

REVIEW ARTICLE

Recent developments and highlights in biomarkers in allergic diseases and asthma

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Abstract

The potential of precision medicine in allergy and asthma has only started to be explored. A significant clarification in the pathophysiology of rhinitis, chronic rhinosinusitis, asthma, food allergy and drug hypersensitivity was made in the last decade. This improved understanding led to a better classification of the distinct phenotypes and to the discovery of new drugs such as biologicals, targeting phenotype-specific mechanisms. Nevertheless, many conditions remain poorly understood such as non-eosinophilic airway diseases or non-IgE-mediated food allergy. Moreover, there is a need to predict the response to specific therapies and the outcome of drug and food provocations. The identification of patients at risk of progression towards severity is also an unmet need in order to establish adequate preventive or therapeutic measures. The implementation of precision medicine in the clinical practice requires the identification of phenotype-specific markers measurable in biological matrices. To become useful, these biomarkers need to be quantifiable by reliable systems, and in samples obtained in an easy, rapid and cost-efficient way. In the last years, significant research resources have been put in the identification of valid biomarkers for asthma and allergic diseases. This review summarizes these recent advances with focus on the biomarkers with higher clinical applicability.

KEYWORDS

asthma, biomarkers, chronic rhinosinusitis, drug hypersensitivity, food allergy

*Both authors equally contributed to this article.

1 | INTRODUCTION

Precision medicine for allergic diseases requires a deep understanding of immunopathology and phenotype heterogeneity in relation to clinically significant outcomes.¹ Precision medicine could also help to limit the socio-economic burden imposed by allergic and chronic respiratory diseases.² According to the National Institutes of Health (NIH), precision medicine is *an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment and lifestyle for each person*. In this regard, asthma and allergic conditions are *ideally suited, as they represent umbrella entities comprising different diseases partially sharing immune mechanisms (endotypes) and presenting similar visible properties (phenotypes), but requiring individualized approaches for a better risk prediction and the identification of treatment responders*.³

The implementation of precision medicine demands measurable indicators of biological conditions usually termed biomarkers. A valid biomarker should be quantifiable in an analytical system with well-defined performance and need to be supported by a body of evidence which sufficiently clarifies the pathological and clinical significance of the test results.⁴ Moreover, the identification of novel biomarkers applicable in daily practice requires clear clinical models with well-established extreme phenotypes, allowing a better understanding of the disease progression along its severity. Another aspect influencing the clinical applicability is the biological matrix ("sample type") where biomarkers are measured (Table 1). Appropriate matrices should be easy to obtain, store and manipulate in standardized and reproducible measuring protocols at a reasonable cost. Moreover, in most cases a single biomarker will not adequately represent the complexity of mechanisms underlying multifactorial diseases. In this regard, the generation of multidimensional biomarker panels displays a greater potential to identify valid markers.⁵

The research efforts in asthma and allergic diseases during the last decades have focused on the identification of biomarkers applicable in clinical practice. Although several markers of allergic inflammation (e.g, IgE, eosinophilia, fractional exhaled nitric oxide [FENO]) have been described, their utility in diagnosis, prognosis and therapy is still controversial.⁶⁻⁸ Different types of molecules (genes, metabolites, etc.) have been also proposed as biomarkers for allergic and chronic respiratory conditions. Some of them display good analytical properties, but overall, they are insufficiently robust to be extrapolated to clinical practice. This fact partially arises from a paucity of clinical models for allergic diseases, which clearly constitutes a limiting factor in the search for biomarkers.

TABLE 1 Characteristics of an ideal biomarker in asthma and allergy

The ideal biomarker

Supported by a body of evidence clarifying its biological significance

Quantifiable in a cost-efficient analytical system with well-defined performance

Detectable in a biological matrix obtained in an easy, rapid and cost-efficient way

Highlights

- 1 The implementation of precision medicine in allergic diseases requires a further clarification of disease phenotypes and endotypes allowing the identification of valid biomarkers.
- 2 Many of the biomarkers of allergic diseases identified to date still require validation in larger cohorts and distinct geographical areas.
- 3 Multidimensional approaches have a greater potential to identify valid biomarkers for allergic and chronic respiratory diseases.

This review will summarize the biomarkers identified to date for allergic and chronic respiratory conditions, with special focus on those with higher clinical applicability.

2 | NEW METHODS TO IDENTIFY BIOMARKERS: THE OMICS

The different omics characterize and quantify biological molecules, which share a common feature and which provide information about the structure and function of organisms. Omics are significantly contributing to the definition of disease endotypes and phenotypes and to the identification of therapeutic targets. Recent technological advances have pushed the omics field forward by allowing higher throughputs, improving detection limits and providing software tools to analyse and visualize the data.

Genomics, transcriptomics and epigenetics have been used to identify genes, RNA sub-types and DNA modifications, respectively. Nevertheless, the validation of these observations requires the investigation of their functional consequences (e.g, the effect in transcription or splicing variants or in the functionality of proteins). Only this step permits valid conclusions for the underlying mechanisms of disease phenotypes (Figure 1).

Metabolomics investigates the nature and concentration of the metabolites generated in living systems and is among the most recent approaches applied to allergy research. Metabolomics displays a high degree of versatility, as it is applicable to a great variety of matrices, whose nature can be tailored to the disease of interest.^{4,7} In chronic respiratory diseases, the metabolic analysis of exhaled breath condensate seems a promising matrix for biomarker identification. Other approaches which could help phenotype chronic airway conditions include electronic nose (eNose) or nuclear magnetic resonance-based metabolomics.⁷ Nevertheless, the lack of standardized procedures for breath sampling, the effect of pH on some metabolites and the low concentrations are well-established limiting factors.

Individual omics display both strengths and weaknesses which overall define the validity and robustness of the results. The

