Current Molecular and Cellular Diagnostic and Prognostic Tests in Food Allergy

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IOM Questions

- What are current diagnostic and prognostic tests for FAs? In multi sensitized individual?

- What are challenges with implementing and interpreting current tests for diagnosis, prognosis, and severity? Any markers to identify patients at risk for severe reactions? Or monitor patients for successful responses to therapy?

- What studies are needed to improve current tests or to identify new tests?

- What are current ideas and approaches to ID those patients refractory vs. responsive to therapy?

- What future research would be recommended in this area to advance allergy prevention and management?

Answers based on peer-reviewed published studies
Potential methods for clinical predictors of diagnosis and prognosis

- IgE and IgG4 tests
- Basophil Activation Test
- Immuno-phenotyping
- DNAmethylation
- Composite/Combination of Markers
- Food Allergen Epitope Mapping
- Other

Potential Markers:
Serum, Allergy Effector Cells and Long-lived Cells (T, B, etc)
Current diagnostics for food allergies

Skin Prick Test (SPT)

Serum IgE titers & Component Resolved Diagnostics (CRD)

Elimination diets

Gold Standard Food Challenge
Variables that affect diagnostic outcomes

- Kinetics of allergic response vary among patients
- Medications
- Age of patient
- Extract preparations
- Sample collection
- Health status (e.g. immune exacerbations)
- Technique of skin prick test
Issues with SPT and IgE diagnostics:

- IgE levels fail to predict allergic responses
  - Indirect assessment
  - Only survey immediate and not late-phase responses
- SPTs do not discriminate between a food sensitivity vs. a true allergy
- Communication of IgE tests to patients can be confusing
- Higher rates of false positives
  - Potential cross-reactivity between allergens
Caveats to current strategies for food allergy detection: SPT and IgE titers

<table>
<thead>
<tr>
<th>Food</th>
<th>Specific IgE</th>
<th>Specificity:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow’s milk</td>
<td>48%</td>
<td></td>
</tr>
<tr>
<td>Hen’s egg</td>
<td>49%</td>
<td></td>
</tr>
<tr>
<td>Wheat</td>
<td>43%</td>
<td></td>
</tr>
<tr>
<td>Soy</td>
<td>38%</td>
<td></td>
</tr>
<tr>
<td>Peanut</td>
<td>59%</td>
<td></td>
</tr>
</tbody>
</table>

Meta-analysis of published studies predicting accuracy of food allergy diagnostics
European Food Allergy Guidelines, EAACI 2014
Clinical History is part of accurate diagnosis

<table>
<thead>
<tr>
<th>Atopic history and challenge results</th>
<th>Eczema</th>
<th>Asthma</th>
<th>Rhinitis</th>
<th>Other food allergy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Milk (n = 129)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With disease</td>
<td>53%</td>
<td>47%</td>
<td>39%</td>
<td>85%</td>
</tr>
<tr>
<td>Passed</td>
<td>42%</td>
<td>40%</td>
<td>46%</td>
<td>45%</td>
</tr>
<tr>
<td><strong>Egg (n = 119)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With disease</td>
<td>71%</td>
<td>65%</td>
<td>46%</td>
<td>95%</td>
</tr>
<tr>
<td>Passed</td>
<td>49%*</td>
<td>48%*</td>
<td>51%</td>
<td>58%</td>
</tr>
<tr>
<td><strong>Peanut (n = 160)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With disease</td>
<td>51%</td>
<td>41%</td>
<td>48%</td>
<td>61%</td>
</tr>
<tr>
<td>Passed</td>
<td>52%</td>
<td>57%</td>
<td>57%</td>
<td>53%</td>
</tr>
<tr>
<td><strong>Wheat (n = 41)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With disease</td>
<td>73%</td>
<td>66%</td>
<td>49%</td>
<td>98%</td>
</tr>
<tr>
<td>Passed</td>
<td>60%</td>
<td>63%</td>
<td>65%</td>
<td>68%</td>
</tr>
<tr>
<td><strong>Soy (n = 75)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With disease</td>
<td>68%</td>
<td>52%</td>
<td>41%</td>
<td>95%</td>
</tr>
<tr>
<td>Passed</td>
<td>73%</td>
<td>74%</td>
<td>71%</td>
<td>72%</td>
</tr>
</tbody>
</table>

