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LEARNING OBJECTIVES

Upon completion of this article, the reader will be able to:

1. Explain how specific IgE blood testing allows primary-care physicians to diagnose and manage allergic diseases in children.
2. Compare specific IgE blood testing — including its methodology, sensitivity, and specificity — with skin testing.
3. Explain how the objective evidence obtained from specific IgE testing contributes to accurate diagnosis and appropriate management of allergic disease.
4. Describe the typical progression of allergic diseases (i.e., the Allergy March).

Specific IgE testing: Objective laboratory evidence supports allergy diagnosis and treatment

By Lee R. Choo-Kang, MD

Two-and-a-half-year-old Mary is being seen by her pediatrician for a persistent cough. It is February and her parents describe a frequent runny nose, nasal congestion, and cough for the past four months. Mary was the product of an uncomplicated full-term pregnancy. She seemed healthy over her first year of life except for eczema, which has gotten better lately. Since entering daycare last winter, she had an episode of bronchiolitis but did well over the summer. On examination, Mary had nasal drainage and swollen turbinates, as well as bilateral wheezing. Mary's mother reports that she takes over-the-counter medication for allergic rhinitis and wonders if her daughter is developing a similar problem.

As the prevalence of asthma and atopic disease increases,¹ primary-care clinicians are seeing more young patients for respiratory disease that may have an allergic component. Clinical manifestations of IgE-mediated disease correspond to age. Eczema and gastrointestinal symptoms secondary to food allergy are often the first clinical manifestations of atopy in an infant or young child. As the atopic child grows, he is more likely to present with recurrent and often chronic upper and lower respiratory illnesses. It has been shown that children sensitized to foods at an early age become sensitized to aeroallergens later,² and they have a higher risk of developing asthma^{3,4} than other children. Yet, without objective diagnostic testing, it is very difficult to differentiate allergic from non-allergic disease on the basis of symptoms, family history, and physical examination alone. In one study, specialists' ability to predict allergic sensitization without diagnostic testing rarely exceeded 50%.⁵

The evolution of specific IgE testing

Two types of tests — *in vivo* skin-prick testing and *in vitro* blood testing — have been used to confirm the presence of

allergen-specific IgE antibodies in patients with suspected allergy. IgE antibodies are a special class of immunoglobulins that are produced by the immune system in response to antigens. Once secreted by specially programmed B-lymphocytes, IgE circulates in the bloodstream until it becomes attached to the surface membranes of mast cells and basophils present in the epithelial surfaces of the body, such as the respiratory and gastrointestinal tracts and the skin. Upon re-exposure, some allergens cross-link the membrane-bound corresponding specific IgE and trigger the release of several inflammatory mediators including histamine, leukotrienes, prostaglandins, and proteases, thereby producing the familiar signs and symptoms of allergies.⁶

Before the introduction of radioallergosorbent (RAST) blood tests in the 1970s, skin-prick testing was the only method of confirming IgE-mediated disease. In skin testing, skin pricks expose small drops of allergen to reactive skin cells. Positive (histamine) and negative (saline) controls are also used. Sensitized patients will develop a wheal-and-flare reaction within 15 minutes. Results of skin testing are reported subjectively on a number scale, or the wheal diameter measured.⁷ Since skin-prick testing depends on the complex interactions among IgE antibodies, immune cells, and the skin, patients must stop taking antihistamines and tricyclic antidepressants (due to their antihistamine properties) seven to 14 days before skin testing. As a consequence of the small but potential risk for anaphylaxis following skin testing, patients should preferably not be taking beta-agonists or monoamine oxidase inhibitors. Skin testing is also contraindicated in pregnant women and patients with unstable asthma. Usually performed by allergists, skin testing requires trained staff, specialized materials, and the ability to manage possibly serious side effects. Medical laboratory personnel were rarely involved in skin-prick testing, unless they prepared the antigens used for skin testing or immunotherapy. The advent of blood-testing technology has moved specific IgE evaluation into the medical laboratory.

By providing an accurate definitive diagnosis, specific IgE testing supports early, appropriate, and targeted therapy, greater patient satisfaction, and better control of costs.

