Learning Objective

- Understand the value of allergy diagnostic testing in everyday practice
- Learn the advantages and disadvantages of in vivo and in vitro testing
- Be familiar with component testing; its value in allergy evaluation

Conflicts of Interest

- Advisory Board-Hycor

“Skin testing, aimless, excessive and misunderstood, has done more than its share in bringing allergy into disrepute.”

Introduction

- There is an explosion of allergy diagnostic testing in the US
- Use of in vitro specific IgE testing in increasing in primary care
- New testing—component testing will have a major impact on allergy diagnosis
- We as allergists are the authorities on diagnostic testing!

Sensitization ≠ Sensitivity

The practical value of allergy skin or blood tests rests in their ability to give accurate and consistent results when used as a confirmatory tool!
Which of the following effect prick skin test results?

- A. Location of the test
- B. Age of the patient
- C. Sun damage to the skin
- D. Proximity to the positive control or other allergens
- E. All of the above

Variables that affect Skin Test Results

- Age
  - Reactivity decreases with age; actually peaks in the late teens to early 20s and then decreases over time

- Histamine sensitivity
  - Inherent inborn sensitivity may increase or decrease skin test reactivity

- Location on the body
  - Which location [upper vs. lower back, forearm vs upper arm, and back vs arm] may vary with device
### Variables that affect Skin Test Results (cont)

- **Allergen immunotherapy**
  - Effective immunotherapy decreases skin test reaction to the treated allergen

- **Allergen extract quality**
  - Weaker extracts may produce false-negative results

- **Proximity to the positive control or other allergens**
  - By-stander effect — if an allergen extract is placed too close to a strong positive extract this may produce a false-positive result


### Variables that affect Skin Test Results (cont)

- **Medications**
  - Some can increase, eg, beta-blockers, and other can decrease skin test reactivity (eg, antihistamines and tricyclic antidepressants)

- **Other diseases**
  - Cancer may suppress skin test reactivity

- **Sun damage of skin**
  - Affects mast cell number and may explain at least some of the loss of skin test reactivity with aging along with decrease in IgE with age


### True or False

- Intradermal testing correlates well with clinical sensitivity to an inhalant allergen.

**Intradermal Tests for Inhalants are rarely helpful**
Skin Prick Test Results and Not Intradermal Skin Test Results Agree With Results of Nasal Challenge


<table>
<thead>
<tr>
<th>Skin Prick Test Results</th>
<th>Intradermal Skin Test Results</th>
<th>Percentage of allergic reactors to nasal challenge by skin test results</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.D.</td>
<td>+</td>
<td>11</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>11</td>
</tr>
<tr>
<td>+</td>
<td>N.O.</td>
<td>68</td>
</tr>
</tbody>
</table>

Predictive Value of Skin Prick Testing, Intradermal Skin Testing and RAST for Cat Allergy


<table>
<thead>
<tr>
<th></th>
<th>Skin Prick Test (95% CI)</th>
<th>Intradermal Skin Test (95% CI)</th>
<th>RAST (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>93.6 (+/-4.3)</td>
<td>60.0 (+/-15.3)</td>
<td>87.2 (+/-6.9)</td>
</tr>
<tr>
<td>Specificity</td>
<td>80.1 (+/-7.1)</td>
<td>32.3 (+/-14.7)</td>
<td>90.5 (+/-6.1)</td>
</tr>
<tr>
<td>Positive predictive value (%)</td>
<td>90.1 (+/-5.3)</td>
<td>12.0 (+/-10.2)</td>
<td>91.1 (+/-5.9)</td>
</tr>
<tr>
<td>Negative predictive value (%)</td>
<td>87.1 (+/-6.0)</td>
<td>84.6 (+/-11.3)</td>
<td>88.4 (+/-7.1)</td>
</tr>
<tr>
<td>Efficiency</td>
<td>9.6/0.08</td>
<td>0.9/1.2</td>
<td>9.4/0.14</td>
</tr>
</tbody>
</table>

