ALLERGY TESTING (AND OTHER HOCUS POCUS)

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Case 1:
Recently transferred to your practice.
• 2 year old AA male with eczema
• Concerns that food allergy might be a trigger
• Request allergy evaluation
• Prior labs show multiple sensitizations

Case 1: What do you advise?

a) Repeat serological testing?
b) Referral to nutrition due to multiple food allergy and restrictive diet?
c) Screen for additional sensitizations with IgG testing?
d) Referral to allergist for skin prick testing?
e) Provide parents with epinephrine and advise them to continue with dietary restrictions?
f) A & E?
• “...treat the patient not the labs...”

- Somebody

slgE Testing Caveats

What you need to know about the new guidelines for the diagnosis and management of food allergy in the U.S.

Overview
• The Guidelines, sponsored by the NIAID and the AAP, are based on expert opinion and a comprehensive literature review. A code of conduct on the use of evidence has been developed by the AAP in consultation with the National Institute of Allergy and Infectious Diseases. The complete Guidelines are available in the AAP's Clinical Practice Guidelines Database.

Definitions
• Food allergy was defined as an adverse health effect occurring from a specific immune response.
• Food allergic reaction is defined as a reaction that is associated with the ingestion of a food allergen.

- Data for food-specific IgE are needed but are not available to aid in diagnosis, but should be collected in future studies to aid in diagnosis.
- Food allergy testing, such as skin tests and blood tests, can be used to identify food allergies.
- Interpretation of test results is based on the clinical presentation of the patient.

For more information, please visit the AAP's website.
**Allergy**

- Inappropriate Immune Response
- Hypersensitivity reaction (Gel Coombs Classifications)
  - Type I: "Classical Allergy"
    - Short Onset
    - Atopy
    - IgE Bound to Mast cells
  - Type II: Antibody Dependent/Cytotoxic
    - IgG/IgM, MAC, Complement
    - Antibody binds to cell
  - Type III: Immune Complex
    - IgG, Neutrophils
  - Type IV: Delayed
    - Cell Mediated

**IgE Allergy**

- Allergic Reactions
  - Exaggerated or inappropriate immune response
  - Typically IgE & histamine mediated
    - Immediate
    - Onset typically within 30 minutes
  - Due to protein or allergen

**Types of Adverse Food Reactions**

- Immune Mediated Reactions
- Organic Disease
- Metabolic
- Non-Immune Mediated Reactions
- Non-IgE
- Infections
- PKU
- Fructosemia
- GERD
- Achalasia
- Hiatal Hernia

- Infectious Food Poisoning
- Lactase Deficiency
- Metabolic
- Organic Disease
- Non-IgE
IgE testing

- Misconceptions
  - All parameters of testing for all types of IgE-mediated allergy are not equal
    - Sensitivity, specificity, predictive values vary
    - Differ for types of reactions
      - Food, drug, insect allergy, aeroallergens
    - Differ for techniques
      - Skin prick, sIgE, intradermal, challenge
    - Age at which testing is valid varies
  - Sensitization does not equal clinical reactivity.

IgE Testing Misconceptions

- Food allergy: significant literature about sensitivity, specificity, predictive values etc.
  - Valid testing well under 3 months of age
  - Intradermal testing is contraindicated
- Drug allergy:
  - Significant information on penicillin testing
  - Other drug testing (serum and skin prick) less well validated
- Insect allergy:
  - Significant information on negative predictive value with skin prick and intradermal testing
  - Serum testing not very sensitive
- Aeroallergens
  - Paucity of information of clinical correlation with positive skin prick, intradermal, serum testing.

IgE Allergy

- Sensitization
  - Production of relevant IgE antibody to allergen, not necessarily indicative of clinical reactions
- Spectrum of Clinical Reactions
  - Localized Reactions
    - Oral Allergy Syndrome
    - Contact Reactions
  - Organ Specific Reactions
    - Eczema
    - Eosinophilic Esophagitis
  - Anaphylaxis
    - Multiple organ systems involvement

Sensitization does not equal clinical allergy!
IgE Allergy Testing

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Diagnostic of IgE Food Allergy</th>
<th>Demonstration of IgE Sensitization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin prick testing</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>• Scratch or percutaneous testing</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Intradermal</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Serological testing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• sIgE (specific IgE)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>• RAST, EIA, Immunocap</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>• Component testing</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>• Microarray</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Clinical testing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food Challenge</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Serum IgG</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Atopic Patch testing</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>ALCAT (antigen leukocyte antibody test)</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

sIgE testing

Scratch/Pinch Testing
- In vitro
- Histamine release from cross-linked IgE bound on mast cells

Serological Testing
- In vivo
- Free serum, unbound IgE molecules collected and measured in assay

IgE Allergy Testing

- Sensitivity
  - True positive rate
    - Number of who test positive divided by the number of truly positive individuals
    - A highly sensitive test has few false negatives
  - Specificity
    - True negative rate
      - Number of who test negative divided by the number of truly negative individuals
      - High specific test has few false positives
  - Positive Predictive Value
    - Number of truly positive individuals results in the total number of test positive.
  - False Positive
    - Incorrect positive result for an individual who does not have clinical symptoms.
In vitro IgE allergy testing

Detection and Count

1 2

Plate for Peanut

IgE in vitro Antibody testing

At normal total IgE level, how many keys?

