The Diagnosis of Food Allergy

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Disclosures

• Research Support
  • NIH
  • ITN
  • DBV

• Advisory Boards
  • Sanofi
  • Stallergenes
The Diagnosis of Food Allergy

- Detailed history
  - Food(s) suspected
  - Specific symptoms
  - Timing of symptoms
  - Reproducibility of reaction

- History may be diagnostic with some acute reactions but overall will be verified only 30 – 40% of the time with further testing (especially in AD and GI syndromes)

- High rate of false positive skin tests and RASTs (poor positive predictive value)
- High negative predictive value (for IgE-mediated syndromes)
- Must be carefully interpreted in the context of the clinical picture
- Oral challenges are the only definitive tests
Interpretation of Food Allergy Test Results

Tests should not be interpreted as just positive or negative but rather as the probability of indicating true food allergy.

CAP-RASTs for Food Allergy Diagnosis (Allergen-Specific IgE in KU/L, Range 0-100)

<table>
<thead>
<tr>
<th></th>
<th>Reaction highly probable (&gt;90%)</th>
<th>Overall</th>
<th>&lt;2 yrs</th>
<th>Challenge level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>15</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Egg</td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Peanut</td>
<td>14</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Tree nuts</td>
<td>10-15</td>
<td></td>
<td></td>
<td>2-5</td>
</tr>
<tr>
<td>Soy</td>
<td>60</td>
<td></td>
<td></td>
<td>10-20</td>
</tr>
<tr>
<td>Wheat</td>
<td>80</td>
<td></td>
<td></td>
<td>10-30</td>
</tr>
</tbody>
</table>
Diagnosis of Food Allergy: Oral Challenges

• May be open, single-blind, or double-blind placebo-controlled
• Only definitive test for food allergy
• Must be used if the history and lab results do not provide clear diagnosis
• Also used to determine when an allergy has been outgrown
• Must be done with considerable caution
### Current Techniques for the Diagnosis of Food Allergy

<table>
<thead>
<tr>
<th></th>
<th>Pro’s</th>
<th>Con’s</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Skin Tests</strong></td>
<td>Simple</td>
<td>POOR specificity</td>
</tr>
<tr>
<td></td>
<td>Cost effective</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sensitive</td>
<td></td>
</tr>
<tr>
<td><strong>Specific IgE</strong></td>
<td>Quantitative</td>
<td>Poor to Limited Specificity</td>
</tr>
<tr>
<td></td>
<td>Sensitive</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time Consuming</td>
</tr>
</tbody>
</table>

→ Better testing methods would be highly desirable

### Molecular Allergology: Component Resolved Diagnostics (CRD)

- **Traditional diagnostics**
- **CRD is designed to distinguish primary sensitization from cross-reactivity**

文化传播："Molecular Allergology: Component Resolved Diagnostics (CRD)"

- 分子过敏学：组件解析诊断（CRD）
- 传统诊断
- CRD旨在区分主要敏感性与交叉反应性

<table>
<thead>
<tr>
<th>Allergen source</th>
<th>Crude Allergenic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unique allergen molecules</td>
<td>Cross-reactive molecules</td>
</tr>
</tbody>
</table>
Component Resolved Diagnostics: Application to Clinical Practice

- Helps to differentiate true allergy from positive tests due to cross-reacting antibodies
- May provide a more accurate diagnosis and lessen the need for oral food challenges
- May help to predict reaction severity
- May help to predict natural history / persistence of allergy
- At present, mostly used as ancillary tests when the diagnosis is not clear based on the history, skin tests, and specific IgE results to help guide food challenge decisions

Specific component testing has been shown to potentially improve diagnostic precision for:

<table>
<thead>
<tr>
<th></th>
<th>Ara h 1, 2, 3 and 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut</td>
<td></td>
</tr>
<tr>
<td>Soy</td>
<td>Gly m 5 and 6</td>
</tr>
<tr>
<td>Wheat</td>
<td>Omega-5-gliadin</td>
</tr>
<tr>
<td>Milk</td>
<td>Bos d 8 (casein)</td>
</tr>
<tr>
<td>Egg</td>
<td>Gal d 1 (ovomucoid)</td>
</tr>
<tr>
<td>Hazelnut</td>
<td>Cor a 1, 8, 9 and 14</td>
</tr>
</tbody>
</table>
Importance of “Allergen Families”

