

It is not yet time to stop skin testing, but...

Key words: Radioallergosorbent test, immunoglobulin E, skin testing

About 40 years ago, the practice of medicine was entering a more scientific era. For better or worse, practitioners were encouraged to rely more on the clinical laboratory and principles of evidence-based medicine rather than on traditional clinical experience and empiricism. Allergists, for years convinced of the efficacy of immunotherapy in atopic diseases, published the first double-blind, placebo-controlled studies that confirmed this efficacy. Yet allergists continued to use crude plant and animal extracts for diagnostic testing and treatment, and the study of the fundamental mechanisms of allergic disease was limited by the fact that the mysterious “reaginic” antibody of immediate hypersensitivity could not be identified despite diligent research efforts.

IgE was the last Ig class to be discovered because it is a homocytotropic molecule present in the serum in nanogram amounts, in an equilibrium with that bound to mast cells, basophils, and other cells. It was thus easy to demonstrate by skin testing but difficult to isolate from the serum. Johansson’s discovery of an untypeable myeloma protein, initially termed *IgND*, in the serum of a Swedish farmer permitted preparation of rabbit antisera for use in immunoassay.¹ Confirmation that IgND was identical to the γ E discovered by Ishizaka and Ishizaka,² and confirmation that both were the elusive reagin of allergy quickly resulted in design and commercialization of immunoassays for allergen-specific IgE. Academic allergists breathed sighs of relief and predicted that such assays would soon replace “old-fashioned” skin testing in clinical practice and raise the specialty to a higher plane of sophistication. However, both the academicians who see patients and the in-the-trenches practitioners of the specialty correctly realized that then-current assay technology did not have the sensitivity and specificity of epicutaneous skin testing, which they considered the clinical gold standard for detection of specific IgE antibody. The Phadebas radioallergosorbent test (RAST)

Abbreviations used

OD: Optical density

RAST: Radioallergosorbent test

appeared in 1974 as an improved testing method, and commercialization of testing proceeded globally. The trademark “RAST” came to be used colloquially to describe all immunoassays for specific IgE.

In the United States, Nalebuff and coworkers³ devised an alternative method for performing the Phadebas RAST. This Fadal-Nalebuff modified RAST used longer incubation times and a different system for reporting results—a “class system” designed to be useful for clinicians accustomed to formulating immunotherapy prescriptions on the basis of results of titrated intradermal skin tests. Sensitivity, compared with that of skin testing, was greater, but with a loss of specificity.⁴ Currently, the modified RAST is not always performed in accordance with its original description. Some methods use substantially different reagents and incubation protocols and convert OD or other units to “counts per minute.”

Multiple commercial immunoassay methods appeared. Practicing allergists familiar with skin testing shunned these initially because of the sensitivity issues. Unfortunately, some of the firms manufacturing test methods began aggressive marketing of test technology to physicians not trained in the use and interpretation of skin testing. A review of advertisements in primary care and some specialty journals in the 1970s indicates that manufacturers had decided to bypass the trained, certified allergist and market not only the testing technology but also short “courses” intended to teach them and their nurses “all they needed to know” without the necessity of formal training in the specialty. Some entrepreneurs attempted to simplify the practice of allergy to the point where patient questionnaires to be completed by a nurse were sent with a tube of blood for testing, test interpretation, and formulation of an immunotherapy extract returned with complete instructions for administration.

The reaction of the community of trained and certified allergists was predictable and swift. Some individuals began an unofficial boycott of all in vitro testing, as well as an allergopolitical campaign intended to relegate such testing to the realm of alternative medicine and to limit third-party reimbursement for testing. This latter aspect of that campaign was not difficult to embrace because on a test-per-test basis, a specific IgE immunoassay is more expensive than a skin test. To an extent, that boycott has lasted to this day, at least for some practitioners. Manufacturers attempted to counter by marketing testing to lay consumers and by promoting “white papers” challenging

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Received for publication Mar 6, 2000; revised Mar 15, 2000; accepted for publication Mar 15, 2000.

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J Allergy Clin Immunol 2000;105:1074-6.

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0091-6749/2000 \$12.00 + 0 1/1/107151

doi:10.1067/mai.2000.107151

the validity of skin testing. The “turf wars” between board-certified allergist-immunologists and other physicians who evaluate and treat patients with allergic disease added fuel to an already hot fire, while some of the test manufacturers quietly continued to improve test technology. The modern methods have a high degree of automation and high binding capacity for antibody, use carefully designed detection reagents and enzyme-substrate detection technology rather than isotopes, have short incubation times (hours rather than days), and report quantitative results.

The Rostrum published in this issue of *The Journal* (Yunginger et al, p 1077) and a related document published last year⁵ suggest that the time to end the boycott and to reassess the role of specific IgE immunoassay in the everyday practice of allergy has long passed. All allergists, and others who evaluate and treat patients with allergic disease, should study these reviews (and the primary articles that they reference) in detail. However, it is not yet time to stop skin testing.