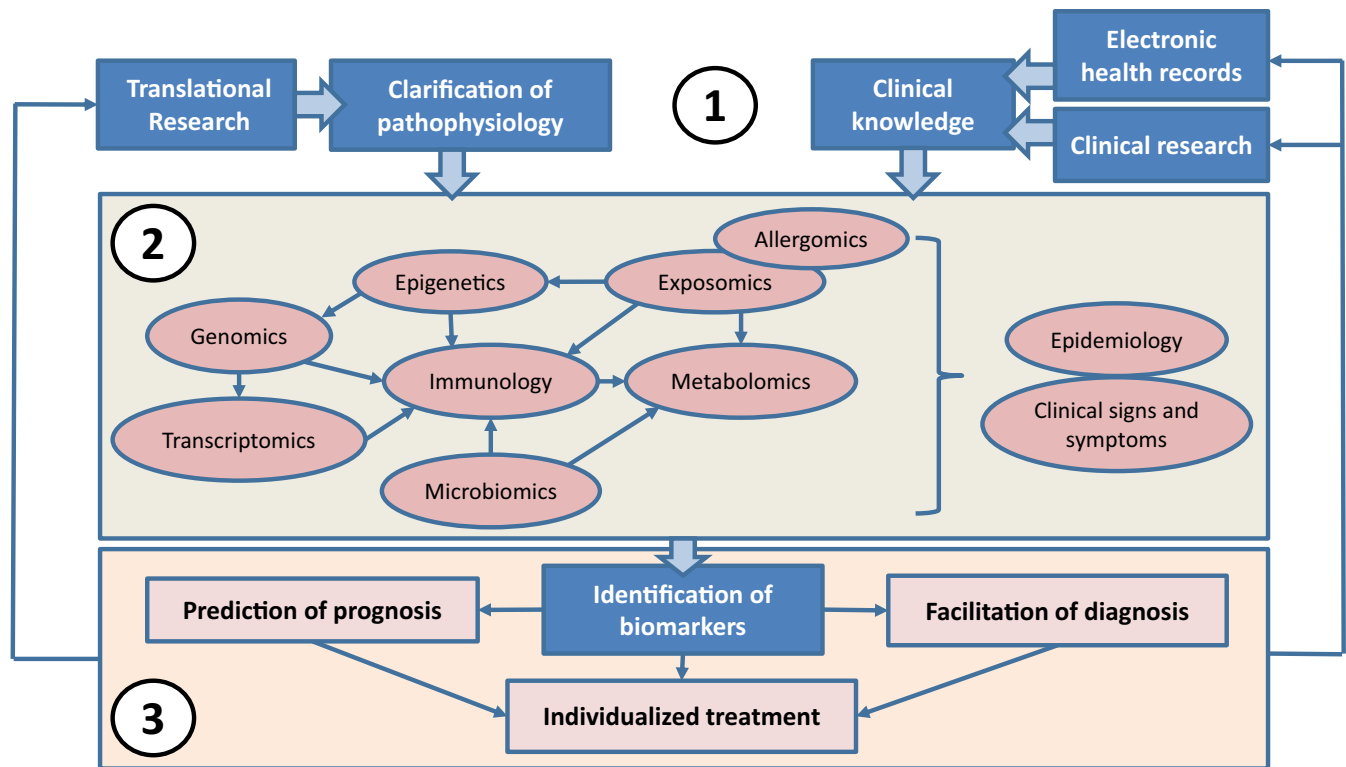


FIGURE 1 Work-flow for the identification of biomarkers. 1: the clarification of disease mechanisms and the knowledge obtained from the clinical research and e-health records generates data about the diseases; 2: these data are interpreted by different disciplines and analysed using their corresponding methodology to generate hypothesis for disease-specific markers; 3: these potential markers need to be clinically validated in order to identify valid biomarkers and implement precision medicine

development of multi-omics and multi-matrix platforms in integrated approaches will probably provide a more holistic picture of biological situations, including allergic and chronic respiratory diseases. Nevertheless, the implementation of these advances in clinical practice requires the identification of biomarkers measurable in biological fluids obtained in an easy, rapid and cost-efficient way.

3 | UPPER AND LOWER AIRWAY DISEASES

3.1 | Rhinitis

Chronic rhinitis is generally divided into allergic rhinitis (AR) and non-allergic rhinitis (NAR).⁹ The NAR category comprises a heterogeneous group of diseases mediated by immune or neurogenic mechanisms.¹⁰ Conversely, AR is a relatively homogeneous entity arising from IgE-mediated inflammation.¹¹ Local allergic rhinitis (LAR) is a disease phenotype not fitting into the AR-NAR dichotomy.¹² The skin prick test (SPT) and/or serum allergen-specific (s)IgE and the nasal allergen challenge (NAC) positively identify AR patients. Subjects with LAR are defined by a positive NAC with negative SPT and serum sIgE, whereas NAR patients test negative for the three biomarkers.

Several inflammatory cells and mediators may also serve as diagnostic biomarkers for AR. Eosinophils, IL-5, IL-6, IL-13 and *macrophage inflammatory protein* (MIP)-1 β increase in the nasal lavage of

AR patients following NAC,¹³ while elevated nasal *endothelin* (ET)-1 and CCL17 at baseline discriminate AR from NAR individuals.¹⁴ Compared to healthy controls, subjects with house dust mite (HDM)-induced AR have increased circulating group 2 innate lymphoid cells (ILC2), which also correlate with serum IL-13 and symptom scores.¹⁵

Allergen immunotherapy (AIT) is an effective treatment for AR¹⁶ and LAR^{17,18} but involves considerable time and cost. Biomarkers assisting the selection of patients most likely to respond to AIT have recently been summarized.¹⁶ A proportion of sIgE to total IgE >16.2% predicted AIT success with 97.2% sensitivity and 88.1% specificity.¹⁹ In children, low serum osteopontin identifies responders to sublingual immunotherapy.²⁰ Serum osteopontin and basophil reactivity increase after NAC²¹ and diminish following successful AIT.¹⁶ Moreover, subcutaneous immunotherapy with HDM armed peripheral T regulatory cells with the ability to inhibit Th2 and Th9 proliferation.²²

3.2 | Chronic rhinosinusitis

Chronic rhinosinusitis (CRS) is defined by nasal and sinus mucosal inflammation. Phenotyping of CRS is typically based on the presence (CRS_{swNP}) or absence (CRS_{snNP}) of nasal polyps on endoscopy or CT scan, whereas examination of nasal samples facilitates the endotyping (Figure 2). In Caucasian populations, nasal polyps generally show an eosinophilic infiltrate, whereas fewer than 50% of Asian patients

display eosinophilic polyyps.^{23,24} One study identified ten CRS clusters, of which six exhibited a type 2 inflammatory profile with raised IL-5 and eosinophilia. Type 2 clusters displayed a higher risk of nasal polyps and asthma.²⁵ The CRSwNP phenotype has been also associated with increased ILC2 at both the tissue level and peripheral blood.^{26,27}

Several matrices have been used to predict CRS prognosis. Blood and tissue eosinophilia correlates with severity as measured by endoscopy and CT scan²⁸ and can predict recurrence following endoscopic sinus surgery.²³ In addition, a small study observed that programmed cell death-1 (PD-1) mRNA expression in nasal polyp tissue correlated with disease severity on CT scan,²⁹ while tissue gene expression of the eosinophil marker—Charcot-Leyden crystal protein—was associated with higher olfactory impairment.³⁰ The level of IL-5 and P-glycoprotein in nasal secretions helped to predict the olfactory and the CT scan scores, respectively.³¹ Nasal nitric oxide (nNO) inversely correlated with CT scan-graded severity and increased after sinus surgery.³²

3.3 | Asthma

Asthma phenotypes are classified into those displaying dominant type 2 inflammation, and those without significant type 2 inflammation,³³ with each group comprising a number of different diseases (Figure 3). Several biomarkers measurable in different matrices have been described for these asthma phenotypes. The relevance of asthma

endotyping is perfectly illustrated by the case of the anti-IL-5 monoclonal antibody (mAb) mepolizumab whose initial lacklustre performance in unclassified asthma³⁴ was followed by excellent outcomes when administered to patients with eosinophilic asthma.³⁵

In any individual, the disease expression may be driven by complex endotypes with numerous mechanistic pathways³; therefore, multidimensional biomarker assessment may be required. Unsupervised statistical analyses examining various blood inflammatory mediators identified unique clusters with different clinical and pathological features. Cluster analysis using sputum mediators at exacerbation also identified distinct biologic clusters with differences in host microbiome. The subsequent paragraphs describe individual biomarkers according to their biological matrices in more detail.