- Polcalcins
- Ole e 1 related
- Pectate lyase
- Poligalacturonase

- Storage proteins
- nsLTP
- TLP

- Profilin
- Bet v1-homologues
- nsLTP
- TLP

- Profilin
- Beta-1,3-glucanase
- Hevein-like domain proteins

Luengo and Cardona Clin Trans Allergy 2014

Allergens of animal origin
Egg (*Gallus domesticus*) Allergens

- Gal d 1 Ovomucoid
- Gal d 2 Ovalbumin
- Gal d 3 Ovotransferrin
- Gal d 4 Lysozyme

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### Gal d 1 (Ovomucoid)

- 10% of total egg white protein
- Stable against to heat and digestion
- IgE to Gal d 1 potentially helpful in the diagnosis and prognosis of egg allergy
- High concentration of ovomucoid-sIgE associated with persistent egg allergy (raw or cooked egg)
- Low concentration of ovomucoid-sIgE associated with tolerance to heated egg
Egg (*Gallus domesticus*)

**Gal d 2 (ovalbumin)**

- Heat-labile, IgE - binging epitopes modified after heating → less allergenic
- Children who have sIgE primarily to Gal d 2 more likely to tolerate heated egg

“Ovomucoid is not superior to egg white testing in predicting tolerance to baked egg”
(Bartnikas et al JACI In Practice 2013)

- 1186 patients with h/o egg allergy
  - 109 eating egg
  - 202 eating baked egg
  - 875 avoiding all egg
    - 169 baked egg challenges 70 at home, 99 in clinic)
  - 84% passed
  - 16% failed
ROC Comparing Egg White IgE, Ovomucoid IgE, and Egg SPT

No difference in predicting baked egg OFC outcome

Bartnikas et al JACI In Practice 2013

Cow’s milk (Bos domesticus)

Table 1 Cow’s milk proteins

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein</th>
<th>Allergen name</th>
<th>Concentration (g/l)</th>
<th>Total proteins (%)</th>
<th>Molecular weight (kDa)</th>
<th>Amino acids</th>
<th>pl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caseins</td>
<td>αs1-casein</td>
<td>~30</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>αs2-casein</td>
<td>12-15</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>β-casein</td>
<td>3-4</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>γ-casein</td>
<td>9-11</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>κ-casein</td>
<td>1-2</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>κ-casein</td>
<td>3-4</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whey</td>
<td>α-lactalbumin</td>
<td>~5.0</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>β-lactoglobulin</td>
<td>1-1.5</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Immunoglobulin</td>
<td>Bos d 7</td>
<td>0.6-1.0</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BSA</td>
<td>Bos d 6</td>
<td>0.1-0.4</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lactoferrin</td>
<td>~0.09</td>
<td>Traces</td>
<td>703</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Caseins= 80% of total protein
Suggested that casein IgE valuable in predicting baked milk OFCs

Curr Opin Allergy Clin Immunol 11: 216-221
ROC’s for Cow’s Milk, Beta-lactoglobulin, and Casein IgE Predicting Baked Milk OFC (N=225)

No difference in predicting baked milk OFC outcome

Allergens of plant origin
Allergenic components in soy: *Gly m 4, 5 and 6*

- **Gly m 4**
  - PR-10 protein family (Bet v 1 homologue)
  - pollen-associated soy allergy (primary sensitization to birch pollen)
  - cross-reactive to *Ara h 8*
  - In the U.S. and Europe approximately 2/3 of patients with true soy allergy are allergic to peanut

- **Gly m 5 and 6**
  - most important in primary sensitization to soy protein
  - sensitization rate to
    - *Gly m 5* 36%
    - *Gly m 6* 43%
  - *Gly m 5* and *Gly m 6* are potential markers for more severe soy reactivity
Wheat (*Triticum aestivum*)

