Serological IgE Analyses in the Diagnostic Algorithm for Allergic Disease

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Overall Purpose/Goal: To provide excellent reviews on key aspects of allergic disease to those who research, treat, or manage allergic disease.

Target Audience: Physicians and researchers within the field of allergic disease.

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List of Design Committee Members: Robert G. Hamilton, PhD, and John Oppenheimer, MD

Activity Objectives

Learning objectives:

1. To apply pre-test probability to interpretation of laboratory measurements of allergen-specific IgE antibody.

2. To understand the potential for discordance in IgE antibody test results and clinical allergy.

3. To appreciate that tests for IgE antibody to individual allergenic proteins may improve diagnostic utility compared to extract-based assays.

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IgE antibody analyses using serological methods are an integral part of the diagnostic evaluation of a patient for allergic disease. They serve to clarify whether a state of sensitization exists in the patient as one of the many risk factors for elicitation of allergic symptoms. This overview examines the role that IgE antibody measurements play in the diagnostic algorithm when considering the pretest likelihood of disease on the basis of the patient's clinical history. Each of the 4 allergen groups (inhalants, venoms, drugs, and foods) are discussed in the context of the various *in vitro* and *in vivo* modalities for evaluating sensitization to allergens. Both the past and present analytical methods for IgE antibody detection and quantification in serum are critiqued. Causes for discordant IgE antibody levels with allergy symptoms are discussed with a special focus on analytically valid but clinically irrelevant positive IgE responses. Finally, applications are discussed where allergenic molecules provide enhanced analytical and diagnostic sensitivity and specificity when compared with results generated with allergen extract—based IgE assays. © 2015 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2015;3:833-40)

Key words: *IgE; Human; Radioimmunoassay; Immunosorbent allergen chip; Microarray; Molecular allergen; Allergen extract; Component-resolved diagnosis; Pretest probability; Clinical history; Skin testing*

Diagnostic measurements of total and allergen-specific IgE level in serum were made possible in 1967 by the discovery that IgE is the reaginic "gatekeeper" of the immediate-type hypersensitivity response.[1-4] Until this time, provocation of the skin, the upper or lower airway, or the gastrointestinal tract with allergen was the only means by which a "state of sensitization" to an allergenic substance could be defined in humans. Today, allergen-specific IgE level, and to a lesser

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Abbreviations used
Bet v 1- Betula verrucosa *(birch) group 1 allergen (PR10 family)*
kUa/L- kilo unit of allergen-specific IgE per liter
PR10- group 10 pathogenesis-related protein family of allergens

extent total serum IgE level, is measured in the serum of patients who provide a history consistent with an allergic disease. A positive IgE antibody response marks an individual as sensitized, which is necessary, but not sufficient, to make the definitive diagnosis of allergic disease. The IgE antibody's concentration, strength of binding or affinity, specificity, and the percentage of specific IgE to total IgE all play a role in translating a humoral IgE response into a clinical symptom. An increased IgE antibody affinity for allergen, increased concentration of IgE antibody relative to that of the non—allergen-specific IgE, increased total serum IgE, and increased IgE antibody clonality or number of epitopes recognized by the IgE repertoire all lead to an increase in basophil and mast cell degranulation. This review will focus principally on serological measurements of allergen-specific IgE that are able to assess its concentration, specificity (clonality), and specific IgE to total IgE ratio. A perspective is provided on IgE antibody assay methods discussed by Adkinson and Hamilton[5] in the preceding chief complaint article and the subsequent critique by Aalberse and Aalberse,[6] which examines the future prospects of *in vitro* molecular allergology.

