36.4%).[244,246]

The value of lymphocyte transformation test (LTT) in NIRs has shown to be unsatisfactory.[14,229] The Tim3/galectin-9 axis functions
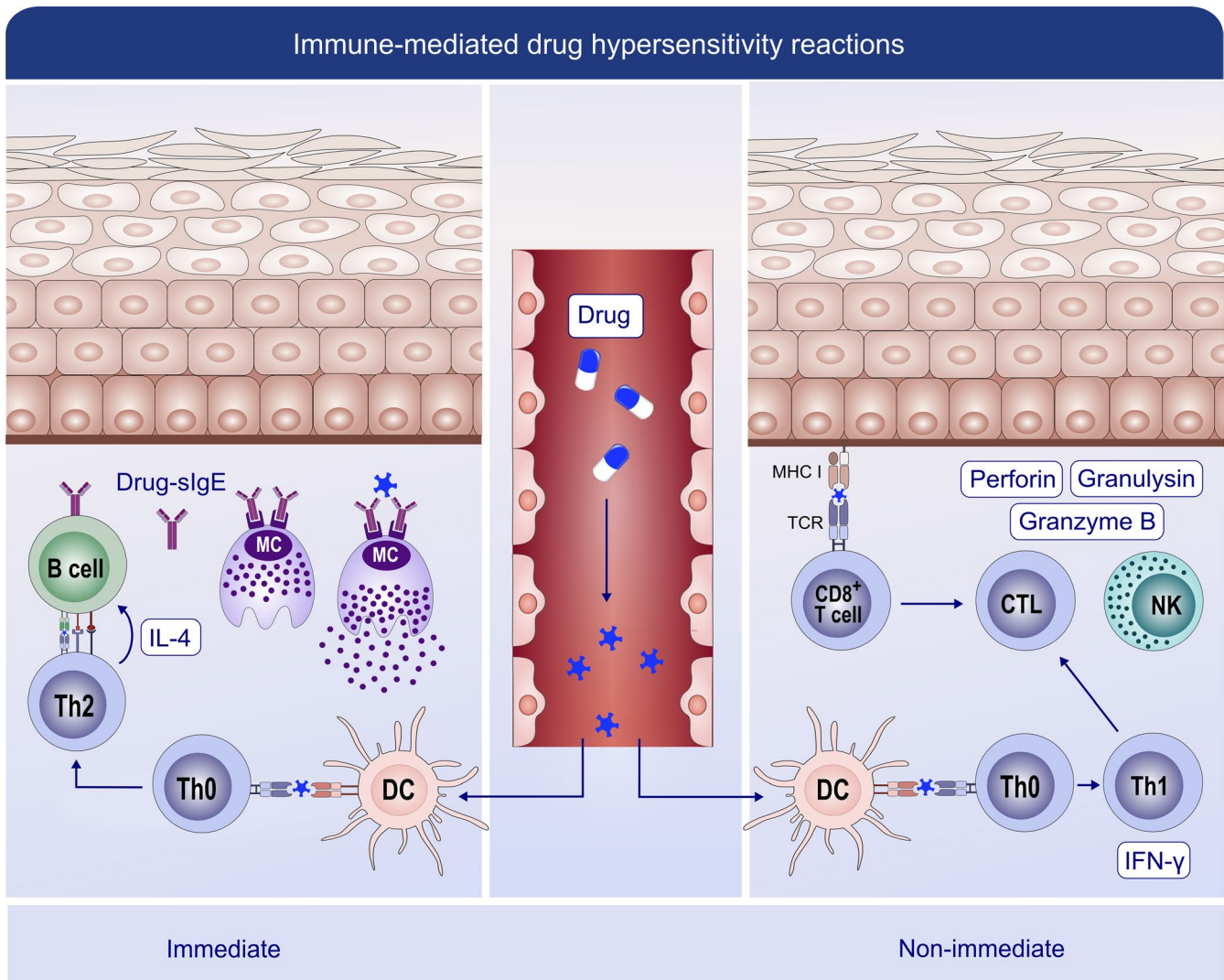
FIGURE 6 Mechanisms of immune-mediated reactions to drugs. These reactions encompass immediate reactions (mediated by IgE) and nonimmediate reactions (mediated by T cells). In immediate reactions, drug-induced polarization of Th2 cells from Th0 cells promotes B cells to produce specific IgE (sIgE). This sIgE binds to the FcεRI receptor on mast cells. In subsequent drug contacts, the simultaneous recognition by at least two sIgE initiates the degranulation and release of mediators. Nonimmediate reactions are generally characterized by a Th1 response with the increased secretion of IFN-γ from Th1 cells and granulysin from NK cells
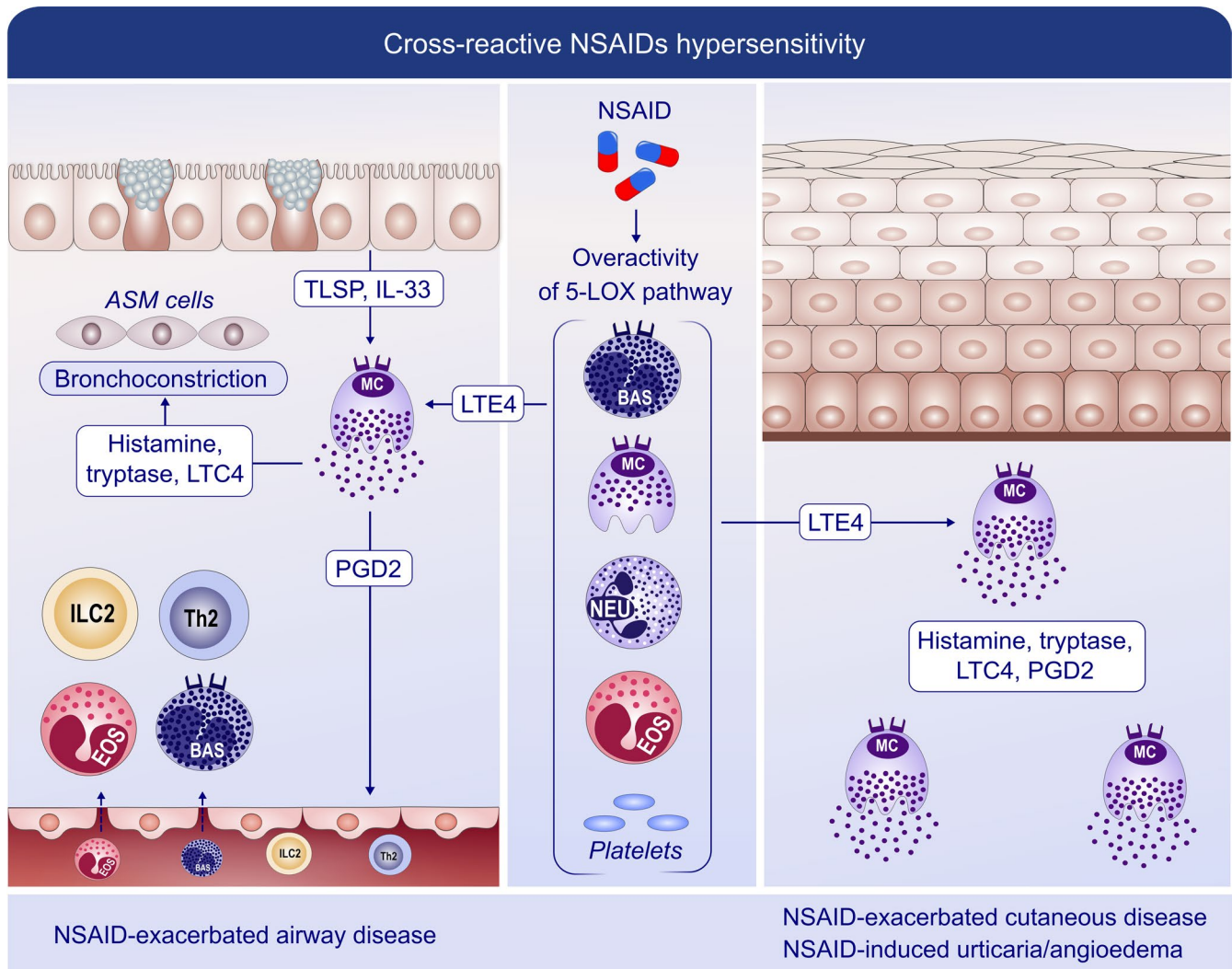
FIGURE 7 Mechanisms of cross-reactive hypersensitivity reactions to NSAIDs. NSAIDs induce reactions relying on their COX-1 inhibitory activity, *i.e*, activation of mast cells and other immune cells without involvement of adaptive immunity. During NSAID-exacerbated respiratory disease (NERD), the administration of NSAIDs permits strong 5-lipoxygenase (5LOX) activation and further generation of leukotriene E4 (LTE4). LTE4 induces the release of IL-33 and TSLP, and consequent mast-cell activation, with bronchoconstriction occurring as a result of the direct effects of leukotriene C4 (LTC4), prostaglandin D2 (PGD2) and other mast cell-derived products. PGD2 recruits effector cells such as Th2 cells, group 2 innate cells (ILC2s), basophils and eosinophils to the airway. Consistently, in NSAIDs-exacerbated cutaneous disease (NECD) and NSAIDs-induced urticarial angioedema (NIUA), increased PGD2 can act on the skin epidermis. In addition, cross-reactive hypersensitivity to NSAIDs may involve additional sources of inflammatory mediators, such as eosinophils and platelets. ILC2: innate lymphoid cells 2; LTE4/C4: leukotriene E4/C4; NSAID: nonsteroidal anti-inflammatory drugs; PGD2: prostaglandin D2; TSLP: thymic stromal lymphopoietin; and 5LOX: 5-lipooxygenase

