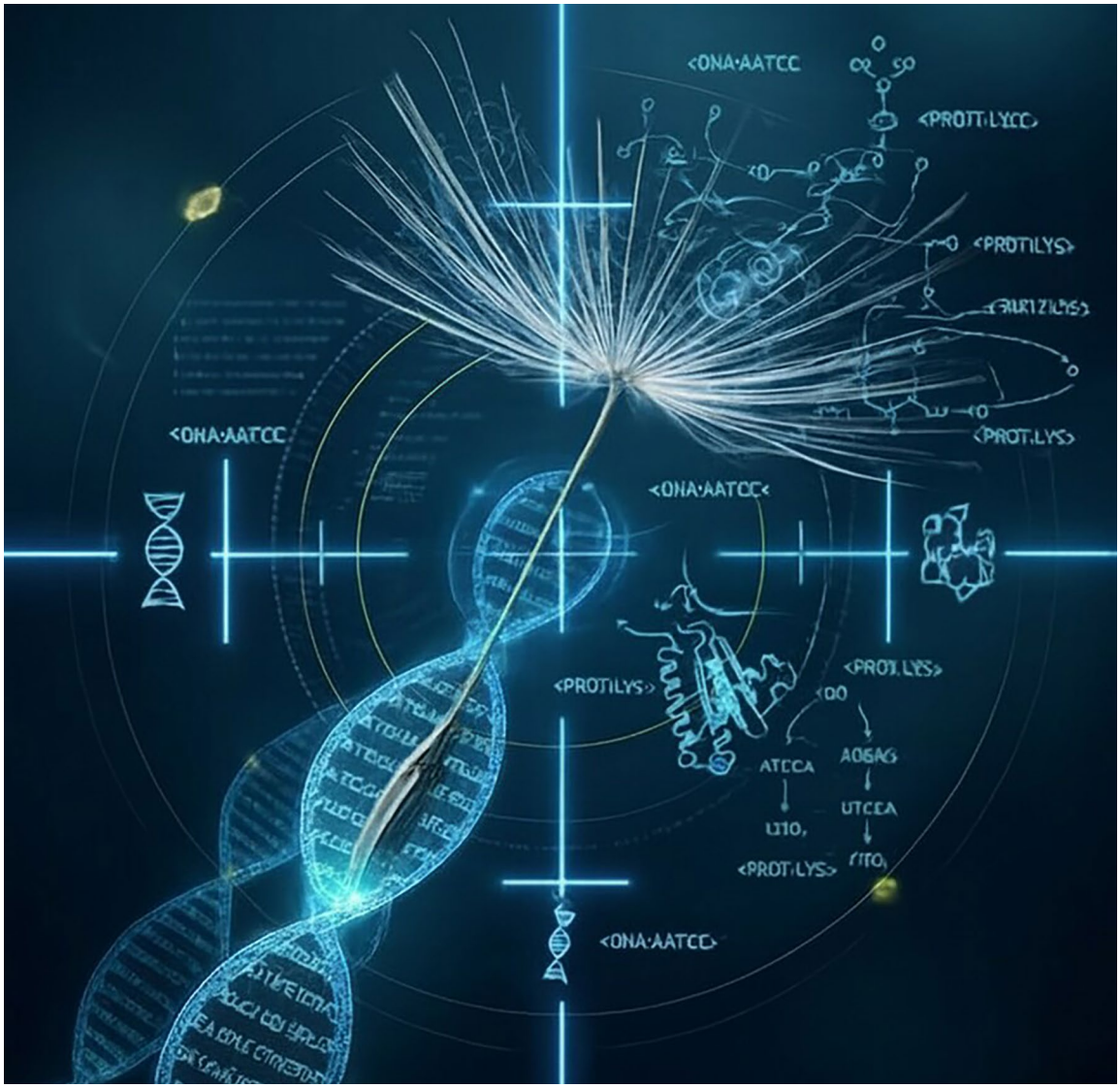
PRECISION ALLERGY

From Clinical Variability to Molecular Certainty



Dr. Ignacio Dávila & Dr. Belén de la Hoz
EDITORS

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Preface: Introduction to Precision Allergy

Ignacio Dávila, Belén de la Hoz Caballer, María Isidoro García

"Ideas do not last long.
One must do something with them."
Santiago Ramón y Cajal

"Science is always worthwhile
because its discoveries, sooner or later,
always find application."
Severo Ochoa

1. MEDICINE: FROM UNCERTAINTY TO PRECISION

Sir William Osler, a preeminent physician of the late 19th and early 20th centuries and widely regarded as a foundational figure of modern Internal Medicine, famously observed: "Medicine is a science of incertitude and an art of probability"[1]. Indeed, since its inception, medicine has navigated the turbulent waters of uncertainty in pursuit of the safe harbor of clinical certainty.

Were we to briefly inhabit the mind of an ancient physician evaluating a patient with abdominal pain, we would grasp the sheer weight of his insecurity when confronted with one of the most labyrinthine differential diagnoses (Figure 1). The skilled practitioner relied solely on his intellect, his perceptions, and his hands — that is, the accumulated wisdom and experience required to conduct and interpret the anamnesis and physical examination (inspection, auscultation, percussion, and palpation, noting that auscultation remained rudimentary until the advent of the stethoscope). At best, he might examine bodily fluids using his own senses. This was further compounded by the fact that for centuries, save for brief interludes, the dissection of cadavers —which would have elucidated human anatomy— was strictly prohibited.

In the modern era, this uncertainty has been markedly diminished; we can now obtain granular imaging of all thoracic and abdominal organs, visualizing many of them from within, and we possess a plethora of biomarkers that can pinpoint specific organ involvement through a simple venipuncture or fluid analysis. Undeniably, the physician's existential angst in the face of uncertainty is far less than that of his ancient counterparts. Nevertheless, in the vast majority of cases, it remains almost impossible to predict with absolute certainty how a patient will respond to a given treatment, whose outcome may be curative, ineffective, or even deleterious. However, some models have demonstrated excellent performance[2]. This aspect is inherent in the staggering breadth of human variability, rendering each patient a singular entity with highly divergent manifestations of the same disease and responses to therapeutic interventions. Osler once again captured this nuance with keen insight: "If it were not for the great variability among individuals, medicine might as well be a science, not an art."[3].

2. PRECISION MEDICINE

Arising from this recognition of human variability is the concept of personalized medicine, i.e., tailoring medical care to each patient's specific characteristics. This stands in stark contrast to the historical norm of generalized diagnostic and therapeutic protocols. However, the necessity of personalizing medicine has been posited since antiquity. Hippocrates' reflections on the paramount role of the sufferer over the sickness are well known, as is his insistence on individualizing the clinical context, given that no two patients are identical. This is epitomized in the dictum

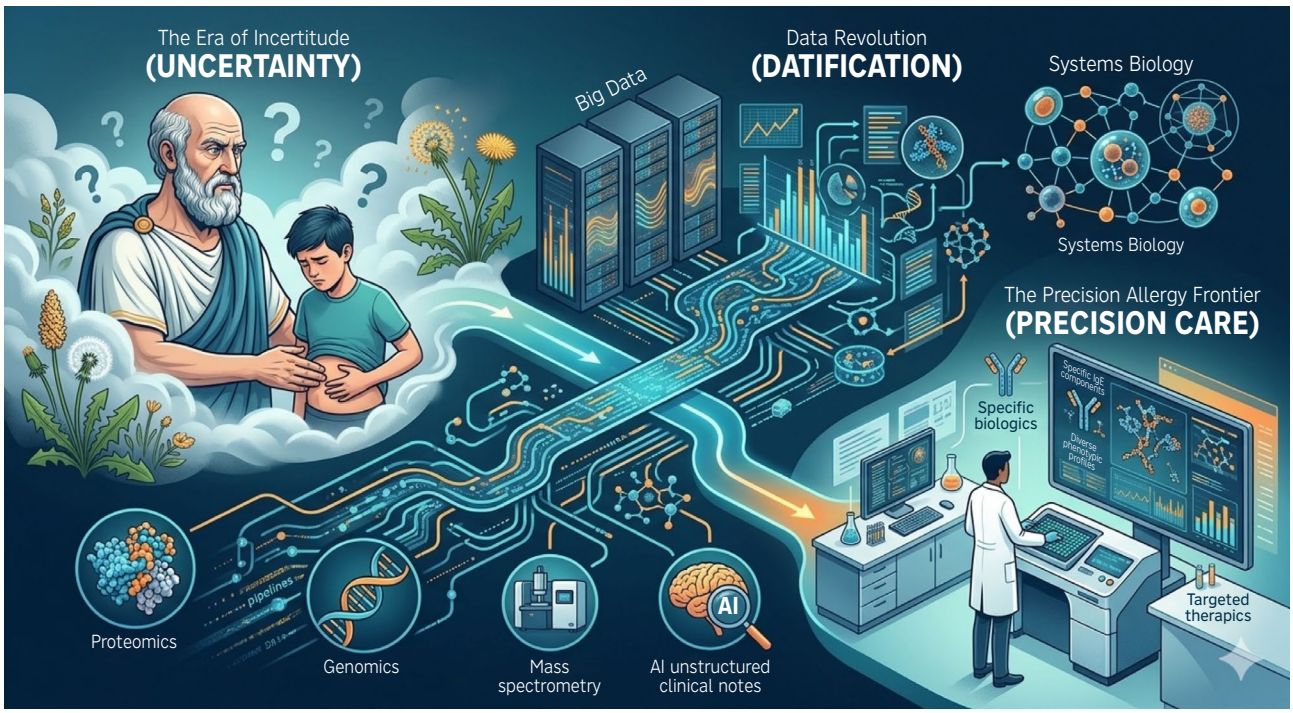

Figure 1. The path to precision allergy (image created with artificial intelligence).

attributed to him: "It is more important to know what sort of person a disease has than to know what sort of disease a person has"[4].

