Starting an Appropriate Immune Evaluation

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Disclosures

- Speaker’s bureau
  - Genentech, AstraZeneca, Regeneron, GSK, CSL Behring
Overview

- Presentation of immunodeficiency
- Laboratory evaluation of immunodeficiency
  - Humoral immunity
- Cases

Awareness

- The National Institutes of Health (NIH) estimate 1 out of every 3 individuals with PIDD are properly diagnosed
- The NIH estimates more than 500,000 individuals remain undiagnosed in the U.S. alone
- Early diagnosis and treatment universally shown to decrease morbidity and mortality

Red Flags for Immunodeficiency

Ten warning signs of PID [11]

1. ≥8 New ear infections within 1 y
2. ≥2 Serious sinus infections within 1 y
3. ≥2 mo on antibiotics with little effect
4. ≥2 Episodes of pneumonia within 1 y
5. Failure of an infant to gain weight or grow normally
6. Recurrent deep skin or organ abscesses
7. Persistent thrush in mouth or on skin after age 1 y
8. Need IV antibiotics to clear infections
9. ≥2 Deep-seated infections
10. Family history of PID

Clinical Presentation

Screening for Immunodeficiency

### TABLE 2: Serum Immunoglobulin Levels in the Study Population

<table>
<thead>
<tr>
<th>Type</th>
<th>Total Number of Patients</th>
<th>Median (mg/dL)</th>
<th>IQ Range (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG 0-0.9 mg/dL</td>
<td>500</td>
<td>541</td>
<td>484-5122</td>
</tr>
<tr>
<td>IgG 0.9-1.9 mg/dL</td>
<td>27</td>
<td>598</td>
<td>460-603</td>
</tr>
<tr>
<td>IgG 1.9-2.9 mg/dL</td>
<td>100</td>
<td>218</td>
<td>164-262</td>
</tr>
<tr>
<td>IgG 2.9-3.9 mg/dL</td>
<td>8</td>
<td>96</td>
<td>84-108</td>
</tr>
<tr>
<td>IgG 3.9-5.9 mg/dL</td>
<td>100</td>
<td>35.5</td>
<td>22-41.4</td>
</tr>
<tr>
<td>IgG 5.9-7.9 mg/dL</td>
<td>23</td>
<td>58</td>
<td>39-74.8</td>
</tr>
</tbody>
</table>

### TABLE 3: Serum Immunoglobulin Levels in Patients with Diagnosis of Antibody Deficiency

<table>
<thead>
<tr>
<th>Age in Years</th>
<th>IgG (70-100 mg/dL)</th>
<th>IgG (50-70 mg/dL)</th>
<th>IgG (15-50 mg/dL)</th>
<th>Diagnosis</th>
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</thead>
<tbody>
<tr>
<td>75</td>
<td>3.516</td>
<td>8</td>
<td>18</td>
<td>Multiple myeloma</td>
</tr>
<tr>
<td>77</td>
<td>254</td>
<td>22</td>
<td>10</td>
<td>Selective antibody deficiency</td>
</tr>
<tr>
<td>56</td>
<td>682</td>
<td>54</td>
<td>42</td>
<td>Selective antibody deficiency</td>
</tr>
<tr>
<td>96</td>
<td>337</td>
<td>79</td>
<td>77</td>
<td>Selective antibody deficiency</td>
</tr>
<tr>
<td>56</td>
<td>0.031</td>
<td>102</td>
<td>&lt;18</td>
<td>Selective Ig deficiency</td>
</tr>
</tbody>
</table>