- *Tri a 19 (GJ-5 gliadin)*
  - associated with risk of IgE-mediated reaction to wheat
  - risk of wheat-dependent exercise induce anaphylaxis

Hazelnut

- Most sensitivity to hazelnut is directed toward Cor a 1, a heat labile protein that is homologous with the major birch pollen allergen, Bet v 1
- The hazelnut ImmunoCAP has supplemental Cor a 1, frequently leading to erroneously high test results
- Systemic reactions to hazelnut are generally mediated by IgE to Cor a 8, 9 and 14
- Assays for Cor a 9 and 14 are not currently available
### Allergenic proteins in peanut

<table>
<thead>
<tr>
<th>Protein</th>
<th>Plant Family</th>
<th>Clinical Implication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ara h 1</td>
<td>Vicilin [7S globulin]</td>
<td>Correlated with reactivity</td>
</tr>
<tr>
<td>Ara h 2</td>
<td>Conglutin [2S albumin]</td>
<td>Best correlation with reactivity / ? severity</td>
</tr>
<tr>
<td>Ara h 5</td>
<td>Profilin [Bet v 2-like]</td>
<td>Not correlated with reactivity</td>
</tr>
<tr>
<td>Ara h 6</td>
<td>Conglutin [Ara h 2 homologue]</td>
<td>Correlated with reactivity</td>
</tr>
<tr>
<td>Ara h 7</td>
<td>Conglutin</td>
<td></td>
</tr>
<tr>
<td>Ara h 8</td>
<td>Bet v 1-like</td>
<td>Not correlated with significant reactivity</td>
</tr>
<tr>
<td>Ara h 9</td>
<td>Non-specific lipid transfer protein</td>
<td>Correlated with reactivity in some studies</td>
</tr>
<tr>
<td>Ara H 10,11</td>
<td>Oleosin</td>
<td></td>
</tr>
</tbody>
</table>

### Examples of studies of peanut food challenge (or convincing clinical reactions) against serum Ara h 2 IgE levels

- Nicolaou 2011, English birth cohort
- N = 81, median age 8 yrs
- Ara h 2 cut-off 0.35 kU/l
  - Sensitivity 1.0
  - Specificity 0.96
  - Likelihood ratio 25
Examples of Ara h 2 studies - continued

- Dang 2012, Australian population based study
- N = 158, median age 14 months
- Ara h 2 cut-off 0.1 kU/l
  - Sensitivity 0.81
  - Specificity 0.88
  - Likelihood ratio 6.7
- Ara h 2 cut-off 3.5 kU/l
  - Sensitivity 0.43
  - Specificity 1.0
  - Likelihood ratio $\infty$

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Food, drug, insect sting allergy, and anaphylaxis

**Increasing the accuracy of peanut allergy diagnosis by using Ara h 2**

![Graph showing the accuracy of peanut allergy diagnosis using Ara h 2](image)

- 98% specificity - ~60% sensitivity
- 98% specificity - <50% sensitivity
- 98% specificity - <30% sensitivity
Examples of Ara h 2 studies - continued

- Keet 2013, U.S. referral population
- N = 60, median age 7 years
- 43% with reaction history, selected for challenge based on relatively low PN-IgE results
- Ara h 2 cut-off 0.25 kU/l
  - PPV 0.7
  - NPV 0.89
  - Likelihood ratio 3.1
- Ara h 2 cut-off 2.0 kU/l
  - PPV 0.75
  - NPV 0.62
  - Likelihood ratio 4.1

Summary of Ara h 2 Results

- Optimal sensitivity and specificity between 0.1 and 0.7 kU/l
- Successful challenge unlikely with levels >2 kU/l (>90 – 95% PPV)
- All results and decisions need to be based on the individual patient profile
- Component testing is not needed for the vast majority of patients with peanut allergy

