Clinical applications of the basophil activation test in food allergy

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MRC & Asthma UK Centre in Allergic Mechanisms of Asthma
Outline

• Diagnosis of food allergy
• Utility of BAT in food allergy
• Bringing BAT to clinical practice
Diagnosis of IgE-mediated food allergy

Clinical History

Allergy tests

Oral food challenge

Gold Standard
The majority of IgE-sensitised children are not allergic
95% PPV cut-offs increase specificity

Skin prick test

Serum specific IgE

Roberts & Lack J Allergy Clin Immunol 2005
Sampson H & Ho J Allergy Clin Immunol 1997
Sampson H J Allergy Clin Immunol 2001

Se = 25.4%
Sp = 98.5%

Se = 28.4%
Sp = 96.8%
The majority of sensitised patients fall into the ‘grey area’

SPT $\geq 8$ mm
Likely to be peanut allergic

SPT $<3$ mm
Very unlikely to be peanut allergic

SPT 3 to 7 mm
Immunological grey area

Adapted from: Roberts & Lack Clin Exp Allergy 2000
A considerable proportion of allergic patients have equivocal allergy test results

Ara h 2-specific IgE <0.35 KU/L

- 82% Peanut tolerant
- 18% Peanut allergic

Oral food challenge to diagnose food allergy

• Risk of immediate-type allergic reaction
• Risk of late reactions
• Resource-intensive
• Increasing demand
• Multiple challenges per patient
• Not fool-proof!
  o 3% false-negatives
  o 3% false-positives
  o 2-9% indeterminate

Perry TT et al JACI 2004; Saleh-Langenberg Allergy 2016
Caffarelli C et al Lancet 2001; Ludman S et al PAI 2013
Nolan RC et al PAI 2007; Niggemann B et al JACI 2012
Up to about 50% of oral food challenges are positive

- Cow's milk
- Baked Egg
- Peanut
- Sesame
- Tree nuts

Lieberman JA et al J Allergy Clin Immunol 2011
Santos AF et al J Allergy Clin Immunol 2014
Severity of allergic reactions during challenges

Perry TT et al
J Allergy Clin Immunol
2004

Milk
Egg
Peanut
Soy
Wheat
Total

Severe
Moderate
Mild

% of patients experiencing a severe reaction

% of challenge food ingested

<25%  25-50%  51-75%  >75%

0%  10%  20%  30%  40%  50%  60%  70%  80%  90%  100%

Severe
Moderate
Mild

Perry TT et al J Allergy Clin Immunol 2004
Outline

• Diagnosis of food allergy
• Utility of BAT in food allergy
• Bringing BAT to clinical practice
Food-induced anaphylaxis occurs without elevated serum tryptase

Samples collected from patients with food-induced anaphylaxis:
- Fatal (patient 3)
- Near-fatal (patient 12)
- ICU (controls 1-3)

Normal range of serum tryptase < 2.5-5 ng/ml
Anaphylaxis usually serum tryptase >10 ng/ml

<table>
<thead>
<tr>
<th>Serum sample</th>
<th>Hours after ingestion</th>
<th>Tryptase (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 3</td>
<td>Post-mortem</td>
<td>2.14</td>
</tr>
<tr>
<td>Patient 12</td>
<td>0.75</td>
<td>1.2</td>
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<tr>
<td></td>
<td>5</td>
<td>&lt;1</td>
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<tr>
<td></td>
<td>8</td>
<td>&lt;1</td>
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<tr>
<td></td>
<td>24</td>
<td>&lt;1</td>
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<tr>
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<td>48</td>
<td>&lt;1</td>
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<td></td>
<td>72</td>
<td>1.5</td>
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<td>Control 1</td>
<td>1</td>
<td>&lt;1</td>
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<tr>
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<td>5</td>
<td>&lt;1</td>
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<td>24</td>
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<tr>
<td>Control 2</td>
<td>0.5</td>
<td>&lt;1</td>
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<tr>
<td>Control 3</td>
<td>2</td>
<td>&lt;1</td>
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<tr>
<td></td>
<td>2.5</td>
<td>&lt;1</td>
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<tr>
<td></td>
<td>4</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>&lt;1</td>
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</tbody>
</table>