as a checkpoint inhibitor for Th1 cells. Interestingly, Th1 cells and dendritic cells of patients with drug-induced maculopapular exanthema expressed lower levels of Tim3/galectin-9 at baseline compared with tolerant individuals.[247] This observation might help identify subjects at risk of NIRs.

10.2 | Immunologically mediated nonspecific reactions

The most important group in this type of reactions is cross-intolerance to NSAIDs (CI),[248,249] in which patients react to NSAIDs from different pharmacological groups related to its COX-1 inhibitory activity.[229,248] CI has been classically divided into phenotypes with exclusive skin involvement (NSAIDs-exacerbated cutaneous disease (NECD) and NSAIDs-induced urticarial angioedema (NIUA)) or with exclusive respiratory involvement (NSAIDs-exacerbated respiratory disease, NERD).[28] Nevertheless, novel data indicate that almost 30% of patients with CI can experience blended reactions, especially involving skin and airways.[250] Genetic predisposition might account for the burden of some CI phenotypes (e.g, variants of *GNAI2* in NIUA) (Figure 7).[251]

As the underlying mechanism is related to arachidonic acid metabolism, potential biomarkers focus on determining leukotriene and

prostaglandin metabolites. Therefore, urinary LTE4 has been reported as a useful biomarker to distinguish between different subphenotypes in NERD,[252] between NERD and aspirin-tolerant asthma[252] and phenotypes with skin involvement (NIUA and NECD).[21] Sensitivity and specificity of urinary LTE4 for identifying NERD have been reported to range from 0.55 to 0.81 and 0.77 to 0.82, respectively, which depends on the detection method used.[253] Serum LTE4 and LTE4/PGF2 ratio have also been reported as potential in vitro biomarkers for NERD[254] and urinary PGF2 for NIUA and NECD (Table 2).[21]

11 | BIOMARKERS IN IMMUNE MONITORING OF ALLERGEN-SPECIFIC IMMUNOTHERAPY

Allergen immunotherapy (AIT) is the only therapy that can alter the natural course of immune responses to specific allergens, directing it toward desensitization and perhaps, toward tolerance. Routes of AIT include subcutaneous (SCIT), sublingual (SLIT), oral (OIT) or epicutaneous (EPIT).[255,256] Licensed products are available for therapy of AR, allergic asthma, stinging insect hypersensitivity, AD.[257–262] AIT for food allergy is currently investigational; however, phase 3 trials are promising.[263] Current evidence indicates that immunological changes associated with AIT differ from permanent tolerance as in healthy nonallergic individuals. AIT-acquired desensitization is often temporary, waning with time, but can be maintained with regular exposure to the allergen.[256]

Biomarkers that can identify responders, monitor treatment, predict durability of desensitization and determine adverse event risk would aid clinical decisions and delivery of targeted and effective treatment. With the advent of data-driven "-omic" technologies, more potential biomarkers have been identified.[264] Here, we briefly review the more promising candidates that are being evaluated for immune monitoring with an expanded number in Figure 8.[265]

11.1 | Specific IgE and IgG

With the discovery of IgE in the 1960s, measurements of IgE have been a first step in diagnosis for atopic diseases. Both allergen-specific IgE (sIgE) and total IgE levels increase during the initial stages of AIT and subsequently decrease[266]; however, decreases may not accompany a positive clinical outcome.[267] Numerous studies indicate IgG1 and IgG4 levels increase with therapy but do not always differentiate between responders and nonresponders. IgG4 increases during AIT may reflect compliance not clinical efficacy.[267] sIgG4 is purported to be a blocking antibody by competing for allergen binding with IgE bound to Fcε receptors on mast cells and basophils, preventing degranulation. IgA and other subclasses of IgG may have similar blocking function.[268] Recent data support skewing toward IgG2 and IgG4 subclasses after SLIT for temperate grass pollen.[193] The IgG4/IgE ratio may monitor AIT progress and outcome, but has demonstrated conflicting utility.[269–271] Flow cytometry-based assay (IgE-FAB) and a

TABLE 2 Biomarkers for drug hypersensitivity

Biomarker	Disease	References
LTE4	Aspirin sensitivity in asthma	253,254
LTE4/PGF2 ratio	Aspirin sensitivity in asthma	254
LTE4 and PGF2	NIUA and NECD	21
Tryptase	Acute phase of IgE-mediated reactions	229,239
Skin tests	Immediate and nonimmediate reactions to drugs	231
Specific IgE	Immediate reactions to betalactams	240
Basophil activation	Immediate reactions to betalactams Immediate reactions to FQ	242,244
Lymphocyte transformation test	Nonimmediate reactions	239
Granzyme B and granulysin	Stevens–Johnson syndrome	238

solid-phase assay enzyme-linked immunosorbent-facilitated antigen binding (ELIFAB) assay can determine IgE-inhibitory activity. Although robust with good clinical efficacy correlation for AIT, these technologies are complex with usage limited to specialized centers.[267,272,273]

11.2 | Immunophenotyping of immune cells

Allergic sensitization is associated with multiple changes in blood immune cells,[187] and high-dimensional immunophenotyping using flow and mass spectrometry (i.e, Cytometry Time of Flight; CyTOF) has contributed greatly to this identification.[274] A study on bee venom AIT provided detailed characterization of allergen-specific B cells before and after bee venom tolerance in allergic patients and bee-keepers with Api m 1-specific B cells showing increased CCR5 expression after high-dose allergen exposure.[275] In a study of OIT for milk allergy, researchers found, using flow cytometry, a significant increase in blood invariant natural killer cells and a shift from a Type 2 T helper (Th-2; i.e, IL-4, IL-13) to a Th-1 (ie, IFN-γ) cytokine profile.[276] Similar studies in other immunotherapy models can assist with determining potential biomarkers for monitoring AIT.