As our access to patient-specific clinical data has expanded, medicine has grown increasingly precise, giving rise to Precision Medicine. This evolution is reflected at varying speeds across medical specialties, which are progressively transitioning into precision-based disciplines defined by three fundamental pillars: (1) The integration of high-throughput technology into clinical workflows; (2) The reflection in medical literature that this transformation extends beyond the realm of research into direct clinical application; (3) The formal incorporation of "precision" as a standard term in daily practice[5].

Historically, medicine was personalized based on generic patient attributes such as weight, age, and disease severity; consequently, many conditions were —and still are— treated with severity-based steps[6]. Today, the integration of proteomics[7], metabolomics[8], epigenomics[9], pharmacogenomics[10], or, more specifically for allergy, immunomics, among others[11], enables the extraction of vast amounts of data regarding complex clinical processes, imbuing medical practice with a precision character.

Supported by advances in Data Science[12], high-throughput technologies have provided an increasingly high-definition map of the molecular and cellular aberrations underlying numerous allergic diseases[13]. This has helped explain the profound inter-individual variability at a molecular level through a holistic, interdisciplinary Systems Biology approach.

3. PERFORMING PRECISION ALLERGY

Allergic patients, as a cohort, exhibit greater variability than other patient groups. They present complex sensitization profiles, with distinct phenotypic manifestations and patterns influenced by their environment[14,15]. This necessitates advanced tools for assessing severity, determining future risk, and selecting treatments, all while accounting for significant regional variability in allergic diseases.

Consequently, from its very beginning, Allergology naturally applied diagnostic and treatment approaches tailored to the patient's profile. Consider the case of allergic asthma: patients undergo an exhaustive, almost detective-like anamnesis to identify potential exposures, which are then correlated with complementary tests to prescribe individualized treatment. Avoidance recommendations depend on the specific allergen sensitization, and therapy is formulated accordingly, prescribing not only inhaled corticosteroids but also immunotherapy tailored to the patient's exposure and sensitivity. In this sense, Allergology was the pioneering specialty in introducing per-

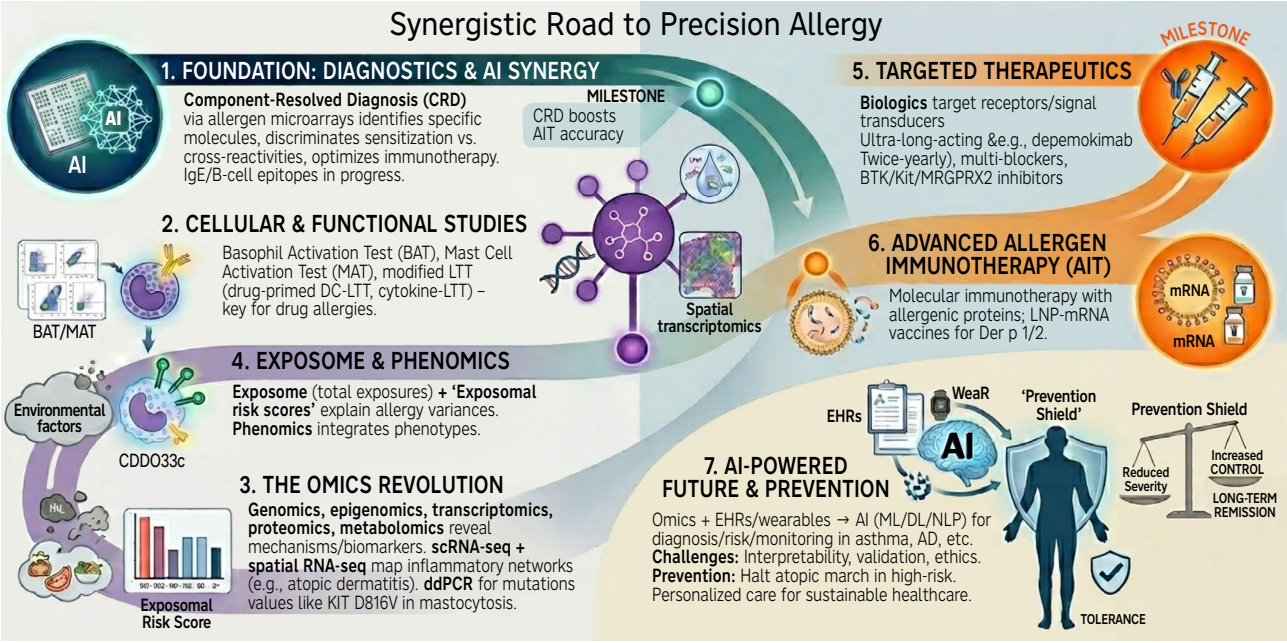

Figure 2. Bases of precision allergy (image created with artificial intelligence).

sonalization to both diagnosis and treatment[16].

Since then, Precision Allergy has branched into several key areas. On one hand, it seeks the etiopathogenic and immunological foundations of allergic diseases, which have not only expanded our knowledge base but also refined diagnostic tools. Through genomics, genetic variants influencing predisposition to allergic diseases have been studied[17]. The study of molecular bases has focused on identifying, validating, and standardizing biomarkers to predict allergic responses. This role extends beyond diagnosis into the therapeutic sphere, using pharmacotranscriptomics[18] as an example to identify potential therapeutic targets or pharmacogenomics to predict adverse drug reactions, thereby enabling the evaluation and indication of specific treatments, such as allergen immunotherapy and biologics targeting diverse immunological pathways[19-21].

The generation of information from clinical histories is fundamental to managing allergic diseases. Traditionally, this data was limited; however, we now have access to a wealth of information that has transformed classic databases into true Big Data, characterized not just by quantity but by a qualitative complexity that exceeds human reasoning capacity. To interpret and transform this information into knowledge, tools such as Artificial Intelligence (AI) are required. These include Natural Language Processing (NLP) for searching unstructured clinical notes and Neural Networks for predicting drug responses, particularly to penicillins or NSAIDs[22].

This shift is reshaping diagnostic testing, which has evolved from traditional skin tests and antibody/cytokine determinations to high-throughput technologies, molecular diagnostics, allergen microarrays, digital PCR, mass spectrometry, and, lately, NGS in pharmacogenomics[23]. Ultimately, this transformation toward Precision Allergy is essential for advancing the efficiency and sustainability of the healthcare system[24].

4. PRECISION ALLERGY

The road toward Precision Allergy is paved by the synergy between the diagnostic/research laboratory and the development of AI (Figure 2). Component-Resolved Diagnosis (CRD) using allergen microarrays is already a reality, enabling the identification of specific allergenic molecules responsible for a patient's symptoms. This enables clinicians to distinguish genuine sensitizations from cross-reactivities, leading to a much more accurate selection of immunotherapy[25]. Furthermore, ongoing studies are exploring the clinical utility of determining IgE[26] and B-cell epitopes[27].

Another critical frontier involves cellular and functional studies, such as the Basophil Activation Test (BAT) and Mast Cell Activation Test (MAT)[28], or modifications of the Lymphocyte Transformation Test (LTT), such as the drug-primed dendritic cell LTT[29] or the cytokine-based LTT[30], which are of particular interest in drug allergies[31].

However, the true revolution lies in the application of multiomics[17], which facilitates the discovery and understanding of disease mechanisms and the identification of new biomarkers[32]. In these fields, advances such as single-cell RNA-seq[33] or spatial RNA-seq —often used in combination— are revealing the complex inflammatory cell networks in conditions like atopic dermatitis[34]. Furthermore, Digital Droplet PCR offers unprecedented precision and sensitivity for quantifying specific mutations, such as KIT D816V in mastocytosis[35], or for describing familial hypertryptasemia[36].

The exposome —the sum of environmental factors to which an individual is exposed— is also vital to understand in Precision Allergy[37]. "Exposomal risk scores" are even being developed to explain variances in allergy diagnoses[38]. Another related field is pharmacophenomics[39].

