ABNORMAL LABORATORY RESULTS

Skin prick testing and *in vitro* assays for allergic sensitivity

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SYNOPSIS

Specific IgE-mediated allergic reactivity can be tested for by an *in vivo* skin prick test or by an *in vitro* enzyme or fluorescence-based immunoassay, commonly called a radioallergosorbent test. Many people have circulating specific IgE but do not have clinical allergic disease. The relevance of a positive or abnormal test result therefore depends on the clinical scenario. Skin prick testing is more sensitive than radioallergosorbent tests for detection of IgE reactivity as the majority of specific IgE in the body is bound to mast cells, or other cells bearing high-affinity IgE receptors, with little in the circulation. In the majority of clinical situations, a negative skin prick test excludes an IgE-mediated allergic basis for a potentially allergic condition, such as asthma or rhinitis.

Index words: hypersensitivity, RAST, immunoglobulin.

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Introduction

Since the early years of the last century, before the aetiology of allergic reactivity had been established, *in vivo* techniques, including conjunctival instillation and skin testing, had been used to identify triggers of allergic reactions. The key mediator of allergic disease, IgE, was the last class of immunoglobulin to be discovered, partly because it is highly bound to mast cells, basophils and other cells and only small amounts are present in the serum. It was therefore easy to detect IgE by skin testing, but difficult to isolate or measure it in the serum. IgE was only conclusively identified and confirmed to be the elusive 'reagin' of allergy in 1967.[1,2] At about the same time, laboratory testing was expanding in all medical disciplines and it was not long before immunoassays for allergen-specific IgE were designed and commercialised. The first radioallergosorbent tests (RAST) appeared in 1974 and tests not unlike the current ones were in use by the late 1970s. Since then the relative merits of *in vivo* skin testing and *in vitro* RAST measurements have been argued by their respective proponents.

Tests for allergen-specific IgE

Skin prick testing

Skin prick testing is the conventional way to test for the presence of allergen-specific IgE and detects IgE bound to the surface of mast cells in the skin. Allergen in solution is applied to the skin, generally the volar surface of the forearm. When the skin is pricked with a lancet the allergen comes into contact with specific IgE, bound to the surface of cutaneous mast cells. The binding of the allergen leads to cell activation and the immediate release of mediators including histamine. Other mediators are released, but histamine appears to be the critical one as skin prick tests become negative after taking antihistamines. The release of mediators results in a wheal and flare type reaction and the test is generally reported as the maximal wheal diameter after 15 to 20 minutes. A wheal with a diameter 3 mm or more greater than control is generally regarded as positive. The amount of specific IgE present can be estimated by the size of the wheal. These tests are simple, quick and the most sensitive method of detecting specific IgE. Skin prick tests are particularly helpful in excluding potential allergens as a cause of symptoms as false negatives are uncommon.

Although these tests are extremely safe, with only rare reports of generalised reactions, the risk of systemic absorption remains and anaphylaxis is a remote possibility in highly sensitised individuals. Testing should therefore always be performed under the supervision of a trained and experienced clinician who has resuscitation equipment immediately available.

Patch testing

As distinct from skin prick testing which measures specific IgE, patch testing is used to detect the presence of antigen-specific T cells. The main clinical application for patch testing is in detecting antigens responsible for contact dermatitis, rather than atopic disorders such as asthma, rhinitis or eczema.

In vitro immunoassays for specific IgE

Although serum tests for specific IgE are still frequently referred to as radioallergosorbent tests, they are generally not performed by traditional radioimmunoassay. They more frequently use a commercial solid phase enzyme-linked immunoassay or ELISA with the antigen bound to some form of solid support, such as a paper disc. Following incubation of the test serum with the bound antigen, specific IgE is detected by adding labelled antibodies specific for human IgE. Results are usually presented in a semi-quantitative fashion with a score of 0 indicating no specific IgE detected; one, low level;

two, significant level; and three, four, five (and sometimes six) indicating increasingly high concentrations. As numerous allergens can potentially be tested for, most laboratories also test for reactivity to batches of somewhat related allergens, for example, food mix or inhalant mix.