Recent evidence shows that AIT modulates the balance between circulating T follicular helper (Tfh) and regulatory cells (Tfr), with Tfr as a potential biomarker for AIT efficacy.[277,278] Upregulation of the activated allergen-specific Tregs and downregulation of dysfunctional allergen-specific Treg cell subset, associated with improved clinical response, were recently described.[279] Responder status was shown to be associated with increased frequency of IgA- and IgG4-expressing allergen-specific B cells, plasmablasts and IL-10+ and/or IL-1RA + Breg cells.[280]

AIT-induced T regulatory cells secreting IL-35 (iTR35) cells promote production of IL-10 from CD19 + B cells, Breg subsets and Tfr cells.[281] Circulating Tfr cells share properties of memory cells and are

distinct from their lymph nodes (LN) counterpart as they suppress B and Tfh cells with a much lower capacity, while circulating memory-like Tfh cells are more potent than LN effector Tfh cells.[282] In addition, circulating memory-like Tfr cells persist for long periods; thus, they could support the long-term immunomodulatory effect of peptide AIT. Besides the accumulative effects, AIT also activated Treg functions.

(A)
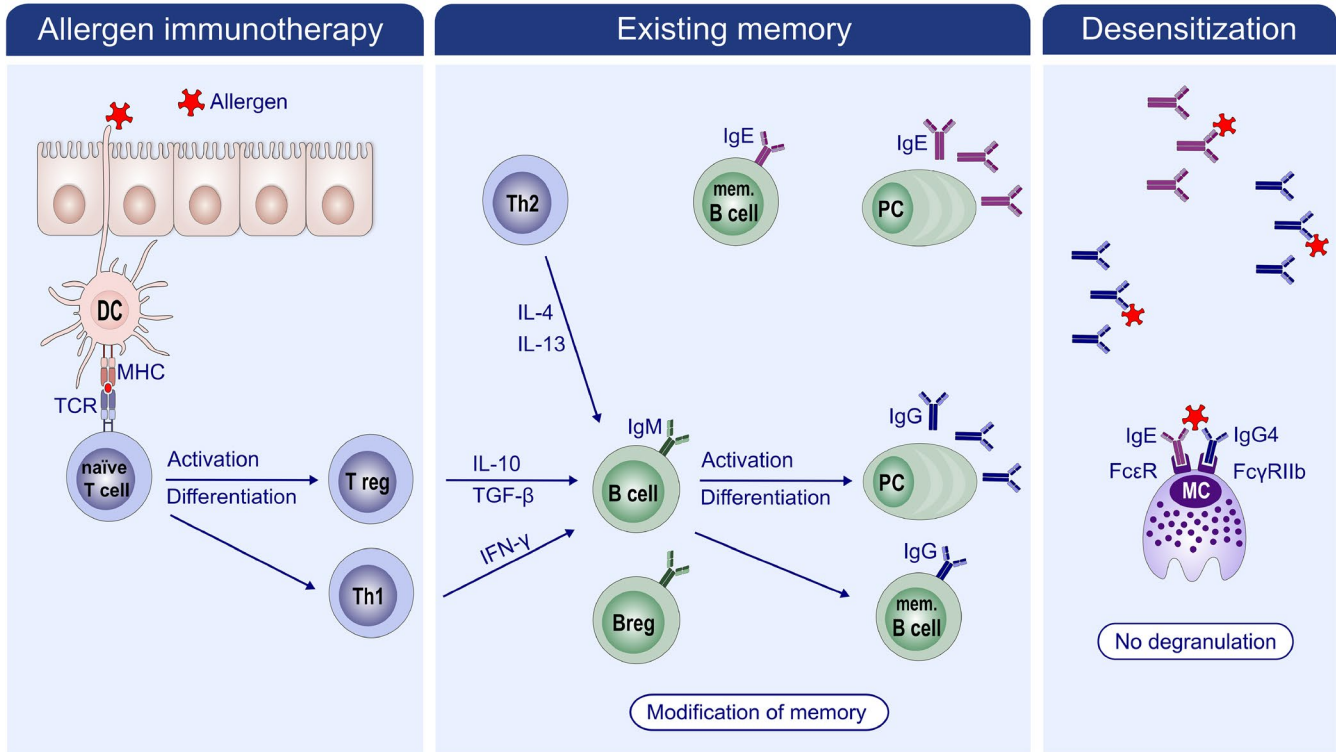
(B)