Clinical Pearls for Diagnosing Peanut Allergy

<table>
<thead>
<tr>
<th>Key Observation</th>
<th>Diagnostic Implication</th>
<th>Caveats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ara h 2 may provide stronger predictive accuracy.</td>
<td>May add diagnostic accuracy if PN-IgE and additional clinical data are not already predictive</td>
<td>Some patients have severe peanut reactions with low or negative Ara h 2</td>
</tr>
<tr>
<td>Binding to Ara H 8 without binding to Ara h 1-3 is usually associated with no or mild reactions.</td>
<td>Component testing may identify individuals with low risk of systemic reactions.</td>
<td>A small subset of patients have systemic reactions despite this profile.</td>
</tr>
<tr>
<td>Persons with isolated Ara H 8 sensitization typically have lower PN-IgE and are birch sensitized.</td>
<td>There may be little utility in testing components when PN-IgE &gt; 25 kU/L, or when it is known that there is no sensitization to birch pollen.</td>
<td></td>
</tr>
<tr>
<td>Diagnostic accuracy and reduced need for OFC can be achieved by combination or serial use of tests.</td>
<td>Pre and post-test probability calculations may be used to incorporate component tests to increase diagnostic accuracy.</td>
<td>Diagnostic with indeterminate history / SPT or history / PN-IgE may be improved with adding component tests</td>
</tr>
</tbody>
</table>

Sicherer and Wood, JACI In Practice 2013
Component Resolved Diagnostics: Application to Clinical Practice

- Case 1:
  - 8 year old, mild eczema, seasonal allergic rhinitis
  - History of vomiting and urticaria to peanut at age 2, no known exposures since then
  - Peanut skin test 8 mm, peanut IgE 9.6 kU/l
  - Peanut challenge → anaphylaxis

- Case 2:
  - 12 year old, asthma, seasonal allergic rhinitis
  - Avoids all peanut, carries epinephrine, because she is “allergic” based on previous testing
  - Peanut skin test 8 mm, peanut IgE 18.6 kU/l
  - Peanut challenge → negative

Reassessment with ImmunoCAP Allergen Components:

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut</td>
<td>9.6</td>
<td>18.6</td>
</tr>
<tr>
<td>Ara h 1</td>
<td>2.3</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Ara h 2</td>
<td>8.5</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Ara h 3</td>
<td>3.0</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Ara h 8</td>
<td>0.6</td>
<td>21.0</td>
</tr>
</tbody>
</table>

Case 1: Ara h 1, Ara h 2 and Ara h 3 – storage proteins
Results indicate persistent allergy with an increased risk of severe reaction

Case 2: Ara h 8 – PR-10 protein
Test results indicate cross-reactivity, possible pollen-related food allergy
Case 3:

- 3 year old, moderate to severe eczema, asthma
- Strictly avoiding milk and peanut based on prior testing, no reaction history
- Test results:

<table>
<thead>
<tr>
<th></th>
<th>Age 12 months</th>
<th>Current</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut PST</td>
<td>8 mm</td>
<td>8 mm</td>
</tr>
<tr>
<td>Peanut IgE</td>
<td>3.2 kU/l</td>
<td>8.1 kU/l</td>
</tr>
<tr>
<td>Milk PST</td>
<td>8 mm</td>
<td>6 mm</td>
</tr>
<tr>
<td>Milk IgE</td>
<td>17.6 kU/l</td>
<td>3.5 kU/l</td>
</tr>
</tbody>
</table>

Further Assessment at Age 3 with Components:

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ara h 1</td>
<td>2.3</td>
</tr>
<tr>
<td>Ara h 2</td>
<td>8.2</td>
</tr>
<tr>
<td>Ara h 3</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Ara h 8</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Casein</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Recall that between age 1 and 3, the peanut IgE rose from 3.2 to 8.1 kU/l and the milk IgE decreased from 17.6 to 3.5

Interpretation:

- Rise in peanut IgE suggests true peanut allergy and the positive Ara h 2 confirms this impression; no challenge is recommended
- Fall in milk IgE suggests favorable prognosis for tolerating baked milk. The casein value did not add value to the assessment