Only nanograms of specific IgE are present in the serum, therefore, even in highly allergic individuals, RAST testing is not as sensitive as skin prick testing and low-level reactivity may not be detected. Tests using the mixes are even less sensitive and more difficult to quantitate than tests for individual allergens so false negative results are common. In addition to this low sensitivity there is variability between the allergen preparations used for RAST testing. Different laboratories may therefore report different results for the same serum sample. Variability is even greater between allergen mixes as standardisation is difficult. This variability between laboratories has been documented by the Quality Assurance Program of the Royal College of Pathologists of Australia.[3] A recent review of testing in US laboratories also showed considerable variation in results between laboratories testing the same serum sample.[4]

When and how to test for allergen-specific reactivity

An underlying atopic state, defined as the capacity to produce specific IgE to ubiquitous allergens, is more common than the presence of symptomatic allergic disease. Consequently, if individuals were randomly tested for allergic reactivity, many irrelevant positive results would be found. Furthermore if a patient is sensitive to one allergen, it is more likely that reactivity will be present to other allergens, even if there is no clinical sensitivity. The detection of specific IgE in the absence of a reasonable clinical suspicion of an allergy is hard to interpret. This may create problems; for example, if tests are used to investigate fairly vague symptoms, such as abdominal bloating or fatigue, and a specific food sensitivity is detected, drastic and unhelpful dietary modification may be advised. It is therefore essential that testing should only be done when there is a reasonable clinical suspicion (pre-test probability) that sensitivity to a particular allergen is present.

An underlying atopic state is more common than the presence of symptomatic allergic disease

Most of the allergens in Table 1 can be tested for by either RAST or skin prick testing.

For perennial respiratory symptoms, the most likely allergens are house dust mite, pet hair and danders and mould spores. For seasonal symptoms, grass pollens, particularly rye grass, are most frequently implicated, although tree and weed pollens, and even mould spores, can cause seasonal symptoms. Food allergens are rarely implicated in respiratory disease but can cause systemic reactions including anaphylaxis and angioedema and, on occasions, can also be relevant in eczema. In the case of serious generalised reactions, the causative food is usually obvious from the patient's history and testing is only undertaken to confirm the clinical suspicion. For severe eczema or for eczema where there is a strong suspicion that particular foods aggravate the condition, skin testing is appropriate and is generally undertaken in specialised multidisciplinary centres. Oral challenge tests are sometimes still used for confirmation of a positive skin prick test.

Table 1

Common allergens

Inhalants	House dust mite, grass pollens, pet (especially cat) hair and danders and mould spores (especially alternaria and cladosporium) are the most commonly recognised allergens.
Foods	Important particularly in children with eczema and in adults where there is a strong clinical suspicion. The most important foods are peanuts and tree nuts, egg, milk, seafood, wheat, soy and fruits. Avoidance is the mainstay of treatment. If doubt exists about the relevance of a particular finding, a double-blind oral food challenge is the most definitive test.
Insects	Honey bee (*Apis mellifera*), European wasp (*Vespula germanica*) and paper wasp (*Vespula polistes*) are the main insect stings tested for in Australia. Allergy to jumper ants (*Myrmecia pilosula*) is also very important in rural South Eastern Australia, but no test is currently available.
Medications	Antibiotics (mainly beta-lactams) and a number of anaesthetic agents.
Others	Latex and a variety of occupational allergens. Whilst tests for latex are now available there are few routine tests for most occupational allergens.

