

LABORATORY TESTS FOR ALLERGY DIAGNOSIS

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The introduction of highly purified natural and recombinant single allergenic molecules represented an important improvement in the diagnosis of IgE sensitization. The identification of specific IgE against cross-reacting molecules such as profilin, lipid transfer proteins, calcium binding proteins or against “genuine molecules”, represents an added value and allows to distinguish between true and false polysensitization. *In vitro* tests add information to recognize patients with sensitization to genuine molecules that cause allergic diseases and to evaluate in childhood the spreading of sensitization for each molecule in order to choose the best treatment and to identify the ideal patient for allergen immunotherapy. Also, in order to detect patients with sensitization to pan-allergens it is important to manage the risk of anaphylaxis for patients allergic to latex and to identify IgE to particular molecules involved in occupational allergy. In patients with negative skin prick tests (SPT), that results in a lower sensitivity compared with *in vitro* tests, the negative test may be caused by the lack of some important allergenic molecules in the extract used for SPT.

Specific IgE antibodies can be detected either *in vivo* by skin prick test (SPT) or *in vitro* by specific IgE assays: both methods usually employ whole extracts from allergenic sources, which contain a mixture of allergenic and non allergenic proteins (1) but the IgE response is specifically directed only towards certain molecules (2).

Specific IgE (sIgE) for inhalant allergens (house dust mites, pollens, molds, latex) and for some food allergens that can induce respiratory symptoms (e.g. serum albumin, wheat flour, casein, fish parvalbumin and vegetables containing lipid transfer proteins (LTP, lysozyme, etc.) are measured to detect the trigger agents of allergic diseases such as conjunctivitis, rhinitis, asthma or occupational allergic respiratory diseases.

The international guidelines from the European Academy of Allergy and Clinical Immunology

(EAACI) and the World Allergy Organization (WAO), as well as the national guidelines from the “Società Italiana di Allergologia Asma e Immunologia Clinica” (SIAAIC) considered *in vitro* tests for allergic diseases as a second level test, to be used after SPT, when a confirmation is needed or if the SPT cannot be carried out because of patient-related factors, such as concomitant use of antihistamines, atopic dermatitis in the site of testing, etc. The measurement of *in vitro* sIgE is an important tool, because it allows to identify in a quantitative way the sensitization towards a complete allergen and/or a specific allergy molecule. The possibility to perform a deeper analysis with the molecular diagnostics gives important information, more specific than the SPT; sIgE are usually measured for common allergens: dust mites, grass, trees, olive,

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cypress, ragweed, Parietaria, Plantago, Alternaria, Cladosporium, dog and cat epithelia. Extractive preparations used for SPT usually contain cross-reactive components which are highly conserved across widely different allergen sources (3). This may complicate the interpretation of the diagnostic results, especially in polysensitized subjects. The introduction of highly purified natural and recombinant single allergenic molecules represented an important improvement in the diagnosis of IgE sensitizations and cross reactivities.

Component Resolved Diagnostics

The identification of specific IgE against cross-reacting molecules such as Profilin, Bet v1-PR10, LTP, calcium binding proteins or against “genuine molecules”, represents an added value and allows to distinguish between true and false polysensitizations. A true polysensitization occurs when specific IgE against genuine components of different allergenic sources are present. The genuine molecules for grass sensitization are Phl p1, usually the first allergen to induce IgE and Phl p 5, Bet v 1 for birch, Par j2 for Parietaria, Pla l 1 for Plantago, Amb a 1 for ragweed, Der p 1, Der p 2, Der p 23, that start the sensitization towards Dermaphagoides, and Fel d1 for cat, a secretoglobulin that is primarily produced in sebaceous, anal and salivary glands and is mainly present in the epidermis and fur. It is spread to the environment mainly through airborne dander, which can be detected both in homes with and without a cat. Pet allergy can induce severe symptoms of rhinitis and asthma that are difficult to control.

False polysensitizations are due to the presence of panallergens like profilins or calcium binding proteins causing the SPT positive results (4, 5). Epidemiological studies and clinical trials showed that the percentage of polysensitized ranges from 20 to 90% with a great variability depending on the populations. Polysensitization may also be associated with different clinical pictures with respect to monosensitized patients, especially those with a more impaired QoL and more severe symptoms. In addition, allergic children seem to display a higher frequency of sensitization than their parents, especially in families with polysensitization. In

some cases they are true polysensitizations, in other cases they are sensitizations to panallergens such as profilin or calcium binding proteins. The component resolved diagnosis (CRD), with either recombinant or purified allergens for SPT or IgE in serum dosage, is a tool to improve the accurate identification of the sensitization allergens. A positive SPT could be due to a sensitization to major allergens or simply to a cross-reaction response to panallergens like profilin Bet v 2 or Phl p 12 present with small conformational change in both pollen species. CRD has an important impact on the management of the patients in term of accuracy of the diagnosis or decision on the therapy (for example specific immunotherapy prescription).