Overview of Laboratory Testing

### TABLE IV: Laboratory tests of immune function

<table>
<thead>
<tr>
<th>Screening tests</th>
<th>Advanced tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humoral immunity</td>
<td>Flow cytometry to enumerate B-cell subsets (eg. naive and switched memory cells)</td>
</tr>
<tr>
<td>Serum immunoglobulin levels</td>
<td>by flow immunoglobulin production in response to mitogens or other stimuli</td>
</tr>
<tr>
<td>Serum specific antibody titers</td>
<td>Antibody response to immunization with antigen</td>
</tr>
<tr>
<td>Autoimmune response to boostersimmunization</td>
<td></td>
</tr>
<tr>
<td>Cellular immunity</td>
<td>Flow cytometry to enumerate T-cell subsets (eg. naive, memory, and activated cells)</td>
</tr>
<tr>
<td>Th1 response screening</td>
<td>by in vitro proliferative response to mitogens and antigens</td>
</tr>
<tr>
<td>Flow cytometry to enumerate CD4 and CD8 T cells and NK cells</td>
<td>T-cell cytotoxicity</td>
</tr>
<tr>
<td>Cytotoxicity against mitogens and antigens</td>
<td></td>
</tr>
<tr>
<td>Spontaneous NK cytotoxicity</td>
<td>Flow cytometry to enumerate surface expression and cytokine production in response to stimuli</td>
</tr>
<tr>
<td>Phenotypic cells</td>
<td>Cytotoxic protein phosphorylation in response to stimuli</td>
</tr>
<tr>
<td>Blood count with differential cells</td>
<td>Chemotaxis and/or phagocytosis assays</td>
</tr>
<tr>
<td>Neutrophil staining, morphology on peripheral blood smear</td>
<td>Enzyme assays (cytochrome c oxidase, G6PDH)</td>
</tr>
<tr>
<td>DR3 reduction in soluble tetramer</td>
<td>WBC count</td>
</tr>
<tr>
<td>Flow cytometry for adhesion molecules</td>
<td>Bacterial or fungal killing</td>
</tr>
<tr>
<td>Complement</td>
<td>Bone marrow biopsy</td>
</tr>
<tr>
<td>C5b9 assay (total hemolytic complement activity)</td>
<td>Level of function of individual complement components</td>
</tr>
<tr>
<td>MPS assay (alternative pathway hemolytic activity)</td>
<td></td>
</tr>
<tr>
<td>Lytic pathway function</td>
<td>Genetic tests</td>
</tr>
<tr>
<td>Micromanipulation for copy number variation</td>
<td>Targeted gene sequencing</td>
</tr>
<tr>
<td>Whole-genome/exome sequencing</td>
<td></td>
</tr>
</tbody>
</table>
Severe Combined Immunodeficiency

- Age < 2 years
- Failure to thrive
- Recurrent infections (bacterial, viral, fungal)
- Diarrhea
- Eczematous rash
- PE: absence of tonsils and/or lymph nodes

Lab Evaluation for SCID

- Screening
  - CBC with diff
  - Chemistry panel
  - ESR/CRP
  - Ig levels
- Additional testing
  - HIV
  - CH50
  - Lymphocyte panel

Images adapted from UpToDate.com
Lymphocyte Phenotypes in SCID

<table>
<thead>
<tr>
<th>Disease</th>
<th>Genes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>T B NK</td>
<td>ADA</td>
<td>89, 90</td>
</tr>
<tr>
<td>Adenylate kinase</td>
<td>AR2</td>
<td>91-93</td>
</tr>
<tr>
<td>Adenylate kinase (reticular dysgenesis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T B NK</td>
<td>DCLRE1C</td>
<td>94, 95</td>
</tr>
<tr>
<td>Arrenis</td>
<td>NHEJ1</td>
<td>96, 97</td>
</tr>
<tr>
<td>Recombinase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA-dependent protein kinase</td>
<td>PRKDC</td>
<td>98</td>
</tr>
<tr>
<td>DNA ligase IV</td>
<td>LG4</td>
<td>99, 100</td>
</tr>
<tr>
<td>RAG1 and RAG2</td>
<td>RAG1, RAG2</td>
<td>101-104</td>
</tr>
<tr>
<td>T B NK</td>
<td>IL28G</td>
<td>67, 105-108</td>
</tr>
<tr>
<td>IL-12 signaling deficiency</td>
<td>JAK3</td>
<td>106, 109</td>
</tr>
<tr>
<td>CD25 deficiency</td>
<td>IL2RA</td>
<td>110, 111</td>
</tr>
<tr>
<td>T B NK</td>
<td>CD3, CD3E, CD3Z</td>
<td>112-115</td>
</tr>
<tr>
<td>CD19 deficiency</td>
<td>CD19</td>
<td>88</td>
</tr>
<tr>
<td>CD45 deficiency</td>
<td>PTPRC</td>
<td>116, 117</td>
</tr>
<tr>
<td>IL-7 receptor deficiency</td>
<td>IL7RA</td>
<td>115, 118</td>
</tr>
</tbody>
</table>

Bonilla. JACI 2015; 136: 1186.