In the therapeutic field, biologics and molecules targeting specific receptors or signal transducers, in the very core of Precision Allergy, have completely altered the prognosis and evolution of allergic diseases[40]. Currently, we are seeing the development of ultra-long-acting biologics (e.g., twice-yearly depemokimab)[41], double or triple or even multiple blockers[42], and targeted molecules such as Bruton's tyrosine kinase (BTK) inhibitors, the Kit receptor[43], or the MRGPRX2 receptor (MRGPRX2)[44], to mention some of the most important.

In the field of Allergen Immunotherapy (AIT), beyond mechanistic advances (reviewed in [45]), we are seeing the rise of molecular immunotherapy using allergenic proteins[46], and the development of lipid nanoparticle (LNP)-encapsulated mRNA vaccines encoding Der p 1 and Der p 2[47].

These various Omics sciences, coupled with electronic health records and data from wearable sensors, generate a gargantuan amount of data that necessitates AI systems[48]. In Precision Allergy, AI provides innovative tools for diagnosis, risk prediction, and monitoring, enabling deeper personalized care. The application of Machine Learning (ML), Deep Learning (DL), and Natural Language Processing (NLP) to pathologies such as asthma, anaphylaxis, atopic dermatitis, allergic rhinitis, eosinophilic esophagitis, and food allergy is yielding significant breakthroughs[49]. Nevertheless, essential hurdles remain, including a lack of model interpretability ("black box" issues), the need for external validation, and the need to navigate ethical and regulatory barriers.

Finally, a cornerstone of Precision Allergy will be its capacity for prevention —either preventing the development of disease in high-risk subjects or halting the progression of the "atopic march," a field that, while still in its infancy, holds immense promise for the future of medicine[50].

The transition toward Precision Allergy is essential for the efficiency and sustainability of global healthcare systems. By integrating high-throughput diagnostics, omics sciences, and AI, the field is moving toward a future in which the prevention and management of allergic diseases are as singular as the patients themselves.

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1 The Allergy Laboratory and Precision Allergy

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1. INTRODUCTION

Laboratory tests in Allergology have progressively evolved and continue to develop towards the concept of Precision Allergy. This approach represents an advanced framework within the allergy laboratory for identifying robust, clinically meaningful biomarkers applicable to diagnosis, disease management, and individualized treatment. Precision Allergy is based on an in-depth characterization of the patient that extends beyond the clinical phenotype, integrating molecular and cellular diagnostic tools and, where feasible, a detailed assessment of the exposome together with high-throughput technologies (e.g., genomics, transcriptomics, proteomics, or metabolomics, among others). On the other hand, integrating these large-scale datasets with artificial intelligence (AI) facilitates the analysis of complex, multidimensional information and the identification of clinically relevant patterns. However, Precision Allergy remains an evolving field; therefore, this chapter focuses on the biomarkers and diagnostic tests currently most widely used in routine allergology laboratory practice.

2. *IN VITRO* DIAGNOSTIC TESTS FOR CLINICAL DIAGNOSIS IN ALLERGOLOGY

In vitro studies in Allergology aim to aid clinical diagnosis and monitoring of allergic disorders by analyzing biological samples, primarily serum and whole blood, although other samples can be used. They complement or substitute *in vivo* tests, such as skin tests (STs) or provocation tests, especially when these are contraindicated or inconclusive.

In general terms, currently validated Allergology *in vitro* assays can be grouped into: 1) immunoassays to detect antibodies or soluble inflammatory mediators, 2) cellular assays to explore cell morphology or physiology, 3) genetic tests, and 4) tissue biopsies. Immunoassays are highly specific bioanalytical methods based on the formation of immune complexes that allow the measurement of the presence or concentration of an analyte in a fluid. Cellular assays include a broad range of methods, based primarily on techniques such as flow cytometry and/or cell culture, but not restricted to, that allow the exploration of cell phenotype or physiology, especially in response to allergen stimulation. Current genetic tests examine variants in the genome that determine or are associated with disease. Finally, tissue biopsies allow exploration of the immune infiltrate and local soluble mediators. In addition to these state-of-the-art methods, several others are available; however, in a research context, such as omics technologies, with the primary aim of discovering new potential biomarkers.

The choice of the *in vitro* assay and the biological sample used is critical for the accuracy and clinical relevance of the assessment. An overview of commonly used sample types (both for clinical diagnostics and emerging research use) and their applications is shown in Table 1.

3. IMMUNOASSAYS FOR ANTIBODIES AND INFLAMMATORY SOLUBLE MEDIATORS

3.1. Principles of the Method

Immunoassays rely on the fundamental principle of antigen-antibody binding, which can be harnessed in various formats to detect either total immunoglobulin (Ig) levels or specific antibodies against defined antigens (allergens in allergy) or other soluble molecules. Multiple tests coexist in the clinical laboratory today, but they all share the fundamental step of incubating a patient's biolog-

TABLE 1. Biological Sample Types and Their Applications in Allergology.

Sample Type	Main Use	Key Considerations
Serum/Plasma	Detection and/or quantification of total and specific antibodies (IgE, IgG, IgG4) and soluble inflammatory mediators (tryptase, cytokines, complement)	• Non-Invasive; Clinically validated • Emerging use: moving into nanoscale technologies/nanofluidics, detection through mass spectrometry, and glycoforms study
Whole Blood	Cellular assays: flow cytometry and cell culture-based assays • BAT • LTT • ELISpot	• Non-Invasive • For BAT, fresh anticoagulated whole blood is used (with heparin or EDTA with calcium supplementation), analyzed within 4–24 h • For LTT and ELISpot PBMC are isolated (e.g., density gradient) from anticoagulated blood (preferably with heparin) • Emerging use: PBMCs can be analyzed using high-dimensional spectral flow cytometry and single-cell analysis
DNA	Genetic studies	• Isolated from anticoagulated blood (preferably not in heparin) or bone marrow • Emerging use: single-cell analysis
Sputum	Airway inflammation monitoring (e.g., asthma)	• Non-invasive; correlates moderately with BAL and biopsies for eosinophilia in asthma
BAL	Distal airway and alveolar immune cell analysis	• Invasive; complementary to sputum; correlation varies by disease (e.g., asthma vs COPD)
Biopsy (bronchial, esophageal, skin, bone marrow…)	Tissue-level/localized immune cell infiltrate and mediator analysis	• Invasive; useful for specific pathologies (AD, CSU, asthma, EoE, mast cell-related disorders) • Emerging use: digitization of biopsies and application of computer tools such as machine learning
Tears/Nasal secretions/Saliva	Exploratory quantification of IgE or soluble mediators	• Limited clinical validation; mainly research; low sample volume and high variability
Urine	Metabolomics, some proteomics	• Non-invasive; low protein content; requires concentration steps

AD: Atopic Dermatitis; BAT: Basophil Activation Test; COPD: Chronic Obstructive Pulmonary Disease; CSU: Chronic Spontaneous Urticaria; EDTA: Ethylenediaminetetraacetic Acid; ELISpot: Enzyme-Linked ImmunoSpot Assay; EoE: Eosinophilic Esophagitis; Ig: Immunoglobulin; LTT: Lymphocyte Transformation Test; PBMCs: Peripheral Blood Mononuclear Cells.

ical sample (mostly serum) to allow antigen-antibody recognition and binding. The second phase involves adding a secondary antibody to capture/detect the antigen-antibody reaction. Detection systems may include enzyme-labeled, fluorescent, chemiluminescent, radioactive, or label-free technologies. The resulting signal or binding event is measured to determine analyte presence or concentration (qualitative and semi-quantitative/quantitative assays, respectively). Assays may be configured as singleplex (one analyte per test) or multiplex (simultaneous detection of multiple analytes)[1–3].

Total and Specific IgE, IgG, and IgG4

Immunoglobulin E (IgE) is the hallmark molecule of type I hypersensitivity reactions. Thus, immunoassays to determine total IgE (tIgE) and/or allergen-specific IgE (sIgE) are essential in allergy diagnosis. For tIgE quantification, a capture antibody is bound to the assay matrix to bind all available IgE. For sIgE, the allergen is immobilized to the assay matrix, allowing specific antibodies to bind. Allergens to test range from whole extracts of allergenic sources (complex mixtures with allergenic and non-allergenic molecules) and synthetic drugs to specific molecular components and drug metabolites. In both cases, tIgE or sIgE bound to the matrix (either solid- or liquid-phase) can be measured with a detectable anti-IgE antibody[1–3]. A reverse format has been employed, in which the patient's IgE is first captured, and then labelled antigens are added to detect bound IgE, but despite its advantages, its implementation remains challenging[2]. While the principle is the same across most systems, they

TABLE 2. Specific IgE Most Widely Used Methods.

