Advances in Allergy Testing: Component Resolved Diagnostics

Immunoglobulin E (IgE)-mediated allergic conditions are increasing worldwide. They affect the quality of life of millions of individuals, causing enormous costs for society. Traditionally the diagnosis of allergic disease is based on a careful and thorough clinical history, skin prick testing, laboratory testing for allergen-specific IgE (sIgE) and elimination-provocation diets. Since the discovery of the IgE antibody in 1967, considerable advances have been made in the development of in vitro allergy testing. Where the allergen-sIgE tests (previously known as RAST® tests) only define the source of the allergen, new molecular tests enable us to measure IgE antibodies to specific components within the allergen source. Information obtained from component resolved diagnostic (CRD) allergy tests enable the clinician to provide the patient with specific information regarding cross-reactivity, prognosis, risks and severity of reactions, effects of specific and patient-individualised allergen avoidance measures, dietary advice and prophylactic and therapeutic treatments. Early detection of sensitisation to new allergens enables the clinician to prescribe the best strategy for managing the allergic disease and avoid development of the disease into a more severe and costly chronic condition. The aim of this document is to enable the clinician to use CRD as a tool to improve the diagnosis and management of allergic conditions, and in doing so improve the quality of life of his/her patients.

Allergy Diagnosis and Testing

According to the World Allergy Organisation (WAO) allergy is defined as a hypersensitivity reaction initiated by immunological mechanisms. These hypersensitivity reactions can be either antibody or cell mediated. Individuals are usually considered to have clinically significant allergy when they have both detectable allergen-specific IgE and have developed symptoms upon exposure to substances containing the specific allergen. Sensitisation refers to the production of allergen-specific IgE only, unrelated to any associated clinical symptoms. Allergy tests should therefore always be interpreted in the context of the patient’s clinical history, as a positive allergen-specific IgE test demonstrates sensitisation and not necessarily clinical reactivity.

From Laboratory to Clinical Application: Component Resolved Diagnostics

Research has shown that one of the key aspects to managing allergic disease is the accurate identification of the causative allergen(s). The diagnostic process, however, should always start with a thorough clinical history followed by allergen-specific IgE (sIgE) tests to confirm the clinically suspected allergen(s). Allergen sIgE tests detect only sensitisation to whole allergen extracts, and not to specific allergenic molecules. The diagnostic challenge is to have a test that can distinguish between sensitisation to clinical irrelevant allergenic components within an allergen source and components known to elicit clinical reactions (Figure 1). Food allergies in aeroallergen-sensitised patients are an excellent example where it is relevant to distinguish between cross-reacting allergens of low clinical significance, and those with a high risk for systemic reactions. With the aid of allergen-component sIgE testing it is possible to create an allergen component IgE profile of each patient identifying the relevant allergen components from different allergenic sources.

Allergens and Components: Clinical Implications

All protein sources possess the potential to elicit an antibody response and cause allergic reactions. Allergen sources (e.g. cow’s milk) contain several different proteins that we refer to as allergen components (e.g. whey and casein in the cow’s milk). Each allergen component or protein consists of different epitopes to which antibodies are formed. These epitopes may be species-specific, thus only present in the specific allergen source. Alternatively the epitopes present in the allergen source may resemble epitopes present in other allergen sources, leading to cross-reacting antibodies between different allergen sources or species. These allergens are often referred to as panallergens. Patients with IgE antibodies to species-specific proteins are more likely to suffer from more severe and systemic reactions as opposed to patients with cross-reacting antibodies to panallergens. Allergen protein stability is another important aspect to take into consideration in multisensitised patients. Allergens that are stable to heat and pepsin digestion are more likely to cause severe clinical reactions, whereas heat and digestion labile proteins are more likely to be tolerated, or cause milder or only local reactions. Please refer to Figure 2 for examples of allergen protein characteristics and their clinical relevance.

1. Increasing the accuracy of tests (detecting clinically relevant allergenic components from allergenic sources);
2. Predicting cross-reactivity to proteins with similar structures;
3. Assessing risk and type of clinical reaction;
4. Providing information important to the dietary management of patient, e.g. food sources containing...
Allergen and Component Nomenclature

Allergen sources are numbered and referred to, according to the allergen group they belong to. The first letter in the allergen reference indicates the allergen group (e.g. t = tree pollen, f = food, g = grass pollen etc.). The number indicates the sequence in which the allergen was identified. An x in the ‘code’ refers to a mixture of allergens (e.g. fx5 = food; mixture; number 5).