Domain	Type of target	Assays	Strengths	Limitations
I: IgE	Serum protein	Total IgE Allergen-specific IgE	Serum markers; sIgE gold standard patient selection for AIT	No correlation of levels with therapy success
II: IgG subclasses	Serum protein	Allergen-specific IgG1 Allergen-specific IgG4	Serum markers; sIgG4 elevated by AIT	Failure in sIgG4 induction might reflect inadequate treatment compliance
III: Serum inhibitory activity for IgE	Serum protein	IgE-FAB IgE-BF	Serum-based assay; functional output	Laborious and requires specialized reagents/equipment
IV: Basophil activation	Cell activation	Flow cytometry of allergen-activated basophils (CD63, CD203c, BHR, DAO)	Requires small amount of blood	*In vitro* assay, which is difficult to optimize and standardize
V: Cytokines and chemokines	Serum protein	ELISA or Luminex for: IL-2, IL-2R, IL-4/5/6, IL-8/9/10, IL-13/18, IFN-γ, ECP, Eotaxin, MCP-1, TARC, transthyretin	Serum-based assay; high-throughput	Low sensitivity for many cytokines in serum
VI: Cellular biomarkers	Cell phenotyping / activation	Immunophenotyping of *ex vivo* or *in vitro* activated cells: CD4+ T cells, Treg, Breg, DC	Multiple cell subsets can be measured in single analysis from small blood sample	Lack of standardization in definitions and evaluation of most cell types. Antigen-specific cells can currently only be measured after *in vitro* culture (laborious)
VII: *In vivo* biomarkers	Allergen reaction	Allergen provocation tests (SPT, NAPT, BAPT, CAPT) Allergen challenge chambers	Key tool to diagnose local allergic rhinitis	Possibility of adverse effects. Nonspecific reactions caused by glycerol, benzalkonium chloride, or phenol.

FIGURE 8 Current view on mechanism and biomarkers in use to monitor AIT. A, Scheme of immune modulation by AIT, where low-dose, repeated exposure to allergen is thought to occur with limited to no inflammation. As a result, Th skewing is balanced toward Th1 and Treg, which subsequently modify the B-cell response. In particular, the production of IL-10 is thought to drive IgG4 class switching. Thus, local and systemic memory is rebalanced, in both the T-cell and the B-cell compartments, and there is a strong increase in allergen-specific IgG4 antibodies. Upon allergen challenge, IgG4 and potentially other soluble factors are thought to inhibit IgE-mediated degranulation of target cells, i,e, desensitization. Together with the loss of Th2 skewing, this underlies the observed clinical tolerance. B, Laboratory biomarkers utilized in diagnostics and clinical trials for AIT (adapted from[267]). Abbreviations: IgE-FAB, IgE-facilitated allergen binding; IgE-BF, IgE-blocking factor; BHR, basophil histamine release; DAO, diamine oxidase; Treg, regulatory T cell; Breg, regulatory B-cell; DC, dendritic cell. Figure reproduced from[265]

Treg cells isolated from *Dermatophagoides pteronyssinus* (DP) AR patients treated with DP-SCIT for 12 months could suppress the function and proliferation of Th2 and Th9 cells, but increased the proportion of IL-10-producing cells.[283] Functional evaluation of T regulatory cells via expression of glycoprotein A repetitions predominant (GARP) and special AT-rich sequence binding protein 1 (SATB1) is also interesting as a future potential biomarker. Tregs uniquely express GARP on their cell surface, and GARP functions as a delivery system for latent TGF-β, which might augment the immunosuppressive role of Tregs on effector cells.[284] GARP expression was described as an activation marker of parasitic infection-induced Tregs that strongly suppress allergic inflammation; thus, it is a novel potential mechanistic pathway for AIT. SATB1 is a genome organizer protein expressed in a lineage-specific manner in CD4 + T-cells. During the early Th2 cell differentiation, IL-5 expression is repressed through direct binding of SATB1 to the IL-5 promoter. Thus, SATB1 modulation might expand the impact of AIT on eosinophilic inflammation. In addition, SATB1-dependent Treg-cell-specific super-enhancers activation is crucial for Treg cell lineage specification in the thymus.

11.3 | Basophil activation test

Blood basophils express high levels of FcεRI that have bound IgE. To determine sensitization, basophils are incubated in vitro with allergen, followed by examination of degranulation. Fusion of granules to the cell membrane leads to upregulation of surface markers CD63 and CD203c, which are indicative of specific IgE functional activity. In patients with AR undergoing SLIT, basophil activation decreased with clinical efficacy.[285] Similar decreases occurred in patients undergoing peanut immunotherapy.[271] However, in grass pollen SLIT, basophil activation did not correlate with clinical efficacy (Table 3).[286]

11.4 | Soluble cytokines

With immunotherapy, there is redirection of Th2 phenotype toward a Th1 and Treg phenotype with decreases in Th2 cytokines (eg, IL-4, IL-19 13, IL-9) and upregulation of Th1 (eg, IFNγ) and regulatory cytokines (eg, TGFβ and IL-10).[193,298] However, serum cytokine measurements are challenging due to low levels, which are often below the detection limit of current methods.[267] Changes in cytokine production by CD4 T cells following AIT are assessable through in vitro stimulation of PBMC from patients using allergen extracts, at both the protein and the transcript levels. In T-cell of patients with HDM allergy, high levels of *IL10* transcripts predicted immunotherapy success.[299]