Results of a Clinical Study of n=391 children undergoing 604 challenges

Component resolved diagnostics as an additional test to IgE and SPT

Caveats:
standardization among reagents, cost, and interpretation

Nicolaou, et al. *JACI* 2011
Caveats to current strategies for food allergy detection: OFCs

• ~40% of OFC induced-anaphylaxis need epinephrine injections
  Noone, Ross, Sampson, Wang, et al. JACI In Practice 2015

• Generates anxiety for the patient

• Variability among challenges

• Requires a specialized setting with highly trained staff
  – Resource-intensive
There is an unmet need for better predicative diagnostics: encouraging diagnostics on the horizon

- Skin Testing with Microneedles with Peptides/Epitopes
- Basophil activation test (BAT): Flow cytometric assay of peripheral blood basophils
- Data mining for antibodies, T cell receptors and peptides
- GWAS DNA methylation: Whole genome scan for methylated CpG motif in peripheral blood
Possible new methods to improve skin prick testing

- Skin prick testing has not advanced in about approximately 100 years.
- A new, quantitative skin prick test using epitopes-peptides to allergens that could possibly correlate with severity, using dissolvable microneedles.
- Quantitative blood flow imaging to measure responses.

Sun, Butte et al. *Acta Biomaterialia* 2013
DNA methylation signatures as possible biomarkers for food allergy

Genome-wide DNA Methylation
Peripheral blood cells with objective OFCs

N=58 food sensitized, Age 11-15 mo
50% reactive on OFC
N=13 non allergic
N=48 validation cohort

Scored from 96 CpG sites

FA status predictive with 79% accuracy even though T cell subset used

Martino, Allen, et al. JACI 2015
Basophil activation test (BAT) potentially predicts the degree of reaction severity

Whole blood is sensitive to temperature fluctuations and anti-coagulant reagents (i.e. EDTA)

Santos, Lack et al. *JACI* 2014
Santos, Lack et al. *JACI* 2015
Comprehensive and Integrative Measur“Omics”: Possible diagnostic data from blood and the microbiome

14 Omics assays + Medical tests
Billions of measurements

Diagnosis over time in all ages: Longitudinal tracking 100 food allergic and control twins over periods of health, stress and other atopic diseases

Year 1
- Infection
- Stress

Year 2 ...
- Diet change

Nadeau and Snyder with Gupta and Wang research groups and others
Challenges to clinical trials and evaluations of therapeutic success

- Nausea
- Inconvenience
- Fear
- Compliance
- Unpredictability
- Risk-of-anaphylaxis
- Life-long Allergy
- Food
- Pain
- No. of visits
- Anxiety
- Duration
Schematic of Oral Immunotherapy (OIT) as a possible method for allergy therapy

Provided by Dr. Wesley Burks
Unmet need for comprehensive prognostics for allergy resolution

• What is allergy resolution?
  – Need to distinguish between desensitization versus immune tolerance
  • Refractory responses or persistence of disease despite therapy: ‘daily allergic symptoms to less than 300mg of allergen for at least 3 months’
  • Desensitization: allergic response upon re-challenge after a period of withdrawal post OIT
  • Immunological ‘tolerance’: no allergic reaction upon re-challenge after a period of withdrawal post OIT

• Currently there is no commercially available test to assess allergy resolution in therapy trials
Basophil studies are possible markers to track response to Anti IgE + OIT

Therapy response seems to be Specific to the allergen used in OIT

Correlating oral immunotherapy (OIT) with frequency of allergen-specific B cells

Use of Controls--health and allergic--is important

Peanut OIT modifies IgE and IgG$_4$ responses to major peanut allergens

Vickery, Jones, Burks, et al. *JACI* 2013
Rapid Multi OIT: Blood & SPT Results for Peanut Baseline vs. Post-OIT

Begin, Dominguez, Nadeau, et al. AACI 2014
Epigenetic studies on peanut specific Treg show FOXP3 hypomethylation in “tolerant” subjects

Determining prognosis based on multivariate analysis of allergen-specific T cells after OIT

Glanville, Ryan, Hovde, Galli, Nadeau, et al. 2015
Determining prognosis based on multivariate analysis of allergen-specific T cells after OIT
Combination Markers: Computational approach to epitope survey, T cell Receptor, B Cell Receptor, antibodies


www.distributedbio.com/abgenesis.html
Next steps: overcoming barriers to facilitate better diagnostics and prognostics