Allergen components are referred to by the allergen source’s scientific name, usually an abbreviation of the Latin name and a number. The letters ‘r’ or ‘n’ that precedes the allergen component name refer to the origin of the allergen component used in the assay, i.e. ‘recombinant’ or ‘native’. Thus r Ara h2 denotes a recombinant Arachis hypogaea allergen number 2 (peanut)³.

Important Allergen Components

Many allergen components from different sources have been identified and characterised, and the list is rapidly expanding. Interpretation of the information gathered from allergen component testing constitutes a huge step forward expanding. Interpretation of the information gathered from allergen component testing constitutes a huge step forward in the field of IgE-mediated allergy diagnosis. Not all the allergen components can be reviewed in this document. A couple of practical examples, demonstrating the advantages of allergen component testing, will be discussed in this document.

1. Inhalant Allergies: Pollen-related sensitisation sharing allergen components in food from plant origin

Allergies to plant-derived foods may occur in individuals with primary sensitisation to pollen allergen components, which may cross-react with allergens in food from plant origin³⁴. These allergen-protein components are classified according to their properties and functions. The most relevant proteins in pollen-related, food-derived allergies are summarised in Figure 2, and include: cross-reactive carbohydrate determinants (CCD), profilins, pathogenesis-related protein-10 (PR-10), non-specific lipid transfer protein (nsLTP) and storage proteins³⁵-⁶.

CCDs are formed by the sugar side-chains of glycoproteins present in pollen and plant-food allergen sources. IgE antibodies to CCDs are extremely common causing a lot of positive allergen-specific IgEs (ImmunoCAP®), but the role of these molecules in inducing clinical symptoms seems negligible⁶.

Profilin and PR-10 proteins are highly cross-reactive, even between distantly related species, increasing the risk of multiple pollen-food allergies⁹,¹⁰. Both profilin and PR-10 proteins are heat labile and destroyed by cooking or processing of food. Symptoms caused by these two proteins are usually mild, and restricted to the oral cavity, referred to as the so-called oral-allergy-syndrome (OAS).

As depicted in Figure 2, individuals with sIgE antibodies to the ns-LTP and storage proteins present in pollen and plant-food are at higher risk of severe clinical reactions, as the proteins are very resistant to heat and pepsin. Ns-LTPs are more prevalent in the peel of fruits, and patients are often able to tolerate peeled fruit.

Seed storage proteins provide the required nutrients to the plant seeds during sprouting, hence the name. Storage proteins are very resistant to cooking and digestion and are the most allergenic food component, responsible for severe anaphylactic reactions in adults. They are generally divided into groups such as the 11S globulin, 2S albumins and 7S vicilins (Figure 2).

All aeroallergen sensitised patients presenting with food allergies should raise a high index of suspicion of the presence of potential cross-reacting antibodies¹¹. An example is the cross-reactivity between grass- and tree-pollen allergen components and wheat (Triticum aestivum), soy (Glycine max) and peanut (Arachis hypogaea). These allergenic protein components may include profilin (Ara h5) and PR-10 proteins (Ara h8, Gly m4) usually associated with local reactions or LTP (Ara h9, Tri a14) and storage proteins (Gly m5, Glym6, Tri a19, Ara h1, Ara h2, Ara h3) associated with more severe clinical reactions (Figure 3)³⁵,⁶,⁸.

2. Inhalant Allergies: House dust mites sharing allergen components present in seafood

Approximately 10% of individuals that are sensitised to house dust mites (Dermatophagoides pteronyssinus and Dermatofagoides farinae) demonstrate sIgE antibodies to the tropomyosin protein (Der p10) present in house dust mites. Tropomyosin is a muscle protein found in crustaceans like shrimp, lobster and crab, arachnoids (house dust mite), insects (cockroach) and parasites (Anisakis simplex)³⁵,⁶. Tropomyosin is a very heat-stable protein, and sensitisation may lead to severe reactions to the above-mentioned allergenic sources. Whether CRD may highlight the importance of management of house dust mite allergic children remains to be elucidated.
3. Food Allergy: Fish

Parvalbumin proteins are major allergens present in fish. Cod (Gadus morhua) and Carp (Cyprinus carpio) parvalbumins (Gad c1 and Cyp c1) are used as markers for fish sensitisation. Because of the high degree of cross-reactivity between the parvalbumins of different fish species, clinically sensitised patients should be advised to avoid all fish species (Figure 4).

