Testing for Food Allergens for Clinicians

BACKGROUND

Food allergies are a subset of adverse food reactions that are increasingly common, potentially fatal, and have a major impact on the lives of patients and their families.[1,2,3] More than 170 foods have been associated with food allergies, although the most common of these are milk, egg, peanut, tree nuts, crustacean shellfish, fish, wheat, soybeans, and sesame.[3,4,5,6] These allergic reactions are caused by the release of inflammatory mediators from degranulating mast cells and basophils. For degranulation to occur, cells have to be initially sensitized by the binding of allergen specific IgE (sIgE) to the high affinity receptors (FcεRI) on cell surfaces. Following re-exposure to allergens, allergens bind to sIgE and hence crosslink the FcεRI to induce degranulation. Symptoms are rapid in onset, typically occurring within minutes to a few hours of ingestion. Although some reactions can be mild and manifest themselves in a single system, such as urticaria in the case of cutaneous manifestations, reactions can also be severe and involve multiple systems, and in the case of anaphylaxis, can lead to life-threatening hypotension, cardiovascular collapse, and death.[5,7] Guided by patient's clinical history, testing for food allergy initially starts with skin prick testing (SPT) and the detection of allergen specific-IgE in serum.[8] If SPT and allergen sIgE results are inconclusive, oral food challenges (OFC), which is the gold standard for the diagnosis of food allergy, can be performed to determine if patients are sensitized-tolerant or sensitized allergic to suspected allergens. As OFC can be associated with severe and potentially fatal reactions, there is increased utilization and reliance on basophil activation tests (BAT) as third-line tests.[3,8,9,10]

Skin prick testing is performed in a provider's office and involves pricking or scratching the skin with a very small amount of allergen extracts or components. Histamine and saline are typically used as positive and negative controls, respectively. The test is considered positive if a wheal is formed, although the size of the wheal does not necessarily correlate with severity of the allergy.[11] The advantage of SPT is that the results are generally immediate (within 15-30 minutes), and are potentially more sensitive, albeit less specific, than serum sIgE tests.[1,5,11] However, the patient has to be free of antihistamines or other allergy medications in the days leading up to the test, which can be problematic in patients with severe allergies, and there is also a small risk of systemic allergic reaction. Allergen sIgE testing, on the other hand, requires a blood draw but does not require the patient to discontinue anti-allergic medications.[11] Compared with SPT, results are not immediate, as they need to be processed and tested in a clinical lab. A generalized approach to food allergy evaluation is shown in **Figure 1**.

Figure 1. Generalized approach to food allergy evaluation

Testing for serum allergen sIgE is performed using immunoassays of various methodologies. Examples include enzyme-linked immunosorbent essays (ELISAs), fluorescent enzyme immunoassays (FEIA), chemiluminescent assays, and immunoblots. Commercial FEIA platforms are widely used by clinical laboratories.[5] In these assays, allergens are bound to activated cellulose foam allergosorbent material. If allergen sIgE is present in patient serum, it will bind to the allergen antigens. After washing steps, enzyme-conjugated anti-human IgE secondary antibodies are added and incubated. A substrate (development solution) is then added resulting in the generation of fluorescence proportional to the quantity of bound allergen sIgE. Measured fluorescence signals are interpolated to a calibration curve to obtain sIgE concentration (**Figure 2**).

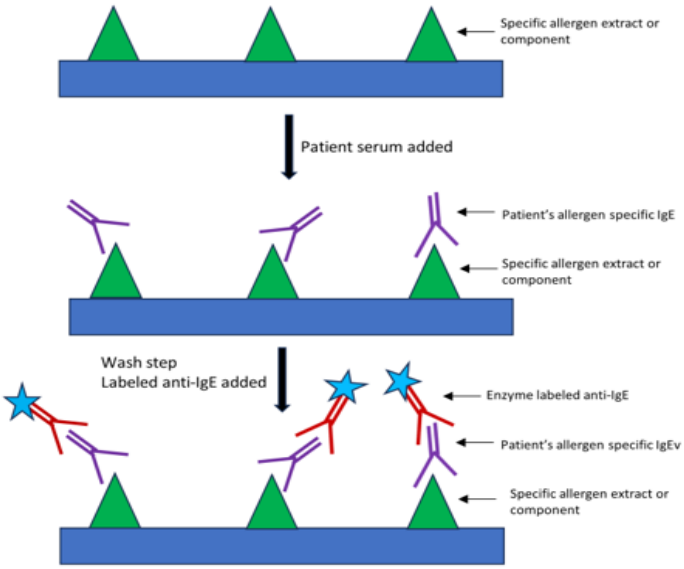

Figure 2. Traditional solid phase specific allergen assay for detecting serum sIgE