- Sample size and study design
  - Multi-site approach, e.g. ITN network, European networks, Australia, FARE Centers of Excellence, NIAID CoFAR, Sean N. Parker Center
  - Community-based participation in clinical studies and in the development of tests

- Comprehensive longitudinal studies
  - Data sharing and bioinformatics of large data sets
    - Normalization of complex and divergent data
    - Open source, secure, democratic and transparent decision making

- Incorporating cutting edge science
  - Nanotechnology, microfluidics, high-dimensional immunophenotyping

- Transparency and education with the food allergy community
  - Improved communication about diagnostics and outcomes to the public, to patients, and their families

- What is the biology behind food allergy?
  - We still need to understand the molecular and cellular underpinnings at blood and tissue levels
With Appreciation to the IOM, Patients and Families, Clinical and Laboratory Team and Collaborators
Back up Slides IOM
Correlating OIT with frequency of somatic mutations in allergen-specific IgG₄ and IgE

Phase 1 Rush Multi Oral Immunotherapy (Rush mOIT) for multisensitized patients

- Baseline visit
  - DBPCFC*
  - IgE measurement
  - Skin Prick Test

- Rapid Desensitization
  - Dose Escalation Visits (Dose increases dependent on patient and safety parameters)

- Week 0
  - Day 1: individual food allergens
  - Omalizumab

- Day 2:
  - (optional)
  - multiple food allergens combined
  - Up to 5 in 1:1:1:1:1

- 2nd DBPCFC:
  - individual food allergens

- N=35 subjects (same eligibility criteria as mOIT study)
  - All subjects had DBPCFCs before entering
  - All subjects had more than one food allergy confirmed on DBPCFC

- PRIMARY GOAL: SAFETY
  - Approach: Rapid dose escalation over time until ready 4 g of each protein allergen

Begin, et al. 2014
Safety Results: Phase 1 Rush Multi Oral Immunotherapy for multisensitized patients

Rush mOIT
Most common symptom: HIVES, Abdominal Pain
Epi Use=.02%

mOIT
Most common symptom: HIVES, Abdominal Pain
Epi Use=.03%

Begin, et al. 2014
Dosing Results of rush mOIT vs mOIT in Phase 1 Studies

[Graph showing the time to a 10-fold increase from original reaction threshold to peanut (mo) for Standard OIT and Rush OIT.]
Dosing Results of rush mOIT vs mOIT in Phase 1 Studies

Time to 300, 1000 and 4000 mg doses
A Desensitization Journey

Prior to Desensitization Baseline

3 months OIT

7 months OIT

9 months OIT

12 months OIT

16 months OIT Negative Skin Tests

Xolair Stopped

Stanford Medicine | Sean N. Parker Center for Allergy & Asthma Research
Biomarkers of Safety and Efficacy over time during immunotherapy

- Study different immune cells involved
  - Basophils
  - B cells
  - T cells
  - Others

- Study mechanisms of immunotherapy are key to our discovering new targets for future therapies

- Tools are being developed to better monitor immune changes during immunotherapy
Longitudinal changes over time during immunotherapy

Mechanisms of peripheral tolerance to allergens

Soyer, Akdis, et al. Allergy 18 DEC
Whole Blood CyTOF

- 26 Ab’s
- Basophil activation by CD63+ CD203c
T cells studies in Anti IgE Therapy

OIT: replacement or reprogramming?

- **Replacement**:
  - Desensitizing anti-milk T-cells
  - Allergic anti-milk T-cells
  - Phenotype-transitional T-cells

- **Reprogramming**:
  - Desensitizing anti-milk T-cells
  - Allergic anti-milk T-cells
  - Phenotype-transitional T-cells
Observation
TCRs against different specificities converge into clusters that are shared across individuals and are antigen specific.

Interpretation
Convergence analysis of TCR bypassing of tetramers to read T-cell specificity from primary sequence.