Screening for SCID

<table>
<thead>
<tr>
<th>TREC results</th>
<th>Genotype</th>
<th>Absorbent T-cell count and mitogen proliferation testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREC critically abnormal or absent</td>
<td>CD3, CD4, CD8</td>
<td>Presence of absence of CD3 T-cell number (&lt;2000/mL) Low T-cell number of normal minimum T-cell counts T-cell proliferation 10-50&lt; lower limit of normal</td>
</tr>
<tr>
<td>TREC abnormal or low</td>
<td>CD4, CD8</td>
<td>T-cell number (&lt;1000/mL) Low T-cell number of normal minimum T-cell proliferation 10-50&lt; lower limit of normal</td>
</tr>
<tr>
<td>TREC abnormal or low</td>
<td>CD3, CD4, CD8</td>
<td>T-cell number (&lt;1000/mL) Low T-cell number of normal minimum T-cell proliferation 10-50&lt; lower limit of normal</td>
</tr>
</tbody>
</table>

Knight. JACI In Prac 2021; 9: 3293.
Case 1

- 27 y/o woman with no PMH admitted to RGH with community-acquired pneumonia. She reports a lifelong history of sinus infections and bronchitis, typically treated with 4-6 courses of antibiotics annually.
- PMH: Transient episode of thrombocytopenia
- PSH: None
- Meds: None
- SH: No tobacco use
- FH: Mother died of gastric cancer
- PE: Normal vitals and normal exam

Case 1 – Evaluation

- CBC wbc = 21.7, hct = 42.1, plt = 87
- Chem normal with cr = 0.7
- EKG: Normal
- CXR: RLL opacity, Mild bronchiectasis
- IgG = 208 (700-1600 mg/dL), IgM = 54 (40-230 mg/dL) IgA < 17 (70-400 mg/DL)
- Vaccine responses:
  - Tetanus: 0.02 IU/mL, s/p vaccination 0.08 IU/mL
  - Diphtheria: 0.01 IU/mL, s/p vaccination 0.02 IU/mL
  - Streptococcus pneumonia: 0/23 serotypes ≥ 1.3 mcg/mL, s/p vaccination 2/23 serotypes ≥ 1.3 mcg/mL
Common Variable Immunodeficiency

**Diagnostic Criteria**
- Clinical Presentation
- 2+ Ig classes < SD from normal
- Failure of vaccine responses
- Exclude causes of SID

**Common Pitfalls**
- IgG subclass deficiency
- Hypogammaglobinemia in the setting of infection
- Sub-optimal response to polysaccharide antigens
- Recurrent infection in the setting of malignancy

---

**Evaluation of Vaccine Responses**

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>T-cell Independent or Dependent</th>
<th>Protective Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemophilus influenza</td>
<td>Dependent</td>
<td>2-fold increase to &gt; 1.0 mg/ml</td>
</tr>
<tr>
<td>Diphtheria</td>
<td>Dependent</td>
<td>2-fold increase to &gt; 0.1 IU/ml</td>
</tr>
<tr>
<td>Tetanus</td>
<td>Dependent</td>
<td>2-fold increase to &gt; 0.15 IU/ml</td>
</tr>
<tr>
<td>Rabies</td>
<td>Dependent</td>
<td>2-fold increase to 0.5 IU/ml</td>
</tr>
<tr>
<td>Meningococcal conjugate</td>
<td>Dependent</td>
<td>2-fold increase to 2.0 mg/ml</td>
</tr>
<tr>
<td>Meningococcal polysaccharide</td>
<td>Independent</td>
<td>2-fold increase to 2.0 mg/ml</td>
</tr>
<tr>
<td></td>
<td>(2 of 4 serotypes)</td>
<td></td>
</tr>
<tr>
<td>Pneumococcal polysaccharide (PPV23)</td>
<td>Independent</td>
<td>• If baseline &lt; 1.3 mg/ml, increase 2-fold to &gt; 1.3 mg/ml OR increase 4-fold</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• If baseline &gt; 1.3 mg/ml, increase 2-fold</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Responses need to be demonstrated by ≥70% of serotypes</td>
</tr>
<tr>
<td>Pneumococcal conjugate (PCV13)</td>
<td>Dependent</td>
<td>• If baseline &lt; 1.3 mg/ml, increase 2-fold to &gt; 1.3 mg/ml OR increase 4-fold</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• If baseline &gt; 1.3 mg/ml, increase 2-fold</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Responses need to be demonstrated by ≥70% of serotypes</td>
</tr>
</tbody>
</table>

Orange. JACI 2012. 130: S1.
Case 2

- 54 y/o AA gentleman with DM presents to the emergency department with fevers, cough, and yellow sputum production. The patient had a similar presentation 4 months previously that required a 4 day ICU admission and treatment with IV antibiotics.
- PMH: DM
- PSH: Sinus surgery at age 28
- Meds: metformin, gabapentin
- SH: No tobacco use
- FH: No hx of malignancy
- PE: HR = 115, thin, dec breath sounds b/l
Case 2 – Evaluation