Method	Company	Singleplex/ Multiplex	E/M	Laboratory Process	Detection Method	Q-Range	tIgE	Others
ImmunoCAP	ThermoFisher	Singleplex	E/M (> 550/> 100)	Automated	Fluorimetry	0.1-100 kUA/L	Yes, Q	Industry reference standard. Allows for allergen-specific IgG/IgG4
3gAllergy (Immulite)	Siemens	Singleplex	E/M (> 450/± 40)	Automated	Chemiluminescence	0.1-100 kUA/L	Yes, Q	Allows for allergen-specific IgG/IgG4
Noveos	Hycor Biomedical	Singleplex	E/M (> 200/± 50)	Automated	Chemiluminescence (Q-correction through fluorescent beads)	0.1-100 kUA/L	Yes, Q	Very low sample volume; allows for allergen-specific IgG/IgG4 (in development)
ImmunoCAP ISAC	ThermoFisher	Multiplex (microarray)	M (112)	Manual	Fluorimetry	SQ (ISU)	No	
Allergy Explorer (ALEX)	MacroArray Diagnostics (MADx)	Multiplex	E/M (> 100/> 150)	Manual/ Automated	Colorimetry	0.1-50 kUA/L	Yes, Q/SQ	Default CCD inhibition
EUROLINE Allergy profiles	Euroimmun	Multiplex (strip-based)	E/M (> 60 profiles combining both)	Automated	Colorimetry	SQ	No	Allows for allergen-specific IgG4 detection

ALEX: Allergy Explorer; CCD: Cross-reactive Carbohydrate Determinants; E: Complete extracts; Ig: Immunoglobulin; ISU: ISAC Standardized Units; kUA/L: kilo Units of Allergen-specific Antibody per Liter; M: Molecular allergens; MADx: MacroArray Diagnostics; Q: quantitative; SQ: semi-quantitative. Table based on references 1 and 2.

differ in the allergens used, the matrix in which the allergen is bound, the detection antibody, and the substrate; all of these factors must be considered when interpreting the results. Extrapolation of values from one method to another is not recommended, and ratio analysis (e.g., sIgE/tIgE or sIgE component/extract) should be done with measurements obtained with the same analytical method[4].

Both quantitative and semi-quantitative systems are commercially available for tIgE and sIgE (Table 2). Methods for tIgE quantification are also broadly available on general clinical chemistry analysers. Additionally, some current systems allow the use of anti-IgG or anti-IgG4 detection antibodies, enabling the measurement of specific IgG/IgG4 (sIgG/IgG4) against the same allergens (see Table 2). Other multiplex systems are available on the market for IgG or IgG4, especially for food allergen panels. However, they are not indicated for IgE-mediated type I hypersensitivity, and their use in other clinical contexts, such as food intolerances, is also discouraged due to the absence of solid evidence[1,5].

Quantitative systems for tIgE and sIgE are calibrated using the World Health Organization (WHO) IgE Standard, 75/502 before and 11/234 now, as a reference material (heterologous calibration for sIgE)[3]. For tIgE, concentration is reported in International Units per milliliter (IU/mL) or kilo Units per liter (kU/L), equivalent to 2.4 ng/mL. For sIgE, results are reported in kUA/L (kilo Units of allergen-specific antibody per liter). Historically, the clinical cut-off for defining a positive sensitization result has been 0.35 kUA/L. Nevertheless, this threshold was set based on the technical detection limits of older systems rather than on clinical significance[6]. Nowadays, with improved detection systems and evidence that values between 0.1 and 0.35 kUA/L may be clinically relevant, especially when tIgE is low, the 0.1 kUA/L threshold has gained support, although it can impair test specificity. Several studies have aimed to establish sIgE cut-off values for diagnostic purposes (e.g., predicting symptoms or challenge outcomes); however, this remains challenging because sIgE cut-offs are allergen- and population-dependent. Semiquantitative systems are calibrated with an internal control, and results are reported in different standardized units.

Inhibition assays are specialized *in vitro* methods used to assess cross-reactivity between allergens and to confirm true sensitization. In particular, the use of CAP inhibition is increasing in

the routine clinical laboratory and is particularly useful for evaluating Hymenoptera venom allergy (HVA), food allergy (FA), pollen-food syndromes, and cases where multiple positive sIgE results complicate diagnosis. It works by pre-incubating patient serum with a suspected allergen, which competitively binds sIgE before exposure to a standardized ImmunoCAP solid-phase allergen. If inhibition occurs, it indicates that the pre-incubated allergen shares epitopes with the solid-phase allergen, suggesting cross-reactivity[7,8].

Precipitins

When IgG immune complexes are suspected, precipitating antibodies (precipitins) can be assessed. Currently, the double diffusion method (Ouchterlony technique) is still the only available method for assessing precipitating antibodies despite its drawbacks. In this assay, the patient's serum and the corresponding antigen extract are placed in separate wells within a porous medium (typically agar or agarose gel). As both diffuse toward each other, visible precipitin lines form at optimal antigen-antibody ratios, indicating the presence of sIgG immune complexes. Precipitins are a poorly standardized manual technique. The lack of validated reagents poses the main challenge when establishing the method. Alternatively, immunoassays such as sIgG ImmunoCAP assays permit the quantification of antigen (avian and fungal) sIgG, although not evaluating its precipitation capacity.

Immunoassays for Inflammatory Soluble Mediators

In allergy, mediators such as tryptase, complement components, and certain cytokines serve as biomarkers of immune activation and inflammation, although they do not indicate the responsible agent. Many of these are detected through classic immunoassays with a capture antibody and a detection antibody (e.g., tryptase, histamine, cytokines). However, other immunoassays are associated with nephelometry or immunoturbidimetry, in which light scattered or transmitted through a sample, respectively, is used to quantify the analyte of interest (e.g., complement system tests).

3.2. Applications in Allergology

Total IgE

Total IgE may reflect an overall atopic status. Thus, it plays a supportive role in the diagnostic work-up of atopic disease and may facilitate the interpretation of sIgE values; however, it has limited diagnostic value on its own[9]. Total IgE interpretation must consider other causes of elevation, such as parasitic or viral infections, an immunodeficiency, neoplasms, or the consumption of certain substances[10]. Total IgE is also used to monitor conditions such as atopic dermatitis (AD) or allergic bronchopulmonary aspergillosis (ABPA) and may help assess anti-IgE therapy[11–13]. In addition, it allows evaluation of selective IgE deficiency, described in patients with extremely low serum tIgE, undetectable with standard total IgE tests with regular calibration curves (generally < 2.0 or < 2.5 kU/L), while all other Ig classes remain normal[14].

Specific IgE

In vitro sIgE testing complements traditional STs in allergy work-up, offering advantages such as precise quantitative measures (particularly when using automated and validated diagnostic systems), avoiding the induction of potential anaphylactic reactions in highly susceptible patients, and suitability for use in patients with skin conditions (e.g., dermographism) or under specific treatments (e.g., antihistamines). The introduction of the use of allergens/molecular components, the so-called molecular diagnosis or component-resolved diagnosis (CRD), in routine allergy practice over the last two decades has significantly revolutionized the specialty, leading towards a more precise diagnosis and personalized patient management (e.g., Precision Allergy). However, the diagnostic complexity associated with it remains a challenge for the proper interpretation of results. Despite pending issues, such as the fact that sIgE still only represents sensitization and the lack of consensus cut-off values for clinical relevance, CRD has led to an improvement in differentiating genuine allergen sensitization from cross-reactivity, patient risk stratification, improved guidance for allergen-specific immunotherapy (AIT) selection and patient management in the need of exclusion diets[1–3].

Specific IgG and IgG4

The use of sIgG and sIgG4 is limited in routine clinical practice. Specific IgG to avian and fungal allergens can aid in the diagnosis and management of hypersensitivity pneumonitis and ABPA, although clear cut-off values for clinical relevance have not yet been established[9].

On the other hand, sIgG4 can be quantified (alone or in combination with sIgE) in the context of AIT, given its role in immune tolerance as a

blocking antibody, although its clinical value in routine allergy practice remains limited[15].

Tryptase

Quantitative measurement of tryptase in peripheral blood is intended to assess mast cell burden and activation. Tryptase exists in several isoforms, with baseline levels reflecting constitutive secretion of immature α- and β-protryptases, and acute elevations indicating release of mature tetrameric tryptase during mast cell degranulation events such as anaphylaxis or mast cell activation syndromes. Acute rises are specific for mast cell degranulation but are not present in all cases of anaphylaxis, and the absence of elevation does not exclude the diagnosis. Test sensitivity varies by context and trigger, but is generally moderate. Pooled meta-analytic data show sensitivity around 0.49 and specificity around 0.82 for anaphylaxis overall, with higher sensitivity in perioperative and venom-induced cases and lower sensitivity in food-induced anaphylaxis and in children[16–18]. For clinical evaluation, paired measurements are recommended: at least an acute sample is collected, optimally between 30 minutes and 2 hours after symptom onset (timing is critical for optimal sensitivity), and a baseline sample is obtained at least 24 hours after symptom resolution. A significant mast cell activation event is supported if the acute tryptase exceeds the baseline by the consensus formula "acute tryptase > (1.2 × baseline tryptase) + 2 ng/mL" (i.e., The 20% + 2 formula)[19].