The Rostrum reports the state of the art in testing technology, reviews current standards for quality assurance and quality control, and describes certain clinical and research situations in which quantitative assays may be useful. The authors carefully review published reports suggesting that quantitative levels of specific IgE may have clinical implications. For instance, in children with atopic dermatitis, high levels of food-specific IgE are so strongly associated with a positive response to double-blind, placebo-controlled food challenge that use of quantitative specific IgE immunoassays could in some cases decrease the need for food challenge by 50%.

The Rostrum also dwells on the challenging issue of interchangeability of skin testing and immunoassay, an issue that in my mind is controversial only because some test manufacturers have made it so. In the ideal circumstance of having a purified venom source for both titrated intradermal skin testing and preparation of an immunoassay solid phase, Ford et al⁶ reported 100% sensitivity and 100% specificity when a background-based cutoff value was used in the immunoassay. In a study in which purified major allergens were used, Witteman et al⁷ found a rather poor quantitative relationship between results of titrated intradermal skin tests and quantitative immunoassay. In the real world of clinical practice, results of conservatively performed and interpreted skin testing and a modern quantitative specific IgE immunoassay are indeed concordant more often than not.⁴ When results are discrepant, the skin test result is generally positive and the immunoassay result is reported as negative, a situation that is thought to be more the result of immunoassay performance issues⁸ than of skin testing itself. Background-based reporting of immunoassay results is one approach that might improve the concordance of results,⁹ but the clinical relevance of a positive skin test result in the face of a negative or very low level positive immunoassay result remains to be established. In some cases an immunoassay can be clearly positive when results of skin testing are clearly negative. The usual and most logical explanation for this situation

has been that there are compositional differences in the extracts used for the immunoassay and skin testing; a frequently noted example is that the major apple allergen Mal d 1 is so labile that it might not be present in an extract used for day-to-day skin testing.¹⁰ A provocative recent case report by Mari et al,¹¹ not reviewed in the Rostrum, describes a patient in whom the presence of cross-reactive carbohydrate determinants accounted for apparently false-positive and clinically irrelevant immunoassay results when skin test results were negative. In the discussion of their article, the authors review the phenomenon of cross-reactive carbohydrate determinate-specific IgE and call for an “overall reappraisal” of commercial systems for measuring specific IgE.

In this issue of *The Journal*, another very disturbing article raises important questions about specific IgE immunoassay testing methods and how they are performed in reference laboratories (Williams et al, p 1221). The authors sent coded serum samples on 3 different occasions to 6 different laboratories that use 5 different commercially available specific IgE immunoassay methods. Some of the sera were a dilution series. Only one method used in two laboratories performed without extensive variability. The authors defined an ideal test as one that is quantitative over a wide range, but several of the test methods studied use a paper disk with known limited binding capacity and report semi-quantitative results over a relatively narrow range. It is thus not surprising that these methods did not meet the defined criteria. The study design did not clearly identify whether the observed discrepancies are due to inherent test characteristics of certain assay methods or to laboratory error. It also made no attempt to assess the prevalence of such discrepancies in all US clinical laboratories.

Physicians tend to trust printed laboratory results, and in a day and age of extensive federal regulation (as reviewed in the Rostrum) clinicians may question the findings of this study. Such wide discrepancies would not surprise a laboratory director who for many years has participated in the Diagnostic Allergy Survey program operated by the College of American Pathologists. In a manner analogous to the design of the study by Williams et al, clinically and laboratory characterized samples are sent to participating laboratories instructed to treat them as routine specimens and report results to the College. A summary report allows them to compare their results with the consensus. Examination of 1999 survey data from the College clearly shows examples of the same kind of discrepancies observed by Williams et al, as well as situations in which results are more concordant. However, the reader should note the College’s admonition that “CAP surveys data do not necessarily indicate the superiority or inferiority of instrument/reagent/testing equipment or materials used by participating laboratories.”

Immunoassays for specific IgE have a clear role in allergy practice as an alternative to (but generally not a substitute or replacement for) skin testing and may have an adjunctive role as well. In conjunction with appropriate educational efforts (spearheaded by trained, board-

certified allergist-immunologists—not the testing industry), they should be promoted as useful tools in certain situations for primary care and other physicians who evaluate and treat patients with allergic disease. To help third-party payers define reimbursement criteria, practicing allergists, working through their national organizations, need to define clinical situations in which specific IgE immunoassays should routinely or exceptionally be used in preference to skin testing or as an adjunct to skin testing and other diagnostic modalities. Doing so will improve patient care, as well as contain costs.

Despite the existence of standards for immunoassay validation and assessment of clinical laboratory performance described in the Rostrum, it is clear that serum for specific IgE immunoassay can not be sent to just any laboratory—whether qualitative or quantitative results are desired. As noted in the Rostrum, all clinicians ordering these tests (and in my opinion, representatives from third-party payers) should meet with the director of the reference laboratory performing the tests to review the laboratory's present and past performance on blinded proficiency test specimens. There is increasing evidence that clinicians should request that results be reported quantitatively in a manner that can be related to the international standard for IgE. Furthermore, academic allergists, practicing allergists, and others with expertise in laboratory medicine need to collaborate with each other (and with federal regulators if necessary) to define clear standards that will continue to improve test and laboratory performance.

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