THE XPLORER

JUNE 2024

SPECIAL EDITION

INTERVIEW: THE IMPACT OF CLONING THE MAJOR BIRCH POLLEN ALLERGEN Bet v 1

With
Univ.-Prof. Dr. Breiteneder, PhD

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INTERVIEW: MOLECULAR ALLERGY DIAGNOSTICS IN CLINICAL PRACTICE

With
Dr. Ramón López Salgueiro

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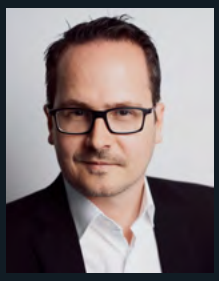
EDITORIAL



DEAR READERS,

Welcome to this special edition of THE XPLORER! After highlighting different allergen sources and the significance of CCDs in molecular allergy testing in previous editions, we are going back to the basics: In this edition of our magazine, we want to introduce readers to the foundations of molecular allergy diagnostics.

Part one of this special edition will focus on the introduction to molecular allergy diagnostics (MAD), explain the most important terms and concepts, show the differences to extract-based diagnostics, and feature an interview with one of the "founding fathers" of MAD, Univ.-Prof. Dr. Heimo Breiteneder, PhD, Professor of Medical Biotechnology at the Medical University of Vienna.

In the second part, it's all about putting theory into practice: We will discuss the concept of surrogate markers, indication for immunotherapy, risk assessment, as well as talk about MAD in clinical practice with Dr. Ramón López Salgueiro.

Lastly, we will also be looking into the future and talk about potential cost-saving effects MAD will have on global health economics.

We hope that this edition of THE XPLORER will serve as a great introduction to the world of molecular allergy diagnostics, so please enjoy reading!

Christian Harwanegg
CEO Macro Array Diagnostics

The impact of cloning the major birch pollen allergen Bet v 1

Interview with Univ.-Prof. Dr. Breiteneder, PhD

Dear Prof. Breiteneder, as a young molecular biology researcher you were instrumental in the cloning of the first pollen allergen, Bet v 1. Can you please describe how events unfolded?

Dr. Breiteneder: It started with Dietrich Kraft, head of the Allergy and Immunology Research Group at the Medical University of Vienna. In autumn 1983, Kraft worked in an outpatient allergy clinic in Vienna together with his colleague Herwig Ebner. Working with patients, he concluded that allergy test solutions and allergen immunotherapy solutions can only be standardised based on pure recombinant molecules. Together with Michael Breitenbach, Otto Scheiner and Helmut Rumpold we started in 1985 to work on Bet v 1 in earnest.

> "Hundreds of allergens were identified and officially recognised after Bet v 1."

Why was Bet v 1 chosen as the target molecule of your research?

Dr. Breiteneder: As around 5 % of the Austrian population suffer from pollinosis induced by birch pollen in early spring, and as there is only one major allergen present in birch pollen extract, this allergen was chosen as the target molecule.

How was Dietrich Kraft's idea received by research funding agencies and industrial partners?

Dr. Breiteneder: There was no interest at all during that time by these funding agencies and companies. Only Jörg Mayerhofer, the owner of a pharmacy in Linz (Upper Austria), financially supported the research on recombinant allergens. This collaboration resulted in the founding of Biomay, a start-up company that funded the research of Kraft's molecular allergology team.

What was the result of your research and when did your efforts bear fruit?

Dr. Breiteneder: The full cDNA sequence of Bet v 1 was published in the EMBO Journal in 1989, representing the most abundant isoform in birch pollen, called Bet v 1.0101. Thus, Bet v 1 became the first cloned plant allergen and also the first allergenic PR-10-like protein that was published worldwide.

> "Immunotherapy can only be standardised based on pure recombinant molecules."

What were the implications of your research in the following years?

Dr. Breiteneder: In the following time hundreds of allergens were identified and officially recognised by the WHO/IUIS Allergen Nomenclature Sub-Committee (1,112 allergens as of March 8th, 2024; www.allergen.org). This led to an explosion of basic and clinical research activities to characterise the allergens themselves, as well as their usefulness in the diagnostic work-up of allergic patients.

After Bet v 1, what were your research topics?

Dr. Breiteneder: My team and I focused our research on allergens from plant (e.g., latex, peanut, tree nuts, kiwi) and animal sources (e.g., fish). Our latest achievement was the biochemical, immunological, and clinical characterisation of various parvalbumins from freshwater and saltwater fish. We could demonstrate that parvalbumin from thornback ray was well tolerated by patients allergic to bony fish and, therefore, might be an alternative dietary option for these patients. Of course, this must be confirmed in each case by a food challenge.

Is there anything you want to emphasise, especially for our readers who work in the medical field?

Dr. Breiteneder: Collaborate as much as possible with basic researchers. It will help you understand the mechanisms of allergy development for the benefit of your patients. Without medical experts, scientific achievements will remain on the test bench and will not be made available for the patients.

ABOUT

UNIV.-PROF. DR. HEIMO BREITENEDER, PHD
is a Professor of Medical Biotechnology at the Medical University of Vienna and known for his research in molecular allergology, particularly focusing on understanding the mechanisms of allergic reactions to plant and food allergens.

> "Collaboration with basic researchers is crucial to understand allergy development."

Introduction to molecular allergy diagnostics

by Dr. Sandra Wieser

The term 'allergy' was first used in 1906 by the Austrian paediatrician, Clemens von Pirquet (1874–1929). At this time, he observed that antibodies were not only part of protective immune responses but could also cause diseases. Von Pirquet already distinguishes between 'allergen' and 'antigen'. The word 'antigen' implies a substance capable of giving rise to an antibody. The term 'allergen' comprises, besides the antigen proper, the ability to induce 'supersensitivity'. Over the years the term allergy has lost its original definition as provided by von Pirquet whereby it just implied a changed reactivity and is now used synonymously with hypersensitivity.

The pathomechanism underlying type I hypersensitivity reactions (Figure 1) refers the major role to immunoglobulin E antibodies. If a genetically predisposed person gets in contact

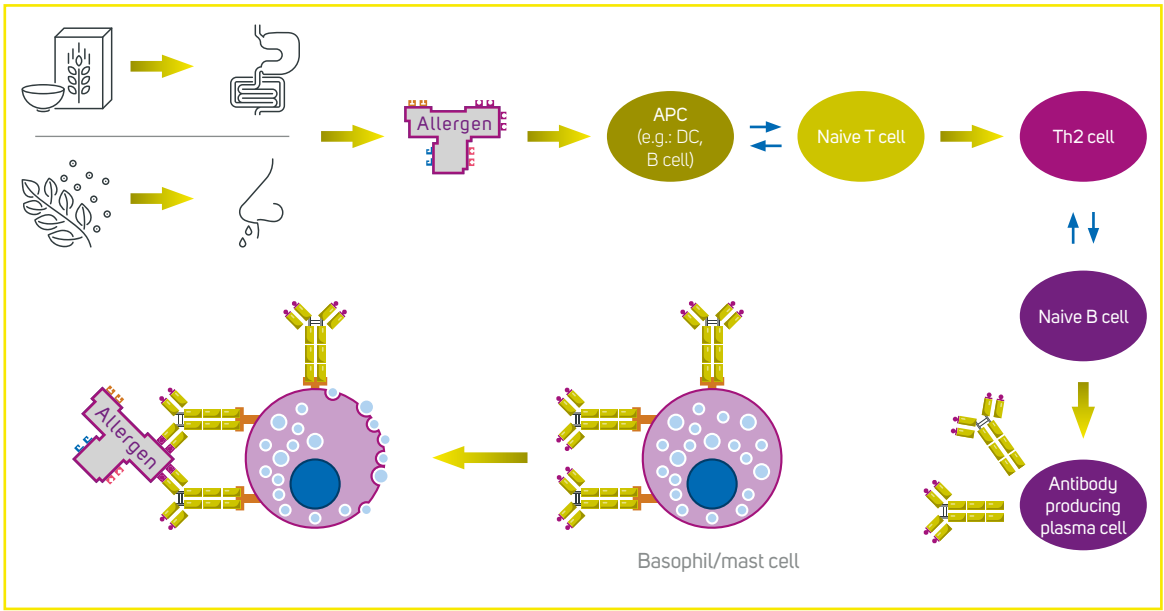
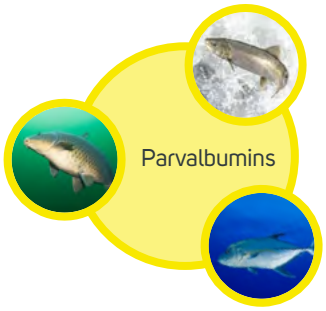


Figure 1: Pathomechanism of IgE-mediated hypersensitivity reactions. APC = Antibody producing cell; DC = Dendritic cell

with a culprit allergen via the gastrointestinal, respiratory tract or the skin, the allergen is absorbed by antigen-presenting cells, processed, and displayed to T cells by means of MHC class II molecules. Activated TH2 cells release IL-4 and IL-13, leading to the generation of allergen-specific IgE-producing plasma cells, a process also termed 'IgE sensitisation'. IgE antibodies bind to mast cells and basophils via the high affinity IgE receptor (FcεRI). Additionally, memory T cells as well as IgE-producing memory B cells are generated. If the individual has repeated contact with the allergen, the latter and mast cell- or basophil bound specific IgE antibodies form immune complexes, which subsequently leads to crosslinking of IgE/FcεRI. This signal activates the cell and triggers the immediate phase of the allergic reaction, by causing degranulation and release of vasoactive amines (e.g., histamine), chemokines, cytokines, and lipid mediators. Chemokines and cytokines cause reactivation and clonal expansion of T cells and their migration from the blood to the place where allergen exposure occurs. IgE that is bound to dendritic cells and monocytes via the high affinity IgE receptor (FcεRI) and to B cells via the low affinity IgE receptor (FcεRII), enhances allergen-uptake by antigen-presenting cells and presentation to T cells, a process called 'late phase of the allergic reaction.'[1,2]



WHAT FACTORS MAKE AN ALLERGEN AN ALLERGEN?

The broadest definition of an allergen is a molecule capable of binding IgE antibodies. Some allergens are sensitising and thus are able to induce allergen-specific IgE antibodies. Others are non-sensitising which is defined as the ability to cause allergic symptoms only due to cross-reactivity. The best example to demonstrate this is a birch pollen-related apple allergy, where birch pollen allergic adults or adolescents react with an oral allergy syndrome to apples. The major birch pollen allergen Bet v 1 is the sensitising protein and the cross-reactive PR-10 protein Mal d 1 from apple is the non-sensitising protein.[3]

Ultimately, there is no single reason why some proteins are allergens while most proteins are not. However, physicochemical properties such as water solubility and extractability (especially for inhalative allergens) or stability (especially for food allergens) play a major role. Studies demonstrate that the proteolytic activity enables easier penetration through epithelial barriers (e.g., group 1 house dust mite allergens – Der p 1).[4] Some proteins also act as ligands of dendritic cells, i.e., they are able to directly bind to receptors of those cells (e.g., group 2 allergens of the house dust mite, e.g., Der p 2, Der f 2; peanut allergen Ara h 1).[5,6]

The mucosal surfaces of the respiratory as well as the gastrointestinal tract are gates by which allergens enter the body. Via these routes, inhalative allergens as part of an airborne particle or aerosol droplet and food proteins included in foods and drinks are absorbed. Not to forget the skin, as it has been documented as a site for sensitisation as well. It directly interacts with applied allergens, potentially influencing systemic allergic reactions. Atopic dermatitis, often linked with food allergies, can precede asthma and allergic rhinitis by several years.[7]

Food allergens can be classified according to their stability. True food allergens are heat-stable proteins that are also resistant to degradation and proteolytic digestion. They induce sensitisation via sequential (i.e., linear) IgE epitopes, consisting of 8 amino acids or more, in the gastrointestinal tract and can cause severe systemic reactions. A prototypic example of true food allergens (primary sensitisers) is the shrimp muscle protein tropomyosin. Examples of true food allergens in plants are the 2S albumins from legumes (e.g., Ara h 2), tree nuts (e.g., Cor a 14, Jug r 1 and Ana o 3) and seeds (e.g., Ses i 1). While these allergens exhibit evident structural similarities due to a shared disulfide-bond pattern, their primary sequences vary considerably, leading to restricted cross-reactivity.

In contrast, cross-reactive pollen-related food allergens are homologous proteins in plant foods and pollen. These are characterised by easy degradation and digestion as well as heat-lability. Due to conformational (discontinuous) epitopes, these allergens commonly lead to milder, local symptoms like the oral allergy syndrome.

CROSS-REACTIVE ALLERGENS

Similar proteins are assigned to common families based on their amino acid sequence. A sequence identity of > 50 % is usually required for cross-reactivity. Proteins with this degree of similarity have many identical sites on their surface that can function as potential epitopes for cross-reactive antibodies.

Specific allergen molecules can act as markers indicating serological cross-sensitisations by binding to cross-reactive IgE. IgE tests relying solely on allergen extracts might show low diagnostic specificity in individuals affected by this phenomenon. Profilins or polcalcins, which belong to plant panallergen families, are examples of such markers with significant cross-reactivity.

Examples of indicators of cross-reactivity

- Serum albumins - Fel d 2, Can f 3, Equ c 3
- Bet v 1 homologues - Bet v 1, Act d 8, Ara h 8, Pru p 1
- Profilins (Panallergen in pollen and plant foods) - Amb a 8, Ara h 5, Art v 4, Bet v 2, Ole e 2, Phl p 12, Pru p 4
- Polcalcins (Panallergen in pollen) - Amb a 10, Art v 5, Bet v 4, Ole e 3, Phl p 7
- CCD (Cross-reactive carbohydrate determinants)

SPECIES-SPECIFIC ALLERGENS

In contrast to cross-reactive proteins, particular marker molecules can serve as markers for a primary, genuine, or species-specific sensitisation. These marker molecules provide higher diagnostic specificity than allergen extracts, especially in affected individuals who potentially experience cross-reactions.