3.4 | Diagnostic biomarkers

3.4.1 | Blood cells

Peripheral blood eosinophilia and neutrophilia in asthma have been associated with different clinical characteristics, with neutrophilia indicating increased sputum production.³⁶ In asthma, neutrophilia is also more prevalent among patients with a smoking history and persistent airflow limitation compared to non-smoking asthma patients (4.5×10^9 vs 3.6×10^9 cells/L),³⁷ suggesting that neutrophilia may

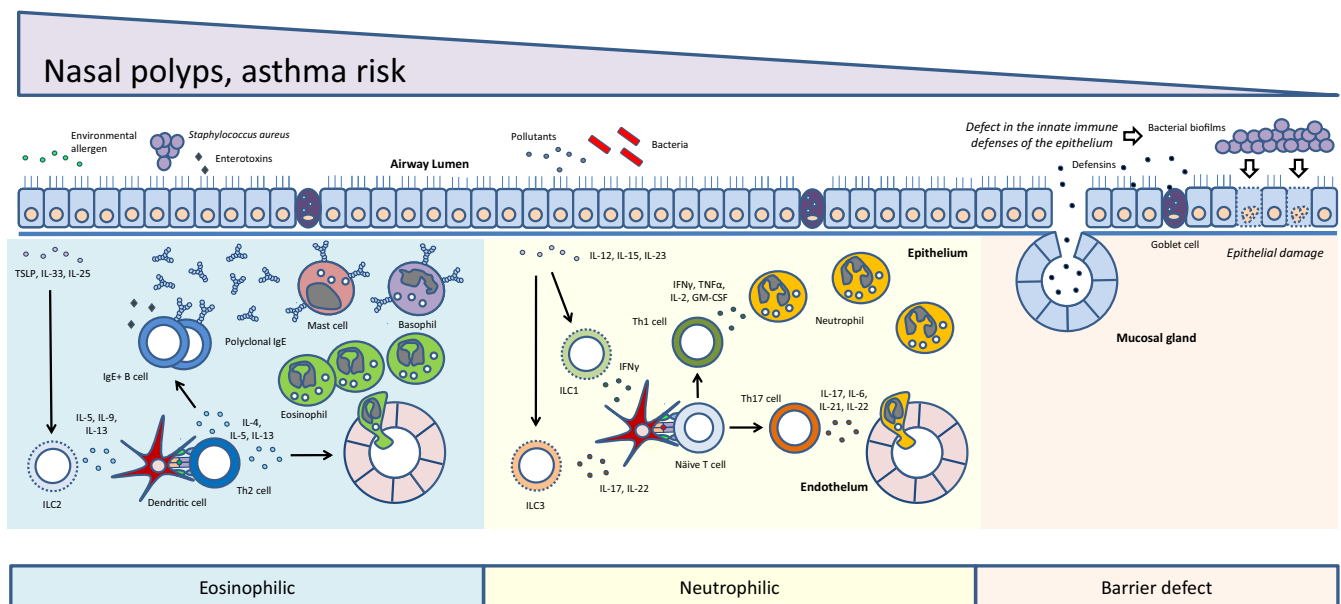


FIGURE 2 Endotypes of chronic rhinosinusitis (CRS). *Eosinophilic CRS*: *Staphylococcus aureus* or environmental allergens activate the epithelium which in turn releases TSLP, IL-25 and IL-33 to stimulate innate lymphoid cells 2 (ILC2). ILC2-activated dendritic cells promote the release of type 2 cytokines by Th2 cells. The enterotoxins from *Staphylococcus aureus* together with the type 2 cytokines drive the activation of polyclonal IgE+ B cells with the subsequent release of vast amounts of IgE. This polyclonal IgE can sensitize resident mast cells and basophils. The IL-5 from ILC2 and Th2 cells mediates the recruitment of circulating eosinophils which accumulate in the nasosinusal mucosa; *neutrophilic CRS*: Pollutants or bacteria stimulate ILC1 and ILC3 through epithelium-derived factors. In turn, ILC1 and ILC3 activate dendritic cells to polarize naïve CD4+T cells to a Th1 and Th17 profile, respectively. Th17 cells drive the recruitment of circulating neutrophils to accumulate in the tissue; *CRS related to a barrier defect of the epithelium*: A defect in the production of innate defensins by mucosal glands, goblet cells and epithelial cells provokes an overgrowth of microbes in the nasal lumen which facilitates the formation of bacterial biofilms with the ability to damage the integrity of the epithelium

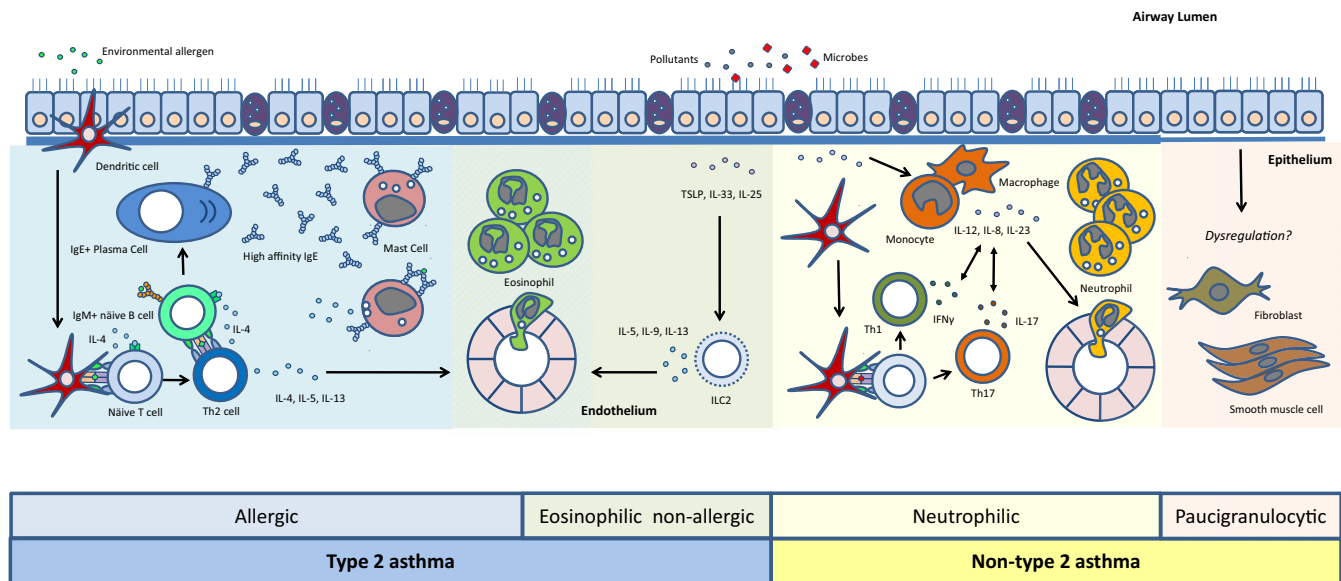


FIGURE 3 Endotypes of asthma. *Allergic asthma*: Allergen-activated mucosal dendritic cells traffic to the secondary lymphoid tissue (not shown) to prime allergen-specific naïve CD4⁺ T cells towards a Th2 phenotype. The IL-4 released by activated Th2 cells stimulates IgM⁺-naïve B cells to perform the class-switch recombination which ultimately results in the generation of IgE⁺ plasma cells. These plasma cells at the lamina propria release high amounts of allergen-specific IgE which sensitizes resident mast cells and basophils for activation. Allergen-specific Th2 cells also release IL-5 which mediates the recruitment of circulating eosinophils to accumulate in the bronchial mucosa; *Eosinophilic non-allergic asthma*: Pollutants or microbes stimulate the bronchial epithelium to release TSLP, IL-33 and IL-25 which in turn activates innate lymphoid cells 2 (ILC2). These cells produce vast amounts of IL-5 which mediates the recruitment of circulating eosinophils to accumulate in the bronchial mucosa; *Neutrophilic asthma*: Unknown factors derived from the epithelium stimulate monocytes, macrophages and dendritic cells. Activated monocytes and macrophages release IL-12 and IL-23 which mediate the priming of naïve CD4⁺ T cells to generate Th1 and Th17 cells, respectively. Monocyte- and macrophage-derived IL-8 drives the recruitment of circulating neutrophils to accumulate in the bronchial mucosa. *Paucigranulocytic asthma*: This endotype is believed to derive from a dysregulation of fibroblast and smooth muscle cells of the bronchial mucosa