First-generation RAST tests yielded a high number of false-negative results and were considered unreliable by allergists and primary-care clinicians.⁸ This perception reinforced the commonly held view that skin testing represented the gold standard for specific IgE testing due to its greater sensitivity. A modified RAST assay was introduced in the late 1970s and is still used by some laboratories today. Its sensitivity was increased by lowering cutoff values, doubling the sample volume per test, and increasing the serum incubation to overnight rather than just three hours. Unfortunately, however, the likelihood of false-positives increased, and the results were mostly qualitative. A third generation of assays, introduced in the late 1980s and early 1990s, had improved sensitivity and improved reproducibility due to automation and use of monoclonal antibodies. And for test methods utilizing calibrators directly

tied to the World Health Organization Reference Preparation for IgE, these assays delivered the first truly quantitative measurements of IgE.

The technology of blood testing

Specific IgE assays differ in their binding capacity, the source, quality, and availability of antigens, reagent stability, and degree of automation.⁸ In the laboratory, serum samples from patients are first incubated with allergens bound to a liquid-phase or solid support.⁹ In early RAST tests, a paper disk served as the support. Later assays improved binding capacity by using cellulose sponges, polystyrene, or liquid-phase carriers. The most commonly used method today utilizes a patented high-capacity reaction vessel, which contains an allergen-coated, porous cellulose sponge that greatly increases the surface area available for the specific IgE in question. The IgE finds and interacts with bound allergen, forming specific antibody-antigen complexes. Allergens attach to the sponge with a robust covalent chemical bond that survives the vigorous washing used to sweep away non-specific IgE. Then labeled anti-IgE monoclonal antibodies are added, resulting in the formation of anti-IgE antibody-antibody-allergen complexes. Finally, unbound anti-IgE-antibody is washed away, and the remaining complexes measured. Early specific IgE methodologies detected these complexes with radiolabeling, which has been replaced with enzyme (fluorescent, colorimetric) or chemiluminescent labeling. The whole process is often highly automated, which contributes to improved reproducibility, depending on the solid phase and the system in use.

Almost all modern assays claim to report quantitative results, although the results may be obtained by extrapolation rather than by calibration to zero.¹⁰ Very low levels of specific IgE can be reported using a calibration curve that includes zero, though the clinical significance of such low levels is not known. Precise low-level detection may offer an early warning of sensitization, which increases the risk for developing more allergic sensitivities. The clinical significance and predictive value of low-level IgE sensitization in children is currently being investigated.

The source, quality, and selection of allergens used in specific IgE testing certainly influences the clinical usefulness of results. Establishing sensitivity to an allergen the patient is not likely to encounter, for example, does nothing to guide clinical management or improve the patient's symptoms but does increase the cost of care. Representative allergen profiles, containing a limited selection of allergens, can provide sufficient evidence to rule out allergy in non-allergic patients and identify pertinent sensitivities in allergic patients.¹⁰ The profiles should include a selection of indoor allergens, outdoor allergens matched to specific geographic regions, and allergens frequently associated with allergic disease. A typical childhood profile would include food and indoor-inhalant allergens, as sensitivities to outdoor allergens take several seasons to develop. An adult profile might include house mite dust (*Dermatophagoides farinae*), dog and cat dander, plus outdoor allergens, such as grasses, pollens, and molds typical of the region. Testing for large numbers of allergens is rarely neces-

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sary. As the link between asthma and allergy continues to be demonstrated, the inclusion of indoor allergens most likely to trigger asthma symptoms rounds out the contents of the test profiles. Some regional respiratory profiles of major inhalant allergens (indoor and outdoor) have been found to accurately identify atopy in up to 99% of cases.¹¹

Today's third-generation technology for blood testing allows primary-care clinicians to approximate the diagnostic preci-

sion of allergists.¹² Wood and colleagues evaluated skin-prick, intradermal, and blood testing for specific IgE, comparing their relative sensitivity, specificity, positive and negative predictive value, and efficiency (a combined measure of sensitivity and specificity).¹³ Results from the skin prick and blood tests were comparable (see Table 1). Poon and colleagues, in a review of the scientific literature, came to the same conclusion.¹⁴ They noted that without an independent gold standard for detecting inhalant allergens, "it is not possible to determine which test is more accurate." They also found that blood testing offered more standardization than skin testing, a finding endorsed in a recent editorial written by Portnoy in the *Annals of Allergy, Asthma & Immunology*.¹⁵

