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Guidelines on Allergy Diagnosis

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The incidence of allergic diseases in Hong Kong has increased several folds in the past 30 years. This phenomenon has been observed worldwide especially in developed countries. Allergy testing aiming at identification of the causative allergen is therefore gaining importance. The results are applicable to clinical management in terms of avoidance of allergens and specific immunotherapy.

Clinical history and examination are of the utmost importance in the diagnosis of allergy. Diagnostic tests can be used to support the clinical diagnosis, but a positive skin or blood test is not sufficient for diagnosis and must be correlated with clinical symptoms and/or challenge testing.

There are several commonly used diagnostic tests for allergy, including prick and intradermal skin tests, patch tests, specific IgE blood tests and challenge tests. There are other laboratory tests that might be useful for very specific situations, which is beyond the scope of this article. The aim of this article is to guide the practitioner in the proper use of diagnostic tests for allergy, and to avoid some common pitfalls.

Skin testing

Allergic symptoms are commonly mediated by immediate-type (Type I) or delayed-type (Type-IV) hypersensitivity responses. The epidermis is rich in mast cells, which carry on their surface allergen-specific IgE. Contact with allergen via skin prick/puncture or intradermal injection leads to mast cell degranulation, which results in a wheal and flare response. This confirms the presence of specific IgE against the allergen, hence sensitization, but does not necessarily indicate that the allergen is causing the patient's symptoms.

Skin prick tests are commonly used to confirm clinical sensitivity induced by environmental, food, and some drug allergens. There are many different devices available for skin prick testing, with significant variability in wheal sizes between them. Proper technique is very important in ensuring reliability of the results, and technicians must be trained for the specific device in use as the techniques required differ between devices. The potency and purity of extracts also vary between different manufacturers, and it is best to obtain the extracts from reliable sources. Standardized extracts should be used whenever possible. Improper storage conditions could affect the potency of the extracts and they should not be left out at room temperature for prolonged periods of time. To ensure proper interpretation, positive (histamine) and negative (saline) controls must be performed with each test. The test results should be read within 15 to 20 minutes, and the diameters of the wheal and the flare should be recorded in mm. Skin prick tests have high sensitivity and specificity in general, but there are limitations especially for food allergens. Knowledge of the natural history of different types of food allergy and the pattern of cross-reactivity would help in the interpretation of skin test results. Patient characteristics such as age, skin type, the presence of concomitant medical conditions and medications must be taken into consideration. Adverse reactions to skin prick tests are very rare, but life threatening reactions have occurred in highly sensitive individuals, usually due to large number of positive food reactions.

Intradermal skin tests are sometimes used to identify patients with low level sensitivity and negative skin prick test results. These are most commonly used for insect venom and drug (e.g. penicillin) testing. Intradermal skin tests are associated with a higher risk of systemic reactions, and should only be performed if the skin prick tests are negative. The starting dilution should be at least 1:100 of the prick test reagent, or 1:1000 in highly sensitive individuals. Intradermal testing could result in more false-positive results due to the irritant effect of the test reagents.

The allergens selected for skin testing should be determined based on the patient's age, history, environment and living conditions, and requires knowledge and experience on the part of the physician. "Screening tests" comprising of panels of allergens with low pre-test probability are not recommended.

Delayed-type hypersensitivity is commonly seen in patients suffering from allergic contact dermatitis. The epicutaneous patch test has evolved as the definitive diagnostic technique for this condition. When clinical evaluations suggest that exposure to specific contact allergens has occurred, patch testing can be used to confirm the diagnosis.

The most common patch test techniques are the individual Finn Chamber and the TRUE Test. The Finn Chamber allows more flexibility in the choice of allergens, whereas the TRUE Test is a fixed panel of 23 allergens. The patch tests should remain in place for 48 hours. After the 48-hour patch test reading, additional readings at 3 to 4 days and in some cases 7 days should be made. Patch tests are indicated in any patient with a chronic eczema and allergic contact dermatitis is suspected. Common indications include exposures associated with the use of topical medications, occupational allergens, plants, cosmetics and personal hygiene products. Again, allergens should be chosen based on pretest probability as determined by clinical history, and the result should be correlated with the patient's specific exposure. Some potent contact allergens such as oleoresins can provoke systemic contact dermatitis or sensitize patients not previous sensitized to these allergens; therefore, patch testing with such allergens are not recommended.

Aside from contact allergens, patch testing is also used as an adjunct for the diagnosis of delayed-type hypersensitivity to drugs and food. Food allergy might be a causative factor for atopic dermatitis and eosinophilic esophagitis/gastroenteritis in the absence of specific IgE, especially in infants and young children. The atopy patch test could be useful in diagnosing these conditions, but it is limited by the lack of standardization.

