

A comparison of techniques for evaluating IgE-mediated allergies

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Abstract

Skin prick testing remains the most popular way to confirm an IgE-mediated allergic response. This study was performed to compare the accuracy and sensitivity of two skin testing modalities (serial endpoint titration and skin prick testing with 2 different in vitro assays.) In 52 atopic patients serial endpoint titration showed a higher degree of sensitivity in evaluating skin response in less sensitive reactors. At higher degrees of reactivity in individual patients there was good correlation between the 3 modalities. Identifying patients who are less sensitive reactors is important, so that they can be started on immunotherapy. In fact, an informal survey of low-reacting patients treated with immunotherapy showed a high degree of success.

Introduction

In 1873, Blackley first described a scratch test to identify an allergy to grass.¹ Allergen-specific immunotherapy was introduced in 1911 by Noon for the treatment of allergic disorders caused by inhaled antigens.² Noon actually used serial dilutions of grass pollen extracts, quantifying the response of the conjunctiva to a given allergen. Based on this sensitivity, he demonstrated that repeated injections of extracts in doses small enough to avoid systemic reactions were effective in protecting the patient.

In 1926, Lewis and Grant first described the skin prick test.³ This test, in various forms, is still the basic

method of diagnosis used by general allergists. A variant of this test is the "multitest," in which a disposable applicator with eight puncture heads is used, allowing for the simultaneous testing of six antigens, a positive (histamine), and a negative (glycerin control).⁴ Reading of skin reactivity is done on a subjective 0 to 4 basis. The advantages of skin prick testing are ease of administration and absence of false positives, but low reactors are missed, and the degree of reactivity is difficult to measure. The multitest was used in the present study; 14 antigens were tested, along with controls.

In 2006, Oppenheimer and Nelson conducted a survey designed to determine how traditional allergists test for aeroallergen sensitivity.⁵ This survey was participated in by 539 members and fellows of the American College of Allergy, Asthma & Immunology. Data from the survey revealed that most allergists performed both skin prick tests and intradermal tests. Data also showed a great degree of variability in the number of tests performed, the skin prick test devices used, and interpretation of test results. The survey showed that most allergists did not depend on history or geographic location in choosing which antigens to use, and that documentation regarding skin test results was poor. Although many used intradermal tests in addition to the skin prick tests, there was no uniformity as to the indications for doing so. Furthermore, most allergists surveyed did not have an objective test protocol for quality assurance.⁵ An editorial by Portnoy in the same journal was highly critical of the variability in the diagnosis and treatment of allergic disease.⁶

Although the intradermal skin test was first described by Robert Cooke in 1915, there is still no standard technique for general allergists to follow when performing this test.⁷

Otolaryngologic allergists

The management of allergy as practiced by otolaryngologists actually began with French K. Hansel, MD, of

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St. Louis. In the 1930s he called attention to the allergic factor in many cases of chronic sinusitis and described the significance of eosinophilia.^{8,9} In 1936, he published his monumental work, *Allergy of the Nose and Paranasal Sinuses*.¹⁰ He also developed a technique of testing by using antigens diluted by a 10-fold method. He found that many of his patients had better symptom relief with moderate dosage levels based on his 10-fold dilution technique than was previously observed using the same dilution for each antigen and increasing the dose until reactions were encountered.

In the late 1940s, Herbert Rinkel, MD, a general allergist from Kansas City who was unhappy with the results in patients treated with the maximum tolerated dose regimen, visited Dr. Hansel. Impressed by his results, he further refined the technique by making 5-fold dilutions and titrating each antigen in individual patients.¹¹ This titration method is the basis for serial endpoint titration (SET), which has been used by otolaryngologic allergists since the late 1950s. The technique was then standardized by Dr. James Willoughby Sr.,¹² and this standardized form has been in use for the past 45 years, in contrast to the unstandardized skin prick testing technique. Basing immunotherapy on the results of SET has proven to be a safe and effective way to treat IgE-mediated allergic disease.^{12,13}

A detailed description of the SET technique can be found in many publications, such as the 2003 article by Krouse and Mabry,¹⁴ and Willoughby's detailed descriptions of this technique can be found in many other publications.^{12,13}

Patients and methods

SET. Following is a brief description of the key concepts of SET. Five-fold dilutions of the allergenic extracts are made, and these are labeled dilutions #1 through #6. The #1 dilution is five times weaker than the concentrate (1:20 in most cases), so the weight per volume designation of the #1 dilution would be 1:100. This dilution is made by taking 1 ml of the concentrate and mixing it with 4 ml of the diluent. The #2 dilution would be made by removing 1 ml of the #1 dilution and mixing it with 4 ml of diluent. The remaining dilutions are prepared in the same manner.

Testing is started with a #6 dilution intradermally. When 0.01 ml of the extract is used, it should produce a 5-mm wheal. If there is no growth of this wheal after 10 minutes, then the next stronger dilution (#5) is injected. Subsequent stronger dilutions are applied until a 7-mm wheal is encountered. This is confirmed by the

next dilution, which should produce a 9 mm wheal. This first growth is then determined to be the endpoint, and immunotherapy is based on this endpoint. The advantages of this method are that the test is quantitative as well as qualitative. It is also highly reproducible, very sensitive, and extremely safe.

SET was the only method of diagnosis taught by the American Academy of Otolaryngic Allergy after it was first described by Dr. Hansel. However, in the past few years the Academy, for reasons unknown, has also decided to include skin prick testing and has combined the multitest with SET in a technique called Modified Quantitative Testing (MQT).