differentiate between patients with asthma alone from those with features of asthma-chronic obstructive pulmonary disease (COPD) overlap (ACO) syndrome. Several genes regulating immune cells such as B lymphocytes, T lymphocytes and granulocytes were up- or down-regulated in severe asthma compared to healthy controls.³⁸ In a paediatric asthma cohort, the expression of five selected genes on CD4 lymphocytes (SRM, HDAC2, SLC33A1, P2RY10 and ADD3) predicted the atopic status with 100% sensitivity and 81.3% specificity,³⁹ while in another paediatric study, a fourteen-gene signature (MCEMP1, AQP9, PGLYRP1, S100P, RNASE2, OLFM4, CAMP, CEA-CAM8, LCN2, MPO, DEFA4, ELANE, BPI, DEFA1B, CTSG, HBD, ALAS2, RPS4Y2 and RPS4Y1) was unique to a neutrophilic phenotypic cluster.⁴⁰ In a recent pilot study, circulating blood microRNA profile (expressed as miRNA ratios) showed promise in differentiating allergic asthma from healthy controls.⁴¹ Lipidomic profile and gene expression after low molecular weight hyaluronic acid stimulation of peripheral blood mononuclear cells were also shown to be different in severe asthma compared to mild asthma and healthy controls.⁴²

Peripheral differential cell counts can also serve as surrogate markers of airway inflammation. In a meta-analysis of 14 studies, the ability of blood eosinophils to predict airway eosinophilia showed an area under the curve (AUC) of 0.78.⁴³ Enumeration of peripheral ILC2 has a similar utility for predicting sputum eosinophilia.⁴⁴

Conversely, blood neutrophilia is less indicative of sputum neutrophilia, with an AUC of only 0.6.⁴⁵ The ex vivo response of blood neutrophils and eosinophils to stimulation with N-formyl-methionyl-leucyl-phenylalanine (fMLP), in combination with relevant clinical parameters, is also able to predict sputum eosinophilia.⁴⁶

3.4.2 | Serum mediators

The chitinase-like protein YKL-40 distinguished asthma from COPD and healthy controls.⁴⁷ Neutrophil expression of Siglec-9 is increased in patients with COPD and may have future potential to diagnose asthma.⁴⁸ Serum (soluble-cleaved) urokinase plasminogen-activated receptor (scuPAR) was found to be higher in severe, non-atopic asthma in a single study⁴⁹ and requires confirmation in further studies. Blood mediators can also predict airway inflammation. Overall, serum periostin moderately correlated with sputum eosinophilia.^{50,51} Eosinophilic cationic protein (ECP) is more predictive of sputum eosinophilia than serum IgE,³⁹ whereas C-reactive protein (CRP) is weakly associated with sputum neutrophilia.⁵²

3.4.3 | Sputum cells and mediators

Sputum quantitative cell count is a reference standard for airway inflammation in asthma. Four inflammatory phenotypes have been

described—eosinophilic, neutrophilic, mixed granulocytic and paucigranulocytic. The neutrophilic subtype has been associated with the obese female asthma non-type 2 phenotype.⁵² The Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (UBIOPRED) study is a multicentre prospective cohort study recruiting patients with severe asthma in various European countries. Sputum analysis of the UBIOPRED cohort identified 3 transcriptome-associated clusters (gene clusters), corresponding to eosinophilic, neutrophilic and paucigranulocytic phenotypes, respectively.⁵³ A six-gene signature (CLC, CPA3, DNASE1L3, IL1B, ALPL and CXCR2) can differentiate asthma patients from controls and distinguish between eosinophilic from neutrophilic asthma.⁵⁴ This method has a practical advantage over sputum differential cell counts, as frozen samples can be batched for processing. Neutrophil myeloperoxidase in sputum has the potential to differentiate ACO from asthma.⁵⁵ Specific microRNAs can discriminate neutrophilic from eosinophilic asthma.⁵⁶ Sputum eosinophil peroxidase (EPX) correlates with sputum eosinophilia,⁵⁷ as does nasal and pharyngeal EPX.⁵⁸ Importantly, nasal sampling may be particularly useful for patients in whom sputum induction is unsafe or not possible.

3.4.4 | Cellular bronchial samples

Patients in the UBIOPRED cohort could be divided into four groups based on the expression of nine gene sets in bronchial cells, each with mixed inflammatory patterns including one with concomitant Th2 and Th17 markers.⁵³ Interestingly, a different study found that Th2 and Th17 gene expression signatures were mutually exclusive in asthmatic airway tissue.⁵⁹ The authors suggested that suppression of Th2 activity by corticosteroids may accentuate Th17 activity. Different gene signatures for Th2 and Th17 activities were used in the two studies, possibly limiting direct comparisons. The interplay between Th2 and Th17 cells is also likely to be complex, and the exact relationship between the two is still uncertain.⁶⁰

3.4.5 | Exhaled breath

The FENO displays an AUC of 0.8 for asthma diagnosis.² Of note, very high or low cut-offs for FENO can, respectively, rule-in or rule-out asthma.²⁷ Conversely, FENO has limited utility to predict sputum eosinophilia,⁴³ as it is confounded by corticosteroid treatment, atopy and smoking status. Volatile organic compounds (VOCs) in exhaled breath can be readily measured using eNose devices. Building on previous work which discriminates between COPD and asthma, eNose has identified label-free clinical and inflammatory clusters among asthma and COPD patients.⁶¹ VOCs and other metabolites in exhaled breath can also differentiate asthma from healthy controls in adults and children.^{62–65} In a paediatric study, metabolomic analysis using nuclear magnetic resonance (NMR) of exhaled breath condensate identified three clusters with different inflammatory profiles based on global spectral patterns of NMR.⁶⁶

3.4.6 | Urine

Urine metabolite analysis can accurately discriminate between asthma and COPD⁶⁷ and also correlates with FENO and blood eosinophilia.⁶⁸

3.5 | Prognostic biomarkers

3.5.1 | Blood cells

Raised blood eosinophils strongly predict the risk of asthma exacerbations in both adults and children.^{69,70} Blood eosinophilia also predicts longitudinal lung function decline, irrespective of smoking status.⁷¹ Blood neutrophilia is linked with airway infections in asthma¹¹ as well as poor symptom control and increased exacerbations.⁷² Circulating blood fibrocytes correlate with asthma severity.⁷³