Recent studies demonstrated that the CRD use in the diagnostic work-up may result in changing the decision on treatment in more than 50% of the patients compared to the diagnosis based only on clinical history and SPT results (6). CRD allows the identification of specific allergens associated with the symptoms and the identification of cross-reactive molecules that are clinically significant. E.g. for Can f 6 or Fel d 4 the physician advises on whether or not to keep a household pet or which species could be tolerated. This may have a huge impact on a child's well-being. CRD gives information on the disease evolution over time; it allows to investigate the relationship between sensitization patterns to cat and dog allergen molecules during childhood and symptoms to these furry animals several years later (7); it highlights the pattern of sensitization to pet IgE components and its association with clinical symptoms.

Specific IgE to Can f 1 was significantly associated with persistent rhinitis, Can f 2 with asthma diagnosis, Can f 3 with moderate/severe rhinitis (M/S-R) and asthma diagnosis (AD) and Can f 5 with persistent and M/S-R. Positive IgE to Fel d 2 was strongly related to M/S-R and AD. Sensitization to ≥ 2 molecules or to pet albumins was associated with more severe respiratory symptoms (8).

Utility of in vitro tests

In patients with positive SPT in agreement with the clinical history, *in vitro* tests add further information (9) and allows:

- 1). to detect patients with sensitization to genuine molecules that cause allergic diseases; to distinguish among patients with positive SPT for more than one allergen, i.e. about 70% of allergic patients, the true poli-sensitization from the sensitization to pan-allergens (10-12);
- 2). to evaluate in childhood the “spreading” of sensitization towards each grass molecule and to obtain prognostic information about the evolution of the disease and the correlation between phenotypes of sensitization and illness severity (13-15);
- 3). to choose the best therapy; if only clinical history and SPT are used, without the support of CRD results, the choice of therapy may prove to be incorrect in more than 50% of cases, with an important cost increase (6);
- 4). to identify the ideal patient for allergen immunotherapy, i.e. the patient with sensitization towards genuine molecules with a high likelihood of improving symptoms and to improve the quality of life with the correct diagnosis (16, 17);
- 5). to detect patients with sensitization to pan-allergens such as profilin or calcium binding proteins, which could induce cross-reactivity with foods and pollens. Additionally, the symptoms are shown for a long period of time also if pollen grains are no longer detected in the air. The profilin, a molecule of grass, trees and ragweed pollen is able to induce nasal and bronchial inflammation for a long period of time (18);
- 6). to manage the risk of anaphylaxis for patients allergic to latex. To detect IgE in latex molecules inducing anaphylaxis, like Hev b 1, Hev b 3, Hev b 5, Hev b 6, or to detect IgE for Hev b8 that is not able to induce anaphylaxis because it is not present in surgery devices;
- 7). to identify sIgE towards particular molecules involved in allergic occupational reactions, such as serum albumin, LTPs or lysozyme.

In patients with negative SPT and a clinical history suitable for allergy, CRD can be a useful tool. The lack of agreement between SPT results and clinical history may be due to the lower sensitivity of SPT compared with *in vitro* tests because the

extract used for SPT could miss some important allergenic molecules such as *Aspergillus* like Asp f 4, Asp f6, Asp f 3 (19) or molecular wheat allergens. IgE mediated responses to wheat can be related to wheat ingestion (food allergy) or wheat inhalation (respiratory allergy). An inhalation associated, IgE mediated wheat allergy can cause baker's asthma or rhinitis, which are common occupational diseases in workers who have significant repetitive exposure to wheat flour, such as bakers. The most important wheat allergens are the α -amylase/ trypsin inhibitor subunits, which are present in all three protein fractions of raw and cooked wheat. Other important allergens are a 9-kDa LTP in the albumin/globulin fraction and several low and high-molecular-weight glutenin subunits in the gluten fraction and gliadin (alfa, beta, gamma, omega fractions). All these allergens showed heat resistance and lack of cross-reactivity to grass pollen allergens. Wheat LTP is a major inhalant allergen associated with baker's asthma caused by wheat flour sensitization. Tri a 14 and peach LTP, Pru p 3, showed a sequence identity of 45%, but the low cross-reactivity between both allergens detected reflected great differences in their 3-dimensional IgE-binding regions (20).

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