Elevated baseline/basal serum tryptase may indicate clonal mast cell disorders, hereditary α-tryptasemia (HαT), or other myeloid malignancies. It should be interpreted in the context of clinical findings and, when appropriate, *TPSAB1* (Tryptase alpha/beta 1 gene) genetic testing by digital droplet polymerase chain reaction –PCR– (ddPCR) assay[20–22].

Complement System

Complement testing uses quantitative assays to measure levels of protein (C3, C4) and activation fragments (C1q, C3a, and C5a), while functional assays such as CH50 and AH50 assess pathway activity. Together, they provide a comprehensive view of complement integrity and function. Complement system testing is central to the diagnosis of hereditary angioedema (HAE) with C1 esterase inhibitor (C1-INH) deficiency and to some hypersensitivity reactions to drugs.

Serum Sickness Reaction is a type III hypersensitivity reaction to drugs, induced by the formation of immune complexes, which produce activation of mast cells through the complement system (C3a, C5a)[16,23].

Additional Inflammatory Mediators

Other inflammatory mediators, such as histamine, cytokines, lipid mediators (e.g., prostaglandins or their urinary metabolites [e.g., prostaglandin D2 (PGD2) or 11β-PGF2α]), platelet-activating factor (PAF), and leukotrienes (particularly urinary LTE4), are central to the pathophysiology of allergic inflammation and anaphylaxis. Nevertheless, their use as biomarkers is limited to research settings or selected clinical scenarios and is not recommended as a first-line diagnostic tool by current consensus guidelines, given that they are not fully clinically validated[24,25].

Histamine has a rapid metabolism and very short plasma half-life; thus, its levels must be measured within minutes of symptom onset, limiting its practical diagnostic utility. Measuring cytokines in serum is increasingly proposed, with IL-6 standing ahead for diagnosing certain drug hypersensitivity reactions, but also for monitoring therapeutic responses and guiding personalized medicine[26,27]. Cytokine quantification presents unique challenges due to their low circulating concentrations, short half-lives, and susceptibility to pre-analytical variables. Several methodologies are currently available with different principles, technical specifications (e.g., quantification range, sample volume request, or hands-on time), strengths and limitations, and clinical applicability[28,29]. Nevertheless, as challenges are overcome and newer technologies become more common, cytokine quantification will become an essential tool for Precision Allergy.

4. CELLULAR AND FUNCTIONAL STUDIES

4.1. Basophil Activation Test

Principles of the Test

The Basophil Activation Test (BAT) is a functional cellular, *in vitro* flow cytometry-based assay that fundamentally detects IgE-mediated allergic reactions by measuring upregulation of basophil activation markers (e.g., CD63, CD203c) after exposure to allergens/drugs. Stimulus concentration varies significantly between allergenic proteins, typically requiring µg/mL, whereas drugs often need mg/mL and must be freshly prepared to avoid degradation.

Results interpretation is based on the percentage of basophils expressing activation markers, but

also as stimulation index (SI) (proportion of stimulated-activated basophils vs. nonstimulated-activated basophils). Basophil sensitivity (CD-sens, the lowest allergen concentration required to achieve 50% of the maximum upregulation of CD63) is helpful for therapeutic monitoring[30].

For outcomes, receiver operating characteristic (ROC) curve analysis is recommended to define cut-off values for positivity, balancing sensitivity and specificity. Standardization of protocols is necessary for widespread clinical implementation with acceptable inter- and intra-laboratory variability[31,32].

External factors that can lead to false negative results include patients' treatment with systemic immunosuppressants (e.g., oral steroids), transient basophil unresponsiveness post-reaction (lasting 3-4 weeks), and a reduction in sIgE levels after the index reaction (critical for drug hypersensitivity reactions [DHRs]). If basophils fail to react to the positive control (non-releasers), the test is deemed inconclusive[30–32].

Two types of BAT assays can be distinguished: the Conventional or Direct BAT, which is the most widely used and requires a fresh whole blood sample to stimulate cells with allergens/drugs directly; and the Passive BAT (pBAT) that uses donor basophils that are stripped of antibodies and passively sensitized with patient serum or plasma. pBAT avoids the need for fresh blood and is helpful for patients with non-releaser basophils; however, it is highly dependent on serum sIgE levels[31,33]. Alternatively, cell lines like Rat Basophilic Leukemia (RBL) Cells, particularly humanized RBL cells (RBL-2H3 or RBL-SX38), can serve as donor basophils in pBAT[33].

Applications in Allergology

BAT complements conventional allergy diagnostics (e.g., STs, immunoassays, or *in vivo* challenges), especially when these are inconclusive or contraindicated[5,30–32,34,35]. Its utility spans diagnosis precision, allowing testing of specific allergenic components or drug metabolites[5,32], as well as monitoring and risk assessment in various allergic conditions.

Immediate Drug Hypersensitivity Reactions (IDHRs)

BAT is highly recommended for IgE-mediated reactions to several drugs and as the first step in the diagnostic algorithm in life-threatening anaphylaxis, where a drug challenge is strictly contraindicated. BAT is not advised for non-allergic reactions to non-steroideal antiinflammatory drug (NSAID) or those mediated by Mas-related G-protein coupled receptor X2 (MRGPRX2)[32].

Food Allergy (FA)

BAT helps distinguish between clinical allergy and asymptomatic sensitization to particular food[5,36]. It is useful when results from STs or immunoassays are unclear, reducing the need for oral food challenges (OFCs), and can predict severe reactions with high sensitivity and specificity[5]. For cow's milk allergy, BAT is the best predictor for reactions to baked and fresh milk[37].

Hymenoptera Venom Allergy (HVA)

BAT supports diagnostics with high predictive value for HVA, complementing STs and being especially important in patients with negative sIgE and in cases of double sensitization, helping to identify clinically relevant allergens and advancing Precision Allergy[34]. BAT also discriminates relevant sensitization from cross-reactive carbohydrate determinants (CCD) reactivity[38].

Respiratory Allergy

BAT is emerging as a tool for phenotyping respiratory allergies (rhinitis and asthma) and evaluating severity, thereby reducing the number of Nasal Allergen Challenges (NAC) required. It has been reported to be particularly relevant in local allergic rhinitis and dual allergic rhinitis[30,35].

Chronic Spontaneous Urticaria (CSU)

BAT, particularly when performed using patient serum to challenge donor basophils (pBAT), is proposed as an *in vitro* alternative to the Autologous Serum Skin Test (ASST) to functionally assess the presence of autoantibodies that can activate mast cells and basophils, helping to identify the Type IIb autoimmune endotype of CSU[30,39].

Disease Monitoring and Activity

In drug desensitization monitoring, BAT can assess the reduction in basophil sensitivity and predict the risk of breakthrough reactions with high predictive values[32]. BAT monitors the natural resolution of FA (e.g., cow's milk allergy) and supports clinical decisions about when a food can be safely reintroduced into the diet[37]. Moreover, it aids in monitoring efficacy and predicting risks in Hymenoptera venom immunotherapy (IT) and food oral or sublingual immunotherapy (OIT/SLIT)[34,37,38]. Basophil sensitivity throughout IT correlates with tolerance. In CSU, BAT helps identify patients with more active disease (higher

Urticaria Activity Score) and can be used to monitor treatment response[39,40].

Other Applications

BAT has also been found helpful in the evaluation of the risk of reactions to (i) novel IgE-based anti-cancer therapeutics before administration[41], (ii) rare IDHRs to polyethyleneglycol (PEG), an excipient found in some vaccines like COVID-19[42],and (iii) discrimination of clinically relevant latex allergy[43].

4.2. Lymphocyte Transformation Test

Principle of the Method

The Lymphocyte Transformation Test (LTT), a cellular *in vitro* test, is increasingly important for Precision Allergy and measures T-cell proliferation in response to a suspected drug. Peripheral Blood Mononuclear Cells (PBMCs) containing drug-specific memory T cells are incubated with the suspected drug, which is presented by antigen-presenting cells (APCs, such as monocytes and B cells)[44–46]. Specific T cells will then activate, typically inducing the secretion of cytokines like Interleukin-2 (IL-2), and undergo proliferation (blastogenesis).