3.5.2 | Serum mediators

The stability of serum periostin over disease progression facilitates its use as a biomarker.⁷⁴ Elevated levels are associated with fixed and more severe airflow obstruction^{75,76} and greater longitudinal lung function decline.⁷⁷ Total serum IgE in children is associated with atopy, airway hyperresponsiveness (AHR) and bronchial wall thickening in CT scan.⁶⁹ In both adults and children, YKL-40 level correlates with severe asthma and poor lung function.^{78,79} The expression of ten selected microRNAs (HS_108.1, HS_112, HS_182.1, HS_240, HS_261.1, HS_3, HS_55.1, HS_91.1, hsa-miR-604 and hsa-miR-638) was higher in children with severe asthma.⁸⁰

3.5.3 | Sputum cells and mediators

Sputum neutrophilia and ILC2²⁷ are associated with asthma severity.^{81,82} Changes in sputum eosinophilia reflect fluctuations in clinical asthma control.⁸³ Human *tumour necrosis factor-like weak inducer of apoptosis* (TWEAK) is an inflammatory mediator whose level in sputum correlated with higher severity, poor symptom control and decreased lung function in children with non-eosinophilic asthma.⁸⁴

3.5.4 | Cellular bronchial samples

Bronchial neutrophilia is present in severe (compared to non-severe) asthma, independent of oral corticosteroid (OCS) intake.⁸⁵ Gene signatures analysed in endobronchial brushing and biopsy specimens predicted persistent airflow limitation in the UBIOPRED cohort.⁸⁶ In bronchoalveolar lavage samples, elevated CD4+ cells expressing both IL-4 and IL-17 predicted greater asthma severity.^{69,87}

3.5.5 | Exhaled breath

In both children and adults, FENO correlates with greater AHR, airway obstruction and exacerbations.^{69,88} Patients with FENO >45 ppb are at greater risk for suffering >2 asthma exacerbations/

year.⁸⁹ Electronic nose-measured VOCs predicted the loss of asthma control upon withdrawal of inhaled corticosteroids (ICS).⁹⁰ Reactive oxygen species (ROS) can also be detected in exhaled breath condensates of patients with asthma and has been shown to be suppressed by anti-inflammatory agents.⁹¹

3.5.6 | Functional imaging of lungs

Functional imaging with hyperpolarized gas magnetic resonance of the lung can predict asthma outcomes; persistent ventilation defects were associated with poorer asthma control.⁹² Greater ventilation defects are also observed in patients with uncontrolled eosinophilic inflammation.⁹³

3.6 | Biomarkers for therapeutic response prediction and measurement

3.6.1 | Blood cells

Blood eosinophilia identifies asthma patients responding to therapies targeting type 2 inflammation. The post hoc analyses of randomized controlled trials with the anti-IgE mAb omalizumab identified blood eosinophilia (≥ 300 cells/ μ L) as a predictor of greater response.^{94,95} Nevertheless, this finding was not reproduced in a real-life study.⁹⁶ There is a direct correlation between blood eosinophilia and the response to mepolizumab,⁹⁷ the anti-IL-5 receptor mAb benralizumab⁹⁸ and the anti-IL-4 receptor mAb dupilumab.⁹⁹ Blood eosinophilia may also predict and monitor the response to corticosteroids. Atopic children with eosinophilia ≥ 300 cells/ μ L respond better to ICS.¹⁰⁰ A decrease in peripheral eosinophilia is observed with the up-dosing of ICS,¹⁰¹ while titration of OCS to maintain blood eosinophilia < 200 cells/ μ L improved asthma control.¹⁰²

3.6.2 | Serum mediators

Elevated serum periostin predicts the response to omalizumab.^{75,103} Interestingly, total serum IgE does not predict the response to omalizumab, despite this molecule being not only the drug target, but also the basis for its dose calculation.¹⁰⁴ On the other hand, a reduction in serum-free IgE after 16–32 weeks on omalizumab is associated with a decrease in exacerbations over two years.⁸¹

3.6.3 | Sputum

Sputum eosinophilia $\geq 3\%$ predicts response to corticosteroids¹⁰⁵ and mepolizumab.³⁵ Sputum eosinophilia as a guide for ICS therapy reduced exacerbations with no associated increase in the total ICS dose.^{106,107}

3.6.4 | Exhaled breath

In patients with symptoms suggestive of AHR, elevated FENO predicts response to ICS.¹⁰⁸ A systematic review concluded that using

FENO to guide ICS therapy in adults reduced the mild but not the severe exacerbations.¹⁰⁹ Among children, FENO also showed unclear benefits on asthma outcomes.¹¹⁰ A FENO level > 19.5 ppb also correlated with response to omalizumab.⁷⁵

3.6.5 | Urine

Urine bromotyrosine correlates with corticosteroid responsiveness, and the predictive accuracy further improves when combined with high FENO levels.⁹⁴

Despite the previous enumeration being made in a matrix-related fashion, the complexity of most asthma phenotypes will require multidimensional approaches to identify valid biomarkers (Table 2). This aspect is exemplified by the greatest benefit from dupilumab being observed in asthma patients exhibiting *both* elevated peripheral eosinophilia and FENO.⁹⁹

4 | FOOD ALLERGY AND ANAPHYLAXIS

The food allergy (FA) phenotypes differ on their IgE dependence and prognosis (Figure 4).¹¹¹ Given this heterogeneity, the search for FA biomarkers has gained significant attention.¹¹²

4.1 | IgE-mediated food allergy

The identification of children at risk of developing FA might help establish preventive strategies.¹¹² The balance between type 2 and type 1 chemokines in cord blood influenced the sensitization to food allergens at the age of 3 years in children from Taiwan.¹¹³ Atopic individuals often display skin prick test (SPT) positivity to foods they tolerate.¹¹² Indoleamine 2,3-dioxygenase (IDO) is a tryptophan-catabolizing enzyme expressed by antigen-presenting cells.¹¹⁴ A high IDO activity was associated with unresponsiveness to food allergens in sensitized children from Turkey.¹¹⁴ Molecular allergology is a useful tool to identify clinically relevant IgE sensitization.^{2,112} Specific (s) IgE to the storage proteins *Cor a 14* from hazelnut or *Ana o 1, 2* or *3* from cashew, correlated with clinically relevant sensitization in children from Denmark¹¹⁵ and the Netherlands,¹¹⁶ respectively. These observations might facilitate the management of patients with FA by limiting the number of oral food challenges (OFC) necessary for diagnosis.^{64,112} Interestingly, a score based on the value of sIgE to *Ana o 3*, the SPT wheal size and the gender of the patient was proposed to predict the outcome of cashew OFC in Dutch patients.¹¹⁷ Basophil activation test (BAT) might also correlate with the OFC outcome in food-dependent NSAID-induced anaphylaxis.¹¹⁸

Anaphylaxis is the most severe phenotype of IgE-mediated hypersensitivity, and the increase in serum tryptase is a helpful biomarker in most cases.¹¹⁹ In Canadian children, milk was the food most likely to increase serum tryptase levels.¹¹⁹ Interestingly, the combination of the serum levels of apolipoprotein A1 and the prostaglandin D2 metabolite $9\alpha,11\beta$ -PGF₂, displayed a good diagnostic performance for food-induced anaphylaxis in German patients.¹²⁰