IgE in vitro Antibody testing

At high total IgE level, how many keys?
IgE in vitro testing

Prescott, et al.
- 49 kids, less than 5 yrs old
- Australia
- Determine sensitization vs clinical reaction.

Skin prick testing and peanut-specific IgE can predict peanut challenge outcomes in preschool children with peanut sensitization.

Clinical II Experimental Allergy

- Skin prick and sIgE curves based on graded food challenges
- Probability curves created from graded challenges.
IgE Allergy

• sIgE (Specific Immunoglobulin E)
• Clinical cutoffs
• Optimal Decision Points
• Positive predictive values
• Used to trend resolution over time

IgE Allergy Testing

• Clinical cutoffs for likelihood of reactions
• Typically based on age and specific for each food
• Classes (I-VI) are arbitrarily assigned and have no clinical reference

sIgE Caveats:

Probability curves vary for food (allergen) to food (allergen).
The relationship is not linear (e.g., 5kU/L does not mean 5% risk of reaction)

Positive predictive value does not equal negative predictive value
IgE Allergy Testing

<table>
<thead>
<tr>
<th>Skin Prick testing</th>
<th>Pros</th>
<th>Cons</th>
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<tbody>
<tr>
<td></td>
<td>High negative predictive value (&lt;95%)</td>
<td>False positive (sensitized but not clinically reactive)</td>
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<tr>
<td></td>
<td>Fairly immediate results</td>
<td></td>
</tr>
<tr>
<td></td>
<td>May reagents react to component allergens, not to whole allergens</td>
<td>Can involve multiple scratches/picks</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serum IgE (sIgE)</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Numerical value to trend</td>
<td>False positive (sensitized but not clinically reactive)</td>
</tr>
<tr>
<td></td>
<td>Assess multiple allergens with one needle stick</td>
<td>Lower specificity than skin prick</td>
</tr>
<tr>
<td></td>
<td>Newer components assays reveal more detailed information</td>
<td>Conventional test measures whole allergens vs component</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Delayed reporting</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Results from various companies may not be equivalent</td>
</tr>
</tbody>
</table>

sIgE Caveats

- Skin and serum testing
  (Sicherer et al. Pediatrics 2011)

<table>
<thead>
<tr>
<th>Recommended Use</th>
<th>Not Advised</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ Confirm allergy to suspected trigger</td>
<td>General screen searching for allergies in symptom-free children</td>
</tr>
<tr>
<td>✓ Monitor course of established food allergies</td>
<td>Drug allergies</td>
</tr>
<tr>
<td>✓ Confirm insect allergy after anaphylaxis</td>
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</table>
IgE Allergy Testing

Component Testing

Milk Components

- **Casein**
  - nBos d 8
  - Heat stable and comprises 80% of the protein in cow's milk.

- **α-lactalbumin**
  - nBos d 4

- **β-lactoglobulin**
  - nBos d 5

- **Lactoferrin**
  - Bos d 6

Caseins* (nBos d 8)

Ovomucoid
- **Gal d 1**
- (11%)

Ovalbumin
- **Gal d 2**
- (54%)

Conalbumin
- **Gal d 3**
- (3.5%)

Lysozyme
- **Gal d 4**
- (3.4%)

Hen's Egg
- Over 20 different proteins

Egg White
- Ovomucoid: heat stable
- Ovalbumin: heat stable
- Conalbumin: heat labile
- Lysozyme: heat labile

Egg Yolk
- Lipovitelin, phospholipids
- IgG4 protein

Hen's egg: Over 20 different proteins

Egg white: Ovomucoid: heat stable
- Ovalbumin: heat stable
- Conalbumin: heat labile
- Lysozyme: heat labile

Egg yolk: Lipovitelin, phospholipids
- IgG4 protein

Which patient better candidate for tolerating boiled milk?

- Patient 1
- Patient 2

β-lac
- Casein
- α-lac

β-lactoglobulin (nBos d5)

Lactoferrin

α-lactalbumin (nBos d4)
IgE Allergy Testing
Component Testing

- Ara h 1, 2, 3 thought to be most relevant in food allergy in USA, many European countries and Asia. Ara h 2 especially.
- Ara h 6 found to correlate to food allergy in Asia.
- Ara h 9 dominant allergy in Spain.
- Ara h 8 is Bet V homologue.

Case 2:

IgE Allergy component Testing

Which patient better candidate for tolerating boiled milk?