In-vitro allergy tests

The causative allergens in IgE-mediated diseases (type I allergy) can be determined in the laboratory by serum specific IgE or basophil activation tests. Serum specific IgE is previously called RAST because it was performed by a radioimmunoassay. There are now various methods that do not involve radioactivity. The reliability of these methods varies and the results from the different tests are not interchangeable. The ImmunoCAP test remains the gold standard in specific IgE testing. In general, only tests that have been validated by the FDA should be employed. These tests are *in vitro* counterparts of skin prick test and intradermal test but are generally considered to be less correlated with symptoms, expensive and time-consuming. Their clinical utilities are less widely accepted than skin tests. Nevertheless *in vitro* tests are preferred in the following conditions:

Severe dermatitis
Pregnant women
Dermatographism
Patients on β-blockers, anti-histamines
Very young and very old patients
Occupational asthma
Dubious skin test results

Positive results of allergen-specific IgE by skin tests or blood tests must be correlated with symptoms. {Pediatr Allergy Immunol. 2011;22(1 Pt 1):50-3} Those who do not have symptoms merely have IgE sensitization and do not have true allergy. Clinical significance is not only determined by the presence of IgE but also the antibody level, strength of antibody binding and proportion of specific IgE to total IgE. Recent studies have shown that the determination of IgE sensitivity to individual allergen components for food such as peanut might give additional information regarding the risk of clinical reactions and likelihood of spontaneous remission[1].

Allergen-specific IgE can also be detected on cell surface of basophils, the blood cells equivalent of tissue mast cells. Activation markers such as CD63 detected by flow cytometry are most commonly employed to determine the causative allergen. Early use was largely confined to drug allergy but now clinical applications in food allergy, chronic urticaria and hymenoptera venom allergy are also recommended[2,3].

Non-IgE mediated allergic diseases are mediated by lymphocytes (type IV allergy) or non-IgE immunoglobulins (type II and III allergy). Lymphocyte proliferation or cytokine release assays are the most often used laboratory tests to determine the responsible agents in cellular type (type IV) of allergy. These tests are the laboratory counterparts of skin patch test and delayed reading of intradermal test. Clinical application is mainly for the investigation of delayed type drug allergy such as maculopapular exanthem[4]. Procedures of these tests are lengthy and expertise in cell culture is required; hence they are only available in reference or research centers.

Anaphylaxis is due to mast cell degranulation caused by specific IgE. Patients with anaphylaxis are often non-atopic. Mast cell degranulation can be confirmed in the laboratory by measuring the serum tryptase level[5]. Tryptase is an enzyme found only in mast cell and is more stable than histamine. After anaphylaxis, tryptase level peaks at around 1 hour and decays with a half-life of 2.5 hours. The level returns to normal after 24-48 hours allowing sufficient time for blood taking. Postmortem blood can also be taken for the diagnosis of anaphylaxis because tryptase remains stable for at least 24 hours after death. The causative agents of anaphylaxis are investigated by skin test or the equivalent blood tests described above.

In recent years, food specific IgG testing has become fashionable. Commercial ELISA kits that test many ethnic-specific food items with tiny amounts of blood are used[6]. One kit can test 96 Asian specific food items within only 3 drops of blood. The results are often positive for multiple foods[6]. Based on these results, with and without ill-informed advice from physicians, parents are eagerly avoiding the food items, albeit without any appreciable beneficial effects. {Hong Kong Med J. 2015;21:574-5; }

To date, IgG to foods has no clinical value in the diagnosis of food allergy and is not recommended by professional bodies[7-9]. Specifically the American Academy of Allergy, Asthma and Immunology opined that IgG and IgG subclass antibody tests for food allergy do not have clinical relevance, are not validated, lack sufficient quality control, and should not be performed[8-11]. Measurement of specific IgG antibodies to foods is unproven as a diagnostic tool. The European Academy of Allergy and Clinical Immunology commented that many serum samples show positive IgG4 results without corresponding clinical symptoms. There is a lack of any controlled studies on the diagnostic value of IgG4 testing in food allergy. Furthermore, the determination of specific IgG-antibodies in serum does not correspond with oral food challenges[12]. There is no evidence that IgG subclasses[13] or the IgE/IgG4 antibody ratio[14] are reliable diagnostic tools. IgG-antibodies to common dietary antigens can be detected in health and disease[15]. Hence, the determination of food-specific IgG is of no clinical relevance and should not be part of the diagnostic work-up of food allergy. In eczema, levels of food IgG do not seem to correlate with any clinical parameters[10]. High levels of IgG4 antibodies to foods during infancy are associated with tolerance to corresponding foods later in life[16].

In conclusion, there are no reliable and validated clinical tests for the diagnosis of food intolerance. Intolerances are non-immune by definition. IgG testing lacks both a sound scientific rationale and evidence of effectiveness. There is a lack of correlation between results and actual symptoms. In light of the lack of clinical relevance, and the potential for harm resulting from their use, allergy and immunology organizations worldwide advise against the use of IgG testing for food intolerance.