RELEVANCE OF IgE ANTIBODY MEASUREMENTS IN THE DIAGNOSTIC EVALUATION

What is the clinical relevance of an IgE antibody measurement in serum? At the outset, it is critical to emphasize a *golden rule* in allergy diagnostics, namely, that the detection of IgE antibody by a serological method or by its companion *in vivo* skin test assay simply identifies whether a state of sensitization (IgE antibody positivity) exists in a patient to a particular allergenic specificity.[7] IgE antibody results do not by themselves prove the existence of an allergic disease state. This fact is independent of whether the source of the allergen used in the analysis is a physiological extract or an allergenic molecule. Allergen-specific IgE antibody can be detected in the blood and skin in an individual who has no objective measure of clinically evident allergic symptoms. It is thus one of many potential risk factors as discussed in the case presented in this issue[7] for the development of an allergy. To minimize unnecessary or inappropriate testing or having to interpret misleading or confusing IgE antibody results, the selection of allergen specificities for testing should be guided by a clinical history of objective symptoms that are consistent with the diagnosis of allergic disease and a physical examination that objectively monitors the patient's indicated symptoms. The clinician then determines a pretest probability of having an allergic disease process on the basis of knowledge gained during collection of the patient's history by considering the strength of the allergen exposure, the type and location of reported allergic symptoms, temporal association of these symptoms with the allergen exposure, the reproducibility of the patient's reported symptoms, the patient's family history of atopy, age, and the presence of other atopic conditions.[8]

High pretest probability of allergic disease

In theory, if the pretest probability is high, then testing is unlikely to improve the probability assessment, and IgE antibody testing is not necessary; therefore, the decision should be to proceed to management (eg, avoidance, pharmacotherapy, or immunotherapy). In spite of a high pretest probability, verification of sensitization with IgE antibody testing is often performed because the potential risk of life-threatening anaphylaxis and/or the prospect of initiating a prolonged course of immunotherapy warrants confirmation. It can also be instructive for the patients to have confirmatory evidence of their sensitization to encourage institution of environmental control measures. Testing also establishes a baseline from which the monitoring of changes in IgE antibody levels over time is possible.

A positive IgE antibody result that is concordant with patient's history and/or a challenge test confirms "allergy" to a particular allergen or structurally similar (cross-reacting) allergen specificity. Such a test result is often labeled as a "true positive," while it should probably be more appropriately considered a "clinically relevant" test result. Likewise, a concordant negative IgE antibody result may support the exclusion of a clinical state of allergic disease. The concern arises with a discordant positive IgE antibody result that occurs in the absence of historical or objective symptoms from provocation testing. This test is often labeled as a "false positive" in reference to its discordant nature with a clinical diagnosis of allergic disease due to the absence of correlated clinical symptoms. However, when IgE antibody testing is performed in a highly regulated clinical immunology laboratory that performs daily quality control with a regulatory agency—cleared assay method, it may be more appropriate to refer to this measurement as a "clinically irrelevant" IgE antibody result. The important point is that IgE antibody, albeit often low in quantity (<0.35 kilo unit of allergen-specific IgE per liter [kUa/L]), has been accurately detected even when no allergic symptoms are reported or observed after a relevant allergen exposure. The IgE antibody measurement simply serves as a marker of "sensitization" in a patient who in this case has no concordant symptoms. The challenge for the clinician is to integrate all the potential risk factors, including this IgE antibody result, to derive an ultimate level of confidence in an interpretation that relies on the patient's history and physical examination as the final arbiter. The case study presented in this issue illustrates this process.[5]

Moderate to low pretest probability of allergic disease

The IgE antibody analysis may have its most useful application when the pretest likelihood of allergic disease is moderate to low. If the history fails to suggest a strong or clear possibility of an allergic disease process, then the performance of an IgE antibody serological evaluation will largely depend on the mode of allergen exposure, disease type indicated, and allergen specificities suspected.

APPLICATION OF SPECIFIC IgE ANTIBODY TESTING TO DIFFERENT ALLERGIC DISEASES

Aeroallergen inhalation-induced disease

Diagnosis of aeroallergen-driven allergic disease begins by historically probing the chronicity and seasonality of nasal and respiratory symptoms. This is followed by a physical examination, with attention to the upper and lower respiratory tract. Although the detection of IgE antibody by skin testing has been