- CBC wbc = 13.4, hct = 38.1, plts = 221
- Chem normal with cr = 1.1
- EKG: Normal
- CXR: RUL and RLL opacity
- IgG = 3049 (700-1600 mg/dL), IgM = 12 (40-230 mg/dL) IgA = 21 (70-400 mg/DL)
- SPEP: Reveals monoclonal gammopathy = 3.2 g/dL
- UPEP: 5% albumin and monoclonal band = 92% of excreted protein, immunofixation reveals free lambda light chains
- Bone marrow biopsy: Hypercellular marrow. Plasma cells markedly increased in number and present in sheets. Plasma cell constitute > 30% of all nucleated cells. Mildly increased reticulin. Giemsa stain normal.

Case

- 58 y/o Caucasian woman with CLL diagnosed in 2013 presents with frequent episodes of sinusitis and bronchitis. Also reports profound fatigue. Episodes of sinusitis and bronchitis are marked with productive cough, documented fevers, swollen lymph nodes, and myalgias. Episodes are treated with antibiotics with eventual improvement, but then recurrence of symptoms. In the last one year, the patient has received 6+ courses of antibiotics. Several have been extended beyond the typical duration.
Case

- PMH: mdd, AR s/p AIT as a child
- PSH: None
- Meds: Bactrim DS (M, W, F), ibrutinib, allopurinol, bupropion, flonase
- SH: No tobacco use.
- FH: Brother with history of CML
- PE: thin, anxious woman

Evaluation

- SPT: negative for common aeroallergens
- CT chest: nonspecific nodules < 3 mm, no bronchiectasis
- IgG = 632 mg/dL, IgM = 55 mg/dL, IgA = 165 mg/dL
- Diphtheria IgG = 0.63, s/p vaccination = 0.60
- Tetanus IgG = 0.97, s/p vaccination = 3.2
- S. pneumo IgG > 1.4 for 6/23 serotypes, s/p vaccination > 1.4 for 5/23 serotypes
Ig Abnormalities

<table>
<thead>
<tr>
<th>Abnormal</th>
<th>Normal</th>
<th>Normal</th>
<th>Normal</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Normal</td>
<td>Elevated</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Low</td>
</tr>
</tbody>
</table>

For any combination of low and normal:

- Many hormonal deficiencies, including common autoimmune deficiency; combined immune deficiencies.
- Specific antibody deficiency in normal individual, either hormonal or combined deficiencies; will provide other primary immune deficiencies.
- Evaluation of antibody and/or vaccine titer response; B-cell enumeration and phenotyping.
- Evaluation of antibody and/or vaccine titer response; B-cell enumeration and phenotyping.
- Evaluation of antibody and/or vaccine titer response; B-cell enumeration and phenotyping.
- Normal

Recurrent or Unusual Infections

- **Trainees**
  - IgG
- **Primary Care & Specialists**
  - IgG, IgM, IgA
- **Clinical Immunologists**
  - IgG, IgM, IgA
  - Vaccine Responses
  - Lymphocyte subsets
  - Complement Studies
- **Hypergam**
  - SPEP
- **Hypogam**
  - Refer
- **Normal**
  - Low suspicion: nothing
  - High suspicion: refer
Genetic Testing


Multi-disciplinary Approach

Summary

- Immunodeficiency is under recognized
- Screening starts with CBC with diff, Ig levels
- Vaccine responses are critical to accurately diagnose antibody deficiency
- Work with colleagues and experts in the field

Thank You

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Making the Most of Laboratory Diagnostics in Allergy

Amanda Michaud, MMS, PA-C
Family Allergy & Asthma Consultants
Jacksonville, FL

History

- 1967: IgE discovered
- 1972: First total IgE test goes to market
- 1974: First RAST test introduced for specific IgE
- 1981: Specific IgE testing using enzyme linked assay
- 1988-89: DNA encoding allergens to hornet venom, birch pollen, and house dust mite cloned
- 1991: First fully automated IgE testing
- 2005: Component-resolved diagnostics
Total IgE

- Elevations of total IgE seen in various disorders
- Total serum IgE level > 100 IU/ml
- Not adequate for diagnosing allergic disease
- Associated with increased risk of atopy
- Patients with atopic dermatitis tend to have highest levels
  - #2: Allergic asthma
  - #3: Allergic rhinitis
- Elevated IgE in childhood can be predictive of developing allergic disease