4. Food Allergy: Milk (Bos domesticus)

Children with milk allergies are often sensitised to several proteins present in cow’s milk (Figure 5). Casein (Bos d8) is the major allergen in milk, with beta-lactoglobulin (Bos d5) and alpha-lactalbumin (Bos d4) constituting the whey protein component in the milk. Other minor protein components present in cow’s milk include serum albumin (Bos d6) and transferrin (Bos d lactoferrin). Testing for individual allergen components is very important in children with milk allergies. It can provide valuable information needed for specific dietary advice. sIgE to the heat-stable casein component in milk are associated with persistent, severe milk allergy. Patients with sIgE to any of the other milk components that are heat labile, are often able to tolerate baked or extensively heated milk products.

5. Food Allergy: Egg (Gallus domesticus)

As with milk proteins, hen’s egg contains multiple allergenic protein components (Figure 6). Ovomucoid (Gal d1) comprises only 10% of the protein in egg white, but appears to be a major allergen associated with persistent egg allergy. Ovomucoid is stable to heat and digestion and sensitised patients may have clinical reactions to very small amounts of this protein in any form of food containing egg. Other proteins present in egg white include ovalbumin (Gal d3), conalbumin (Gal d3) and lysozyme (Gal d4) all of which are heat labile, indicating that patients may be able to tolerate well-cooked/boiled eggs. This information may be important when considering a food challenge in clinical practice. The main protein present in egg yolk is livetin or serum albumin (Gal d5). Serum albumin in egg yolk cross-reacts with bird epithelia and is associated with the so-called bird-egg syndrome. Serum albumin is partially heat labile and patients may react less to cooked egg or cooked meat. Patients may have an egg yolk allergy, but may then also develop respiratory symptoms when they are exposed to birds and feathers.

Allergen Component Assay Systems

Many different in vitro immunoassays have been developed worldwide. In South Africa, two systems are mainly used for the measurement of allergen-specific components:

Quantitative measurement of individual allergen component-specific IgE: An immunoassay method is used to quantify IgE levels to a native or recombinant allergen component in IU/L. ImmunoCAP® (ThermoFisher, Sweden) is the most frequently used test for identifying IgE to allergen components. Not all allergen components are available as individual tests, but the most clinically relevant components are available.

Semi-Quantitative measurement of allergen component-specific IgE by means of a microarray-based immunoassay with a predefined test panel such as the ISAC® (ThermoFisher, Sweden) immuno solid-phase allergy chip with 112 components from 51 allergen sources. This is a semi-quantitative assay (ISU units) which is best indicated in multisensitised patients with both food and inhalant allergies. The ISAC® microarray will be able to identify cross-reactions between pollen- and food-related sensitisations.

Summary

Advances in the field of recombinant allergens have allowed for the development of a new concept in allergy diagnosis – Component Resolved Diagnostics (CRD). CRD is based on natural or recombinant allergen components with structural and immunobio-logical properties comparable with their natural sources, establishing a detailed IgE reactivity profile for each patient. By classifying the allergen components into protein families, it provides an overview and insight into the clinical relevance of the IgE sensitisation pattern of each patient. Identification of cross-reactive allergens between aeroallergens and food allergens can explain clinical phenomena such as oral-allergy-syndrome. On the other hand, the presence of primary or species-specific allergen...
components may help to identify the risk of reaction on exposure to the different allergen sources.

Although CRD may improve the specificity of allergy testing, care must still be taken in the interpretation thereof. The age-old quote, “treat the patient not the paper,” still holds true. All in vitro tests should always be evaluated together with the clinical history of the patient, because allergen sensitisation does not necessarily imply clinical responsiveness.

References


Mariana Lloyd is a Chemical Pathologist (MMed (Chem Path) (UFS)) and FCFPath (Chem)(SA)) currently practising at a private pathology group in South Africa. She also holds qualifications in Family Medicine (FCFP (SA)) and Tropical Medicine (DTMH (UP)). Her interest in diagnosis of allergic conditions stems from her clinical experience in otolaryngology, dermatology and family medicine. She is currently the coordinator of the Allergy and Immunology discipline in her pathology practice. Email: mariana.lloyd@pathcare.org.