Serum sIgE based food allergen testing has high sensitivity and negative predictive value but somewhat low specificity.[8,13,14] It is important to understand that several factors translate the presence of sIgE to allergic responses, including properties of the IgE antibodies (avidity, affinity, ratio specific to total IgE), interindividual variability in cell surface expression of the high-affinity FcεRI receptors and downstream intracellular signaling pathways.[15] A positive sIgE result (ie, sensitization) therefore does not necessarily indicate that the patient has a clinical food allergy. The level of positivity also does not correlate with severity of reactions. This emphasizes that serum sIgE based testing should be only performed in patients with clinical history suggestive of IgE-mediated food allergy.

Food allergen screening panels are a feature of many laboratory test menus. Panels can be designed for screening to include common food allergens, or by groups and categories of allergens (eg, tree nuts, meats, and fish). A major disadvantage of such panels is that they can lead to over testing, misinterpretation of results and unnecessary dietary restrictions, especially in patients with intolerance symptoms due to non-IgE mediated reactions.[2,16] As previously stated, testing for sIgE should be guided by clinical history. Panels however can be appropriate in some situations; for instance, in the case of a confirmed shrimp allergy, a larger panel including other shellfish may be helpful due to cross-reactivity.[1]

Component-resolved diagnostics (CRD) refers to the utilization of purified native or recombinant major allergenic proteins to detect serum specific-IgE against the individual allergenic molecules.[17] Components are more standardized than whole allergen extracts used for serology and SPT. Since components are present in higher concentrations in assay allergosorbent materials than in whole allergen extracts, they significantly improve the analytical sensitivity and specificity for sIgE detection **(Figure 3).**[18] Testing for individual components can also allow working up cross reactions between different foods and can be helpful in risk stratification of patients (eg, eligibility for

OFC and immunotherapy; **Figure 3**). As an example, detection of high sIgE concentrations to the storage protein Ara h2 provides high diagnostic accuracy in patients with suspected peanut allergy. An expanding menu of components are now available for clinical validation and testing.[10,14,18] CRD can be offered as standalone assays or in panels in combination or reflexive to detectable sIgE to whole allergen extracts.

Figure 3. Component resolved diagnostics in allergy diagnosis

Alpha-Gal syndrome (AGS) is allergy to red meat that nowadays is increasingly diagnosed by clinicians. This is partially driven by the availability of component testing for sIgE to the culprit allergen, alpha-Gal (galactose-alpha-1,3-galactose) glycans. Alpha-Gal is present in mammalian red meat (eg, pork, beef and lamb) and derived products, including gelatins in food and vaccines. It is now established that Lone star ticks (*Amblyomma americanum*) play the central role in AGS pathogenesis. Patients can develop AGS symptoms 1-3 months after exposure to tick bites. It is understood that tick saliva contains alpha-Gal, which in addition to the local and systemic inflammation effects induced by tick bites, can promote sIgE response to alpha-Gal. sIgE to alpha-gal then sensitizes effector cells (basophils and mast cells) and on re-exposure to alpha-gal present in consumed red meat, can lead to severe allergy symptoms. Interestingly, detection rates and positivity for serum alpha-Gal sIgE is higher in the Midwest and Southeastern regions of the country, matching the known geographic distribution of Lone star ticks. In a key publication that established our understanding of AGS, unusual clusters of hypersensitivity cases to Cetuximab (anti-EGFR monoclonal therapeutic) reported in Southeast US states were attributed to the presence of alpha-Gal-specific IgE in treated patients and the presence of alpha-Gal moieties on the Fab portion of the antibody molecules.[19] One difference to note between AGS and typical food allergies is the delayed onset of symptoms (2-6 hours) after ingestion of red meat. This has been explained by the time needed for the digestion of glycolipids rich in alpha-Gal moeiteis.[10,14]

There is no scientifically proven role for IgG-based testing in the diagnosis of food allergies.[12] The American (AAAAI), Canadian (CSACI), and European (EAACI) allergy and immunology societies have put out statements or communications against specific IgG testing for the diagnosis of food allergies.[20,21,22]

INSIGHTS

1. Serum sIgE testing for food allergies is extensively available but should be limited to targeted testing based on clinical history.
2. IgG-based food allergen testing is of no known value to the diagnosis of food allergies.
3. Component-resolved testing to specific food proteins can be useful in some situations.

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