Challenge tests

While skin and specific IgE tests could give useful information regarding allergen sensitization, their predictive value for the risk and severity of clinical reactions could be quite poor. Therefore, challenge tests are sometimes needed to determine whether an allergen can provoke clinical symptoms, and remain the gold standards in allergy testing. Challenge tests commonly performed include conjunctival, nasal, bronchial and oral.

Conjunctival challenge tests are evaluated by symptoms of itching and objective indices, including tear production and erythema. Nasal challenge responses are evaluated by subjective symptoms and objective measurements of nasal airway resistance, sneezing, and the measurement of inflammatory

mediators in nasal secretions. Both tests are more commonly performed for research purposes, but might provide additional useful information under certain clinical situations.

Bronchial hyperresponsiveness (BHR) is the abnormal increase in airflow limitation following the exposure to a stimulus[17]. Direct stimuli, such as histamine and methacholine, act directly on effector cells in the airway smooth muscle and the bronchial mucosa to cause airway narrowing. Indirect stimuli act on other effector cells to release pharmacologically active mediators including histamine which in turn lead to bronchoconstriction. Non-isotonic aerosol, hyperventilation, exercise, and mannitol cause osmotic mast cell mediator release whereas adenosine acts via non-osmotic mechanism. Chemicals such as bradykinin and metabisulphite causes increased airway responsiveness via activation of sensory nerves. BHR is a pathophysiologic characteristic of bronchial asthma but is also present in patients with COPD, bronchiectasis, allergic rhinitis without asthma and after viral respiratory tract infection[17]. Up to 10% of asymptomatic normal individuals have been shown to have increased BHR[18,19].

Bronchoprovocation with methacholine is historically the most widely performed bronchial challenge test. Interpretation requires current symptoms and normal baseline spirometry. It is often used as an aid to the diagnosis or exclusion of bronchial asthma. Indirect bronchial challenge has increased diagnostic specificity and is more useful in the confirmation of exercise induced bronchoconstriction. Hyperventilation and mannitol challenge have become the methods of choice in many centers in recent years.

Bronchial challenge using specific allergens is time consuming and requires 3-4 days to capture the early and late bronchoconstrictive responses as well as the allergen induced increase in airway responsiveness. It is a risky procedure and can cause severe bronchospasm. There is a close correlation between skin prick test sensitivity to an allergen and the allergen PC20, the provocative allergen concentration of allergen necessary to induce a 20% drop in FEV1[20]. This implies that the response to allergen bronchial challenge can be predicted by the non-specific BHR and the intensity of the skin test response to the allergen. Fabbri *et al* stated that atopic subjects with airway hyperresponsiveness to methacholine or histamine often respond also to allergens to which they are skin test positive but which may not be relevant for their asthma, and thus the real value of allergen provocation in the management of the single patient remains unclear[21]. As allergen bronchial challenge is potentially hazardous and does not provide useful information in an individual patient, it is seldom indicated in clinical practice[22].

In occupational asthma where low molecular weight sensitisers such as isocyanates induce bronchospasm via non-IgE mediated mechanisms (and thus skin test cannot be used), bronchial challenge remains the confirmatory test of choice. However, occupational challenge requires experienced staff to perform the test and interpret the result. False positive results may be apparent in patients with unstable baseline asthma where it may be difficult to distinguish between irritant and hypersensitivity response. False negative results may occur if the sensitized subject has been away from work for prolonged periods. In general, management of occupational asthma and bronchial challenge test with occupational agents should be performed in tertiary and research centers only[23].

Oral challenge tests remain the gold standards in diagnosing food allergy. Since it can provoke life-threatening anaphylaxis in highly sensitized individuals, it should only be performed by experienced personnel. If the patient has experienced clearcut allergic symptoms following

exposure to a food, and sensitization to that food is demonstrated by skin or specific IgE testing, oral challenge should not be needed. It should be performed if the diagnosis is doubtful although skin/IgE test shows sensitization, or if spontaneous remission is suspected but the skin or IgE test remains positive. In patients with delayed-type hypersensitivity reactions, such as atopic dermatitis and/or eosinophilic GI disorders, an elimination diet avoiding the suspected foods should first be implemented, preferably for two weeks, before oral challenge. Be aware that patients without prior history of immediate-type reactions are at risk of anaphylaxis following food elimination if they have positive skin test or specific IgE.

Drug challenge remains the gold standard for ruling out drug allergy, and is the only option for testing certain drugs. Drug challenge is absolutely contraindicated if severe cutaneous reactions, such as Stevens-Johnson syndrome, toxic epidermal necrolysis and drug-induced hypersensitivity syndrome, are suspected. The oral route is preferred, as the risk of parenteral administration is much higher. The starting dose of graded oral challenge should be no greater than 1/10th of the usual dose, with 3 to 4 dose escalations to reach full dose. Given the risk, only patients determined to have a low probability of being allergic to a given drug after careful evaluation should undergo drug challenge.

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