Conclusion: SPT relate closely to disease, while ICT do not. (There are presently no available data in children or in allergens other than cat and grass)

SPT vs ICT

Comparison Using Evidenced Based Medicine

<table>
<thead>
<tr>
<th>Test</th>
<th>Allergen</th>
<th>+ Likelihood</th>
<th>- Likelihood</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPT</td>
<td>Cat</td>
<td>4.93</td>
<td>0.08</td>
</tr>
<tr>
<td>IDT</td>
<td>Cat</td>
<td>0.89</td>
<td>1.24</td>
</tr>
<tr>
<td>SPT</td>
<td>Grass</td>
<td>6.82</td>
<td>0.28</td>
</tr>
<tr>
<td>IDT</td>
<td>Grass</td>
<td>1.05</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Bottom line

- In inhalant ST, no published body of evidence favors routine use of a (large) predetermined panel of IDs when SPT is negative.
- Some modern evidence that a positive ID in the face of a negative SPT is not clinically relevant, in the case of grass and cat.
- Some consensus (but not evidence) that when there is a strong clinical history for sensitization, but the SPT is negative, an ID test might provide additional useful information.
**Points in Food Allergy Testing**

- Egg white is the major source of egg allergen
- Cow’s milk is all that is needed in testing for cow’s milk allergy
- When possible, fresh food prick tests are best
- There is no reliable validated IgE test for additives and colors

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**Foods tolerated should NOT be tested**

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**In Vitro Allergy Diagnostics**

- RAST-disc allergosorbent
- ELISA methods
  - ImmunoCAP (Thermo Fisher)
  - HyTec (Hycor)
  - Immunlite (Siemens)
- The antibody binds to the allergen on the solid phase
  - Enzyme anti-IgE detects bound IgE
  - All assays report in similar units (kUa/L) with comparable analytical sensitivities of 0.1 kUa/L
  - All assays primarily use allergens from extracts

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**True or False**

- The ALCAT has value in the detection of food allergy.
ALCAT

- ALCAT test does not detect IgE Food Allergy
- ALCAT detects IgG antibodies to foods and other substances
- According to the company: The ALCAT is the most effective Food Intolerance Test available

Be familiar with PPV for food allergen

When to Test/What to Test

IgE associated clinical disorder?  
Is testing for food allergy appropriate?  
Yes  
Determination of potential triggers  
• Requires careful history, consideration of epidemiology, pathophysiology  
• Foods tolerated (should not be tested)  
• Foods not often ingested, more likely triggers  
• Foods commonly associated with severe reactions:  
  • Peanut, nuts from trees, fish, shellfish, seeds  
  • Common allergens for children with moderate-severe atopic dermatitis:  
    • Eggs, milk, wheat, soy

No  
Alternative tests/advice

Selection of serological or skin tests  
• select tests to confirm/exclude suspicions  
• avoid “panels” of food allergens  
• avoid testing tolerated foods
Diagnosis of food allergies with the use of 95% PPV for s-IgE and SPT

<table>
<thead>
<tr>
<th>Food</th>
<th>95% PPV for s-IgE (kU/L)</th>
<th>95% PPV for SPT wheal diam.(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>Tree nuts</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>Milk</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>Egg</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Fish</td>
<td>20</td>
<td>7</td>
</tr>
</tbody>
</table>

Lack G. NEJM 2008;359:1252-60

Molecular-based Allergy Diagnostics

- This is an approach used to map the allergen sensitization of a patient at a molecular level, using purified natural or recombinant allergenic molecules (allergen components) instead of allergen extracts
- Resolving genuine versus cross-reactive sensitization in poly-sensitized patients, thereby improving the understanding of triggering allergens
- Assessing, in selected cases, the risk of severe, systemic versus mild, local reactions in food allergy, thereby reducing unnecessary anxiety for the patient and the need for food challenge testing
- Identifying patients and triggering allergens for specific immunotherapy (SIT).