Examples for markers of genuine (species-specific) sensitisation

- Fel d 1 (cat)
- Api m 1, Api m 3, Api m 4, Api m 10 (honeybee venom)
- Ves v 1, Ves v 5 (Vespula species)
- Bet v 1 (fagales)
- Ole e 1 (olive tree, plane tree)
- Phl p 1, Phl p 5 (grass)
- Art v 1 (mugwort)
- Amb a 1 (ragweed)
- Par j 2 (pellitory)

MAJOR VS. MINOR ALLERGENS

Among allergens found in a source, certain ones trigger IgE sensitisation in a larger proportion of individuals compared to others. It seems like an obvious choice to deem these as 'major' allergens with greater clinical significance. By official definition, a major allergen is one that is recognised by IgE antibodies in ≥ 50 % of patients allergic to that specific allergen source, while a minor allergen is recognised by < 50 % of patients.

Typically, major allergens are assigned lower numbers in the nomenclature system, primarily because researchers historically tended to identify the most prevalent allergens first.

Examples of allergen families[8]

- Pollen panallergens (Profilins and polcalcins)
- PR-10 proteins (Bet v 1 homologous proteins)
- nsLTPs
- Seed storage proteins
- Parvalbumins
- Tropomyosin
- Lipocalins
- Serum albumins
- Gibberellin-regulated proteins
- Oleosins
- Defensins
- CCDs

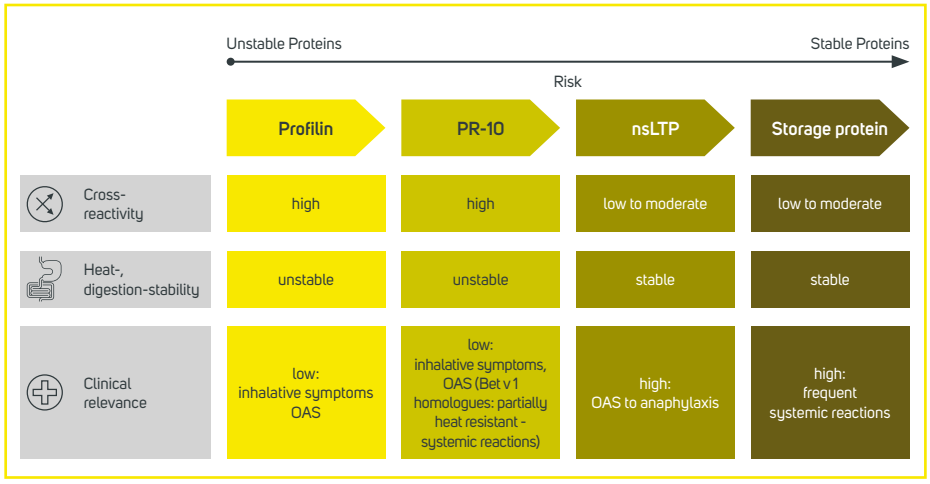


Figure 2: Examples of protein families classified based on their cross-reactivity, stability, and clinical manifestations.

Profilins

- Actin-binding proteins with great homology and cross-reactivity even between distantly related species (pollen & plant food)
- Pollen allergens: clinical relevance is variable but potentially present, up to half of patients allergic to pollen are sensitised to profilin
- Food allergens: present in most higher organisms (fruits, vegetables), up to 50 % of primary sensitised pollen allergic patients may have secondary food allergies (e.g., melon, tomato, banana): majority of them is suffering from oral allergy syndrome
- Stability concerning heat and digestion is low: patients tolerate processed foods

Polcalcins

- Calcium-binding proteins
- Minor allergens in patients sensitised to grass, tree or weed pollen (found in about 5 % of pollen allergy sufferers)
- Highly cross-reactive proteins present in most pollen (panallergen), but not in plant foods
- Can be considered markers of polysensitisation with unknown clinical relevance for respiratory symptoms
- Diagnosis of patients sensitised to polcalcins can be performed with specific IgE to Phl p 7 or Bet v 4
- If a conventional sensitisation test shows multiple pollen allergies, the result might be caused by polcalcins
- Only molecular allergy diagnostics can detect sensitisation to polcalcin

PR-10 proteins

- The major birch pollen allergen Bet v 1 acts as archetype of PR-10-like allergens
- Bet v 1 is the primary sensitiser in birch pollen endemic regions
- Heat-labile (cooked foods are often tolerated)
- Consumption of raw fruits, nuts, vegetables and legumes containing PR-10-like allergens can lead to mild and local symptoms (e.g., OAS) and sometimes severe allergic reactions in Bet v 1-sensitised patients: pollen-associated food allergy in birch-endemic countries

nsLTPs

- Non-specific lipid transfer proteins
- Exist in all branches of the plant kingdom (panallergens) – pollen, fruits, vegetables
- Relevant nsLTP-containing plant foods belong not only to the Rosaceae family but also to the nut group and to cereals, such as wheat, maize and rice
- Most prevalent plant-food allergens in Southern Europe
- Pronounced thermal and proteolytic stability: allergic reactions to raw and to cooked foods observed
- Clinical reactions can be mild (oral allergy syndrome) to severe and systemic (i.e., anaphylactic reaction)
- In Southern Europe IgE reactivity to peach LTP Pru p 3 (major allergen of peach) is frequently diagnosed: marker allergen

Seed storage proteins (Figure 3)

- Very stable and heat-resistant: severe reactions to fresh and cooked foods
- 2S albumins, 7S globulins, 11S globulins are marker allergens for clinically relevant sensitisations to legumes, tree nuts and seeds
- IgE cross-reactivity occurs between members of the same protein family but may also occur between allergens from different families of seed storage proteins
- 2S albumins, prevalent allergens in peanuts and tree nuts, carry a potential for severe allergic reactions, including anaphylaxis; as marker allergens, they characterise sensitisations to various seeds and nuts, emphasising the importance of precise diagnosis using IgE antibody tests for effective allergy management and preventive measures
- 7S globulins, known as vicilins, are major allergens found in legumes like soy, pea, lentil, and lupine, serving as marker allergens for identifying sensitisations to legumes; there is a risk of cross-reactivity between peanuts and peas, as well as peas and lentils, highlighting potential challenges in managing allergies to these proteins

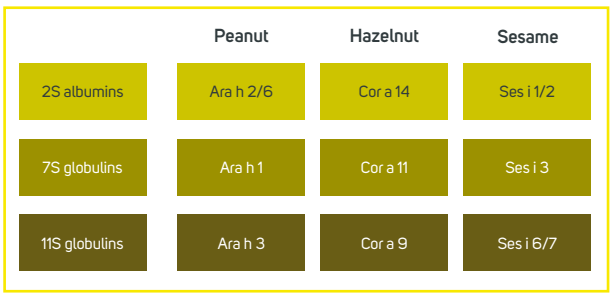


Figure 3: Important representatives of seed storage proteins.

- 11S globulins, or legumins, are significant allergens in hazelnut and almond, serving as marker allergens for sensitisations to these nuts and peanuts; IgE responses to these allergens can result in severe symptoms, highlighting the need for precise diagnosis and effective management of allergies

Parvalbumins

- Food, respiratory and contact allergen
- Resistant to heat and digestion (also reactions to cooked foods)
- Marker for cross-reactivity among different fish species: clinical cross-reactivity is based on the presence of highly conserved IgE epitopes
- Beta-parvalbumin is the major fish allergen: low cross-reactivity between beta-parvalbumins from bony fish and alpha-parvalbumins from cartilaginous fish
- Fish allergy in children starts early, mostly during the first two years of life: children with less severe reactions in general have lower levels of sensitisation and good chances to outgrow fish allergy
- Those who continue being allergic may still tolerate several fish species: thornback ray, shark, tuna and swordfish
- Tolerance to at least one fish can be important for allergic children because fish has beneficial effects on health due to the high omega-3 content and its consumption is associated with a lower risk of coronary heart disease

Tropomyosins

- Thermostable protein
- High allergenicity
- High degree of immunological and clinical cross-reactivity: invertebrate panallergen
- Seafood allergy is mostly induced by tropomyosins
- Patients sensitised to Der p 10 (inhalative allergen) may also react with Pen m 1 when consuming shrimps or even when inhaling cooking fumes
- Therapy: dietary or sanitary intervention

Lipocalins

- One of the most important animal allergen families
- Present in body fluids and secretions of furry animals: airborne, easily spreading into indoor environment
- β-barrel fold with a central molecular pocket is similar among human and animal lipocalins
- Sensitisation to multiple components is associated with higher disease severity
- Cross-reactive subgroup with high sequence identity

Serum albumins

- Highly conserved sequences with high amino acid sequence identity: cross-reactivity between serum albumins of various mammalian species (e.g., cat-dog, cat-pork)
- Sensitisation may give rise to airway reactions to mammalian animals, as well as food reactions to meat and milk
- Minor respiratory allergen of animal dander
- Food allergen of milk and meat: common proteins present in different biological fluids and solids (e.g., cow's milk, beef, chicken)
- Allergen implicated in cat-pork and bird-egg syndrome

Cat-pork syndrome (Figure 4) arises from an IgE-mediated hypersensitivity to serum albumin from cats, cross-reacting with serum albumin from pork due to a shared similarity between both proteins.

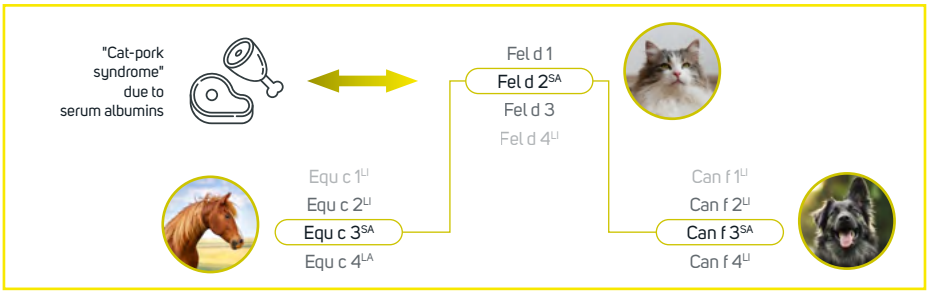


Figure 4: Cat-pork syndrome.

Bird-egg syndrome (Figure 5): After coming into contact with birds, a patient may develop sensitisation to airborne avian allergens, which leads to the manifestation of respiratory allergy symptoms. The cross-reactive allergen that is responsible for producing both respiratory and gastrointestinal allergy symptoms in bird-egg syndrome was identified as serum albumin, which is found in bird feathers and droppings and in egg yolk as well (Gal d 5).

Gibberellin-regulated proteins

- Small, cationic, non-glycosylated monomeric proteins with anti-microbial activity, present in plant foods and pollen
- Resistant to heat and digestion: may induce severe systemic reactions
- Cross-reactive and involved in pollen food allergy syndromes (main fruits involved are peach, citrus, apricot, cherry, and pomegranate)
- Until now, Cupressaceae is the only tree family shown to express allergenic pollen GRP

Oleosins

- Unique structure: a central hydrophobic domain flanked on each side by relatively hydrophilic domains
- Oleosins are lipophilic, therefore, they are underrepresented in aqueous extract-based in vitro- and in vivo routine diagnostic tests
- Oleosins are resistant to heat and enzymatic processing
- An increase of allergenicity has been observed for peanut and hazelnut oleosins after roasting when compared to raw seeds
- Oleosins are potential marker allergens for allergy severity after peanut and hazelnut consumption: risk assessment of anaphylaxis is possible by the detection of IgE to oleosins

Defensins

- Prevalent in Asteraceae pollen
- Described as potent allergens, exemplified by the major mugwort pollen allergen Art v 1
- While their allergenicity is well-established in certain pollen, only a few defensins with allergenic properties have been identified in plant foods like peanut and celery
- Api g 7 is a novel celeriac defensin, demonstrating allergenicity in celeriac-allergic patients[9]:
  - Heat-stable
  - IgE reactivity has been found in 60 % of mediator release assays
  - Underrepresented in celery extracts (celery extract-based allergy diagnosis missed 5 out of 8 patients reactive to Api g 7)

CCDs

CCDs are covered in depth in the second issue of THE XPLORER

- Cross-reactive carbohydrate determinants
- CCDs are sugars located on natural allergens (e.g., plants, insects, and molluscs)
- CCDs are able to induce production of IgE

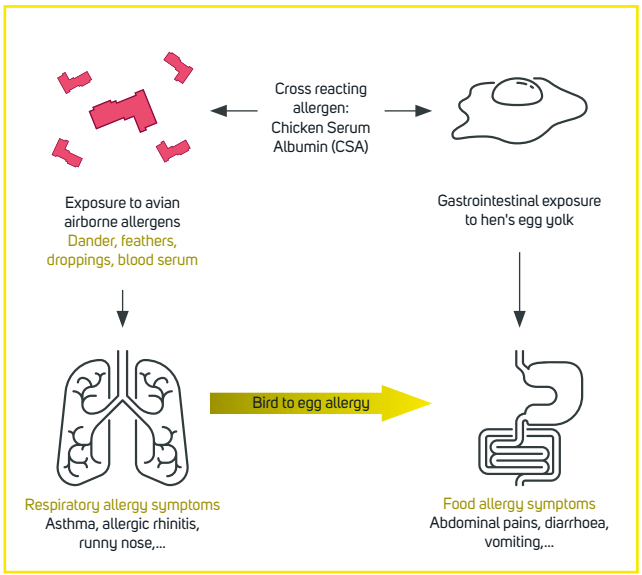


Figure 5: Bird-egg syndrome.