Conditions associated with elevated IgE

- Infectious disease
  - Parasitic, HIV, Epstein-Barr, TB, Leprosy, Candidiasis
- Atopic disease
  - ABPA, AFRS, atopic dermatitis, asthma, allergic rhinitis
- Immunodeficiency
  - Hyper-IgE, Wiscott-Aldrich, Netherton, atypical DiGeorge, etc
- Inflammatory conditions
  - EGPA, Kawasaki disease
- Neoplasm
  - Hodgkin lymphoma, IgE myeloma
- Others
  - Tobacco smokers, CF, nephrotic syndrome, graft vs. host disease, bullous pemphigoid
- Drug effect
  - Aztreonam, penicillin G
IgE in Specific Diseases

▷ Asthma
  ○ Higher IgE levels associated with risk of developing asthma
  ○ Levels may correlate with disease severity
  ○ Serum levels correlate with lower FEV1 in asthmatics

▷ ABPA
  ○ Total IgE values used for diagnosis and guiding treatment

▷ Allergic rhinitis
  ○ Specific IgE to relevant allergens; Total IgE less useful

▷ Atopic dermatitis
  ○ Elevated total IgE seen in 80-85% of patients

▷ Food allergy
  ○ Food-specific IgE plus history suggestive of IgE-mediated reaction

▷ Venom allergy
  ○ No role for total IgE; Venom-specific IgE can be done if skin testing inconclusive or negative with convincing history

Food-Specific IgE

▷ Patients can have positive food sIgE and still be tolerant
▷ Levels do not correlate with reaction severity
▷ Higher concentrations correlate with likelihood of reaction
▷ Widely available, unaffected by antihistamines/meds, useful in patients who cannot do/tolerate SPT
▷ More costly than SPT, results not immediate
Predicting Food Allergy – 95% predictive decision points

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Decision Point (kU/L)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Efficiency</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>6</td>
<td>64</td>
<td>90</td>
<td>69</td>
<td>96</td>
<td>39</td>
</tr>
<tr>
<td>Milk</td>
<td>32</td>
<td>34</td>
<td>100</td>
<td>56</td>
<td>100</td>
<td>44</td>
</tr>
<tr>
<td>Peanut</td>
<td>15</td>
<td>57</td>
<td>100</td>
<td>66</td>
<td>100</td>
<td>36</td>
</tr>
<tr>
<td>Fish</td>
<td>20</td>
<td>25</td>
<td>100</td>
<td>89</td>
<td>100</td>
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<tr>
<td>Soybean</td>
<td>65</td>
<td>24</td>
<td>99</td>
<td>79</td>
<td>86</td>
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<tr>
<td>Wheat</td>
<td>100</td>
<td>13</td>
<td>100</td>
<td>77</td>
<td>100</td>
<td>76</td>
</tr>
</tbody>
</table>

Component Resolved Diagnostics

- Pinpoint specific allergic triggers
- Explain symptoms due to cross-reactivity
- Improve diagnostic accuracy
- Assess risk for reactions
What is component testing?

▷ Single allergenic molecule
▷ Component names:
  ○ First 3 letters of genus + first letter of species + order of allergen identification
  ○ 1st allergen identified in peanut \((Arachis hypogaea)\) = Ara h 1
▷ Commercially available for peanut, tree nuts, milk, egg; Coming soon for sesame and wheat
▷ Also available for venom, furry pets

PROs of CRD

▷ Cross-reactivity vs. true allergy
▷ Reduce need for oral food challenges
▷ Assess risk of severe food-allergic reactions
▷ Information re: whether the allergy will be outgrown
▷ Guides decision making for baked milk/egg challenges
▷ Guidance for VIT
CONs of CRD

- Study design and selection bias
- No ability to infer true reaction severity
- Unclear if highly specific test is truly advantageous over traditional diagnostics
- Many not tested as screening measure
Peanut

▷ Peanut IgE of 14 kU/L has 95% PPV allergy
▷ Consider Peanut OFC if:
  ○ Peanut IgE is ≤ 2 kU/L (if no history of reaction)
  ○ Peanut IgE is ≤ 5 kU/L (if history of reaction)