Case 3:

IgE Allergy component Testing

Which patient more likely to tolerate peanut challenge?
Which patient is likely most sensitized to birch pollen?
IgE Allergy Component Testing

Ara h 2

Ara h/Bet v1

LTP

Component testing

Pairing for Systemic Reactions (Anaphylaxis)

Pairing for Localized, Contact Reactions (Oral Allergy Syndrome)

Cross Reactive Proteins Across Species

<table>
<thead>
<tr>
<th>Bet v homologues (Bet V homologues)</th>
<th>Almonds, apple, apricot, carrot, celery, cherry, coriander, fennel, hazelnuts, kiwi, nectarines, peaches, pears, plums, prunes, potatoes, peppers, parsley, peanuts, soy, &amp; peanut.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeds (ragweed/mugwort)</td>
<td>Apple, banana, carots, celery, chamomile tea, coriander, cucumber, dandelions, fennel, kiwi, melons (cantaloupe, honeydew, watermelon), peanut, parsley, sunflower, peppers, &amp; zucchini.</td>
</tr>
<tr>
<td>Poison Ivy/laurel (evergreen family)</td>
<td>Cashew, peanut, mango, &amp; plum.</td>
</tr>
<tr>
<td>Grasses</td>
<td>Apple, avocado, banana, celery, cherry, kiwi, melons, oranges, peaches &amp; tomatoes.</td>
</tr>
<tr>
<td>Dust mites (Impomycosis)</td>
<td>Cockroach, crab, fiddler, shrimp</td>
</tr>
</tbody>
</table>
IgE Allergy Component Testing

Worldwide variations in the proteins associated with anaphylaxis as well as the number of proteins patients were sensitized to.

Advantages

• More detailed information about relevant proteins
• Help identify candidates for desensitization or challenges

Disadvantages

• Can still be affected by high total IgE levels
• Sensitization patterns may vary based on geography/ethnicity
• Positive predictive clinical thresholds not yet establish

sIgE Caveats
**sIgE Testing Caveats**

- Clinicians and laboratories should be aware of inherent problems with currently available allergen-specific sIgE tests. Following pre-market review, FDA allows these tests on the market but suggests that manufacturers and distributors include limitations in the labeling (package insert) that accompanies each test kit to the clinical laboratories/physicians.

- A definite clinical diagnosis should not be made solely on the basis of an in vitro allergen-specific sIgE result. Diagnosis should be made by the physician only after all clinical and laboratory findings have been evaluated.

- False positive test results in persons who are tested for food allergies may lead to inappropriate dietary restrictions while false negative results in food sensitive persons may result in anaphylactic reactions of varying severity.

- Identical results for different allergens may not be associated with clinically equivalent manifestations, due to differences in patient sensitivities and sIgE binding capacities.

- The results of an allergen-specific sIgE antibody test should not be used as a definitive guide to select an initial dose for immunotherapy. Prior to implementing such therapy, a skin test with the planned initial dilution of the immunotherapy solution should be performed to prove that the patient tolerates in vivo administration of this allergenic extract.

- Very low levels of allergen-specific sIgE antibodies should be evaluated with caution when total sIgE values are above 1000 kU/L.

- A positive result may be due to cross-reactivity with other similar allergens and may not be specific. The user should be aware of the possibility of immune cross-reactivity within an allergen family.

- In food allergies, circulating sIgE antibodies may remain undetectable despite a convincing clinical history because these antibodies may be elicted toward allergens that are revealed or altered during industrial processing, cooking or digestion and therefore do not exist in the original food for which the patient is allergic.

- Latex specific sIgE antibodies may show cross-reactivity with ragweed and certain food allergens such as banana, avocado, kiwi and chestnut. Since a latex assay measures allergen-specific sIgE, type IV delayed reaction or irritation from latex will not be detected.

**FDA sIgE caveats**

- The clinician might consider food allergens as triggers of AD more commonly in young infants and children. The clinician should be aware that for children less than 5 years of age with moderate-to-severe AD, the Food Allergy Expert Panel suggested consideration of limited food allergy testing if the child has experienced AD in spite of optimized management and topical therapy, has child has a positive history of an immediate reaction to a specific food, or has a positive history of a delayed reaction to the same food.

- The clinician should not recommend extensive elimination diets based on positive skin or specific sIgE test results, because potential nutritional deficiency can occur, and even with multiple positive skin test results, most patients will react to few foods on oral challenge.

**When should patient have food allergy testing?**

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IgE Allergy Testing

- Take Home Points
  - Diagnosis of "allergy" is a clinical diagnosis, not laboratory.
  - Serum and skin prick testing is not diagnostic for allergy.
  - Sensitization ≠ Allergy
  - Cross reactions can occur.
  - Skin good at ruling out systemic IgE mediated allergy
  - Currently, serological assays good for trending or confirming.