- IgE antibodies to CCDs result in a broad cross-reactivity which rarely elicits clinical symptoms, leading to poor clinical relevance
- IgE antibodies directed against CCDs give the impression of polysensitisation, leading to falsely elevated or false positive results
- CCD-like inhibitor is an essential tool in molecular allergy diagnostics to rule out detection of CCD-specific IgE

METHODS FOR THE DETECTION OF ALLERGEN-SPECIFIC IgE

Measurement of allergen-specific IgE (sIgE) can be conducted using different validated in vitro diagnostic (IVD) test kits. The principle of all currently available serum sIgE assays is based on the Enzyme-Linked Immunosorbent Assay (ELISA) method (Figure 6), where patient's serum IgE antibodies bind to specific allergens. Allergen extracts or molecular (natural or recombinant) allergens are either suspended in the liquid phase or bound in the solid phase (e.g., cellulose paper, cellulose sponge, polymers, glass) of the test. If the patient's serum contains specific IgE antibodies, allergen/IgE complexes form, which are then visualised using labeled antibodies and a substrate, with intensity correlating to specific IgE concentration. Qualitative, quantitative, and semi-quantitative methods primarily use colorimetric or fluorescent markers.

Benefits of molecular allergy diagnosis

- Facilitates accurate identification of IgE sensitisation at the molecular level
- Discrimination between cross-reactivity and true sensitisation, accurate risk assessment, and prescription of AIT (allergen-specific immunotherapy)
- Valuable tool for precise, individualised, and cost-effective allergy diagnosis, leading to more effective treatment and allergen avoidance

ALLERGEN EXTRACTS

Allergen extracts can be produced from specimens of all kinds of allergen sources, including, for example, pollen, moulds, animal dander, food allergens as well as insect venoms. Extracts are heterogenous mixtures of allergenic and non-allergenic molecules. Most clinical diagnostic tests have used extracts from allergen source materials for skin prick testing as well as for the determination of specific IgE in blood. For skin prick testing, clinicians rely on licensed commercial extracts[10]. However, the validity of a test that only uses allergen extracts is limited – due to various factors:

- Allergen extracts contain multiple proteins, i.e., allergens, allergen-derived material, and non-allergenic material (e.g., CCDs).
- Another major concern is that allergen extracts may contain contaminants from other allergen sources. House dust mite allergens can occur in extracts derived from animal dander. Similarily, pollen extracts might contain other unrelated pollen or fungi. Additionally, recent findings have indicated the presence of IgE-reactive bacterial antigens in house dust mite allergen extracts.[11 12 13]
- Allergen extracts are essentially unselective aqueous extraction products. The choice and handling of the original allergen source material as well as the selection of the extraction buffer is crucial. The primary challenge in allergen extract production stems from variations in allergen sources, with content, concentrations, and ratios differing significantly based on factors like environmental conditions and pollution. Factors affecting pollen allergen content

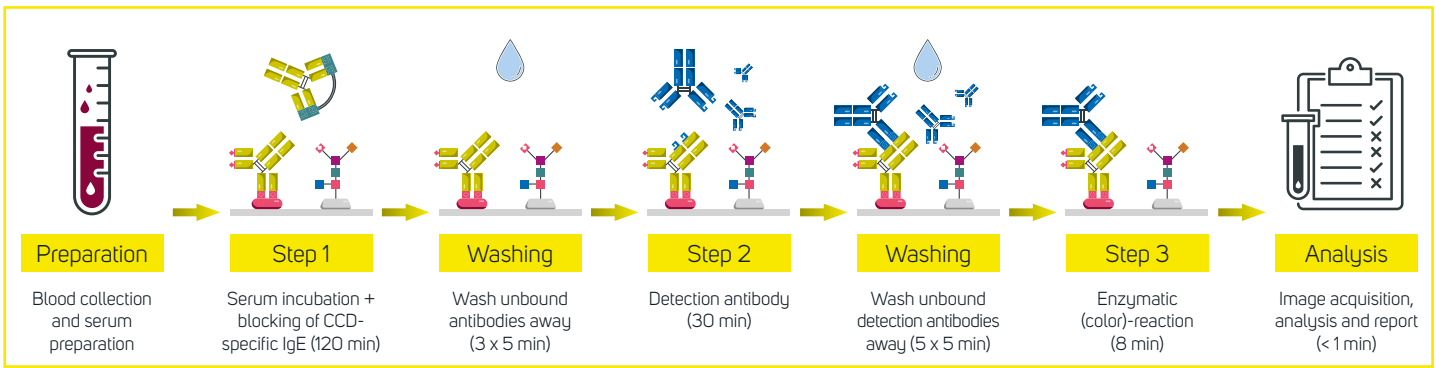


Figure 6: ELISA-based allergy diagnostics.

Advantages	Limitations
Preparation avoids extensive purification steps	May contain non-allergenic components
Contain several allergens of the allergen source, including isoforms of the same allergen	May be contaminated with allergens from other sources
Often reflect the allergen contents of the natural allergen sources	May present variable contents and ratios of allergens, or even lack particular allergens
	May present batch-to-batch variations due to manufacturing procedures and raw materials
	May be unstable and degrade
	Do not provide molecular information when used for diagnosis

Table 1: Advantages and limitations of using allergen extracts in allergy diagnostics.

include ozone exposure and pollution, leading to a lack of uniformity in natural allergen preparations.

- Food allergen extracts exhibit protein variations in different fruit parts and cultivars, with extraction methods influencing the outcomes. Thus, aqueous extracts often do not contain water-insoluble proteins, which can lead to underdiagnosing food allergies when extracts are used. Essential major and/or marker allergens with high clinical relevance are often underrepresented. Thus, false negative IgE test results may be the consequence.
- Lipophilic allergens have historically been overlooked, contributing to the challenges in obtaining homogeneous natural allergen preparations.
- The presence of proteases in allergen extracts poses a significant issue, leading to allergen degradation and impacting allergenic activity, immunogenicity, and immunomodulatory capacity.

ALLERGEN MOLECULES – ESSENTIAL FOR MAD

Allergen molecules are among the most well-defined groups of molecules in biomedical research in terms of their function, structure, and biologic effects. Molecular allergy diagnostics is essential to improve the specificity of allergy testing[13] [14] [15]. The use of molecular allergens gives more detailed information on the actual sensitisation status of the patient. Clinical cross-reactivities can be resolved. The knowledge on the stability of a protein provides additional information and thus differentiated dietary recommendations can be discussed. Thus, IgE sensitisation to heat-labile proteins includes the information that cooked foods may be tolerated, whereas raw foods lead to symptoms.

Marker molecules for severe clinical manifestations (e.g., anaphylactic reaction) are available, enabling risk assessment. Molecular allergy diagnostics also makes it possible to determine whether immunotherapy is indicated.

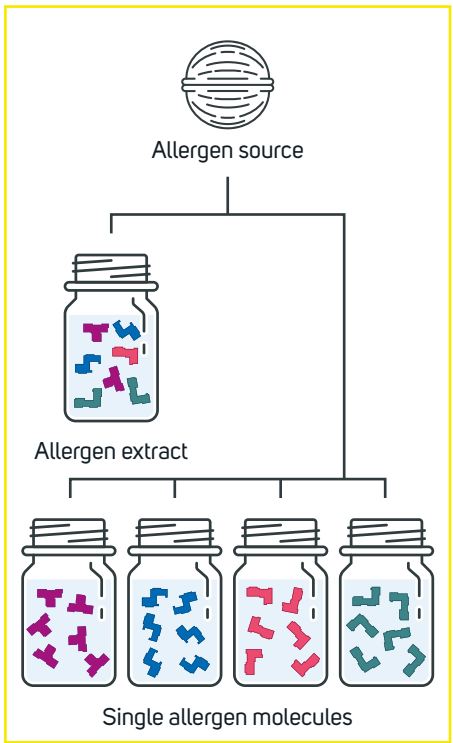


Figure 7: Allergen extracts and single molecular allergens.

Advantages	Limitations
Pure proteins of desired quality and properties	Require knowhow
Can be produced and reproduced in defined amounts and concentrations, independent of allergen raw material	Require modern recombinant or synthetic production process
Allergenic, immunogenic, and tolerogenic properties are predefined	A recombinant allergen represents only one isoform
Multiple advantages when used for diagnosis: • Identification of culprit allergen molecules • Revealing cross-reactivity • Actual molecular sensitisation profile • Personalised treatment options	

Table 2: Advantages and limitations of using recombinant allergens in allergy diagnostics.

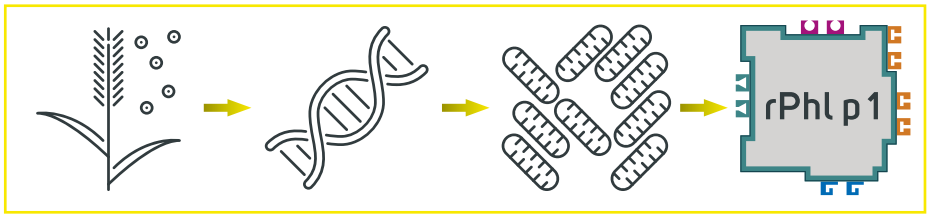


Figure 8: Schematic representation of the production of recombinant allergens.

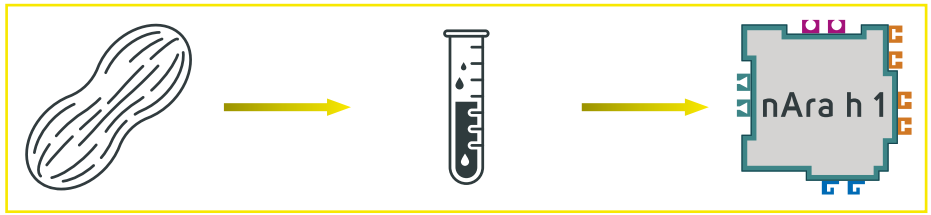


Figure 9: Schematic representation of the production of natural allergens.

RECOMBINANT ALLERGENS (e.g., rPhl p 1)

Recombinant allergens are produced in high-level expression systems like Escherichia coli. While many allergens are produced as soluble proteins, some accumulate in so-called inclusion bodies and require refolding during the purification procedure. While recombinant allergens that were expressed in E. coli are not glycosylated, which is considered advantageous for allergy diagnosis, another expression system, the yeast Pichia pastoris overglycosylates proteins. In a third system that is based on insect cells, well-folded, secreted proteins are produced, but handling complexity and higher costs limit their use. Irrespective of the selected expression system, the end product is a pure, well-characterised recombinant allergen or derivative thereof, ensuring consistency through detailed physicochemical and immunologic characterisation in each batch.

NATURAL ALLERGENS (e.g., nAra h 1)

Natural allergens are purified from natural allergen sources through chromatographic steps and analysed for identity, quantity, homogeneity, folding, and stability. However, a drawback is the need for substantial starting material, especially when allergens constitute a small percentage of total protein content, as seen in some pollen or house dust mite allergens.

Traditional vs. targeted diagnosis

by Anna Ringauf, MSc ETH

In the landscape of allergy diagnosis, precision is paramount. The choice between extract-based and component-based methodologies profoundly impacts the level of information and subsequent success for the selected type of therapy. Patient care and outcome is therefore directly affected by the choice of testing method.

Extract-based IgE testing has been the cornerstone of allergy diagnosis for decades, owing to its simplicity and widespread availability. However, in the past 35 years, since the first plant allergen, Bet v 1, was cloned by Heimo Breiteneder (read more), substantial progress has been made in the field of molecular allergy diagnosis, allowing for in-depth information of patient sensitisation profiles[16 17 18]. This article will explore the advantages of molecular allergy diagnosis over extract-based diagnosis.

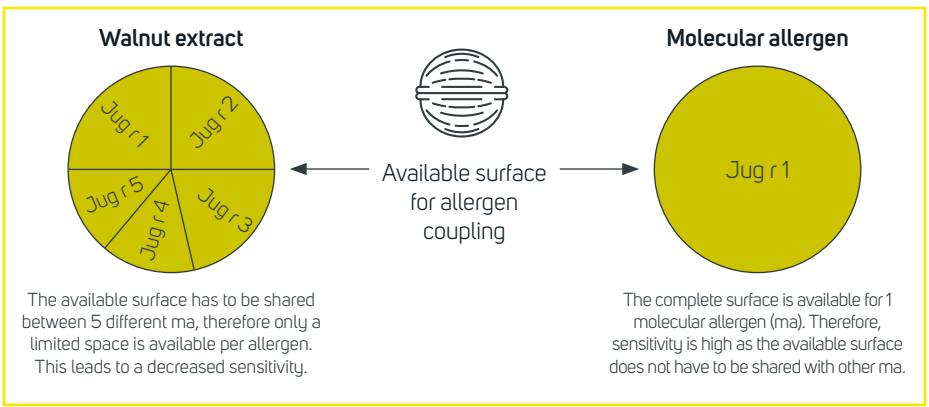


Figure 10: Allergen extracts vs. molecular allergens.

EXTRACTS VS. ALLERGEN MOLECULES

An allergen extract is composed of a mixture of proteins from its source material. This includes glycosylated- and non-glycosylated proteins, some of which are non-allergenic[17]. If an extract is coupled to the solid phase of the test system, lower amounts of each allergen are coupled when compared to tests which use molecular allergens (Figure 10). Therefore, the sensitivity of such an extract-based test can be lower.

The composition of the extract largely depends on the extraction method. Depending on the buffer used, some allergens solubilise

easier than others due to their physicochemical properties (e.g., Omega-5-gliadin vs. wheat extract)[19]. While some allergens will be overrepresented, others will be underrepresented, which can reduce analytical sensitivity of the test with respect to IgE sensitisation to the latter. In case of allergens with a high potential to induce severe allergic reactions, underestimation of IgE reactivity might represent a substantial risk for the patient (e.g., Gly m 4 vs. soy extract)[20].

Molecular components on the other hand are purified allergenic molecules, either produced recombinantly or from natural sources (read more). For molecular components, only the respective allergen is immobilised on the solid phase, increasing sensitivity as well as adding resolution to the test result. Research in molecular allergology has made major progress, currently listing 1,112 officially recognised molecular allergens[21]. In contrast to singleplex testing, multiplex tests generate a comprehensive sensitisation profile of the patient in one step with low sample consumption. Those results facilitate choosing the right type of therapy. Molecular allergy diagnosis is therefore important for clinical practice. For a correct interpretation of the result, an in-depth knowledge about molecular allergology is necessary[13]. RAVEN Interpretation Software developed by MADx helps to draw the right conclusions about the patient's sensitisation status. Having the full picture of the patient's sensitisations allows to make the right decision and improve the patient's quality of life.