With blood testing, there can also be variations in results among laboratories, testing technologies, and allergens. Williams and associates conducted a well-controlled study of the accuracy and precision of specific IgE blood tests on 26 masked serum samples sent to six laboratories that used five testing procedures for 17 aeroallergens.¹⁶ Analysis of 12,708 test results showed that one third-generation technology used in two laboratories proved consistently superior to other commercially available assays. This technology measured specific IgE antibodies over a large range with precision and accuracy. The authors — and other allergy experts — support

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Table 1. Comparison of results from skin-prick and blood tests for specific IgE

	Skin-prick testing	Specific-IgE blood testing*	Intradermal skin testing
Sensitivity (%)	93.6	87.2	60
Specificity (%)	80.1	90.5	32.3
Positive predictive value (%)	90.1	91.1	12
Negative predictive value (%)	87.1	86.4	84.6
Efficiency	89.2	88.8	36.8
* Study processed with ImmunoCAP technology			

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its use as the current standard for quantitative measurement of specific IgE.¹⁷

The Diagnostic Allergy Proficiency Survey, administered by the College of American Pathologists, is one means of evaluating diagnostic performance. In addition, the Clinical and Laboratory Standards Institute¹⁸ publishes standards for quality-control and minimal-performance targets, including recovery of antibodies, precision, linearity, and parallelism over the measuring range. Assay manufacturers can also be consulted regarding recommended validation and calibration procedures. Henry Homburger, MD, a pathologist at Mayo College of Medicine, recently published an excellent review of specific IgE testing that includes practical recommendations about whom should be tested, which tests should be ordered, and what the results mean.¹⁹

Reporting results

Quantitative results for specific IgE testing are most often measured in kilo units per liter (kU_A/L , where $_A$ represents the amount of antibody), and calibration should be linked to the World Health Organization 75/502 IgE standard. Many laboratories also categorize kU_A/L measurements using a simplified class system with reactions ranging from Class 0 (no reaction) to Class VI (very high specific IgE levels). This antiquated method of grouping results by class is slowly being phased out in favor of quantitative specific IgE results. This is an important direction for the presentation of specific IgE results, because the results of assays from various manufacturers are not interchangeable, although the class system tends to create that impression.^{8,20} Consequently, an increasing number of experts believe the class system should be abolished. In fact, in 1992, the Executive Committee of the American Academy of Allergy and Immunology recommended "the arbitrary reference systems with myriad class-scoring schemes should be abandoned in favor of quantitative-reporting methods where test results are reported in units that are proportional to antibody content."²¹

Negative results can prevent unnecessary trials of allergy medication, direct further diagnostic efforts, and spare the inconvenience of avoidance for patients who would not benefit.

Just as history and physical examination alone are insufficient for a definitive diagnosis, specific IgE test result must be interpreted in the context of symptoms and clinical signs. For example, low levels of specific IgE for one allergen certainly have less clinical significance than higher levels for multiple allergens. In individuals with a positive result, identifying specific allergens guides management. Because the clinical effect of any number of allergens is dependent upon cumulative exposure, rather than an all-or-nothing phenomenon, the avoidance of certain key allergens may lower an individual's allergic burden sufficiently to relieve or eliminate symptoms.

The value of a negative result should not be overlooked either. Many non-allergic conditions, such as vasomotor rhi-

nitis or infection, mimic allergic respiratory conditions. As many as two-thirds of patients who present with allergy-like symptoms test negative for specific IgE.^{22,23} For these non-allergic patients, the search for other etiologies should continue. A thorough diagnostic work-up can spare them unnecessary, ineffective, or costly treatment.

The clinical benefits of specific IgE testing

A definitive diagnosis of confirmed allergic disease offers several benefits for patients. First, specific IgE test results can be used to predict the course of atopic disease. IgE antibodies can be measured by three months of age, before clinical symptoms appear. Infants who develop food sensitization are much more likely than other children to later develop sensitivities toward inhalant allergens.^{24,25} So, back when little Mary's eczema was more acute, her pediatrician could have used the results of specific IgE blood testing to determine if it had a potentially avoidable food trigger as well as to predict if she was at risk for subsequent respiratory problems, such as allergic rhinitis and asthma.