Skin prick testing remains more sensitive and more specific than *in vitro* tests for allergen-specific IgE and, in general, remains the method of first choice for detection of reactivity. It is quicker and simpler than undertaking a RAST but, on the negative side, it requires a trained clinician with access to resuscitation equipment. These requirements may result in delays before the test is carried out. If a RAST is requested it is important to specify which allergens are to be tested, as a positive result to an allergen mix does not identify the specific sensitivity and further tests are required to find the relevant (or most relevant) allergen. There are some situations where a RAST may be preferable to a skin prick test (Table 2).

Table 2

Indications for *in vitro* RAST measurement rather than skin prick testing

1. Patients with extensive skin disease with no suitable site for testing
2. Dermatographism where wheals are produced by any minor trauma
3. Current administration of antihistamines
4. Risk of anaphylaxis, especially certain foods and latex
5. Confirmation of an unexpectedly negative skin prick test
6. Lack of availability of an allergist or appropriately trained clinician

Conclusion

Either skin prick tests or RAST can accurately determine the presence of allergen-specific IgE. Skin prick testing is the preferred method as it is more sensitive, quicker and simpler. False negatives are very unusual and a negative skin prick test makes the presence of IgE mediated allergic reactivity most unlikely. Conversely specific IgE may well be present in the absence of clinical sensitivity and positive tests must always be interpreted in conjunction with the clinical findings.

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Conflict of interest: none declared

Self-test questions

The following statements are either true or false (answers on page 99)

5. The usefulness of skin prick tests is limited by the large proportion of false negative results.
6. Skin prick testing should only take place when resuscitation equipment is immediately available.

Web site review

Database of Individual Patient Experiences (DIPEx) web site: www.DIPEx.org

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DIPEx is an internet-based multimedia resource. It tries to respond to the needs of people recently diagnosed with an illness by providing both clinical information and the experiences of individual patients. 'To be diagnosed with an illness can be bewildering and frightening, especially if there is no-one around to tell you the things you really want to know'. DIPEx includes video clips, sound (testimonies of patients), and links to web sites which are reliable, but have a more specific focus, such as cancer. DIPEx itself represents an unusual collaboration between health professionals and consumer groups. It is a not-for-profit organisation funded by the UK Department of Health, Macmillan Cancer Relief, the Citrina Foundation, the Consumers Association and the Lord Ashdown Trust.

Scope

The web site is divided into modules based on particular conditions. As funding becomes available it is intended to include 'experiences of all the main illnesses'. Topic information is organised into categories of diagnosis, such as colorectal cancer, together with relevant tests, investigative procedures and links to condition-specific web sites, for example Cochrane and CancerBACUP. Links to patient experiences are a key feature of the site which also invites people to volunteer to tell their own story. The focus of these 'stories' is patient responses to particular treatments, yet the web site does not include evidence about risks of these treatments or procedures. The patient comments do include concerns and experiences of, for example, adverse effects.

Audience

Although its stated aim is to meet the needs of patients, DIPEx is also intended to play an educational role for health workers. It is likely that the site will be more successful in achieving this aim than in its more ambitious aims. In particular it is questionable to what extent it can substitute as a support group for people who are looking for timely answers to non-medical questions. However, links are provided to various support groups.

Limitations

The web site does not acknowledge that what people often need is immediate support and information about what might be available. In addition, because DIPEx aims at that 'window of opportunity' between diagnosis and treatment it is health-system focused and does not cater for the concerns of people with long-term illness.

The site uses DISCERN quality criteria for evaluating medical information on treatment choices. DIPEx claims to provide 'balanced encounters between patients and health care professionals'. However, the site content appears to be written by health professionals accompanied by links to patient testimonies. A more robust approach might be to establish an advisory group for each illness dealt with, giving both patients and practitioners equal say in the content and design of the site.

The partnership approach is badly let down in two further ways. Firstly, the background provided by health professionals is not supported by evidence or referenced. Secondly, patient testimonies consist of one person's experience rather than a range of experiences. Yet the experience of one patient invariably differs from the experience of another person. There is no evidence or discussion about factors that may influence different experiences of the same procedure or diagnosis, for example socio-economic status, current health status and life experiences.