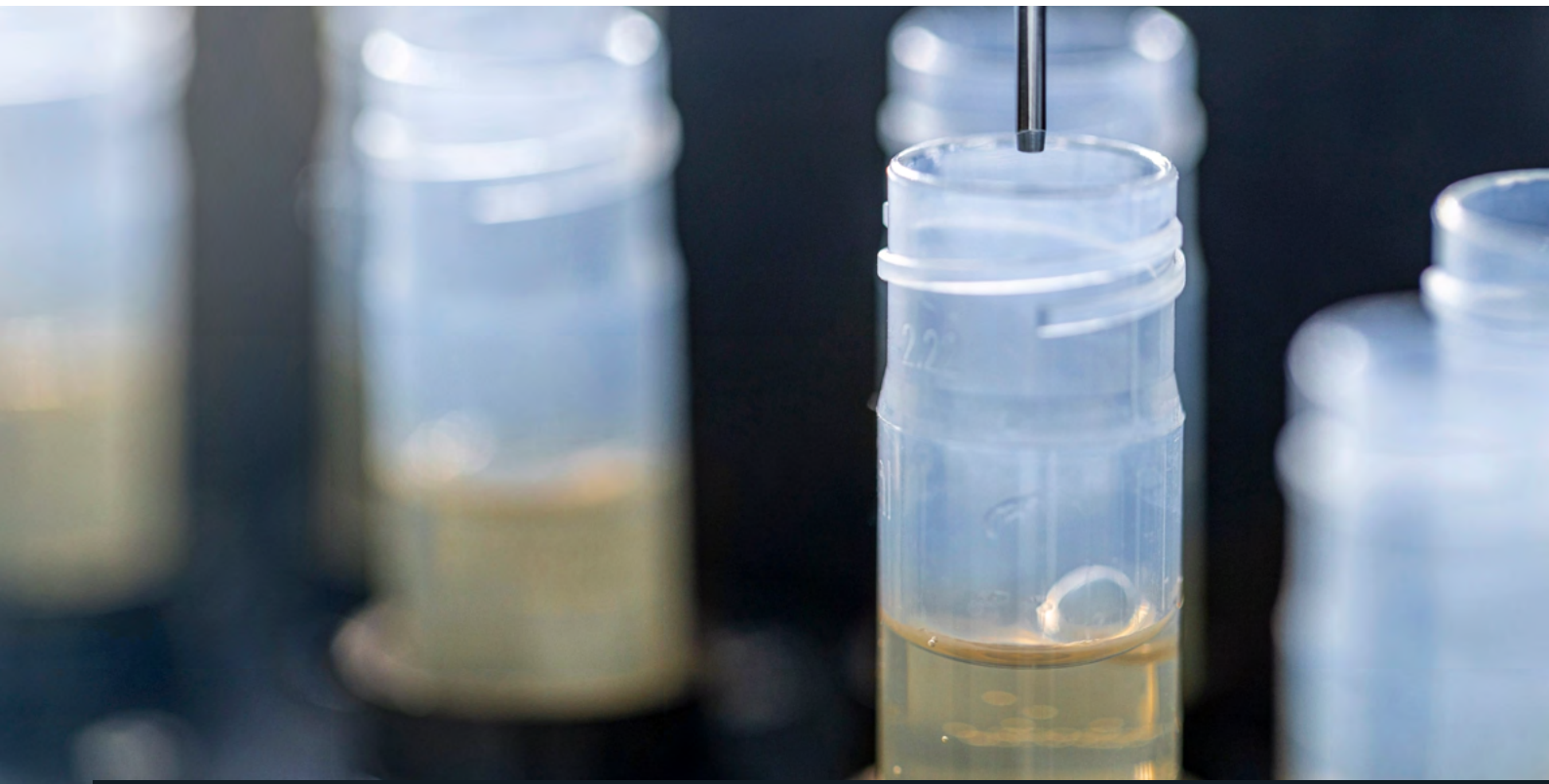
ALEX² is the largest Multiplex Test on the market

- 178 molecular allergens
- 117 extracts
- CCD-control spot (Hom s LF)
- Automatic CCD inhibition

Molecular allergens bear more information in themselves compared to extracts, regardless of the extract composition. The type of protein, knowledge about the primary sensitiser, cross-sensitisation, and co-sensitisation as well as the potential efficacy of allergen immunotherapy (AIT) can only be understood through molecular allergy diagnosis.

CROSS-REACTIVITY

There are two different types of cross-reactivity found in molecular allergology. The first one relies on panallergen families, which are universal among many species (allergen families) such as profilins, polcalcins, or cyclophilins. Panallergens are highly conserved and known



to bear a specific risk. For example, profilins are very conserved and known to provoke very mild reactions while the reaction to PR-10 can range from mild to severe. Positive signals to extracts from apple, hazelnut, peanut, walnut, celery, and soy could lead to major restrictions in the patient's diet when it is unclear whether the patient is prone to exhibit a severe allergic reaction upon exposure. Thus, a positive result to a mostly harmless allergen family like profilins can be relieving, whereas in case of PR-10 reactivity, stronger reactions might occur, e.g., triggered by Gly m 4 from soy. However, since PR-10 proteins are heat-labile, all PR-10 bearing foods will be tolerated after heat-processing and mostly cause mild symptoms only[17].

Another type of cross-reactivity relies on particular sugar moieties that are found on many natural glycoproteins. Cross-reactive carbohydrate determinants (CCDs) are very conserved and found in plant extracts, insect venoms, and molluscs. Glycoproteins purified from these sources, or produced by eucaryotic organisms such as yeast will also exhibit these structures. A study found that around 30 % of all pollen allergic individuals are IgE positive to CCDs[22]. The IgE antibodies to CCDs, however, are regarded as clinically irrelevant. Therefore, diagnostic specificity is significantly reduced for CCD positive patients reporting clinically irrelevant signals, when extracts or natural allergens are used. High diagnostic specificity is only achieved by blocking these anti-CCD antibodies[23].

Currently, ALEX^2 is the only commercially available test that automatically blocks anti-CCD antibodies during the incubation step. To ensure complete blocking of CCD-specific IgE, a control spot (Hom s LF) is included on the ALEX^2 test. If no signal to that CCD-marker is detected, IgE reactivities to all spots on the test can be interpreted without constraint[23].

Another type of cross-reactive sugar moiety is alpha-gal. Anti-alpha-gal IgE antibodies can provoke clinically relevant symptoms, with a delay of some hours. It is an important marker for delayed meat allergy[24].

PRIMARY SENSITISATION AND SEVERITY ASSOCIATED MARKERS

While panallergens are found in various species and indicate cross-reactivity, other allergens are markers for genuine, family- or species-specific sensitisation. These sensitisations are regarded as primary sensitisations and help to distinguish co- from cross-sensitisation in case of allergy to multiple sources. The use of extracts does not allow for such a distinction. The following proteins are examples for markers for genuine sensitisation: Amb a 1; Api m 1, 3, 4, 10; Art v 1; Bet v 1; Fel d 1; Ole e 1; Phl p 1, 5; Par j 2; Ves v 1, 5[17 25].

Stability is an important property of allergens when considering the severity of reactions. Allergens that resist heat and conditions encountered in the digestive tract (acidity, proteases) are more likely to cause severe clinical reactions, whereas those that are sensitive to heat and digestion are more likely to be tolerated or cause only mild or local symptoms[17]. Therefore, knowing the type of protein and its characteristics will allow to estimate the severity of the reaction. Very severe reactions are associated for example with seed storage proteins. They are abundant in seeds and very stable to heat and digestive degradation. Roasting even increases their allergenicity[26].

PREDICTION OF EFFICACY OF AIT

Emerging data suggests that AIT (allergen-specific immunotherapy) may be more effective in individuals who are only sensitised to markers of genuine sensitisation, while it appears to be less effective in those sensitised to cross-reactive components or panallergens[23]. Furthermore, IgE reactivity to allergen molecules that are often not contained in extracts used for AIT (e.g., Der p 23 from Dermatophagoides pteronyssinus) indicate a lower probability of successful AIT. Therefore, in addition to supporting the accurate prescription of AIT, detecting the patients' detailed sensitisation pattern also helps to identify patients who are likely to benefit from AIT[23].

Molecular allergy diagnostics in practice

Interview with Dr. Ramón López Salgueiro

Dear Dr. López, you have been practicing and teaching allergology for 18 years. During this time, you witnessed the introduction of molecular allergology into clinical practice in Spain firsthand. Was the use of molecular allergens (components) embraced wholeheartedly by the medical community, or was there a big amount of scepticism in the beginning?

Dr. López: The introduction of molecular diagnosis in allergy drove a paradigm shift in the management of patients, especially in the performance and interpretation of complementary in vitro tests. Therefore, this deep change of understanding allergy diagnosis pushed many colleagues outside of their comfort zone. So, in the beginning, attaining knowledge about molecular allergology and applying it to the clinical routine was considered with scepticism by many allergists, mainly for those with higher years of clinical experience. However, a smaller group of professionals considered this change as an opportunity to improve the diagnosis and clinical management of patients. Thanks to the enthusiasm and effort of these persons the use of molecular allergens could be introduced, step by step, into the daily routine of allergy departments until we reached the status we have today. Now, molecular allergology is accepted and used by the majority of allergists in Spain.

When did you start using molecular allergens as part of your diagnostic work-up of allergic patients and what was your first impression?

Dr. López: I started to use component resolved diagnosis in 2008 as part of an epidemiological research project about identifying sensitisation profiles in allergic patients from the geographical area of our hospital. But, from the very beginning of this project, we realised the diagnostic strength of this tool allowed us to have a better understanding of cross-reactivity and, in consequence, to make better diagnoses and prescribe treatments fitted to the genuine allergy of the patients. So, after the authorisation of the management team of our hospital, we began to use molecular components in the daily routine of the allergy department.

> Molecular diagnosis drove a paradigm shift in patient management.

What is your favourite allergen family (e.g., Profilin) and why?

Dr. Lopéz: Without any doubt, my favourite family of allergenic components is the non-specific lipid

transfer proteins (nsLTPs). This allergen group is responsible for almost 75 % of food anaphylaxis in patients older than 14 years in our geographical area. As you might know, nsLTPs are associated with moderate and/or severe reactions related with the consumption of fresh fruits and vegetables, nuts and cereals like corn or wheat. Thanks to the use of molecular components of this family we could identify different sensitisation profiles to nsLTPs and individualise the diet of the patients (avoiding unnecessary restrictions) according to these profiles.

So, molecular diagnostics is not only useful for the diagnosis of our patients but also to detect new allergy aspects to be investigated to improve the understanding and management of our patients.

What is the strength of a diagnostic work-up that includes molecular allergens?

Dr. Lopéz: In my opinion, the real strength of molecular diagnosis in allergy is that these kinds of tests allow us to know the real sensitisation profile of the patient. Sensitisation profile is described as the set of specific and cross-reactive molecular components to which the patients are really sensitised. In allergy diagnosis, the clinicians have a big problem with cross-reactivity. This phenomenon can be responsible for the presence of several "false positive" results in traditional complementary tests like skin prick tests. The determination of specific IgE levels to whole extracts could drive allergists to make an inaccurate diagnosis and, in consequence, to prescribe treatment not fitted to the real allergy of the patient. So, today to make the best diagnosis possible for our allergic patients it is necessary to know their real sensitisation profile, and the only way to do it is using a diagnostic work-up with molecular allergens.

Is there a downside of a diagnostic work-up that includes molecular allergens?

Dr. Lopéz: Obviously, not everything about molecular diagnostics is perfect, and some disadvantages exist. For example, the occurrence of some positive results without clinical relevance due to CCDs (cross-reactive carbohydrate determinants) present on native components makes it difficult to obtain an accurate interpretation of the results. The use of CCD-blocking agents is an advantage that minimises the interference of cross-reactivity and makes the result interpretation easier.

Secondly, the presence of some false negative results in multiplexed tests due to a lack of sensitivity in samples with low levels of total IgE (<20 kU_A/L). And thirdly, in multiplexed tests, sometimes a high number of positive results gives a lot of information that could make the result interpretation more difficult for allergist colleagues without enough knowledge about molecular diagnosis. But, in general, the benefits of molecular diagnosis far outweigh the disadvantages.

What are your wishes for the in vitro diagnostics industry regarding new developments?

Dr. López: I would like to have a multiplexed test with a lower number of allergens than available tests with a focus on allergen families like foods or inhalants, or multiplexed tests with maximum 15 to 20 components specific to allergen groups like LTPs, seed storage proteins or thaumatin like proteins (TLPs).

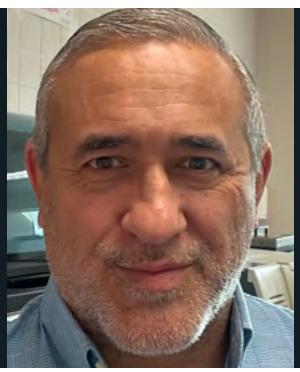
I also wish for an increased sensibility of multiplexed tests regarding samples with low levels of total IgE and to have more allergenic components from other groups such as thaumatins, gibberelins, cyclophilins and oleosins available.

Also, in my opinion, I think that a joint task force composed by industry representatives and clinicians should work on developing AI tools to identify sensitisation profiles to specific allergens (LTPs, seed storage proteins, thaumatins, house dust mites, etc.) and link these profiles with clinical features to increase the diagnostic accuracy and therefore improve the treatment of our patients.

ABOUT

DR. RAMÓN LÓPEZ SALGUEIRO
is a doctor at the Hospital Universitari I Politècnic la Fe in Valencia, Spain. He has been working as a clinician in allergology for 18 years and is also involved in training doctors in the field.

> "The benefits of molecular diagnostics far outweigh the disadvantages."