Relevance
- Identification of allergen-specific “indicator” TCR convergent groups
- Identification of “cross-over” TCRs between correlated allergens
- Directly survey Treg and other populations for allergen-related clonotypes
- Early detection of allergic response vs. therapy response
High-throughput antigen stimulation and TCR sequencing

- T cell proliferation
- RNA extraction
- TCR sequencing
- Clone enrichment analysis
Phase 1 Open Label Single Center Study in Single and Multi OIT

Type of Food Allergens:
- peanut n = 35
- cashew n = 12
- walnut n = 13
- egg n = 9
- milk n = 8
- pecan n = 8
Phase 1 Open Label Single Center Study in Rapid Single and Multi OIT
71 – Kaplan–Meier for time until densitization to a FA

Number of Food Allergens
- 1 FA, n = 20
- 2 FA, n = 6
- 3 FA, n = 9
- 4 FA, n = 2
- 5 FA, n = 6

Percentage of subjects having reached dose

Time to 2000 mg dose (mo)
72 – Kaplan–Meier for time until densitization to a FA

Number of Food Allergens
- 1 FA, n = 4
- 2 FA, n = 6
- 3 FA, n = 5
- 4 FA, n = 8
- 5 FA, n = 7
Type of Food Allergen
- cashew n = 10
- egg n = 8
- milk n = 5
- peanut n = 23
- pecan n = 8
- walnut n = 12
72 – Kaplan–Meier for time until SPT neg to a FA

Type of Food Allergen
- cashew n = 15
- egg n = 11
- milk n = 7
- peanut n = 18
- pecan n = 8
- walnut n = 10
72 – Kaplan–Meier for time until SPT neg to all FA

Number of food allergens
- 1 FA, n = 4
- 2 FA, n = 5
- 3 FA, n = 4
- 4 FA, n = 5
- 5 FA, n = 3
Phase 1 Open Label Single Center Studies in OIT

Percentage of patients with negative repeat food challenges to 2 g of each FA

Non Rapid
- 55.81%

Rapid
- 60%
- 30%
- 10%
- 4.65%

Dose:
- eating 2g after reaching maintenance
- eating between 300 mg and 2g after reaching maintenance
- eating other amount after reaching maintenance
Biphasic response:
An obstacle for diagnostic development

IgE-mediated
(Early phase)
Onset: minutes-hours

Cell-mediated
(Delayed-phase)
Onset: hours-days
Risk of Anaphylaxis during oral food challenges

38% of patients developed an anaphylactic response during peanut challenge

<table>
<thead>
<tr>
<th>Challenge outcome</th>
<th>n (%)</th>
<th>Reaction threshold amount</th>
<th>Cumulative amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;6 g (n)</td>
<td>&gt;6 g (n)</td>
</tr>
<tr>
<td>Anaphylaxis</td>
<td>21 (38)</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Non-Anaphylactic*</td>
<td>6 (11)</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Tolerant*</td>
<td>28 (51)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Control Group = Non-anaphylactic + Tolerant.

Wainstein et al. *Pediatric Allergy and Immunology*. 2010. 21:603-11
Greater IgE epitope recognition correlates with clinical reactivity

Flinterman et al. JACI Volume 121, Issue 3, Pages 737-743
IgE/IgG4 ratio may positively predict outcome of a food challenge

Basophil activation test (BAT)

Flow Cytometric based test:

[Diagram showing in vitro stimulation process]
DBPCFC reduces biases in severity reporting

<table>
<thead>
<tr>
<th></th>
<th>OFC versus AF</th>
<th>OFC versus PL</th>
<th>AF versus PL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 37 symptoms)</td>
<td>(n = 12 symptoms)</td>
<td>(n = 12 symptoms)</td>
</tr>
<tr>
<td></td>
<td>OFC</td>
<td>AF</td>
<td>P value*</td>
</tr>
<tr>
<td>Score (severity)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>3.6 ± 1.4</td>
<td>4.5 ± 2.1</td>
<td>0.015</td>
</tr>
<tr>
<td>Median</td>
<td>3.0</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1-7</td>
<td>2-8</td>
<td></td>
</tr>
<tr>
<td>Onset (h)</td>
<td>NS</td>
<td></td>
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</tr>
<tr>
<td>Mean (SD)</td>
<td>4.3 ± 5.8</td>
<td>2.6 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.5-24</td>
<td>1-8</td>
<td></td>
</tr>
<tr>
<td>Duration (h)</td>
<td>NS</td>
<td></td>
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</tr>
<tr>
<td>Mean (SD)</td>
<td>24.5 ± 26.5</td>
<td>20.4 ± 25</td>
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</tr>
<tr>
<td>Median</td>
<td>12.0</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1-96</td>
<td>1-96</td>
<td></td>
</tr>
</tbody>
</table>

Summary of all pooled symptom data from all patient challenges (see Results). NS, not significant.
*Wilcoxon signed rank test.

De Yun Wang et al. *Otolaryngology -- Head and Neck Surgery* 2007