The LTT methods are classified based on the readout method: 1. Conventional LTT (C-LTT) readout relies on measuring tritiated thymidine (H^3-thymidine), a radioactively labeled DNA base, incorporation in the dividing cells using liquid scintillation counting. This technique is hampered by the use of radioactive tracer and the inability to identify proliferating cell subpopulations[45]; 2. Flow Cytometry-Based LTT (CFSE-LTT) uses fluorescent markers like Carboxyfluorescein Diacetate Succinimidyl Ester (CFSE), whose content decreases in proliferating cells ($CFSE_{Dim}$) and is measured by flow cytometry. CFSE-LTT allows the analysis of proliferation of specific cell subpopulations ($CD3^+$, $CD4^+$, $CD8^+$, NK cells…) as well as cytokine production patterns within each[47]. A modification of this method to enhance sensitivity is the drug-primed-DCs LTT (dDC-LTT), which incorporates monocyte-derived dendritic cells (mo-DCs) as professional APCs into the culture, significantly improving drug presentation and, consequently, the proliferative response[47,48], demonstrating higher sensitivity in patients with delayed DHRs (DDHRs)[47]. Moreover, the analysis of the real effector cells for each endotype using CFSE-LTT improves the sensitivity[49]; 3. Cytokine-based LTT (Cyto-LTT) measures the secretion of multiple cytokines and markers (IL-5, IL-13, IFN-γ, granzyme B, and granulysin) in supernatant to capture T cell responses, including Th1/Th2, and cytotoxic activity[50]. Cyto-LTT not only identifies the culprit drug but, because it correlates with severity, also quantifies the strength of immune activation, especially important for more severe reactions like Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS)[50]; 4. Early Activation Markers: Measuring the upregulation of T cell surface markers (e.g., CD69 or CD25) via flow cytometry, which occurs early after stimulation (often 48 hours), can also be used as an *in vitro* marker for DDHR[51].

Results are commonly expressed as a percentage of proliferating or activated cells, or as SI (the ratio of results in drug-stimulated cultures to those in unstimulated control cultures). Typically, a cut-off SI > 2-3 is generally considered for a positive result, depending on the drug involved. This should be calculated after a ROC analysis[45].

Applications in Allergology

LTT is particularly used in the diagnosis and risk stratification of DHRs, which often present complex and heterogeneous clinical manifestations[52]. It is primarily used for DDHRs, which are often T cell-mediated and are important for achieving precision in DHR diagnosis and management, stemming from its role in accurately identifying culprit drugs/drug metabolites, understanding immunopathological mechanisms, and stratifying risk based on the strength of the immune response. LTT could be recommended when the "gold standard" Drug Provocation Test (DPT) is contraindicated due to the high risk of inducing a severe or life-threatening reaction, such as Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) or DRESS or acute generalized exanthema (AGEP). The LTT enables investigation of underlying pathological mechanisms, a key step in precision medicine. Given its limited sensitivity, LTT remains a complement to other tests (such as STs) and increases overall diagnostic sensitivity.

In DDHRs, the C-LTT showed a significantly lower sensitivity (29.4%) compared with dDC-LTT (61.8%), which was confirmed in each particular clinical entity: SJS-TEN (62.5% vs. 87.5%), MPE (15% vs 47.4%), and AGEP (33% vs. 80%)[44]. Moreover, when analysing the proliferation of the particular effector cells for each phenotype, it can increase the sensitivity of the *in vitro* test to 100% in SJS-TEN when assessing $CD4^+$Th1 or NK cells, 68.4% in MPE when analysing $CD3^+CD4^+$Th1, and NK cells, and 100% in AGEP when evaluating $CD3^+$+NK cells[47].

The LTT is the most commonly used diagnostic method for T cell-mediated organ damage, such

as drug-induced allergic hepatitis (liver injury), where other *in vivo* tests are difficult or contraindicated. It has also been used in interstitial lung disease and pancreatitis[53,54].

Moreover, LTT has also been used to monitor the immunological response, with a switch in the pattern profile (from Th2 to Treg) during IT towards tolerance[55–58].

4.3. Cell Subset Analysis in Sputum and Bronchial Lavage

Cell subset analysis in sputum and bronchoalveolar lavage (BAL) is a central method for evaluating airway inflammation and immune profiles in asthma, chronic obstructive pulmonary disease (COPD), and other lung diseases. Induced sputum, a non-invasive technique, yields higher counts of neutrophils and eosinophils, whereas BAL samples the distal airways and alveoli, yielding higher counts of macrophages and lymphocytes. Flow cytometry and cytospin remain the gold standards for differential cell counts, though BAL flow cytometry has emerged as a rapid and reliable tool for classifying interstitial lung diseases and guiding personalized therapies. More advanced approaches, such as DNA methylome-based deconvolution, confirm that sputum can accurately reflect pulmonary cell populations[59–61].

5. GENETIC STUDIES IN ALLERGOLOGY

5.1. Principles of the Method

Genetic testing approaches have evolved significantly in recent years. In routine clinical laboratories, classical techniques such as Sanger sequencing coexist with advanced methods based on Next-Generation Sequencing (NGS), with the primary goal of identifying DNA sequence variants that can be confidently associated with the patient's presenting signs and symptoms. Each offers distinct advantages and limitations. The choice between these approaches, which often depends on factors such as cost, turnaround time, and resolution, is central to achieving the best diagnostic accuracy.

Sanger sequencing is a classical method to detect single-nucleotide variants with high accuracy. Despite the rise of high-throughput technologies, it remains widely used for targeted sequencing and validation of variants identified by other methods. Compared to NGS, Sanger offers superior accuracy for small regions but lacks scalability and efficiency for more comprehensive genomic studies. Droplet Digital PCR (ddPCR) is an advanced molecular technique that allows for absolute quantification of nucleic acids without the need for standard curves, offering extremely high sensitivity and precision. Clinically, ddPCR is particularly useful for detecting low-frequency variants, copy number variations, and minimal residual disease in oncology or rare mutation studies. Based on the diagnostic scope, a clinical NGS test can be designed to target a panel of selected genes linked to specific clinical phenotypes, the exome, or the entire genome. Each approach differs in genomic coverage and carries a distinct risk of false negatives[63,64].

Integrating genetic data enables precise molecular diagnosis, improved understanding of disease mechanisms, subtype classification, and personalized management strategies in Allergology.

5.2. Applications in Allergy

Hereditary Angioedema (HAE)

Genetic studies in HAE have established that the majority of cases are caused by heterozygous pathogenic variants in the *SERPING1* gene, which encodes C1-INH, leading to either reduced quantity (type I HAE) or dysfunctional protein (type II HAE). Functional studies have shown that some SERPING1 variants exert dominant-negative effects, further reducing C1-INH activity. For HAE with normal C1-INH levels and function (nC1-INH-HAE or type III HAE), genetic studies have identified causative mutations in several genes: *F12* (factor XII), *PLG* (plasminogen), *ANGPT1* (angiopoietin-1), *KNG1* (kininogen 1), *MYOF* (myoferlin), *HS3ST6* (heparan sulfate-glucosamine 3-O-sulfotransferase 6), *DAB2IP* (DAB2 Interacting Protein) and *CPN1* (Carboxypeptidase N Subunit 1). These mutations affect the regulation of bradykinin or vascular permeability, resulting in angioedema despite normal C1-INH levels. NGS and whole-exome/genome sequencing are now standard approaches for identifying pathogenic variants in both *SERPING1* and non-*SERPING1* genes, especially in cases with atypical presentation or negative family history. Genetic testing is recommended as a first-tier diagnostic tool by international guidelines[65,66].

Hereditary Alpha Tryptasemia (HαT)

The latest evidence demonstrates that HαT is a significant heritable risk factor for severe anaphylaxis and mast cell activation disorders. Screening for HαT is recommended in patients with unexplained elevated basal serum tryptase, recurrent anaphylaxis, or mast cell activa-

tion symptoms, and in first-degree relatives with similar clinical presentations. The optimal baseline serum tryptase cut-off for predicting HαT in symptomatic patients without other causes of elevated tryptase is 9.2 ng/mL, with high sensitivity and specificity[67]. Accurate identification of HαT can guide further diagnostic workup, avoid unnecessary bone marrow biopsy, and inform management of mast cell-mediated symptoms.

The state-of-the-art method for HαT is primarily ddPCR assays that quantify copy-number variation (CNV) of the *TPSAB1* in a single reaction. This method has been validated to distinguish HαT genotypes reliably and is suitable for implementation in standard laboratory workflows, with significant reductions in material costs and turnaround time compared to previous duplex assays[68–70].

Mastocytosis and Related Mast Cell Disorders

KIT D816V mutation analysis in bone marrow is a highly sensitive and specific diagnostic tool for mastocytosis and related mast cell disorders, and its detection confirms the presence of clonal mast cell disease. It is considered a cornerstone of the diagnostic workup and also provides prognostic information. The mutation may be found in mast cells and, in advanced cases, in other myeloid lineages, reflecting multilineage involvement and a more aggressive disease.