The impact of MAD for allergy diagnosis and therapy

by Dr. Raffaela Campana

This chapter will discuss the concept and importance of surrogate allergen markers and how molecular allergy diagnostics using multiplex assays impacts the accuracy of allergy diagnosis and therapy prescription.

ALLERGY DIAGNOSIS, ALLERGEN FAMILIES AND ALLERGEN CROSS-REACTIVITY

The discovery of IgE by Ishizaka (1966) and Johansson (1967) groups independently had a significant effect on the understanding, diagnosing and management of allergic diseases[27] [28] and for decades, serum IgE has been considered a marker for allergic sensitisation.

Traditionally, allergy diagnosis is based on (i) clinical history trying to relate the occurrence of clinical symptoms to a possible causative allergen source, (ii) provocation testing with selected allergens and (iii) in vitro IgE measurements to confirm the presence of IgE antibodies specific for the suspected allergen sources in the blood or other body fluids of the patient[29] [30].

Since the beginning of allergy diagnostics, allergen extracts have been used for those tests. The problem is that allergen extracts are limited in composition and vary considerably regarding concentrations of allergen molecules, often producing different quantitative and qualitative results. Another limitation is that extracts do not allow to differentiate between co-sensitisation (simultaneous genuine sensitisation to allergenic molecules from different allergen sources) and cross-sensitisation (ability of an allergen to bind with an antibody that was raised to a different allergen) when a patient shows IgE reactivity to several allergen sources[31].

To overcome those limitations the concept of molecular allergy diagnosis was conceived. In this technological innovation, allergen-encoding DNAs have been isolated[18] [32] [33] and well-defined recombinant allergen molecules can be produced allowing IgE-based serological diagnosis with high sensitivity and specificity[34] [35].

These allergen molecules have been identified and characterised with respect to their structural and immunological properties. Each allergen molecule can be assigned to one distinct allergen family.

Allergen families are defined by similar amino acid sequence and comparable three-dimensional structures. Similarities in sequences and 3D structures of allergenic proteins provide important information to identify clinically relevant IgE cross-reactivity[36] (see allergy families). The prediction of cross-reactivity is normally

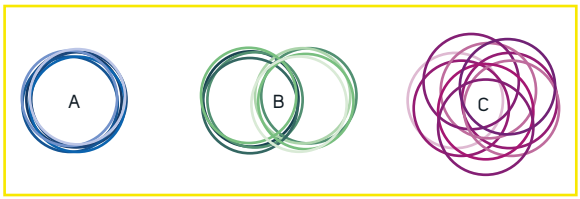


Figure 11: Patterns of cross-reactivity. The amount of cross-reactivity between allergens from the same family varies between protein families. (A) allergen families with very strong cross-reactivity (e.g., profilins), (B) allergen families with moderate cross-reactivity (e.g., grass group 1 allergens) and (C) allergen families with very poor cross-reactivity (e.g., 2S albumins).

achieved considering three points: one is the full-length amino acid sequence alignment, where allergens with sequence identity > 50 % indicates potential cross-reactivity; the second is that when allergens share > 35 % homology in an 80 amino acid sequence, the chance of cross-reactivity is high; and the third is search for an exact match of 8 amino acids[37] [38]. Cross-reactivity is mostly confined to members of the same family and hardly occurring between members of different families. However, not all allergen families show the same degree of cross-reactivity. Some families exhibit very strong or even close to 100 % cross-reactivity (e.g., profilins) while others show moderate (e.g., PR-10) or very poor (e.g., 2S albumins from the group of seed storage proteins) cross-reactivity implying that there are shared epitopes but also a varying number of epitopes specific for the individual allergens of the same family (Figure 11).

Of note, cross-reactivity occurs mainly in aeroallergens and food allergens. For example, polysensitisation to animal dander such as cat, dog and horse can in part be explained by cross-reactive lipocalins and albumins. The dog lipocalin, Can f 6, is a candidate for cross-reactivity with Fel d 4 from cat and Equ c 1 from horse with clinical relevance[39]. However, not all IgE cross-reactions translate into clinical cross-reactivity.

Based on the different degrees of cross-reactivity in the different allergen families, it is possible to define surrogate markers. A surrogate marker consists of one allergen of a particular allergen family that is used to detect IgE sensitisation to the whole family or a subgroup thereof. The 'ideal' genuine allergen marker should be recognised by 100 % of the sensitised patients without cross-reactivity to other allergens.

It is important to keep in mind that when defining specific markers and markers for cross-sensitisation, regional differences in the exposome should be considered. While an allergen serves as marker for genuine sensitisation to a particular allergen source in one region, in another part of the world, where this allergen source is not prevalent, it might be used as a surrogate marker for sensitisation to other sources that comprise allergens of the same allergen family.

SURROGATE MARKER ALLERGENS

In case of strong cross-reactivity, IgE reactivity to one member of that family implies that those antibodies will bind to all other members as well. Therefore, it is sufficient to test for IgE reactivity to only one member of such families. Examples for such allergen families are profilins and polcalcins (Figure 12).

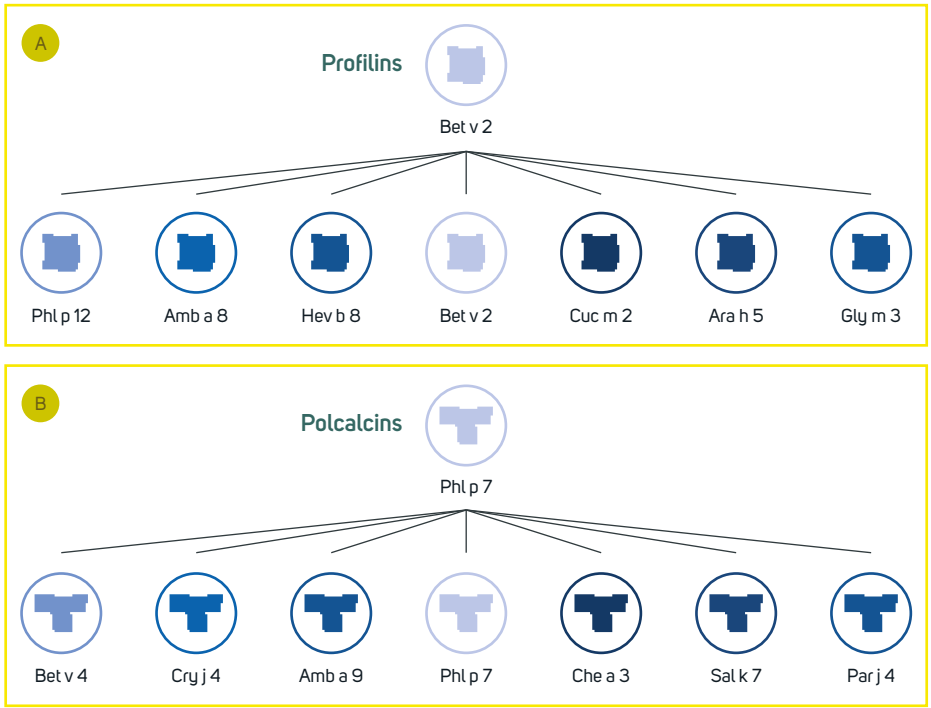


Figure 12: Surrogate markers in allergen families with strong cross-reactivity. (A) Surrogate marker for profilins, (B) Surrogate marker for polcalcins.

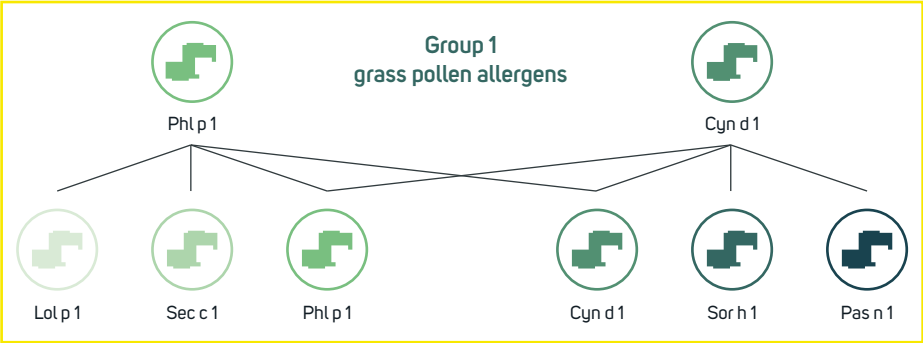


Figure 13: Surrogate markers in allergen families with sub-groups of cross-reactive allergens. Shown are surrogate markers for group 1 grass pollen allergens.

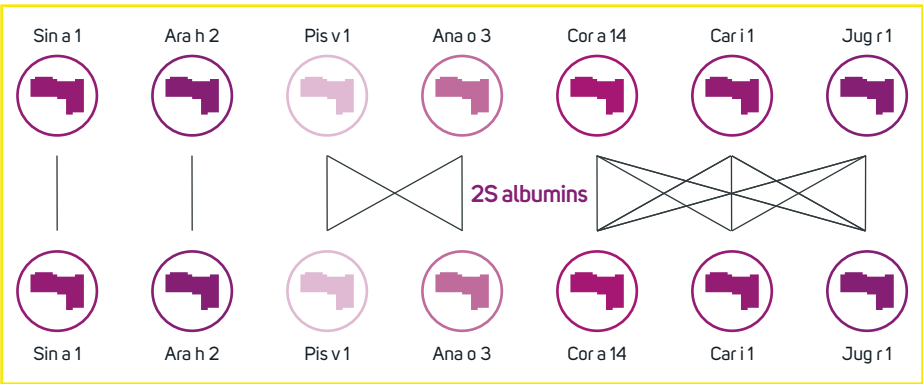


Figure 14: Surrogate markers in allergen families with limited cross-reactivity. Shown are surrogate markers for storage proteins 2S albumins.

For families with subgroups of cross-reactive allergens that show strong cross-reactivity within but less so between the subgroups, it is necessary to use one member of each sub-group as surrogate marker for IgE testing. This is the case, for example, for lipocalins or group 1 grass allergens (Figure 13).

For families with greatly limited cross-reactivity, no surrogate marker can be defined. In such a case it is necessary to test for IgE reactivity to several members of this family or, at least, to the one from the allergen source that is suspected to cause the clinical symptoms. Especially for high-risk allergens, reliable detection with high sensitivity is very important. This is the case of the seed storage proteins 2S albumins, 7S globulins and 11S globulins[40]. Therefore, ALEX^2 contains a high number of lipid transfer proteins and storage proteins (Figure 14).

The advantage of a suitable surrogate marker is that testing for one allergen covers all allergen sources that contain a member of that family. The allergen source from which the surrogate marker is derived does not even need to be prevalent in the region where the test is used. Bet v 4 from birch pollen, as an example, can be used as surrogate marker for any other polcalcin that is found in pollen around the globe, even in regions where birch trees are not prevalent (Figure 16 on the next page).

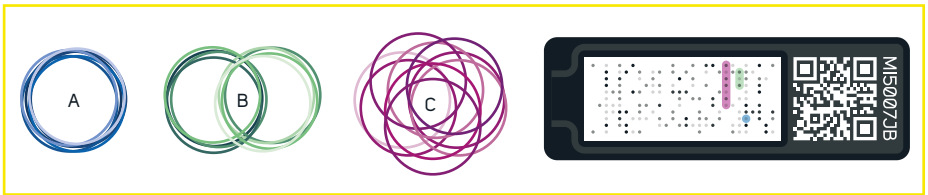


Figure 15: Scheme showing patterns of cross-reactivity (left side) and representative surrogate markers from the different allergen families on ALEX^2 (right side). The selection of how many surrogate markers should be included in a multiplex text is based on the cross-reactivity pattern of each family.

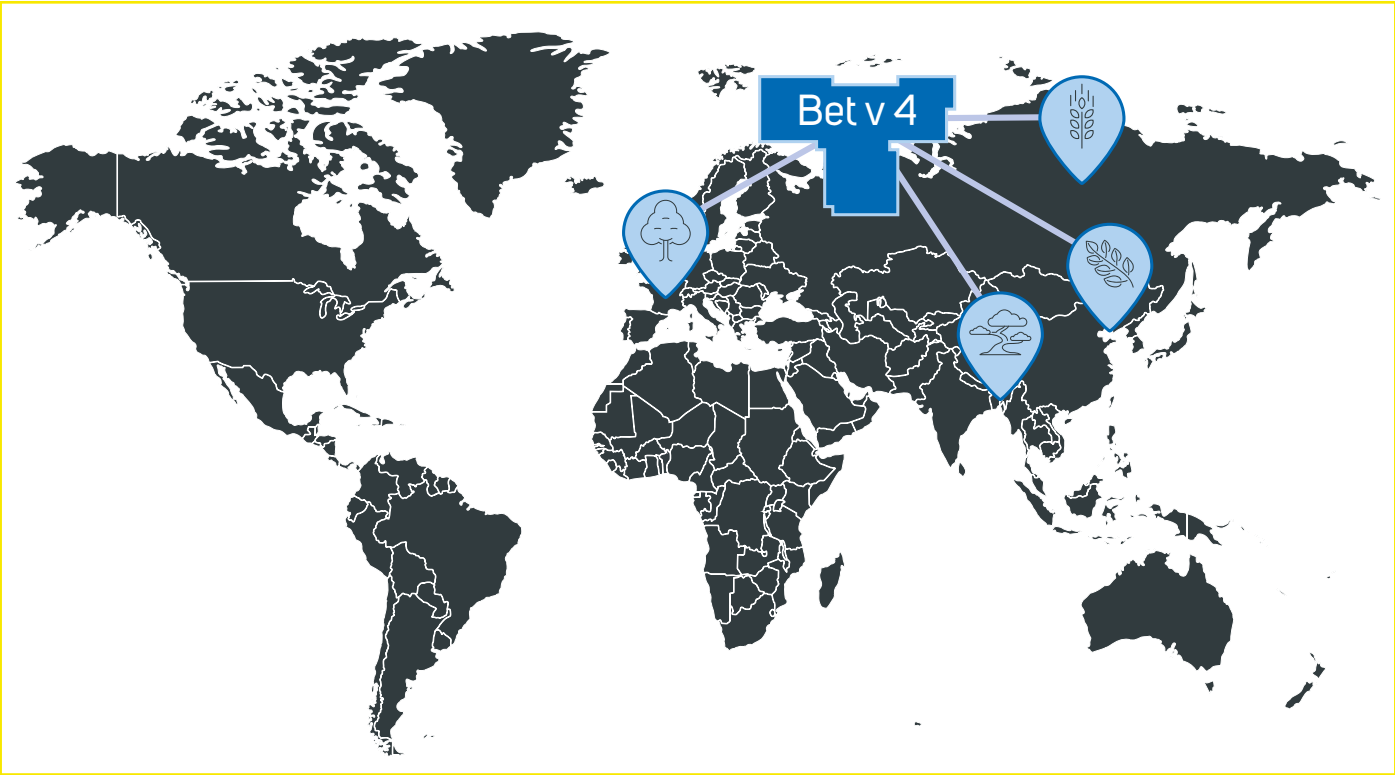


Figure 16: Concept and advantage of surrogate markers for the diagnosis of allergy.