Characteristics of Peanut Protein families

Increasing risk to cause systemic symptoms and reactions

CCD  Profilin  PR-10  LTP  Storage Proteins

Ara h 8  Ara h 9  Ara h 1, 2, 3, 6
Peanut Components

- Ara h 1, 2, 3, 6: higher risk of systemic reaction and/or anaphylaxis
  - Ara h 2 sensitization nearly always associated with clinical reactivity
  - Ara h 2 is superior test
- Ara h 9: variable risk; usually accompanied by sensitization to other components
  - Cross-reactive with pitted fruits (e.g., peaches)
- Ara h 8: lower risk; mild, localized symptoms
  - Cross-reactive with pollens (e.g., birch)
- Bet v 2 (Profilin): cross-reactive with pollens, lower risk
- CCD: Lowest risk; highly cross-reactive

Ara h 2

- Superior for diagnosis of peanut allergy vs. PSIgE or SPT in high-risk infants
- Using cut-point of $\geq 0.1$ kU/L:
  - Sensitivity 94%/Specificity 96%
  - PPV 92%
  - NPV 98%
### Management Decisions

<table>
<thead>
<tr>
<th>Ara h 8</th>
<th>Ara h 9</th>
<th>Ara h 1, 3, 6</th>
<th>Ara h 2</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>OFC recommended</td>
</tr>
<tr>
<td>+/-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Depends on clinical history</td>
</tr>
<tr>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
<td>Likely allergic</td>
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</tbody>
</table>

### Peanut Diagnostics and Testing Strategy

<table>
<thead>
<tr>
<th>Diagnostic test(s)</th>
<th>Testing strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut SPT</td>
<td>Food challenge if SPT wheal size 3-8 mm; deem allergic if SPT wheal size ≥8 mm</td>
</tr>
<tr>
<td>Ara h 2-sIgE</td>
<td>If ≥0.1 kUA/L, deem allergic</td>
</tr>
<tr>
<td>Ara h 2-sIgE</td>
<td>Food challenge if Ara h 2-sIgE is between 0.1 and 1 kUA/L; deem allergic if Ara h 2-sIgE &gt;1 kUA/L</td>
</tr>
<tr>
<td>Peanut-sIgE, Ara h 2-sIgE</td>
<td>Food challenge if peanut-sIgE is ≥0.1 kUA/L and Ara h-2-sIgE is &lt;0.1 kUA/L; deem allergic if Ara h-2-sIgE is ≥0.1 kUA/L</td>
</tr>
<tr>
<td>Peanut SPT followed by Ara h 2-sIgE</td>
<td>Food challenge if SPT wheal size 3-8 mm and Ara h 2-sIgE &lt;0.1; deem allergic if Ara h 2-sIgE ≥0.1 or SPT wheal size ≥8 mm</td>
</tr>
</tbody>
</table>
Tree Nut Specific-IgE

- In general:
  - ≥15kU/L is 95% predictive for clinical allergy
  - 63-89% ≤ 2 kU/L will pass OFC

  - Cashew/pistachio – no 95% PPV reported, optimal cut-off ranges from 0.35-2.62 kU/L
  - Walnut/pecan—95% PPV cut-point ≥5.07 kU/L in one study; Ranges from 0.46-2.58kU/L in others
  - Hazelnut—No optimal cut-point; Best study is ≥3.15kU/L (91%)
  - Almond—No optimal cut-point; one study showed ≥10kU/L
  - Brazil nut—No optimal cut-point; ≥1.51kU/L in one study but poor sensitivity
  - Macadamia nut—No studies

Weinberger & Sicherer, 2018; Brettig et al. 2021; Elizur et al. 2020; Buyuktiryaki et al. 2016; Baker et al. 2019;

Cashew-Pistachio

- Most cross-reactive of all tree nuts

- Pistachio-allergic = 100% also allergic to cashew
- Cashew-allergic = 67% also allergic to pistachio

- Ana o 3 ≥ 2.0 can be indicative of BOTH cashew and pistachio allergy

Elizur et al. 2018; Andorf et al. 2017; Cox et al. 2021
Walnut-Pecan

- Pecan-allergic = 100% allergic to walnut
- Walnut-allergic = 67-91% allergic to pecan

Elizur et al. 2018, Andorf et al. 2017; Cox et al. 2021
Hazelnut

- **Cor a 1**
  - Profilins
  - Homologous to Bet v 1 (birch)
  - Causes food pollen syndrome

- **Cor a 2**

- **Cor a 8**
  - LTP
  - Heat-stable
  - Not cross-reactive to pollen

- **Cor a 9**
  - Seed storage proteins
  - High risk of systemic reaction
  - Cor a 9 > 2.0kU/L or Cor a 14 > 1.0kU/L highly specific and sensitive