Second, early intervention allows better management of allergic disease. Although the majority of children who wheeze with respiratory infections under three years of age are likely to outgrow this tendency, there is a subgroup of children who will have persistent asthma.²⁶ These at-risk children can be identified by their propensity for allergic sensitization.²⁷ Furthermore, observations of children with asthma suggest that treatment in the first five years of life is necessary to minimize progressive loss of lung function.²⁸ Clinical evidence suggests that early diagnosis followed by appropriate treatment may interrupt or ameliorate the progression of allergic disease.²⁹⁻³¹ Implementing avoidance, which can reduce the need for medications,³² is impractical if specific allergens have not been identified. If Mary's specific IgE results are positive, her pediatrician can use those results to recommend avoidance of specific inhalant allergens or use pharmacotherapy to prevent or treat symptoms.

Finally, negative results can prevent unnecessary trials of allergy medication, direct further diagnostic efforts, and spare the inconvenience of avoidance for patients who would not benefit. A negative specific IgE result for Mary would prompt her pediatrician to look for other causes of her symptoms and reassure her mother that she is at less risk of developing chronic childhood asthma. By providing an accurate definitive diagnosis, specific IgE testing supports early, appropriate, and targeted therapy, greater patient satisfaction, and better control of costs. □

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The progression of allergic disease

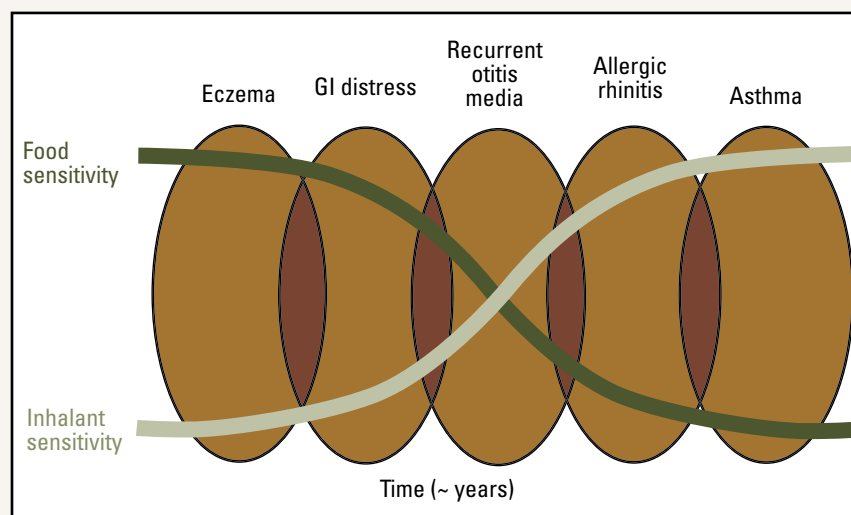
By Lee R. Choo-Kang, MD

Atopic children, probably genetically predisposed to allergic disease, develop symptoms in a predictable pattern known as the Allergy March. The formation of IgE antibodies begins early in life and sensitization can often be detected before clinical symptoms appear. Sensitization usually occurs first to food allergens, such as cow's milk, egg white, wheat, and soy. Sasai and associates demonstrated a positive predictive value of hen's egg antibodies in children as young as six months of age.¹ In the youngest patients, hypersensitivity to food allergens can manifest itself as atopic dermatitis, gastrointestinal symptoms (colic), chronic otitis, or rarely, wheezing.² These allergic conditions typically have their highest incidence at age two. In children younger than age three, atopy can be diagnosed with positive IgE results to food allergens and perennial inhalants such as dust mites, as sensitization to outdoor seasonal allergens rarely occurs before then. Even prior to the onset of wheezing, the significance of early atopy is important, as there is an inherent risk of later developing asthma. For example, young children who have atopic dermatitis and a positive family history of asthma have a 40% risk of later developing asthma.³ Nevertheless, it is important to recognize that food allergies themselves are not entirely the cause of later inhalant allergic sensitization but merely serve as a clinical marker in a child who is genetically predisposed for atopic disease.

In children older than three years, food sensitivities tend to decrease and sensitization to inhalant allergens typically increases during the preschool years.⁴ In most children with asthma, symptoms commence before age five. Sensitization to perennial allergens, such as house

dust mites, dog and cat dander, occurs before sensitization to seasonal allergens. Risk factors for development of allergic asthma include a family history of allergy, sensitization to food allergens, total serum IgE >100 kU/L before age six, living in an allergen-rich environment, and smoking.⁵ Understanding the progression of allergic disease lays the foundation for early intervention, a strategy shown to improve the long-term prognosis.⁶ □

Symptom manifestations of the Allergy March



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