11.5 | Epigenetic biomarkers

Beyond cells, proteins and transcripts, epigenetic biomarkers may suggest prognosis.[300,301] DNA methylation of promoter regions is associated with gene silencing, and *FOXP3* gene expression is in part controlled through this. FoxP3 is the master regulator of Treg cells, and Syed et al found that peanut OIT resulted in hypomethylation of *FOXP3*, indicating increased gene expression with immunotherapy.[302]

In conclusion, technological advances inform novel methods for monitoring immune responses with increased sensitivity and specificity. Studies indicate dysregulation of a number of molecular

TABLE 3 Identification of changes in immune cells associated with allergic sensitization

Immune cell type	Biomarkers	Assay	References
Basophils	Decrease in basophil activation post-allergen-specific OIT (CD63, CD203, histamine release)	BAT, iBAT	287–289
B cells	Induction of Breg cells on AIT, serological readouts: food, and allergen-specific IgE, IgG4 epitope-specific IgE and IgG4	Flow cytometry, ImmunoCAP, epitope mapping	182,271,280,290
T cells	Functional changes in tetramer positive/CFSElo/CD69 + CD40L+ allergen-specific CD4 + T-cells, tracking Th2A cells Decrease in T-cell-derived Type 2 cytokines Epigenetic changes in T-cell subsets (Tregs)	Flow and mass cytometry, Luminex/ELISA, pyrosequencing	291–296
Dendritic cells	Changes in costimulatory potential (CD80, CD86)	Flow cytometry, DC-T cell co-culture	297,302

markers with AIT. Detection method optimization continues and should facilitate precision immunotherapy.

12 | BIOMARKERS OF THE EPITHELIAL BARRIER

Environmental factors, microbiome, epithelial cells and immune cells show a dynamic cross talk at the skin and mucosal barriers in the development of AD, AR, CRS, eosinophilic esophagitis and asthma.[174,303–308] Studies on the pathogenesis of these diseases have clearly demonstrated a barrier defect in the skin and involved mucosas and a systemic inflammation.[307–310] Defects in the epithelial barriers, caused by both environmental risk factors and a genetic predisposition, may represent the starting point of a chronic inflammation and allergen sensitization.[307–310] A significant number of studies have reported that environmental factors directly affect the barrier function of epithelium.[162,311,312] In addition, T helper 2 cells, ILC2s and their cytokine IL-13 damage skin and lung barriers.[307,313] In addition, mast cells and their enzyme chymase damage the epithelial barrier.[304,314] The effects of environmental factors may, at least in part, be mediated by epigenetic mechanisms. Histone deacetylase activation by Type 2 immune response has a major effect on leaky barriers, and blocking of histone deacetylase activity corrects the defective barrier in human air–liquid interface cultures and mouse models of allergic asthma with rhinitis.[107,307,315] The assessment of the barrier function of the skin and mucosas in vivo has an extremely high value in the clinics to identify barrier leakiness for an individual patient and requires the discovery of biomarkers.

The value of barrier detection in allergic diseases

- Early prediction of atopic dermatitis development in babies
- Patient selection for certain therapies, endotyping and personalized medicine
- Early prediction and monitoring response to treatments
- Early and rapid analyses of environmental hazards to skin and mucosas before severe lesions start

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To date, there are a few noninvasive methods to assess the skin epidermal barrier function in vivo. The quantification of transepidermal water loss (TEWL) in the skin across the stratum corneum has received some interest for early prediction of atopy prone children and detection of skin barrier.[316] Although TEWL increases in proportion to the level of damage, it is also affected by environmental factors such as humidity, temperature, season and moisture content of the skin.[317] The noninvasive and rapid measurement of natural moisturizing factor by Raman spectroscopy provides a method suitable for use in children. The association of natural moisturizing factor, filaggrin null mutations and AD suggests Raman spectroscopy as a promising approach for stratification of endotypes in AD in the clinics. Other noninvasive methods currently used include assessment of the stratum corneum hydration, colorimetry, skin surface pH and sebometry, but they only provide information on different characteristics and/or the condition of the skin and do not directly measure the barrier function.[318]

Recently, electrical impedance spectroscopy (EIS) has been identified as an effective and stable tool for the detection of epidermal barrier.[175] This method works by transmitting a harmless electrical signal through the skin at several depths and frequencies and measuring the electrical resistance and impedance response of the tissue. EIS reflects particularly the tissue barrier status by collecting the electric impedance information from extracellular and intracellular tissue.[319] Recently, Rinaldi et al have studied the impairment of the epidermal barrier in mice by the epicutaneous administration of barrier-damaging proteases, such as papain, trypsin, Vibrio cholera toxin or by tape stripping.[175] They showed the barrier-damaging effects of these substances which correlate with the electric conductivity of the skin, causing a decrease in electrical impedance. According to these results, EIS shows a broad range of possible clinical applications in AD and atopic march, including early prediction of atopy-prone children, early diagnosis of the disease, stratification and endotyping of patients, evaluation of the overall therapy response as well as single lesions and assessment of disease severity.