CONCLUDING REMARKS – SURROGATE MARKERS

- Primary sensitisation can be distinguished from cross-sensitisation by using marker allergens for genuine and for cross-sensitisation.
- For allergen families with strong cross-reactivity, an IgE microarray does not need to contain allergens from all possible allergen sources. Instead, a few surrogate markers are sufficient to detect sensitisation to those families.
- For clinically relevant allergen families with limited cross-reactivity, more representatives need to be included in the panel of the microarray.
- Identification of the causative allergen by allergen-based tests is particularly important because it facilitates accurate diagnosis and precise treatment prescriptions.
- ALEX^2 contains relevant surrogate markers from different allergen families and includes a wide range of allergens from families with limited cross-reactivity such as storage proteins and LTPs.

Implications of MAD

The prescription of allergen-specific immunotherapy (AIT)

by Dr. Raffaela Campana

Allergen-specific immunotherapy (AIT, SIT or ASIT), also called desensitisation, hyposensitisation, or specific immunotherapy, is the only treatment for allergic diseases with sustained therapeutic effect.

The work of Leonhard Noon[41] demonstrating that subcutaneous injections of aqueous grass pollen extracts into a few patients reduced seasonal symptoms of pollinosis and asthma is considered the first official report describing allergen-specific immunotherapy.

Today, AIT is based on repeated administration of increasing doses of allergen extracts either subcutaneously (SCIT) or sublingually (SLIT) to allergic individuals, until a maintenance dosage is achieved[42]. The treatment normally takes three years to complete and the selection of the route of AIT application depends mainly on the preference of the patient, availability of allergen preparations and severity of disease and associated comorbidities.

CLINICAL EFFICACY AND SAFETY OF AIT

Since the publication of Noon's work, several studies have been conducted to evaluate the safety and efficacy of AIT in various patient subgroups. Overall, different double-blind placebo-controlled trials demonstrated that AIT treatment provides significant improvement of allergic symptoms and patients' quality of life, reduces the need for pharmacotherapy and has long-term clinical effects, including reduction in allergic disease progression from rhinitis to asthma. Moreover, AIT can prevent new sensitisations and/or prevent clinical manifestation of latent sensitisations[43].

MECHANISMS OF AIT

The mechanism of action of AIT is complex and still not fully understood. The primary goal of AIT is to restore immune tolerance to allergens. Successful immunotherapy includes a very early desensitisation effect and a shift of Th2 immune responses, which is associated with atopic conditions, to a more balanced Th1 immune response. AIT is also associated with an early increase of specific IgE followed by late decrease that goes in accordance with induction of allergen-specific regulatory T cells (Tregs) which downregulate allergic inflammation by the production of anti-inflammatory cytokines IL-10 and TGF-beta. A relatively early increase in allergen-specific IgG_4 levels in serum is also observed. Some studies also showed an increase in IgG_1 and IgA production together with upregulation of IFN-gamma antagonistic type 1 responses. In addition, AIT is also associated with a decrease in mast cells, basophils and eosinophils recruitment to the skin, eye, nose, and bronchial mucosa after allergen exposure. This reduces the release

of mediators (e.g., histamine) from mast cells and basophils, decreasing the tendency for systemic anaphylaxis, and suppression of effector T cell migration to the skin, decreasing late-phase skin reactions[44 45].

INDICATIONS FOR AIT

AIT is indicated in patients with IgE-mediated moderate-to-severe allergic rhinitis, allergic conjunctivitis, and allergic asthma with symptoms that cannot be controlled by avoidance measures and pharmacotherapy, or, in case of substantial adverse effects, with symptomatic medications. In addition, there are studies showing that AIT can be beneficial to patients suffering from atopic dermatitis that is associated with sensitisation to aeroallergens[25 45 46]. Furthermore, AIT showed to be safe and effective in mono- and polysensitised patients. Thus, it may be initiated regardless of the type of sensitisation (mono-/oligo-/polysensitisation).

Allergen-specific immunotherapy is contraindicated in patients with severe and uncontrolled asthma, with significant co-morbid cardiovascular disease as well as in patients with use of beta-blockers due to the risk of anaphylaxis. However, according to the EAACI and AAAAI guidelines, the risk-benefit of hymenoptera venom immunotherapy should be considered for patients with anaphylaxis to stinging insects who also have cardiovascular disease since the immunotherapy benefits may outweigh the potential risks associated with beta-blockers[25 45 46].

Of note, it is recommended to pursue AIT for at least three consecutive years to achieve a sustained effect.

IMPACTS OF MAD FOR AIT

The current allergen-extract-based forms of allergen-specific immunotherapy proved to be cost-effective and the only disease modifying form of allergy treatment. However, to be beneficial to the patient AIT requires accurate prescription and monitoring.

In this context, different studies showed that patients sensitised to genuine components have a better AIT result compared to patients sensitised to cross-reacting components[47]. In addition, there are studies showing that the decision on specific immunotherapy prescription was changed/improved significantly by the availability of CRD[48 49 50]. In summary, advances of molecular diagnosis and the use of multiplex arrays can help in identifying the most relevant sensitisations and cross-reactions, thus, a detailed IgE profile may improve AIT in terms of higher accuracy of indication and risk assessment.

MAD ASSAYS FOR AIT PRESCRIPTION

Marker allergens allow for differentiation between genuine sensitisation and cross-sensitisation which is particularly advantageous in diagnosis of polysensitised patients and may influence the doctor's AIT prescription.

If a patient shows clinical and IgE reactivity to two allergen sources "A" and "B", it is possible to differentiate if the patient is truly sensitised to "A" and cross-sensitised to "B" or vice versa, or if he is truly sensitised to both, in terms of co-sensitisation. The first two cases could be an indication for immunotherapy only to A or to B but not to both, while in the latter case, immunotherapy to both allergen sources might be indicated. With this approach, accuracy of allergy diagnosis and therapy recommendations can be immediately improved (Figure 17). Using a multiplex IgE test such as

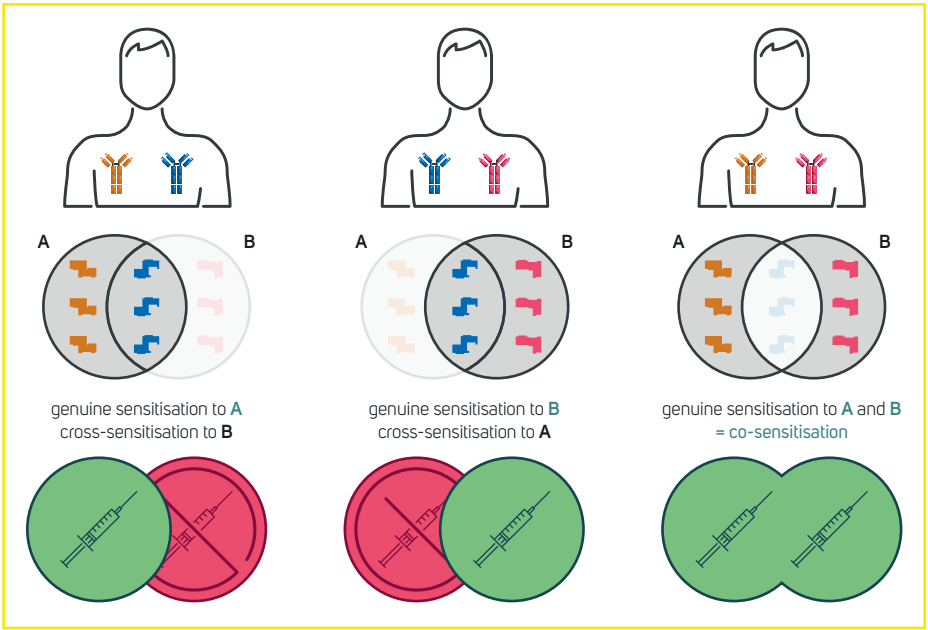


Figure 17: Differentiation between co- (genuine) and cross-sensitisation by molecular allergy diagnosis (MAD) and the implication for AIT indication.

ALEX² which includes many marker allergens, the genuine sensitisations can be identified which is in fact of extreme importance for AIT prescription, since unnecessary AIT treatment may result in new IgE sensitisation.

In this clinical case, the patient suffered from strong allergic rhinitis during early and late spring and had a positive SPT to birch and grass pollen extracts. Based on the results of the extract-based SPT, allergen-immunotherapy to birch and grass would have been prescribed.

The use of diagnostic multiplex tests based on allergen molecules (e.g., ALEX²) provides a more accurate IgE sensitisation profile of the patient allowing for differentiation between genuine and cross-sensitisation. In Figure 18, different scenarios of IgE reactivity to marker allergens for genuine and for cross-sensitisation are shown, including the respective indications for AIT.

CASE STUDY: AIT SELECTION THROUGH MAD

Based on the results of the extract-based SPT, allergen-immunotherapy to birch and grass would be prescribed. However, the use of MAD may change the AIT indication. Here three molecular diagnostic scenarios are exemplified. Scenario 1 shows a genuine sensitisation to the allergen markers from birch and grass, thus AIT to both allergen sources is indicated. Scenario 2 shows a cross-reactivity between birch and grass pollen. MAD shows a genuine sensitisation to birch and cross-sensitisation to grass via profilin. In this case AIT to birch but not to grass is recommended. Scenario 3 shows a genuine sensitisation to birch (Bet v 1) and grass (Phl p 1) with additional reactivity to panallergens (Bet v 4 and Phl p 7) therefore, AIT to birch and grass might be indicated, as in scenario 1.

Selection of AIT based on extract-based diagnostics

Clinical history	Extract-based diagnostics	AIT indication
Strong rhinitis during early and late spring	SPT: Birch extract (+) SPT: Grass extract (+)	Birch (+) Grass (+)

Component-resolved diagnostics (CRD) (co- or cross-sensitisation)

Bet v 1 - allergen marker for birch pollen sensitisation

Phl p 1, 2, 5, 6 - allergen markers for grass pollen sensitisation

Added value of MAD for AIT selection

	Molecular allergy test	Diagnostics: co- and cross-sensitisation	AIT indication
Scenario 1	Bet v 1 (+) Phl p 1 (+)	co-sensitisation to birch and grass	Birch (+) Grass (+)
Scenario 2	Bet v 1 (+) Phl p 1, 2, 5, 6 (-)	cross-sensitisation	Birch (+) Grass (-)
Scenario 3	Bet v 1 (+) Bet v 4 (+) Phl p 1 (+) Phl p 7 (+)	genuine sensitisation (co-) to birch and grass with reactivity to panallergens	Birch (+) Grass (+)

Figure 18: Comparison of molecular and extract-based allergy diagnosis for prescription of allergen-specific immunotherapy.

MAD ASSAYS TO PREDICT THE EFFICACY OF AIT

Predicting the efficacy of AIT and identifying patients who are likely to respond to the treatment would be very important but still, there is no suitable biomarker available. The use of molecular-based allergy diagnostic tests may help to overcome this constraint. In this context, insect venom allergy and house dust mite (HDM) allergy could be named.

Reports show that patients with strong IgE sensitisation to Api m 10, a marker allergen for honeybee venom may not benefit from venom AIT because Api m 10 is frequently underrepresented in venom preparations[51]. A similar constraint is observed in patients with house dust mite allergy. Der p 1, Der p 2 but also Der p 23 are

major HDM allergens and studies demonstrated that diagnosis and AIT performed with HDM extracts containing mainly Der p 1 and Der p 2 can lead to misdiagnosis and will not be beneficial for patients who were sensitised to other allergens than Der p 1 and Der p 2[52].

The importance of diagnostic tests based on marker allergens to improve the choice and predict the efficacy of allergen immunotherapy was also demonstrated in patients sensitised to polcalcins such as Bet v 4. Kazemi-Shirazi et al. showed that patients sensitised to Bet v 4 without reaction to the major birch pollen allergen, Bet v 1, may not benefit from birch pollen extract-based immunotherapy[53].

THE USE OF MAD ASSAYS FOR RISK ASSESSMENT

Another advantage of using allergen molecules instead of extracts is that it is possible to distinguish between sensitisation to allergens which are associated with a high risk of severe systemic allergic reactions and sensitisation to mostly harmless allergens of the same source (Figure 19).

Attention should be given to IgE sensitisation to seed storage proteins (2S albumins, 7S globulins and 11S globulins), oleosins and pathogenesis-related (PR) proteins like PR-12 (defensins) and PR-14 (non-specific lipid transfer protein) related to tree nuts, legumes, and seeds. Those are heat-stable proteins linked to severe and sometimes fatal anaphylaxis. For instance, the major peanut allergens Ara h 1, 2, 3 and 6 showed to be high risk allergens related to severe reactions whereas the minor peanut allergen Ara h 8 is normally responsible only for mild reactions such as oral allergy syndrome[40].