Cashew/Pistachio

- **Ana o 3**
  - Seed storage protein
  - Correlate with systemic reactions
  - 2.0kU/L cut-point diagnostic of cashew/pistachio allergy

- **Ana o 1**
  - Storage proteins
  - Not considered clinically useful

- **Ana o 2**

- **Pis v 1**
  - Storage proteins
  - Associated with systemic reactions
  - Homologous to Ana o 3 (Pis v 1)

- **Pis v 2**
  - Homologous to Ana o 2 (Pis v 2)
Walnut/Pecan

- Jug r 1
  - Seed storage proteins
  - Correlate with systemic reactions
  - Jug r 1 has best specificity
- Jug r 2
- Jug r 4
- Jug r 3
  - LTP
  - Associated with local reactions
- Jug r 5
  - Profilin
  - Associated with mild reactions and oral allergy syndrome
- Car i 1
- Car I 4
  - Seed storage proteins
  - Associated with systemic reactions
  - Cross-reactive with Jug r 1 & Jug r 4

Egg White

- In children ≥ 2 years old, egg white IgE > 6-7 kU/L had 95% PPV at predicting egg allergy
- In infants younger than 2 years old, egg white IgE ≥ 2 kU/L had 95% PPV at predicting egg allergy
- For all ages, egg white IgE > 2 had 50% PPV
- 70% of egg-allergic children tolerate baked egg
Egg Components

- Ovalbumin (gal d 2)
  - Less heat-stable
  - Higher risk of reaction to uncooked egg
  - Lower risk of reaction to baked egg
  - Can be predictive of likelihood of outgrowing allergy

- Ovomucoid (gal d 1)
  - More heat-stable
  - Higher risk of reaction to baked egg
  - Higher levels associated with lower likelihood of outgrowing egg allergy

Baked Egg Cut-Points

- Higher Gal d 1/Ovomucoid is associated with persistence of egg allergy
- Egg white IgE of 10 kU/L and SPT > 15mm wheal has 60% PPV for reactivity to baked egg
- Typically, Ovomucoid IgE < 0.35 kU/L is 95% predictive at tolerating baked egg
Egg: Medical Decision Making

<table>
<thead>
<tr>
<th>Ovalbumin</th>
<th>Ovomucoid</th>
<th>Management</th>
</tr>
</thead>
</table>
| +         | -         | Avoid uncooked eggs  
Consider baked egg challenge |
| +/-       | +         | Avoid all forms of egg  
More likely to react to baked egg |

Milk

- Milk IgE predictive values:
  - 95% PPV = 32 kU/L
  - 90% PPV = 15 kU/L
  - 50% NPV = 2 kU/L

- 75% of milk-allergic children can tolerate baked milk

Milk Components

▷ α-lactoglobulin (Bos d 4) and β-lactoglobulin (Bos d 5)
  ○ Susceptible to heat denaturation
  ○ Higher risk of reaction to fresh milk
  ○ Lower risk of reaction to baked milk
  ○ More likely to outgrow allergy

▷ Casein (Bos d 8)
  ○ Resistant to heat denaturation
  ○ Higher risk of reaction to all forms of milk
  ○ Unlikely to outgrow allergy if levels are high


Baked Milk Cut-Points

▷ Casein IgE 10 kU/L is 95% PPV for positive OFC to baked milk
▷ Casein IgE 5 kU/L is 50% PPV for positive OFC to baked milk *(considered best decision-point)*
▷ Casein IgE , 0.35 kU/L is 95-100% NPV
  ○ Casein IgfE < 1.21 kU/L is 94% NPV
Milk: Medical Decision Making

<table>
<thead>
<tr>
<th>α-lactoglobulin</th>
<th>β-lactoglobulin</th>
<th>Casein</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Avoid fresh milk</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Consider baked milk challenge</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
<td>Avoid all forms of milk More likely to react to baked milk</td>
</tr>
</tbody>
</table>


Wheat

- Likely reactive if wheat IgE > 80kU/L
- Wheat IgE decision point is 100 kU/L with 100% PPV
- Wheat IgE decision point at 26 kU/L with 74% PPV and 87% NPV
- Components:
  - Gliadins and glutenins
  - Omega-5-gliadin (Tri a 19) predictive for wheat-dependent exercise-induced anaphylaxis and OFC-proven wheat allergy
  - Tri a 19 (omega-5-gliadin) not yet commercially available in US (coming soon)