TABLE 2 Summary of biomarkers for diagnosis, prognosis and response prediction and monitoring in asthma

	Diagnosis	Prognosis	Response prediction and monitoring
Blood cells	Distinguish asthma from COPD - Blood neutrophil - Distinguish asthma from healthy controls - Gene expression - Inflammatory phenotyping - Blood eosinophils - Blood neutrophils - Responsiveness of blood neutrophils and eosinophils to fMLF	Exacerbations - Eosinophils - Neutrophils - Symptoms - Neutrophils - Lung function - Eosinophils - Asthma severity - Fibrocytes	Predict response to anti-IL-5 - Eosinophils - Predict response to ICS - Eosinophils - Monitor response to corticosteroids - Eosinophils
Blood mediators	Distinguish asthma from COPD - YKL-40 - Siglec-9 - Determine atopy status - scuPAR - Inflammatory phenotyping - ECP - CRP	Lung function - Periostin - YKL-40 - Airway remodelling - IgE - Asthma severity - IgE - MicroRNA - YKL-40	Predict response to omalizumab - Periostin - Reduction in serum-free IgE
Sputum cells	Inflammatory phenotyping - Quantitative cell count - Gene signature	Lung function - Neutrophils - Asthma severity - ILC2 - Loss of asthma control - Eosinophils	Predict response to mepolizumab - Eosinophils - Predict response to corticosteroids - Eosinophils - Guide ICS titration - Eosinophils
Sputum mediators	Distinguish asthma from COPD - MPO - Inflammatory phenotyping - MicroRNA - Sputum/nasal/pharyngeal EPX	Asthma severity TWEAK	
Bronchial tissue	Inflammatory phenotyping Gene expression	Lung function - Gene signatures - Asthma severity - Neutrophils	
Exhaled breath	Asthma diagnosis - FENO - VOC - Metabolites - Inflammatory phenotyping - eNose	Exacerbations - FENO - Lung function - FENO - Loss of asthma control - eNose	Predict response to ICS - FENO - Guide ICS therapy - FENO (in adults)

(Continues)

TABLE 2 (Continued)

	Diagnosis	Prognosis	Response prediction and monitoring
Urine	Distinguish asthma from COPD		Predict response to corticosteroids
	- Metabolites - Inflammatory phenotyping		Urine bromotyrosine
	Urine metabolites		

COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; ECP, eosinophilic cationic protein; eNose, electronic Nose; EPX, eosinophil peroxidase; FENO, fractional exhaled nitric oxide; fMLF, N-formyl-methionyl-leucyl-phenylalanine; ICS, inhaled corticosteroid; IgE, immunoglobulin E; IL-5, interleukin-5; ILC2, group 2 innate lymphoid cell; LTE4, leukotriene 4; MPO, myeloperoxidase; NERD, aspirin-exacerbated respiratory disease; scuPAR, serum soluble-cleaved form of the urokinase plasminogen-activated receptor; Siglec-9, sialic acid-binding immunoglobulin-type lectins-9; TWEAK, tumour necrosis factor-like weak inducer of apoptosis; VOC, volatile organic compounds.

IgE-dependence

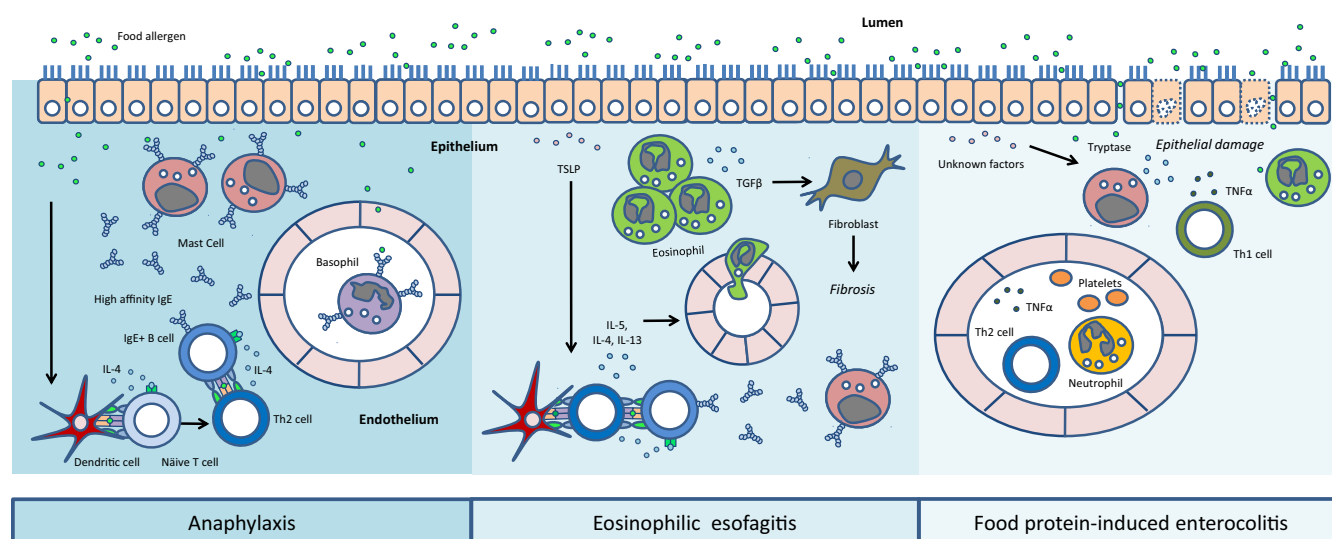


FIGURE 4 Phenotypes and endotypes of food allergy. *IgE-mediated food allergy and anaphylaxis:* Food allergens are taken up by intestinal dendritic cells which travel to the secondary lymphoid tissues (not shown) to prime naïve CD4 + T cells and generate allergen-specific Th2 cells. Th2 cells and IL-4 activate in turn naïve IgM+ B cells (not shown) to generate IgE+ B cells. B cell-derived allergen-specific IgE sensitizes resident mast cells and circulating basophils; *Eosinophilic esophagitis:* Food allergens induce the release of TSLP from the oesophageal mucosa which in turns stimulates dendritic cells to re-activate Th2 cells. Th2 cells also reactivate IgE+ B cells to generate allergen-specific IgE. Th2 cell-derived IL-5 mediates the recruitment of circulating eosinophils. Eosinophil-derived TGFβ activates resident fibroblasts to induce the fibrosis of the oesophageal mucosa; *Food protein-induced enterocolitis syndrome:* Epithelial unknown factors stimulated by food proteins mediate the activation of resident mast cells, Th1 cells and eosinophils. These cells release tryptase and TNFα which damage the integrity of the epithelium and increase its permeability, thus furthering the trans-epithelial traffic of food proteins. The intestinal inflammation is reflected at the peripheral level by the increase in TNFα, and activated Th2 cells, platelets and neutrophils