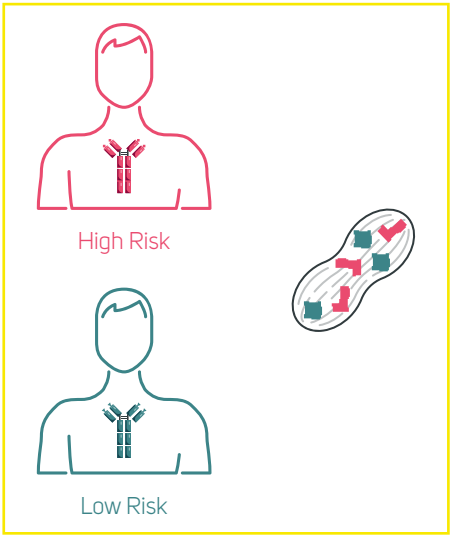


Figure 19: Risk assessment by MAD. High risk allergen (represented in pink) is related to severe allergic reactions. Low risk allergen (represented in blue) is related to mild allergic reactions.

ALEX² MULTIPLEX IgE TEST IN THE CONTEXT OF SURROGATE MARKERS, DIAGNOSIS, AND TREATMENT (i.e., AIT) OF ALLERGY

- ALEX² uses a microarray technology that enables the simultaneous determination of specific and total IgE to a broad panel of different allergen molecules and extracts (178 components + 117 extracts) from a single test run requiring only a small amount of serum (100 - 200 µl).
- ALEX² is the most comprehensive diagnostic allergy test for the detection of sIgE on the market. It includes a wide range of marker allergens facilitating the identification of co- and cross-sensitisation in one step.
- ALEX²'s wide spectrum of allergens allows for patient risk assessment and supports clinical risk management.
- ALEX² helps to assess the risk and benefit of allergen-specific immunotherapy.
- ALEX² increases accuracy of prescription of allergen-specific immunotherapy, particularly in cases of polysensitisation.

MAD changes the choice of medication and therapy

An economic perspective

by Anna Ringauf, MSc ETH

Allergic diseases pose major challenges to public health. Symptoms caused by allergies consist of a broad spectrum including specific types of asthma, rhinitis, conjunctivitis, urticaria, eosinophilic disorders and potentially life-threatening disease manifestations such as anaphylaxis. All these conditions are unpleasant, if not dangerous and many people are affected. Asthma alone affects 300 million individuals globally. 200 to 250 million people have food allergies, even more people suffer from rhinitis (about 400 million)[54]. According to the European Parliament's Interest Group of Allergy and Asthma, asthma and allergic rhinitis cause more than 100 million work and school days lost in Europe each year[55]. If patients were treated appropriately, around 142 billion euros could be saved each year with cost-effective treatments[55]. The indirect costs of not treating allergies properly in the EU are estimated to be between 55 - 151 billion euros each year. Alongside the health-related constraints, these figures highlight the significant economic impact of allergic diseases on a global scale[55].

RISING ALLERGIC POPULATION

The number of patients suffering from allergies is still on the rise. Increase of allergic individuals correlates with global developments. As societies become more affluent and urbanised, as outdoor and indoor pollution increases, as lifestyle and dietary habits change, prevalences for allergies increase accordingly. Climate change and reduced biodiversity support this development[54]. With the rise of allergy prevalences, rising costs for healthcare systems are expected. Direct and indirect costs of allergies and asthma are already high[54]. When it comes to economic optimisation and cost saving for healthcare systems, precise and efficient diagnosis of allergies is key.

TESTING IS KEY

Classic tests for allergy diagnostics are designed to detect IgE sensitisation against allergenic extracts either by skin prick test (SPT, in vivo) or by detecting specific IgE in the blood of the patient (sIgE, in vitro)[56]. Until

now, SPT remained the first-line diagnostic tool for allergy diagnosis due to its low cost and its minimally invasive nature[57]. However, the performance is far from optimal. The sensitivity is fair (70 - 100 %) but specificity is poor (40 - 70 %)[58], which means that there is a high number of false positive results. In the case of food allergies, this often results in the need for oral food challenges, which has several disadvantages: Limitation of available diagnostic centres, high personnel costs, and a potentially dangerous situation for the patient[59]. Given these challenges and risks, one should opt for methods to assess IgE sensitisation with higher sensitivity and specificity in order to reduce numbers of oral food challenges.

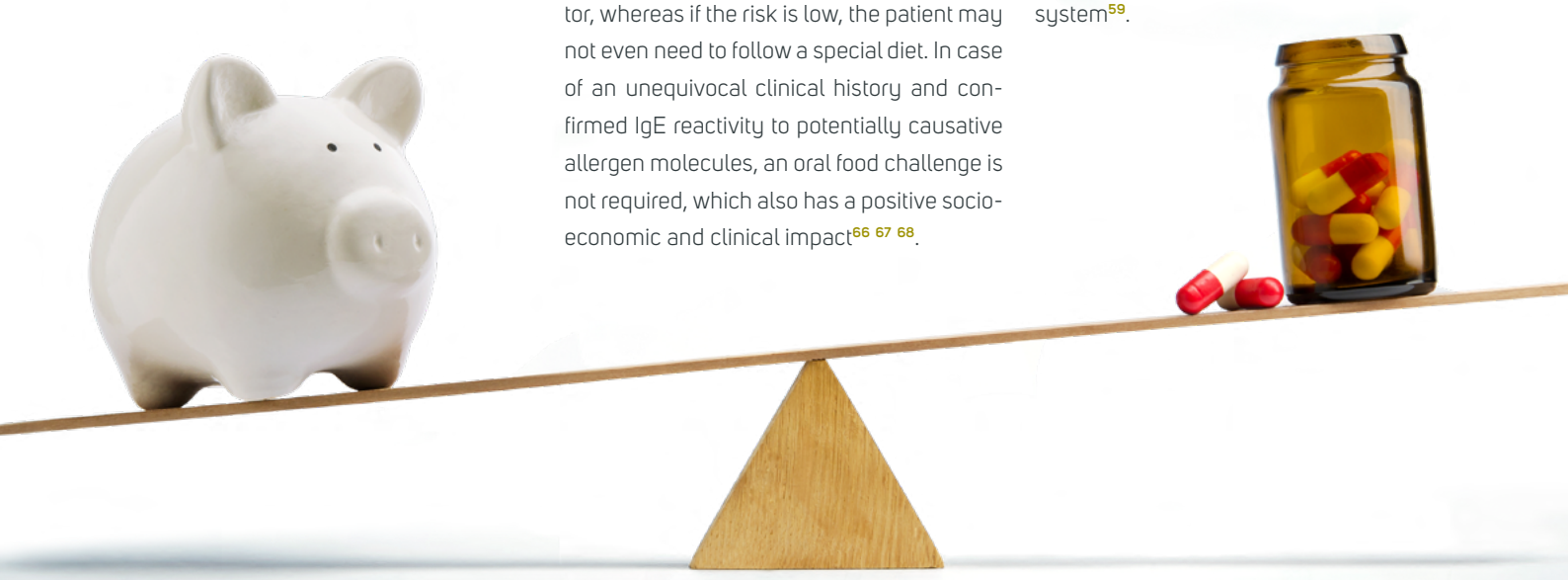
MAD VS. EXTRACT-BASED TESTING

In contrast to molecular allergy diagnosis (MAD), allergen extract testing provides limited information regarding differentiation between genuine sensitisation and cross-sensitisation to a particular allergen source. This may lead to the inaccurate prescription of allergen-specific immunotherapy (AIT) which may be indicated in the first, but not in the latter case[60 61 62]. Therefore, detecting IgE reactivity to markers of genuine sensitisation and to panallergens is key to selecting an appropriate allergen-specific immunotherapy (AIT) or oral immunotherapy (OIT). In particular, the advantages of MAD become apparent in polysensitised patients, as interpretation of extract-based test results and differentiation between cross-reactivity and genuine sensitisation becomes very difficult. Higher accuracy of AIT prescription also conveys economic implications, since AIT is more cost-effective compared to symptomatic therapy, due to disease-modifying effects[63 64 65].

In food allergy, it is particularly important to precisely identify the type of sensitising molecule and its properties (e.g., degree of heat stability and resistance to digestion). If the risk of a severe reaction is high, the patient will need to follow an avoidance diet and may need to carry an adrenaline auto-injector, whereas if the risk is low, the patient may not even need to follow a special diet. In case of an unequivocal clinical history and confirmed IgE reactivity to potentially causative allergen molecules, an oral food challenge is not required, which also has a positive socio-economic and clinical impact[66 67 68].

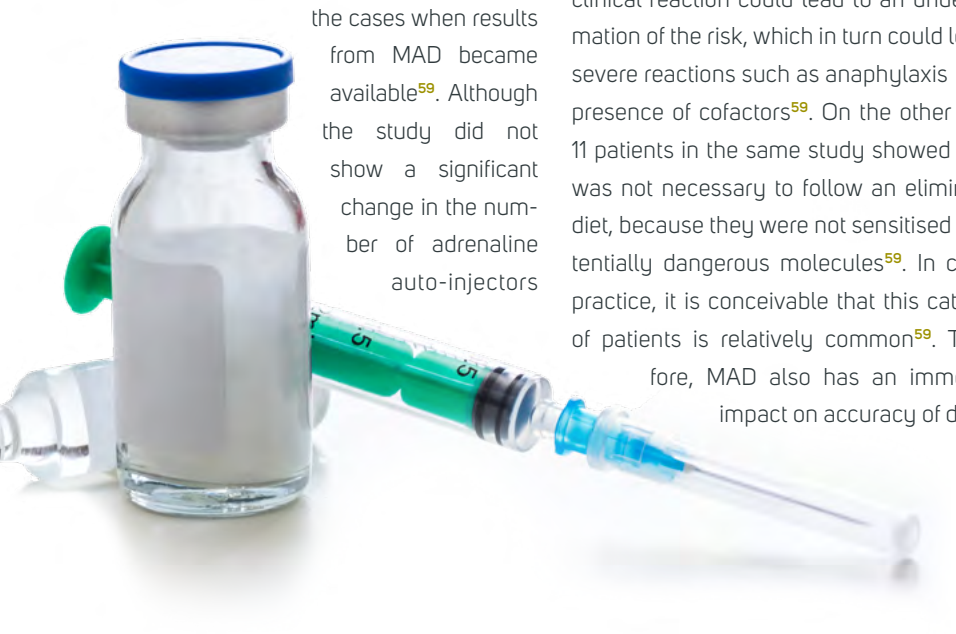
RESPIRATORY ALLERGIES

Several studies have shown that the use of MAD has a profound impact on the diagnosis and therefore the choice of treatment, when compared to traditional allergy testing with SPT. Different authors reported changes in the result of allergy diagnosis between 32 % and 54 % of cases[48 59 69 70]. In an Italian study involving a total of 275 patients with clinical respiratory symptoms, a total of € 42,250 could be saved by switching from SPT to molecular allergy diagnosis[59]. Considering current costs for AIT from Central Europe and ALEX^2 as the test platform, the total savings for the same patient group were estimated at € 56,500 in the year of 2024 (based on the costs of € 1,500 per immunotherapy and € 100 for one ALEX^2 test). While the initial detection of IgE to molecular allergens is more costly than traditional tests, the outcome of MAD increases accuracy of AIT prescription, ultimately leading to a substantial decrease in overall costs borne by the healthcare system[59].



FOOD ALLERGIES

The same study from Italy[59] found that in 38 % of patients (n=82) with food allergies, the diagnosis and therapy were changed after performing MAD compared to SPT (total group of 215 patients). This includes the prescription and type of elimination diet and adrenaline auto-injectors[59]. In this study, recommendations of carrying such an auto-injector were altered in about half of the cases when results from MAD became available[59]. Although the study did not show a significant change in the number of adrenaline auto-injectors prescribed (from 112 to 111), 19 cases were only confirmed as clinically significant and clinically dangerous after molecular diagnostics were used[59]. Therefore, although for food allergies, the economic impact seems to be limited with respect to costs for emergency medication (approx. € 100 per adrenaline auto-injector in central Europe), it has a major positive impact on the quality of life of the patient. For example, a presumed mild clinical reaction could lead to an underestimation of the risk, which in turn could lead to severe reactions such as anaphylaxis in the presence of cofactors[59]. On the other hand, 11 patients in the same study showed that it was not necessary to follow an elimination diet, because they were not sensitised to potentially dangerous molecules[59]. In clinical practice, it is conceivable that this category of patients is relatively common[59]. Therefore, MAD also has an immediate impact on accuracy of dietary recommendations, compared to results based on SPT[59].



All those studies show that MAD is cost-effective and helps to make healthcare systems more economically sustainable. MAD can prevent misdiagnosis and assist doctors in choosing the right type of therapy, especially for polysensitised patients. As climate change continues, the pollination seasons of different plant species become longer and might show increasing overlap, making it difficult to draw the right conclusion based on symptoms alone. Clinically relevant sensitisation to panallergens makes the prescription of AIT error-prone, therefore, MAD is essential for efficient and cost-effective allergy management.

CONSENSUS PAPER BY WAO (2013)[71] STATES THAT MAD IS ...

- Distinguishing genuine from cross-reactive sensitisation in polysensitised patients, thereby improving the knowledge of the real causative allergen source.
- Assessing the risk of severe, systemic, versus mild local reactions in food allergy, thus reducing anxiety for the patient and the need to perform oral food challenges.
- Identifying the allergens responsible for symptoms and therefore the indication for allergen immunotherapy (AIT) and oral immunotherapy (OIT).

From extract-based to molecular allergy diagnostics

CEO talks #5 with Dr. Christian Harwanegg

What are the advantages of molecular allergy diagnostics over traditional extract-based methods, and why are doctors or laboratories still reluctant to use them?

CH: In my view, there are three main reasons: lack of expertise, low market share, and lack of reimbursement.

Firstly, many physicians do not have the required expertise and therefore do not "dare" to use it. Molecular allergy diagnostics is much more knowledge-intensive because it is based on components. The doctor must therefore already know which components are available and understand when it makes sense to use which ones.