Bone marrow testing using ultrasensitive methods such as allele-specific oligonucleotide quantitative PCR (ASO-qPCR), ddPCR, and Flow-SuperRCA (Rolling Circle Amplification) achieves a limit of detection as low as 0.001% variant allele frequency, enabling identification of low-burden disease and improving diagnostic yield compared to conventional sequencing or NGS. Alternatively, KIT D816V mutation analysis in peripheral blood is also available in clinical practice. Flow-SuperRCA, ddPCR, and ASO-qPCR are also leading peripheral blood assays, with Flow-SuperRCA currently demonstrating the highest sensitivity and specificity, approaching 100%, although it is not yet widely available in the clinical laboratory. Detection rates in systemic mastocytosis range from 85-94%, but are lower in indolent forms without skin involvement and in cases with low mast cell burden. Thus, a negative peripheral blood result does not exclude mastocytosis, and bone marrow investigation remains mandatory in patients with high clinical suspicion. Sensitivity can be further improved by analyzing purified myeloid cell populations from blood, but this is not yet standard practice[62,71–73].

Human Leukocyte Antigen (HLA) Typing for Drug Hypersensitivity

Population-specific adverse reactions to therapies linked to HLA gene variants are best exemplified by severe cutaneous and systemic drug hypersensitivity reactions (SCARs). The clinical utility of HLA testing is determined by both the strength of the association and the allele frequency in the population, and test interpretation must consider clinical context and alternative therapies. Healthcare systems that have adopted population-specific HLA screening protocols for drugs with known SCAR risk alleles have demonstrated marked reductions in SCAR incidence, improved patient safety, and substantial cost savings compared to unscreened populations. Currently, HLA screening protocols for drug hypersensitivity are best practice for abacavir (HLA-B*57:01, global), carbamazepine (HLA-B*15:02, Southeast Asia; HLA-A*31:01, Europe/Japan/Korea), allopurinol (HLA-B*58:01, Asia), and dapsone (HLA-B*13:01, China/Southeast Asia), with recommendations tailored to population allele frequencies and risk profiles.

Currently available HLA genotyping methods include high-resolution sequence-based typing (SBT), sequence-specific oligonucleotide probe (SSOP) assays, real-time PCR with melting curve analysis, and NGS. SBT and NGS provide the highest accuracy and allele resolution but require advanced laboratory infrastructure, trained personnel, and are costly and time-consuming. Point-of-care and low-resource HLA genotyping methods have advanced rapidly. Rapid PCR-based assays offer optimal diagnostic performance, reproducibility, minimal manual labor, and scalability for population-specific screening[74–78].

6. TISSUE BIOPSIES ANALYSIS IN ALLERGOLOGY

6.1. Principle of the Method

The main classical methods used are histopathology with hematoxylin and eosin staining, special histochemical stains, and immunohistochemistry. The latter is employed to detect specific antigens in tissue sections using labeled antibodies, enabling the identification and quantification of cell types and the assessment of protein expression relevant to allergic and inflammatory processes. *In situ* hybridization and immunofluorescence are also used for more detailed molecular and cellular analyses in selected cases, offering individualized tissue information.

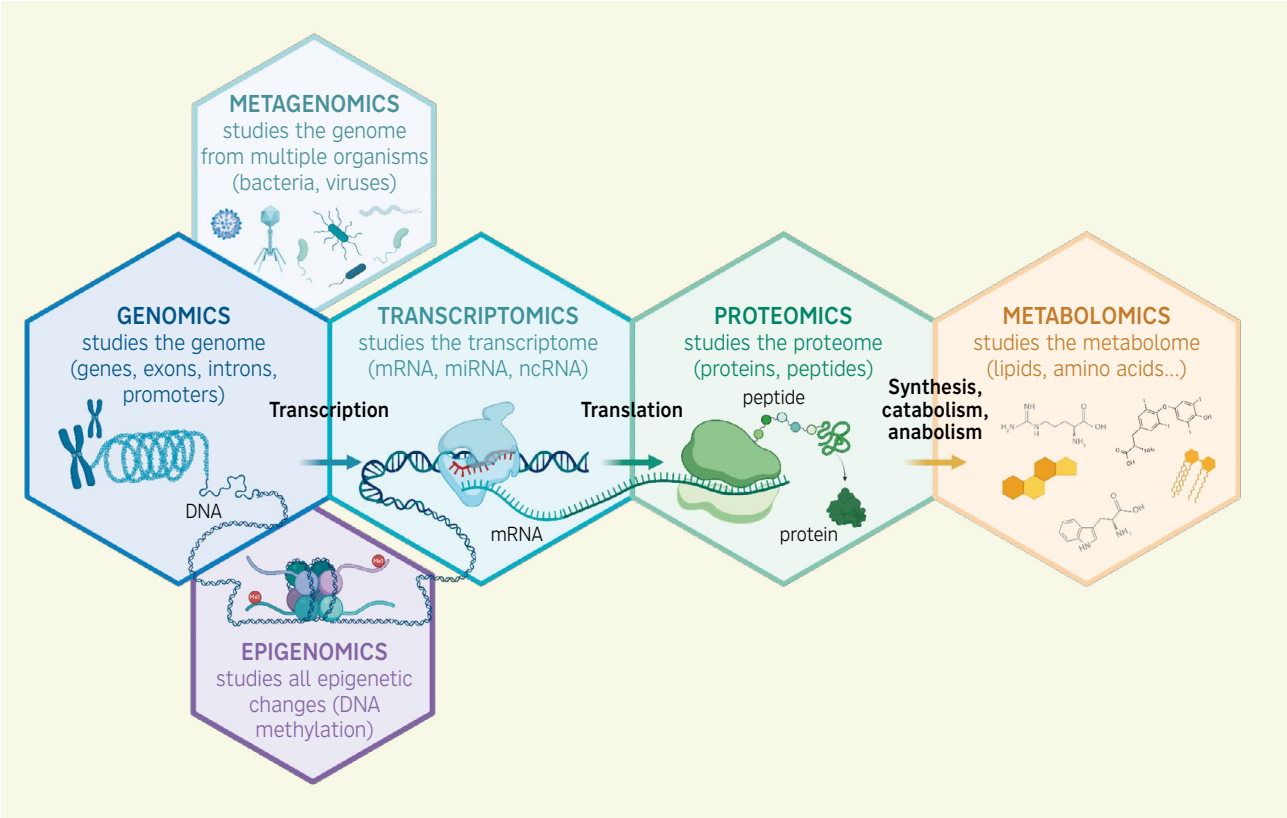

Figure 1. Schematic representation of the main omics technologies and the biological information they assess.

6.2. Applications in Allergology

The main use is to assess for the presence, density, and activation state of mast cells and eosinophils in tissue to support the diagnosis of specific allergic and eosinophil/mast cell-mediated diseases, especially when noninvasive testing is inconclusive or when tissue involvement is suspected based on clinical presentation. In the gastrointestinal (GI) tract, biopsies are used to quantify and characterize mast cells and eosinophils, which is essential for diagnosing EoE, eosinophilic gastritis/enteritis, and GI involvement in systemic mastocytosis or mast cell activation syndrome. In the skin, biopsies can help distinguish urticarial vasculitis from CSU and exclude other mimickers[79].

7. OMICS AND THE FUTURE OF THE LABORATORY TOWARDS PRECISION ALLERGY

7.1. Principles of Omics

Omics technologies have revolutionized our understanding of allergic diseases over the years[80], with an increasing number of studies adopting techniques like genomics, proteomics, and metabolomics. Integrating omics into the allergy laboratory offers an unprecedented opportunity to move beyond one-size-fits-all treatments and develop diagnostics that can predict who will respond best to which therapy, thereby advancing the Precision Allergy approach. However, at present, translating new findings from omics discoveries into clinical practice remains a challenge[80,81].

The term "omics" encompasses several high-throughput techniques that analyze a molecular profile in a single run. In addition, these techniques can nowadays be performed at the single-cell level or in combination with microscopy in the field of spatial omics, offering high precision.

These technologies can help process and analyse large amounts of biological data and, consequently, decipher the mechanisms underlying the development of complex diseases, such as allergy. These innovative approaches offer unparalleled opportunities for endotyping diseases, discovering biomarkers, and developing drugs, thereby advancing personalised medicine[81]. A schematic of the different omics technologies and their focus on different biomolecules is shown in Figure 1.

All omics technologies can be applied in two main analytical approaches. First, untargeted analysis —or non-targeted analysis— provides

TABLE 3. Omics Technologies and Their Main Analytical Platform.