In vitro testing. In 1966 and 1967, the discovery that antibodies of the IgE class are responsible for allergic disease was made, both by Ishizaka and colleagues^{15,16} and by Johansson and Bennich.^{17,18} The radioallergosorbent test (RAST) that detects specific IgE antibodies in human serum was developed.^{19,20} RAST has now been accepted worldwide for the estimation of specific IgE antibodies and has been used for the detection of IgE antibodies to animal dander, pollens, stinging insect venoms, viruses, bacterial components, and chemicals. Its advantages are: no risk to the patient, quantitative results, and low incidences of false-positive results.

In the 1980s, the radioactive labels used in RAST were replaced with enzymatic markers. These enzyme labels remain stable longer and avoid the hazards of radioactive materials. Precision and ease of operation are shown to be comparable when isotopic and enzymatic labels have been compared.

Importance of location. It is important to test patients for airborne antigens found in your geographic location. Obviously there is no point in testing a patient for an antigen with which he will not come into contact. New allergy patients in our office are tested for ragweed mix, grass mix, tree mix, dust, dust mites, animal dander, and mold spores.

The pathologic fungi *Trichophyton*, *Candida albicans*, and *Epidermophyton floccosum* are tested separately and not as a mix. Each patient is given a ruler and is called the next day for recorded delayed reactions. These delayed reactions usually only happen in mold testing. For each 2 mm of delayed growth, the endpoint is moved 1 point to the left.

In our office, when pollen mixes react at an endpoint of 3 or greater, the individual weeds, grasses, and tree pollens that are in our geographic location are tested separately. We also test the five most common mold spores found in our geographic area, and if the end-

points are 3 or above, the next six most common mold spores are tested.

For purposes of this study, all mixes were eliminated so that only individual antigens were compared. The pollen mixes were replaced by Bermuda grass, June Kentucky bluegrass, common/short ragweed, birch, and white oak. Patients were also tested for allergy to standardized cat hair, dog epithelium, and three common molds (*Hormodendrum*, *Aspergillus fumigatus*, and *Alternaria tenuis*). *Dermatophagoides pteronyssinus* mites and *Dermatophagoides farinae* mites, house dust, and American cockroach rounded out the 14 antigens.

Patient population. All 52 patients in this study were adults who had never had allergy testing or immunotherapy before. When tested, they were not receiving any allergy therapy such as antihistamines, steroids, etc. All patients were tested within a 2-month period in the summer. None of the patients had asthma or other life-threatening diseases.

Results

SET results are reported by the endpoint dilution number. Multitest results are reported using the modified multitest scoring system. *In vitro* test results are reported using the Fadel and Nalebuff modified RAST scoring system, with the sandwich-type immunoassay with radiolabeled antihuman IgE antibodies (Sero Lab). The second *in vitro* technique (Diagnostic Products Corporation) utilized monoclonal antibodies with enzyme-enhanced chemiluminescence. The results were reported in classes, starting with Class 0, where no IgE can be measured, up to Class 5, which shows a high amount of specific IgE. For the purposes of this study, any class above 0 was considered as positive.

All of the 52 patients were found to be positive on SET testing, having endpoints of 1 to 6. All of the test patients had a typical history of allergic rhinitis, and some had recurrent sinusitis over a period of years. Because of their strong history and lack of other nasal problems, such as polyps and chronic sinusitis, these 52 patients were included in the study group.

Twenty-two of the 52 patients had no reaction to either prick testing or *in vitro* testing. Eight of these 22 patients had endpoints of 1 for all antigens, and 4 had endpoints of 1 and 2. The remaining 10 patients in this group had endpoints of 2 or above.

Of the remaining 30 patients, 11 had positives by *in vitro* and negatives by prick testing. Five patients had positive prick tests and negative *in vitro* tests, and the remaining 14 patients had both positive prick tests and

positive *in vitro* tests. In the patients who were positive to both *in vitro* and prick testing, the best correlation with SET was in patients in whom the SET endpoint was 3 or above. However, prick testing and *in vitro* testing will not pick up the patients whose SET endpoints are 1 and 2. In the group of patients who had negative prick testing and positive *in vitro* tests, the *in vitro* test frequently picked up the lower endpoints, while the prick test did not.

Discussion

Over the years, many leading otolaryngic allergists using SET as a diagnostic modality believed that the number 1 endpoint and even the number 2 endpoint could be due to glycerin and phenol irritation rather than representing a true immunologic response. This opinion was strengthened after the development of *in vitro* testing, when these clinicians came to believe that if no specific IgE was found, then there could not be any allergy to that specific antigen. Unfortunately, no one has done any formal or informal study to validate this opinion.

In our practice, we have always treated those patients whose endpoints were 1, 2, or 1 and 2. For this study, 230 patients were identified in our practice whose endpoints were either 1, 2, or a combination of 1 and 2. These patients were all interviewed when they came to the office for immunotherapy, and they all reported either complete or significant relief of their symptoms. All these patients had been under treatment for at least 1 year, and most of them for 2 or 3 years. If these 230 patients had been diagnosed only by skin prick or *in vitro* testing, they would have been considered negative for allergic disease and would not have received immunotherapy. Instead of being cured or having their disease controlled without drugs, they would have treated their symptoms with drugs for the rest of their lives.

In summary, it is obvious that neither prick testing nor *in vitro* testing will pick up the number 1 or 2 endpoints seen with SET. If SET is not utilized as a diagnostic technique, there will be a significant number of patients who are treated medically when they should be treated by desensitization. This study shows that SET should be the basic technique for diagnosing IgE-mediated inhalant allergy.

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