Soybean

- Soy IgE of 65 kU/L has 86% PPV
- Soy IgE of 30kU/L has 73% PPV
- Components
  - Gly m 4, 5, 6, 8 all shown to correlate with clinical allergy in various studies
  - Gly m 8 cut-points at 3.55kU/L: 82% PPV, 78% specificity and sensitivity
  - Gly m 8 > 7.00kU/L: 91% specificity
  - Gly m 4 at 12.00kU/L 91% specificity
  - Gly m 5 at 2.74 kU/L, 89% sensitivity

Fin Fish

- Parvalbumin: major fish allergen
- 95% PPV for any fish IgE is 20 kU/L
- Codfish, salmon, pollock, herring are most allergenic and cross-reacting; Isolated allergy reported
- Cod IgE levels ≥ 8.2 kU/L had 75% PPV for cod allergy
- Salmon IgE levels ≥ 5.0 kU/L had 79% PPV for allergy to cod, salmon, and mackerel
- Higher sensitization = more likely to be allergic to multiple fish types
- Studies on components lacking, ongoing
Shellfish

- Specific IgE to shellfish less reliable
- Major allergen = Tropomyosin (Pen a 1)
- In general, $\geq 20$ kU/L has 95% PPV
- Shrimp IgE $\leq 3.55$ kU/L is 100% sensitive at ruling out shrimp allergy in patients NOT sensitized to dust mite
- Overall, shrimp IgE cutpoint $0.35$ kU/L has 42% PPV and 91% NPV
- IgE to shrimp-specific tropomyosin more reliable

Pedrosa et al, 2015; Yang et al. 2010.

Sesame

- In general, SPT more reliable
  - $\geq 14$mm 95% PPV
- Sesame sIgE does not correlate with OFC outcome
- No 95% PPV for sesame-specific IgE
- Some studies demonstrate sesame IgE of $\geq 50$ kU/L is 86% PPV
- Components (Ses i 1 most useful)
  - Ses i 1 $\geq 0.30$ kU/L associated with failing OFC
  - Ses i 1 $\geq 3.96$kU/L 86% sensitivity/89%specificity
  - Ses i 1 $\leq 0.13$kU/L = at-home introduction

Saf et al. 2020; Yanagida et al 2019
Venom

Stinging Insect Allergy

▷ Apidae: honey bee, bumble bee, sweat bee
  ○ Limited cross-reactivity
▷ Vespidae: yellow jacket, yellow hornet, white faced hornet, paper wasp
  ○ Extensive cross-reactivity within subfamilies
▷ Formicidae: harvester ant, fire ant
▷ Testing limitations:
  ○ Cross-reactivity
  ○ False positives
  ○ Sensitization without clinical reactivity
  ○ Many “double sensitized” to honey bee and vespids

Tankersley & Ledford, 2015; Kotuba & Greenburger, 2012
**Venom Components**

<table>
<thead>
<tr>
<th>Api m 1</th>
<th>Api m 2</th>
<th>Api m 3</th>
<th>Api m 5</th>
<th>Api m 10</th>
<th>Ves v 1</th>
<th>Ves v 5</th>
<th>Pol d 5</th>
<th>VIT/Management</th>
</tr>
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<tbody>
<tr>
<td>+</td>
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<td></td>
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<td>Honey bee</td>
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<td></td>
<td></td>
<td></td>
<td>Yellow jacket and paper wasp</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Honey bee, yellow jacket, and paper wasp</td>
</tr>
</tbody>
</table>

Mitterman et al, 2010; Blank et al, 2018
Furry Pets

Pet Allergy

- Allergies to cats and dogs affect 10-20% of the population worldwide
- 50% of people with exposure to horses/horse barns show respiratory symptoms
- Sensitization to multiple pet components predictive of asthma and severity
- Sensitization to multiple pet components at age 4 predictive of rhinitis, conjunctivitis and asthma at age 16

Pet Components

Monosensitization to Can f 5:
- Can f 5 is androgen-regulated protein expressed in prostatic fluid
- Up to 30% of dog-allergic patients may be monosensitized
- May tolerate female dogs or castrated male dogs
- If history of seminal fluid allergy, consider evaluating Can f 5


Take-Home Points

- Patients can have positive specific IgE to foods without clinical reactivity
- Allergy testing is imperfect
- Component derived diagnostics can aid in clinical decision making
- Provocation testing or oral challenge remains gold standard for diagnosis
- Testing to venom components could provide more accurate results and targeted VIT
- Monosensitization to Can f 5 in dogs could allow patients to tolerate female or castrated male dogs