The second reason is related to the logistics of referral. Many laboratories do not even offer molecular allergy diagnostics as part of the menu which doctors can choose from. This is another reason why the market share is still low – in the single-digit percentage range globally. As MADx, we are pioneers: if you multiply the number of our tests by the number of components, the cumulative share is significantly higher than the rest of the industry combined.

> "The market share of molecular allergology is still low."

Another reason why many people hesitate is that molecular allergy diagnostics are not reimbursed by health insurance due to the scope of the test. With grasses, for example, you must test several components, and even more with food. Those responsible do not see the added value here – in contrast to available evidence – because they do not have the training.

Molecular allergy diagnostics contributes to better personalised medicine in allergology. How does this benefit individual patients and society?

CH: Individual patients benefit from receiving comprehensive results that cover all parameters and achieve the highest level of resolution possible. By the highest level of resolution, we mean that the clinical literature and research work has already been done to define exactly what can be derived from

the results for the benefit of the patient. This includes individualised avoidance recommendations for food allergies, more precise prediction of cross-reactions and better coordination of therapies. The aim is to better assess the risk – some forms of allergy never get significantly worse; others develop further and can cause asthma or anaphylaxis. The predictive value is crucial, and this must be communicated to the referring doctor so that they can maximise the outcome for their patients. Only when this process functions smoothly will there be a significant benefit for society.

> "There is huge potential for cost savings if allergies are correctly diagnosed."

We are dealing with a dogma as to how diagnostics should be carried out: Should we test predictively and extensively to recognise and mitigate problems early on, or should we test specifically after problems have already occurred? I believe that if you wait for a problem to occur and then look at it in detail and treat it, you can never derive any great benefit from it – after all, the damage has already been done. There is huge potential for cost savings for healthcare systems if patients are correctly diagnosed and treated from the outset, but this is currently not being realised. According to model calculations by the European Parliament Interest Group on Allergy and Asthma, the potential savings in the EU amount to 142 billion euros per year. These calculations are based on existing treatment options – if better, more meaningful diagnostics were to be used, the potential would be even higher.

How would you address concerns about the cost-effectiveness and accessibility of molecular allergy diagnostics?

CH: As far as cost-effectiveness is concerned, these concerns cannot be dispelled across the board. There are data, figures, and facts that the cost-effectiveness of allergy treatment is inefficient from a holistic perspective – i.e., from diagnosis to therapy – and represents a huge cost factor for healthcare systems worldwide. We are talking about several percent of a country's GDP.

Prospective comparative studies with significant population groups would have to be carried out to assess the cost-effectiveness holistically. One example is the mother-child health passport in Austria: A risk assessment should already be carried out in early childhood development using questionnaires, anamnesis, and family history to proactively start not only with a diagnostic concept, but also with a treatment concept. The diagnosis with the patient's test result should not be the end point; it must be followed by adequate treatment. If this fails, it is difficult to prove the effectiveness of improved diagnostics. Diagnostics must not be seen as a means to an end, but as a starting point.

How has the switch to molecular diagnostics in allergology been received by the medical community, and what steps can be taken to ensure that doctors feel well trained in these new methods?

CH: The changeover has not taken place in this sense. We assume that the majority of allergy diagnostics are still done with extracts – in vivo using SPT (skin prick test), and not in vitro. The SPT is still the dominant test on the global market.

Although MADx has a high single-digit, perhaps soon double-digit share of the global market, most in vitro diagnostics are still carried out with extracts. Molecular allergy diagnostics is therefore still a long way from becoming routine. Only ALEX^2 can be considered as a routine,

automated and affordable molecular allergy test – there is no alternative. But here again there are a few hurdles, starting with the information chain: the referring doctor must know that such a test even exists. This is not always the case, as many laboratory referral forms simply state "food panel", for example, and not the name ALEX². In addition, the test must be available and billable in the respective country.

> "ALEX² is the only routine, automated & affordable molecular allergy test."

The transfer of information from laboratory to doctor is also crucial. The goal is to offer physicians a tool that is easy to understand and work with, and from which the benefits for the patient can be clearly read. Doctors need to feel that they don't have to invest more time in selecting the right test, communicating with patients, or training to use the test.

For educational purposes, the MADx Academy, our digital training and certification platform, is certainly a pioneer when it comes to molecular allergy diagnostics. Of course, there are other online training courses and seminars, but the Academy is unique due to its scope, language availability and free access for physicians worldwide and is a great asset to provide a deeper understanding of the method.

What trends and developments could characterise the field in the coming years?

CH: In terms of trends, the digitalisation of the entire chain – from the laboratory to the doctor to the interpretation of the results to the patient – is at the top of the list. We are thinking in the direction of a patient portal that will provide patients with even better support after receiving their test results and encourage them to make the most of their findings. The better a patient understands their results, the easier it is for them to adapt their lifestyle to achieve the best possible treatment outcome.

The second major trend that we are establishing as MADx is that we will be able to work with big data in allergology for the first time. We collect millions of patient profiles every year and the next step will be to collect clinical data. In the future, we will therefore be in a much better position to recognise global and regional patterns and define algorithms with the help of machine learning models. The aim is to derive information from this large amount of data that will clearly benefit the individual patient. It would be very useful to be able to make clear statements such as "Sensitisation to allergen X and Y in region Z leads to allergic asthma in 98 % of cases".

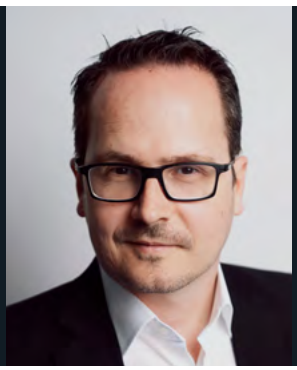
We can initiate this process by making the data available, but of course we can't do it alone – it must be supported by the whole scientific and medical community.

ABOUT

DR. CHRISTIAN HARWANEGG

studied Molecular Genetics at the University of Vienna, Austria. He joined a team of entrepreneurs in 1999 and graduated with a PhD in 2003. He has spent his entire professional education and career working in the development of all aspects of allergy testing in a multiplexed setup.

> "Diagnostics are not a means to an end, but a starting point."



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RELEVANT MOLECULAR ALLERGENS FROM DIFFERENT ALLERGEN FAMILIES

Allergen source	Scientific name	Allergen	Biochemical designation
Timothy	Phleum pratense	Phl p 12	Profilin
Melon	Cucumis melo	Cuc m 2	
Alder	Alnus glutinosa	Aln g 4	Polcalcin
Timothy	Phleum pratense	Phl p 7	
Birch	Betula verrucosa	Bet v 1	PR-10
Alder	Alnus glutinosa	Aln g 1	
Celery	Apium graveolens	Api g 1	
Peanut	Arachis hypogea	Ara h 8	
Hazelnut	Corylus avellana	Cor a 1.0401	
Soy	Glycine max	Gly m 4	

Allergen source	Scientific name	Allergen	Biochemical designation
Olive	Olea Europaea	Ole e 7	
Plane tree	Platanus acerifolia	Pla a 3	
Mugwort	Artemisia vulgaris	Art v 3.0201	
Glasswort	Parietaria judaica	Par j 2	
Hemp	Cannabis sativa	Can s 3	
Celery	Apium graveolens	Api g 2	
Celery	Apium graveolens	Api g 6	
Tomato	Solanum lycopersicum	Sola l 6	
Maize	Zea mays	Zea m 14	
Sunflower seeds	Helianthus annuus	Hel a 3	
Wheat	Triticum aestivum	Tri a 14	nsLTP
Pea	Pisum sativum	Pis s 3	
Peanut	Arachis hypogea	Ara h 9	
Hazelnut	Corylus avellana	Cor a 8	
Lentil	Lens culinaris	Len c 3	
Walnut	Juglans regia	Jug r 3	
Apple	Malus domestica	Mal d 3	
Strawberry	Fragaria ananassa	Fra a 3	
Kiwi	Actinidia deliciosa	Act d 10	
Peach	Prunus persica	Pru p 3	
Grape	Vitis vinifera	Vit v 1	

Allergen source	Scientific name	Allergen	Biochemical designation
Buckwheat	Fagopyrum esculentum	Fag e 2	
Sesame	Sesamum indicum	Ses i 1	
Mustard	Brassica / Sinapis spp.	Sin a 1	
Cashew	Anacardium occidentale	Ana o 3	
Peanut	Arachis hypogea	Ara h 2	
Peanut	Arachis hypogea	Ara h 6	
Hazelnut	Corylus avellana	Cor a 14	2S Albumin
Brazil nut	Bertholletia excelsa	Ber e 1	
Pecan nut	Carya illinoinensis	Car i 1	
Pine nut	Pinus pinea	Pin p 1	
Pistachio	Pistacia vera	Pis v 1	
Soy	Glycine max	Gly m 8	
Walnut	Juglans regia	Jug r 1	
Pea	Pisum sativum	Pis s 1	
Pea	Pisum sativum	Pis s 2	
Peanut	Arachis hypogea	Ara h 1	
Hazelnut	Corylus avellana	Cor a 11	
Coconut	Cocos nucifera	Coc n 1	
Lentil	Lens culinaris	Len c 1	7/8S Globulin
Pecan nut	Carya illinoinensis	Car i 2	
Pistachio	Pistacia vera	Pis v 3	
Soy	Glycine max	Gly m 5	
Walnut	Juglans regia	Jug r 2	
Walnut	Juglans regia	Jug r 6	

Allergen source	Scientific name	Allergen	Biochemical designation
Cashew	Anacardium occidentale	Ana o 2	11S Globulin
Peanut	Arachis hypogea	Ara h 3	
Hazelnut	Corylus avellana	Cor a 9	
Almond	Prunus dulcis	Pru du 6	
Pecan nut	Carya illinoinensis	Car i 4	
Pistachio	Pistacia vera	Pis v 2	
Soy	Glycine max	Gly m 6	
Walnut	Juglans regia	Jug r 4	
Atlantic cod	Gadus morhua	Gad m 1	β-Parvalbumin
Atlantic herring	Clupea harengus	Clu h 1	
Atlantic carp	Cyprinus carpio	Cyp c 1	
Salmon	Salmo salar	Sal s 1	
Mackerel	Scomber scombrus	Sco s 1	
Swordfish	Xiphias gladius	Xip g 1	
Tuna	Thunnus albacares	Thu a 1	
Thornback ray	Raja clavata	Raj c Parvalbumin	α-Parvalbumin
American cockroach	Periplaneta americana	Per a 7	Tropomyosin
Blomia tropicalis	Blomia tropicalis	Blo t 10	
European house dust mite	Dermatophagoides pteronys-sinus	Der p 10	
Tyrophagus putrescentiae	Tyrophagus putrescentiae	Tyr p 10	
Anisakis simplex	Anisakis simplex	Ani s 3	
Black Tiger Shrimp	Penaeus monodon	Pen m 1	
Giant Freshwater Prawn	Macrobrachium rosenbergii	Mac r 1	

Allergen source	Scientific name	Allergen	Biochemical designation
Djungarian Hamster	Phodopus sungorus	Phod s 1	
Dog	Canis familiaris	Can f 1	
Dog	Canis familiaris	Can f 2	
Dog	Canis familiaris	Can f 4	
Dog	Canis familiaris	Can f 6	
Rabbit	Oryctolagus cuniculus	Ory c 2	
Cat	Felis domesticus	Fel d 4	
Cat	Felis domesticus	Fel d 7	Lipocalin
Mouse	Mus musculus	Mus m 1	
Guinea pig	Cavia porcellus	Cav p 1	
Horse	Equus caballus	Equ c 1	
Rat	Rattus norvegicus	Rat n 1	
Cattle	Bos domesticus	Bos d 2	
Pigeon tick	Argas reflexus	Arg r 1	
Golden hamster	Mesocricetus auratus	Mes a 1	
Dog	Canis familiaris	Can f 3	
Cat	Felis domesticus	Fel d 2	
Horse	Equus caballus	Equ c 3	Serum Albumin
Chicken egg yolk	Gallus domesticus	Gal d 5	
Beef	Bos domesticus	Bos d 6	
Pork	Sus domesticus	Sus d 1	
Peach	Prunus persica	Pru p 7	Gibberelin-regulated protein
Peanut	Arachis hypogea	Ara h 15	Oleosin
Mugwort	Artemisia vulgaris	Art v 1.0101	
Ragweed	Ambrosia artemisiifolia	Amb a 4	Defensin
Celery	Apium graveolens	Api g 7	

GLOSSARY OF MOLECULAR ALLERGOLOGY

Allergen	Molecule (e.g., protein) that can trigger an allergic immune response
Allergen extract	Mixture of allergenic and non-allergenic components extracted from an allergen source (e.g., pollen, nuts)
Allergen source	Biological species producing allergens
IgE Epitope	Binding site for IgE antibodies
Linear (sequential) IgE epitope	Epitope – a binding site on an allergen – that is recognised by IgE antibodies by its linear sequence of amino acids
Conformational IgE epitope	Epitope – a structure-dependent binding site for IgE antibodies – that has a specific three-dimensional shape
Cross-reactivity	Similarity-related, immunological reaction with molecular structures that were not responsible for the original sensitisation; ability of an allergen to bind with an antibody that was raised to a different allergen
Major allergen	Allergen that is recognised by ≥ 50 % of patients that are sensitised to the source
Minor allergen	Allergen that binds IgE in < 50 % of the affected allergy sufferers
Multiplex test	In vitro diagnostic test with simultaneous determination of IgE antibodies against numerous allergens
Panallergen	Ubiquitous or present in many allergen sources; mostly highly conserved (evolutionarily little changed) allergen
Protein family	Relationship between proteins, based on similar sequence and structure
Recombinant allergen	Protein expression using a genetically modified organism (e.g., bacteria - E.coli, yeast - P.pastoris)
Singleplex test	In vitro diagnostic antibody test against a defined allergen molecule or allergen extract
Species-specific allergen	Allergen that only occurs in one biological species

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Publisher
MacroArray Diagnostics
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