Omic	Focus	Analytical Platforms
Genomics	Genetic material (genes) and their functions	GWAS and NGS are the most used
Transcriptomics	RNA: including protein-coding RNAs, ncRNAs	Microarrays for predefined RNA and RNAseq for transcriptome profile
Proteomics	Proteins from structure to function	Immunoassays, protein microarrays, MS, and Olink® High throughput flow cytometry (spectral or mass cytometry)
Metabolomics	Metabolites, molecules of metabolic pathways, including lipid (lipidomics)	MS —usually coupled with a separation technique, e.g., LC-MS and GC-MS— and NMR

GC-MS: Gas Chromatography coupled to Mass Spectrometry, GWAS: Genome-Wide Association Studies, LC-MS: Liquid Chromatography coupled to Mass Spectrometry, MS: Mass Spectrometry, ncRNAs: Non-Coding RNAs, NGS: Next-Generation Sequencing, NMR: Nuclear Magnetic Resonance Spectroscopy, RNAseq: RNA Sequencing.

a complete profile of each type of data for all the samples in the study, with the highest number of data possible and in the most robust and reproducible way, with the final purpose of applying a differential analysis between conditions. This approach, often exploratory, aims to detect omics changes to understand better the molecular mechanisms underlying the pathology[81].

The other main strategy is targeted analysis, in which a panel of biomolecules of interest is selected and analyzed with higher accuracy, using more specific separation and detection techniques. This type of analysis is usually performed after an untargeted analysis that has yielded a set of potential biomolecules. These are then analyzed to confirm their identification or to perform their precise quantification. Due to the untargeted approach's potential to discover biomarkers for diagnosis, prognosis, or patient stratification in complex diseases, this approach has gained popularity in the research laboratory, as robust biomarkers are essential for the development of truly personalized therapeutic interventions. However, to increase omics potential, integrating different omics data types is often recommended[81]. The principles of the main omics strategies and their main techniques are summarized in Table 3. In addition, the integration of AI and machine learning (ML) algorithms into the management of different allergy diseases, including asthma, is rapidly advancing, offering new opportunities for early diagnosis, risk stratification, and personalized management[82].

7.2. OMICs Findings in Allergology

Omics studies have been applied to various allergic diseases, including allergic rhinitis, AD, FA, and DHRs, but primarily to asthma[80,81]. The SNPs (rs2872507, rs7216389) in orosomucoid-like protein 3 (*ORMDL3*), the first and rate-limiting enzyme for sphingolipid biosynthesis, have been associated with asthma susceptibility and development, and are now being validated for their use in clinical settings[80]. Moreover, thanks to multiomics integration, a set of biomolecules with potential for monitoring mepolizumab treatment has been identified, helping towards personalized medicine[83].

8. LIMITATIONS OF CURRENT *IN VITRO* TOOLS IN ALLERGOLOGY

Current *in vitro* tests still face several challenges that restrict their use in clinical settings. For some tests, specificity and sensitivity remain limited because the biomarkers are not exclusive to hypersensitivity reactions. In other cases, certain biomarkers require rapid sampling and strict processing conditions to avoid degradation, and the use of cellular tests is restricted due to demand for fresh samples and lack of fully standardized protocols across laboratories. For these reasons, laboratories must apply analytical quality control standards. Advanced approaches, such as multi-omics and advanced genetic testing, require significant infrastructure and bioinformatics resources, making them costly and less accessible. These limitations underscore the need for improved standardization, integration of multi-omics data, and development of robust biomarkers to enable precision *in vitro* allergy diagnostics. On the other hand, rapid technological advances and the rise of AI-based tools offer significant opportunities. Main limitations and emerging opportunities are summarized in Table 4.

TABLE 4. Overview of the Most Relevant Limitations and Emerging Opportunities for Each Analyte/Technique.

Type	Limitations	Emerging Opportunities
Immunoassays for Soluble Analytes		
Total/Specific IgE	• Not all components are equally represented in extracts • False positives due to CCD interference if no inhibition is included (for some native/purified components, some extracts, and even some platform-related elements) • False negative results may occur when levels of allergen-sIgE are below the assay's detection threshold, especially in cases of recent onset allergy, waning sensitization, or after immunotherapy. Other causes include the use of non-standardized or incomplete allergen extracts, interference from high concentrations of other immunoglobulin isotypes (e.g., IgG), and technical limitations of the assay. • Singleplex systems: larger sample volume requirements, less cost-efficient per analyte • Multiplex systems: defect of allergen, competence for binding between different isotypes of antibodies, competence of binding for extract/component in the same platform	• Better characterization/design of extracts and molecular components • Adoption of microfluidic and nanofluidic technologies to reduce sample volume • Use of AI-based tools to assess sensitization patterns and their association with clinical parameters (especially for multiplex systems) • Incorporation of allergen epitopes
Specific IgG/ IgG4	• Not all components are equally represented in extracts • Singleplex systems: larger sample volume requirements, less cost-efficient per analyte • Multiplex systems: defect of allergen, competence of binding for extract/component in the same platform	• Development of clinically-relevant cut-off values (e.g., AIT response, ABPA)
Tryptase	• Not elevated in all anaphylaxis cases (especially food-triggered or in children) • Timing critical: acute phase sampling within 1-2 hours • Baseline variability and genetic factors (e.g., HαT) • Serial measurements are logistically challenging	
Complement	• Pre-analytical errors and handling issues • Lack of assay standardization and inter-lab variability • Limited specificity for disease mechanisms • Labile proteins; improper storage can cause false activation	• Biomarkers under investigation (C4a, cleaved kininogen) • Factor H as a potential disease activity marker • Stratification of allergic endotypes and monitoring therapy response
Histamine	• Very short plasma half-life (1-2 min) • Must measure within minutes of onset • Not specific for allergy (also elevated in non-allergic mast cell activation)	• Urinary metabolites (e.g., N-methylhistamine) are helpful in systemic mastocytosis • Measurement in nasal secretions (investigational)
Cytokines	• Low circulating concentrations • Short half-life • Highly susceptible to pre-analytical variability	• Details not specified in the document
Lipid mediators: Prostaglandins, Leukotrienes, and PAF	• Low specificity (produced by multiple cell types) • Lack of standardized cut-offs • Variable stability • Limited commercial assay availability (especially PAF)	• Non-invasive measurement in urine • Elevated when tryptase is not • May guide therapy (e.g., aspirin for PGD2, leukotriene antagonists for LTE4)

(Continued)

TABLE 4 *(continued)*. Overview of the Most Relevant Limitations and Emerging Opportunities for Each Analyte/ Technique.

Type	Limitations	Emerging Opportunities
Cellular Tests		
BAT	• Depends on sIgE affinity and allergen epitope density • Requires fresh blood (within 4–24 hours) • Lack of protocol standardization • Sensitivity decreases over time post-reaction • 10–15% non-releasers • pBAT less sensitive; RBL-BAT inconsistent • Patients' treatment can interfere with the results	• MAT and TAT as emerging alternatives for multicentric studies and to overcome invalidity in non-releasers basophils
LTT	• Lack of standardization • Time-consuming and technically demanding • Low and variable sensitivity (~56%) • A positive result may only indicate sensitization • Corticosteroids can suppress proliferation and interfere with results	• Inclusion of professional APCs • Assessment of the results on effector cells • Multi-omics integration to improve specificity and sensitivity for precision medicine
Genetic Tests	• Limited clinical validation for allergy-related genes • Complex interpretation of polygenic risk • Ethical and privacy concerns • High cost and limited accessibility	• Identification of genetic predisposition to allergic diseases • Pharmacogenomics for personalized therapy • Integration with clinical and omics data for precision medicine
Multi-Omics Approaches		
Genes (DNA), Transcripts (RNA), Proteins, and Metabolites	• High complexity and cost • Require advanced bioinformatics and infrastructure • Limited standardization and clinical validation • Data interpretation challenges	• Comprehensive profiling of allergy endotypes • Discovery of novel biomarkers • Enables precision medicine and targeted therapies

ABPA: Allergic Bronchopulmonary Aspergillosis; AI: Artificial Intelligence; AIT: Allergen-specific Immunotherapy; APCs: Antigen-Presenting Cells; BAT: Basophil Activation Test; CCD: Cross-Reactive Carbohydrate Determinants; C4a: Complement Component 4a; HαT: Hereditary Alpha-Tryptasemia; Ig: Immunoglobulin; LTT: Lymphocyte Transformation Test; LTE4: Leukotriene E4; MAT: Mast Cell Activation Test; PAF: Platelet-Activating Factor; PBMC: Peripheral Blood Mononuclear Cells; PGD2: Prostaglandin D2; pBAT: Passive Basophil Activation Test; RBL-BAT: Rat Basophilic Leukemia Cell Basophil Activation Test; sIgE: Specific IgE; TAT: T-cell Activation Test.

9. CONCLUSIONS AND FUTURE OF THE CLINICAL LABORATORY FOR ALLERGOLOGY

In summary, the clinical routine of *in vitro* diagnostic tests is essential for obtaining reliable, accurate diagnoses and achieving precision allergy. Their main limitations will be overcome over time, e.g., through the establishment of cut-off thresholds or the standardization of protocols. In parallel, new technologies (e.g., omics, single-cell approaches, AI-based tools) are being developed for implementation in clinical practice over the coming years.

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