Oral immunotherapy (OIT) is a promising tool for persistent forms of IgE-mediated FA.^{111,121-123} In anaphylactic children from the United States, successful milkOIT induced the increase in peripheral invariant natural killer T (iNKT) cells and skewed milk-stimulated iNKT cells from a type 2 to a type 1 profile.¹²⁴ Furthermore, successful OIT reduced blood eosinophils and increased several mediators functionally related to type 1 immunity (adipokines, leptin or resistin) in milk-allergic children from Finland.¹²⁵ A higher baseline sIgA and a rapid increase in sIgG1 after OIT initiation identified good

responder egg-allergic children from Japan.¹²⁶ The adverse reactions (AdR) during OIT limit its use in the clinics.¹²³ In children undergoing peanutOIT, the presence of allergic rhinitis and the SPT wheal size were associated with systemic and gastrointestinal AdR.¹²⁷ Adjuvant therapy with omalizumab might reduce AdR during OIT,¹²³ and the combination of basophil reactivity and sIgE/total IgE ratio at baseline could identify patients more likely to benefit from omalizumab during milkOIT.¹²⁸ Beyond the oral route, other administration routes are under investigation for severe FA.¹²³ Sublingual immunotherapy

with Pru p 3, the lipid transfer protein from peach, induced anti-inflammatory PDL-1+ dendritic cells and IL-10+ T regulatory cells in responder patients from Spain.^{129,130}

4.2 | Other types of food allergy

The diagnosis of eosinophilic esophagitis requires the demonstration of >15 eosinophils/high-power field in the oesophagus of individuals with suggestive symptoms.¹³¹ Oesophageal eosinophilia correlated with male gender and the number of positive food sIgE tests in American children.¹³² This observation might help to limit the number of endoscopies required for diagnosis.¹³¹ The management of food protein-induced enterocolitis syndrome (FPIE) patients involves consecutive OFCs to assess for disease resolution.¹³³ In Japanese children with FPIE, the OFCs induced the activation of intestinal

and peripheral eosinophils.¹³⁴ Interestingly, the peripheral level of C-reactive protein and of eosinophilia correlated with a poor and good prognosis, respectively, in Japanese patients with FPIE.¹³⁵

Despite the progress made in recent years, most biomarkers remain to be validated in larger populations and distinct geographical areas. Moreover, growing evidence suggests that airway allergy influences many of the parameters identified as FA biomarkers.^{136,137} In this regard, the clarification of atopic phenotypes and their relationship with FA will improve the interpretation of biomarkers.^{68,138}

5 | DRUG HYPERSENSITIVITY

A summary of the different drug hypersensitivity phenotypes can be seen in Figure 5.

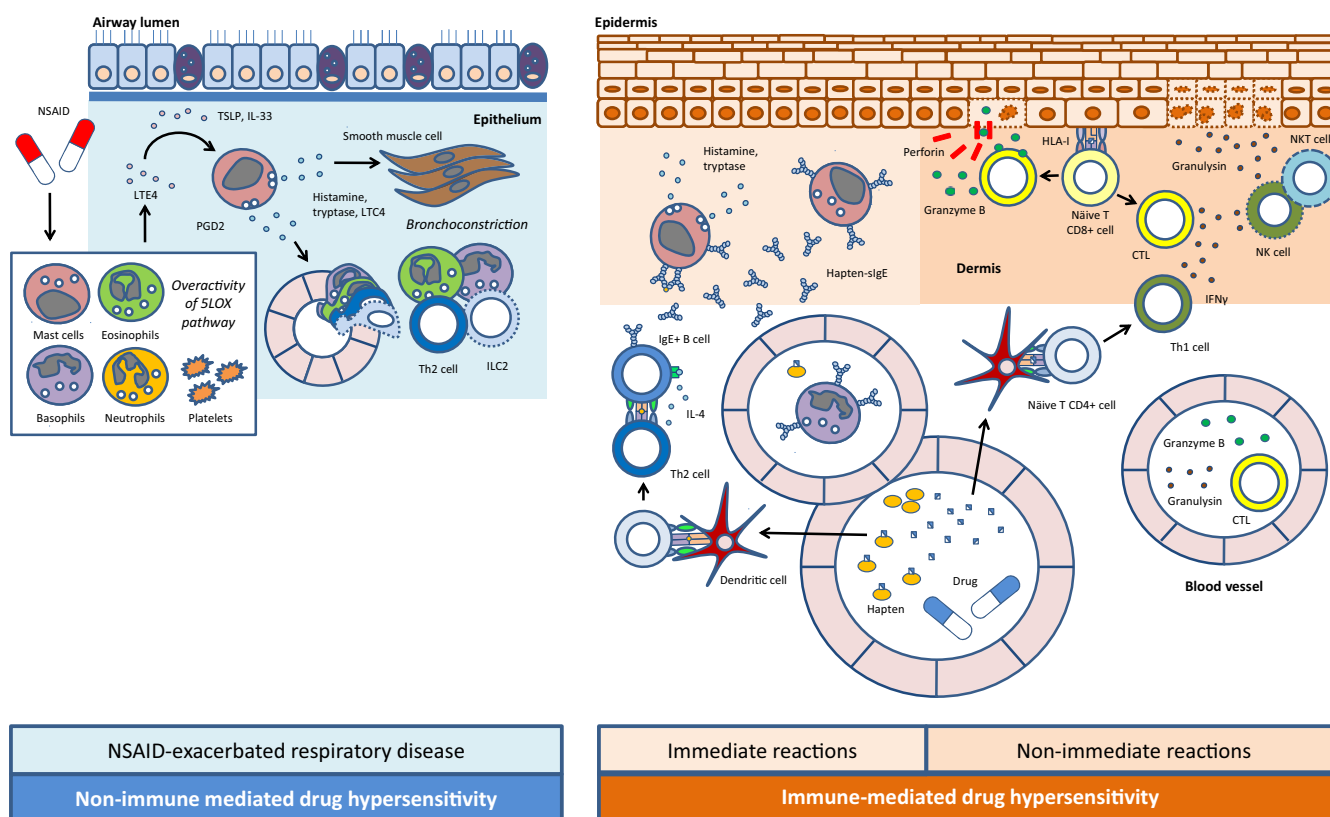


FIGURE 5 Phenotypes and endotypes of drug hypersensitivity. *NSAID-exacerbated respiratory disease*: In individuals with dysregulated activity of 5-lipoxygenase, the intake of NSAID induces the overproduction of LTE4 and other arachidonic acid metabolites by mast cells, eosinophils, basophils and platelets. LTE4 stimulates the airway epithelium to release TSLP and IL-33 which in turn mediates the IgE-independent activation of resident mast cells. Activated mast cells release histamine, tryptase and LTC4 which induce the constriction of bronchial smooth muscle cells. Mast cell-derived PGD2 induces the recruitment of eosinophils, basophils, Th2 cells and innate lymphoid cells 2 (ILC2) to accumulate in the airway mucosa; *Immune-mediated drug hypersensitivity, immediate reactions*: Once the drug reaches the bloodstream, it covalently binds to a circulating protein to become immunogenic (hapten). The hapten-carrier complex is taken up by dendritic cells which mediate the activation of naïve CD4+ T cells to generate hapten-specific Th2 cells. Th2 cells stimulate B cells to produce hapten-specific IgE which sensitizes resident mast cells and circulating basophils for activation; *Immune-mediated drug hypersensitivity, non-immediate reactions*: Some drugs can non-covalently bind to HLA-II on dendritic cells without active uptake or intracellular processing (p-i concept) to be presented to naïve CD4+ T cells which in turn acquire a Th1 profile. Some drugs can also non-covalently bind to HLA-I/peptide complexes on keratinocytes to generate a new antigenic determinant, activate naïve CD8+ T cells and generate cytotoxic lymphocytes (CTL). CTLs release perforin and granzyme B which exert cytotoxic activity over the keratinocytes expressing the new antigenic determinant. Th1 cells and CTLs also release IFNγ which activates in turn NK and NKT cells. These cells release vast amounts of granulysin which further damages the skin barrier. The inflammatory events of the skin are reflected at the peripheral level by increased granzyme B, granulysin and activated CTLs