A critical feature of the gastrointestinal epithelium is intestinal barrier permeability as it must allow an efficient passage of nutrients while restricting the entry of larger molecules to avoid food allergy development. Multiple autoimmune diseases have been identified to arise or be exacerbated by a leaky gut, such as in inflammatory bowel disease, celiac disease, Type 1 diabetes, systemic lupus erythematosus, multiple sclerosis.[320–323] Recent studies point to a leaky gut as the initiator of Type 1 diabetes because a subclinical intestinal barrier dysfunction was already detected before clinical onset of Type 1 diabetes.[324] Zonulin is a prehaptoglobulin protein and a biomarker for gut barrier leakiness that downregulates TJ function, and it has been proposed to play a role in several autoimmune diseases.[325]

Translocation of bacterial endotoxin (lipopolysaccharide, LPS) from the gut microbiota to blood circulation stimulates systemic inflammatory responses.[326] Measurement of intestinal permeability is often used in the examination of inflammatory gastrointestinal disorders. It can be assessed by measurement of urinary recovery of ingested nonmetabolizable lactulose and mannitol. Urine L/M ratio measured by ^{1}H NMR spectroscopy showed high correlation with the standard measurement of the urinary recoveries by enzymatic assays. In conclusion, NMR metabolomics enables simultaneous intestinal permeability testing and discovery of biomarkers associated with an impaired intestinal permeability.[327] In conclusion, identification of clinically reliable biomarkers for skin, respiratory and intestinal barrier measurements represents an important future research area.

13 | IN VIVO BIOMARKERS IN AIT DEVELOPMENT PROGRAMS: EUROPEAN IMPLICATIONS

An increasing number of clinical trials in AIT have been published underlining both efficacy and safety of AIT as the only disease-modifying treatment option for patients with IgE-mediated respiratory allergic diseases.[328] With country-specific exceptions, AIT products are regulated by authorities such as the European Medicines Agency[329] and others on the basis of methodological guidelines.

In its guideline "*Clinical Development of Products for Specific Immunotherapy for The Treatment of Allergic Diseases*",[329] the European regulatory authority (European Medicine Agency (EMA)) has provided strict guidance for designing and performing clinical development programs in AIT.[113,330] In Germany, these principles were followed in the "*Therapy Allergen Ordinance* (TAV)" for future registration of allergens and allergen mixtures (derived from grass pollen, early flowering trees pollen, house dust mites, and bee and wasp venom) based on the main prevalent respiratory allergies in Germany.[331–333] First registrations have been granted fulfilling the TAV demands by the German Paul Ehrlich Institut (PEI).[334] Besides, a sufficient body of evidence exists in the clinical documentation of both SLIT and SCIT AIT products.[335–337]

An important unmet need for further harmonization of methodological principles in AIT study design[338] has been followed in a series of Task-Force initiatives of the European Academy of Allergy and Clinical Immunology (EAACI)[339] as overviewed in the 2019 report of our group.[113] As such, position papers and guidelines on allergen-challenge procedures including clear standardization of procedures (SOPs) through the nasal[340] or conjunctival[341] route have been provided by the EAACI. As aforementioned, the safety, reproducibility and cutoff points for NAC have also been proven recently.[96,97]

In addition, the combined symptom and medication score (CSMS) as defined by the EAACI as standard primary endpoint for future (pivotal) trials in AIT[342] has been recently used recently in an increasing number of key trials in AIT.[343–345] However, further formal validation and amendments especially for the pediatric population are needed.[334,346] As another example, a further Task Force initiative aimed to provide clear clinically and aerobiologically justified definitions of pollen counts for onset, peak and duration of pollen seasons[347] and they have been confirmed to be robust[348] and clinically relevant as reflecting patients' symptom load in different countries in Europe in recent reports.[348,349] Recently, the EAACI has published a Position Paper reporting the impact of the placebo effect in AIT from different methodological perspectives and outlining possible strategies to minimize this bias in clinical trials.[350]

Taken together, further emphasis should be put on international collaborations of clinical experts, methodologists and regulatory authorities to optimize methodological standards for AIT clinical development programs aimed to increase the level of evidence of AIT as the only disease-modifying therapy available.[334,337,351]

14 | BIOMARKERS ON THE CLINICAL USAGE OF BIOLOGICALS IN THE EAACI

The EAACI recently published the Guidelines on the use of biologicals in severe asthma. Recommendations were formulated following the GRADE approach[352–354] for each biological (benralizumab, dupilumab, mepolizumab, omalizumab, reslizumab) and each outcome (decrease in asthma exacerbations, oral and inhaled corticosteroids and rescue medication use; improvement in asthma control, quality of life and lung function; and safety).[33] The recommendations were informed by three systematic reviews.[355–357] FeNO, sputum and blood eosinophils were scored as outcomes with low importance; however, the evidence was analyzed for subgroups based on biomarkers and comorbidities. The higher the blood eosinophils, the higher the expected impact of benralizumab, dupilumab and mepolizumab in reducing severe asthma exacerbations. The improvement in asthma control was significantly better in the high-eosinophil subgroup for benralizumab and reslizumab. Lung function increased significantly more in the high eosinophil subgroup for benralizumab, dupilumab and reslizumab. Benralizumab improved QoL better in the high-eosinophil subgroup. The effect of omalizumab on exacerbations did not depend on blood eosinophils. Neither the atopic status nor total IgE predicts the magnitude of effect of benralizumab, and serum IgE thresholds (within regulatory limits) did not influence the response to omalizumab (Table 4).