CAP RAST levels and challenge results for peanut allergy (N=80)

<table>
<thead>
<tr>
<th>CAP RAST kU/L</th>
<th>% Pass Challenge</th>
<th>% Fail Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 5</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>0.35-2</td>
<td>52</td>
<td>48</td>
</tr>
<tr>
<td>≥0.35</td>
<td>73</td>
<td>27</td>
</tr>
</tbody>
</table>


Table 3: High- versus low-risk molecules from foods giving rise to anaphylaxis

<table>
<thead>
<tr>
<th>Source</th>
<th>High risk</th>
<th>Low risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut</td>
<td>Ara h 1, 2, 3, 9</td>
<td>Ara h 8, profilin, CCD</td>
</tr>
<tr>
<td>Hazelnut</td>
<td>Cor a 8, 9</td>
<td>Profilin, CCD</td>
</tr>
<tr>
<td>Walnut</td>
<td>Jug r 1, 2, 3</td>
<td>Profilin, CCD</td>
</tr>
<tr>
<td>Soy</td>
<td>Gil m 5, 6, 8 (4)</td>
<td>Profilin, CCD</td>
</tr>
<tr>
<td>Rosacea fruits</td>
<td>Pru p 3, Mal d 3</td>
<td>Pru p 1, Mal d 1, profilin, CCD</td>
</tr>
<tr>
<td>Wheat</td>
<td>Tri a 14, Tri a 19</td>
<td>Profilin, CCD</td>
</tr>
</tbody>
</table>

KEY: CCD = Cross-reactive Carbohydrate Determinant
Rule of Thumb

- Profilin and PR10 proteins
  - Highly cross reactive (PR 10 especially to Birch)
  - Often associated with less severe reactions e.g. OAS
- nsLTP’s and Storage Proteins
  - Associated with more severe reactions
  - More heat/digestive enzyme resistant and therefore can be more often associated with OAS and well as digestive problems

pathogenesis-related (PR)-10 protein family

Peanut

Peanut Allergen Components

| Ar a h 1 | Storage proteins |
| Ar a h 2 | Storage proteins |
| Ar a h 3 | Storage proteins |
| Ar a h 5 | Profilin |
| Ar a h 8 | PR-10 |
| Ar a h 9 | LTP |

- Ar a h 1-3 are the major peanut allergens

Which is the major allergen in egg which is a risk factor for persistent egg allergy, including reactions to cooked/heated egg?

- A. Gal d 1
- B. Gal d 3
- C. Tri a 14
- D. Tri a 18
- E. Bos d 8
Egg

- High levels of sIgE antibodies to ovomucoid (Gal d 1) have been identified as a risk factor for persistent egg allergy, including reactions to cooked/heated egg, while undetectable levels indicate tolerance to cooked egg.

Wheat

- Sensitization to ω-5-gliadin (Tri a 19) is a risk factor for immediate allergic reactions in children and for systemic exercise-induced reactions in adults.
- The wheat LTP (Tri a 14) shows some degree of cross-reactivity with other food LTPs, however more knowledge is needed about its prevalence and clinical implication.
- Patients with profilin or cross-reactive carbohydrate determinant (CCD) sensitization to wheat alone usually exhibit no, or local oral, symptoms and heated wheat may be tolerated.

Milk

- Casein (Bos d 8) and beta-lactoglobulin (Bos d 5) sIgE antibodies are markers of persistent allergy to milk, including heated milk.
- In milk allergic patients while undetectable levels indicate tolerance to baked milk products.
The only definitive test for food allergy is an oral food challenge.

Review of Pearls

- Sensitization ≠ Sensitivity
- Intradermal Tests for Inhalants are rarely helpful
- Foods tolerated should NOT be tested
- Be familiar with PPV for food allergens
- Rationale use of component testing
- The only definitive test for food allergy is oral food challenge