5.1 | Cross-intolerance to NSAIDs

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most common triggers of drug hypersensitivity reactions, and in most cases, these reactions are not mediated by immunological mechanisms.¹³⁹ In non-selective or cross-intolerant reactions, NSAIDs from different groups provoke skin or respiratory symptoms.¹⁴⁰ In these cases, the reaction-inducing potential does not rely on the chemical structure of the drug, but on its COX-1 inhibitory activity.¹⁴⁰ Aspirin- or NSAID-exacerbated respiratory disease (AERD and NERD, respectively) is the most studied phenotype of cross-intolerance. This entity is defined by the onset of respiratory symptoms upon intake of NSAIDs and is related to a dysregulation of arachidonic acid (AA) metabolism with overproduction of leukotrienes (LT) and prostaglandins (PG).¹⁴¹ Many NERD subjects have concomitant CRS and asthma.¹⁴¹ In a Korean study, NERD patients were divided into four sub-phenotypes based on the presence of CRS, urticaria and atopy.¹⁴² Interestingly, significant differences existed in asthma severity, total serum IgE, sputum and peripheral eosinophilia, and urinary LTE4 (uLTE4).¹⁴² In American patients, uLTE4 helped to identify aspirin sensitivity in patients with different nasal inflammatory conditions.¹⁴³ A recent meta-analysis reported that the sensitivity and specificity of uLTE4 for identifying aspirin sensitivity in asthma ranged from 0.55 to 0.81 and from 0.77 to 0.82, respectively, depending on the detection method.¹⁴⁴ Serum LTE4 in combination with LTE4/PGF2 α ratio might help to detect NERD among other asthma phenotypes.¹⁴⁵ Aspirin provocation increased 8-iso-PGE2 in the exhaled breath condensate of NERD patients and correlated with uLTE4.¹⁴⁶ Platelet activation was also associated with overproduction of AA metabolites and to a reduced lung function in NERD patients.¹⁴⁷

Other biomarkers beyond AA metabolites have been related to NERD. The serum sphingosine-1-phosphate was higher in NERD patients than in other asthmatics.¹⁴⁸ American patients with NERD displayed higher activation of mast cells, basophils and platelets measured in nasal microparticles than other CRS individuals.¹⁴⁹

Overall, these biomarkers might facilitate the diagnosis of NSAID hypersensitivity by decreasing the need for drug provocations. Some cross-intolerance phenotypes resolve over time,¹⁵⁰ and these biomarkers might help determine the most adequate timing to test for aspirin tolerance.

5.2 | Immune-mediated reactions

These conditions can be divided into immediate and non-immediate reactions arising from IgE- and T cell-mediated mechanisms, respectively (Figure 5).¹¹²

5.3 | Immediate reactions

Betalactams (BL) and fluoroquinolones (FQ) are the most common drugs involved in immediate reactions.¹³⁹

5.3.1 | Betalactams

Skin testing displays a diagnostic sensitivity of up to 70%.¹⁵¹ Available in vitro tests include immunoassays to quantify serum BL-sIgE, including the commercial ImmunoCAP $\text{\textcircled{C}}$ (Thermo-Fisher, Uppsala, Sweden).¹⁵¹ Its sensitivity shows a high variability (0%-50%),¹⁵² depending on the reaction severity and the time gap at the moment of measurement.¹⁵³ Moreover, ImmunoCAP $\text{\textcircled{C}}$ can induce false-positive results when testing for Penicillin-V.¹⁵⁴ Increased serum tryptase during the acute phase of reactions can confirm mast cell activation¹¹² and correlates with the severity.¹⁵⁵ The sensitivity of BAT for BL allergy ranges from 22% to 55% with a specificity of up to 96%.^{156,157}

5.3.2 | Fluoroquinolones

Skin testing is not useful for the diagnosis of FQ allergy,¹⁵⁸ and there are no available immunoassays. The CD63-based BAT displayed 83.3% sensitivity and 88.9% specificity for ciprofloxacin allergy.¹⁵⁹ Surprisingly, CD203c outperforms CD63 as BAT-activation marker for moxifloxacin allergy, yet its sensitivity was low (36.4%).^{159,160} These data question the role of basophils in moxifloxacin allergy, but identify BAT as a promising tool for ciprofloxacin allergy.

5.4 | Non-immediate reactions

Patch testing and intradermal test with delayed reading are useful in vivo biomarkers. The sensitivity of the in vitro lymphocyte transformation test (LTT) is lower than that of BAT for immediate reactions.^{63,155} A combination of granzyme B and granulysin expression in blood cells can detect lymphocyte activation in the setting of severe cutaneous reactions like Stevens-Johnson syndrome.¹⁶¹

The screening for HLAB*57:01 before abacavir prescription is recommended by regulatory agencies,¹⁶² as it showed 100% of negative predictive value for immunologically confirmed abacavir hypersensitivity.¹⁶³ The screening for HLAB*15:02 is also recommended before carbamazepine treatment in patients at high risk (Han Chinese, Vietnamese, Cambodians, etc.).^{164,165}

6 | CONCLUSIONS

The potential of precision medicine in the fields of allergy and chronic respiratory diseases has only started to be explored. A better definition of disease phenotypes and endotypes based on treatable traits and other clinically significant aspects is a prerequisite to progress in individualized therapies. In the last decade, we have seen an improvement in the definition of allergic respiratory disease, and we have gained insights into other eosinophilic phenotypes of rhinitis, CRS and asthma. This knowledge has translated into a significant broadening of therapeutic options, including (but not limited to) new biologicals. Because precision

medicine needs to be performed in a cost-efficient way, there is a need to identify responder patients to these new drugs. On the other hand, the available therapies for NAR and non-eosinophilic CRS and asthma are much more limited, reflecting the important knowledge gaps in the pathophysiology of those phenotypes. Similarly, there is a need to progress in the definition of EoE and FPIES in order to improve the clinical management of the patients. The clarification of the disease mechanisms behind IgE-mediated food allergy and drug hypersensitivity will help identify patients at risk of developing allergic reactions, limit the number of required provocations and establish preventive strategies.

Nevertheless, the clarification of disease phenotypes per se does not guarantee the implementation of precision medicine in the clinical practice, as this step requires the detection of valid biomarkers. This search will be a long and resource-consuming path requiring large population cohorts. Among the different disciplines applied to biomarker identification, metabolomics appears as a promising tool, yet growing evidence indicates that valid biomarkers will be detected by multidimensional strategies. Valid biomarkers do not only need to accurately reflect the phenotype-specific disease mechanisms, but also to be quantifiable in a rapid, easy and cost-efficient way. Only under these premises, the research in biomarker identification will be able to impact the clinical practice and translate into an improved diagnosis, management, and treatment of patients with allergic and chronic respiratory diseases.

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CONFLICT OF INTEREST

None of the authors have any conflict of interest in relation to this article.

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