In addition, biomarkers were used to characterize the population where a strong recommendation is made instead of a conditional one, for example for dupilumab in improving lung function for asthmatic and adolescent adults with blood eosinophils > 300 cells/μL and/or with FeNO levels > 50 ppb. This upgrade was possible as in this subgroup the addition of dupilumab to standard of care improved FEV_1 above the minimal important difference threshold.

The Guideline Development Group (GDG) formulated a three-pillar decision tree aiming to help the clinician in reaching the decision to start on a particular biological based on the combination between phenotypic traits, biomarkers (blood and sputum eosinophils, FeNO) and clinically relevant asthma-related endpoints. For suboptimal response, the GDG recommends to re-assess airway inflammation and airway hyperresponsiveness (conditional recommendation, expert opinion based). Induced sputum is the preferred option to re-assess airway inflammation (conditional recommendation, expert opinion based) as a noninvasive validated tool. The GDG strongly recommends joint efforts from academia, industry and healthcare systems to develop both educational tools and resources supporting a wider use of induced sputum evaluation in severe asthma. If at re-evaluation for suboptimal response there is no airway eosinophilia and neutrophilic inflammation is present, the biological should be interrupted and measures addressing non-T2 asthma such as macrolides should be considered (conditional recommendation, expert opinion based). In cases with no airway inflammation, addressing airway hyperresponsiveness (LABA/LAMA combinations or bifunctional drugs) or airway remodeling (bronchial thermoplasty in selected

TABLE 4 Current biologicals, biomarkers, asthma outcomes and GRADE recommendation

Biological	Asthma-related outcome	Biomarkers	Recommendation as per GRADE methodology
Benralizumab Dupilumab Reslizumab	Decrease in severe asthma exacerbations	The higher the blood eosinophils, the higher the expected impact	Conditional recommendation
Omalizumab	Decrease in severe asthma exacerbations	The effect does not depend on blood eosinophils	Conditional recommendation
Benralizumab Reslizumab	Improvement in asthma control	The higher the blood eosinophils, the higher the expected impact	Conditional recommendation
Benralizumab	Improvement in asthma-related QoL	The higher the blood eosinophils, the higher the expected impact	Conditional recommendation
Dupilumab	Improvement in lung function (FEV_1)	Clinically significant effect in asthmatic and adolescent adults with blood eosinophils > 300 cells/µL and/or with FeNO levels > 50 ppb	Strong recommendation
Benralizumab Reslizumab	Improvement in lung function (FEV_1)	The higher the blood eosinophils, the higher the expected impact	Conditional recommendation
Benralizumab	All asthma-related outcomes	Neither the atopic status or total IgE predicts the magnitude of effect	Conditional recommendation
Omalizumab	All asthma-related outcomes	Serum IgE thresholds (within regulatory limits) do not influence the response	
HDM immunotherapy	All asthma-related outcomes	No biomarkers sufficiently predict response to HDM-AIT in HDM-driven allergic asthma	Conditional recommendation

cases) is recommended (conditional recommendation, expert opinion based).

Identification of clinically relevant biomarkers in order to select responders to the currently available biologicals was identified as high priority, and proof-of-concept prospective studies evaluating patient selection based on biomarkers were proposed as a tool to overcome this barrier in severe asthma research.

Another recently published EAACI Guideline formulated recommendations for the use of AIT with HDM for HDM-driven allergic asthma. To date, there are no biomarkers that sufficiently predict response to HDM-AIT that can be used to decide on initiation or cessation of HDM AIT in HDM-driven allergic asthma.[358]

15 | CONCLUSIONS

In our discipline, a huge amount of omics research has been employed during recent years that needs to be analyzed in depth. Allergic diseases are a group of complex and heterogeneous diseases. Specific, sensitive and reliable (point-of-care) biomarkers are critical for selecting the proper treatment for the proper patient and enabling precision medicine. Precision endotyping is linking pathobiological mechanisms with visible properties via specific biomarkers. It is expected to be translated into pathway-specific diagnostic tests and opens a pathway to accurate disease classification and individualized targeted treatment options. We are expecting to change the diagnostic and therapeutic landscape in our specialty as well as of all medical disciplines. However, endotype-driven treatment still needs to face multiple challenges before its implementation in daily

practice in allergic diseases and asthma. Most of the disease endotyping research is conducted in severe forms of allergic diseases nowadays. Over the past few years, the biomarker discovery field has gained great advances, which facilitates the identification of novel methods for disease diagnosis and therapy. With these fast growing efforts, we can imagine that in the near future, we will be able to find better ways to treat allergic diseases, with a therapeutic approach targeted to the individual patient.

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