Sampson HA et al. New Engl J Medicine 1992
Basophil activation concomitant with delayed anaphylaxis to mammalian meat
**Basophil activation testing using flow cytometry**

### Basophil identification markers
- CD123/HLA-DR
- CD203c
- CCR3
- CRTH2 / CD3
- IgE or FceRI

### Basophil activation markers
- CD63
- CD107a
- CD203c
- CD164
- CD11b
- CD13
- CD69

MacGlashan DW Jr, J Allergy Clin Immunol 2013
Santos AF & Lack G. Clin Transl Allergy 2016
Santos AF & Shreffler WG. Clin Exp Allergy 2017

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**Legend:**
- Isotype control
- Unstimulated basophils
- Stimulated basophils

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### Flow Cytometry Analysis

**No stimulation**

**Peanut extract 10 ng/ml**

**Anti-IgE 1μg/ml**

**SI = 1.0**

**SI = 3.4**

**SI = 3.3**
CD63 expression correlates with histamine release

# BAT to diagnose food allergy

<table>
<thead>
<tr>
<th>Food allergy</th>
<th>Food extract or allergen component</th>
<th>Study</th>
<th>Cut-offs</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow's milk allergy</td>
<td>Cow's milk extract</td>
<td>Rubio (2011)&lt;sup&gt;63&lt;/sup&gt;</td>
<td>&gt;6% CD63+</td>
<td>91%</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sato (2010)&lt;sup&gt;46&lt;/sup&gt;</td>
<td>SI CD203c ≥ 1.9</td>
<td>89%</td>
<td>83%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Casein</td>
<td>SI CD203c ≥ 1.3&lt;sup&gt;46&lt;/sup&gt;</td>
<td>67%</td>
<td>71%</td>
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<tr>
<td>Egg allergy</td>
<td>Ovalbumin</td>
<td>Ocman (2009)&lt;sup&gt;46&lt;/sup&gt;</td>
<td>≥5% CD63+ SI CD203c ≥ 1.6</td>
<td>77% for CD63</td>
<td>100% for CD63</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>63% for CD203c</td>
<td>96% for CD203c</td>
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<tr>
<td>Baked egg allergy</td>
<td>Egg white extract</td>
<td>Sato (2010)&lt;sup&gt;46&lt;/sup&gt;</td>
<td>SI CD203c ≥ 2.4 SI CD203c ≥ 1.7</td>
<td>74%</td>
<td>62%</td>
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<tr>
<td></td>
<td>Ovomucoid</td>
<td></td>
<td></td>
<td>80%</td>
<td>73%</td>
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<tr>
<td>Raw egg allergy</td>
<td>Egg white extract</td>
<td>Sato (2010)&lt;sup&gt;46&lt;/sup&gt;</td>
<td>SI CD203c ≥ 1.7 SI CD203c ≥ 1.6</td>
<td>77%</td>
<td>63%</td>
</tr>
<tr>
<td></td>
<td>Ovomucoid</td>
<td></td>
<td></td>
<td>83%</td>
<td>83%</td>
</tr>
<tr>
<td>Wheat allergy</td>
<td>Wheat extract</td>
<td>Tokuda (2009)&lt;sup&gt;50&lt;/sup&gt;</td>
<td>&gt;11.1% CD203c+ &gt;14.4% CD203c+</td>
<td>86%</td>
<td>58%</td>
</tr>
<tr>
<td></td>
<td>Omega-5 gliadin (nTri a 19)</td>
<td></td>
<td></td>
<td>86%</td>
<td>58%</td>
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<td></td>
<td>Omega-5 gliadin (rTri a 19)</td>
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<td></td>
<td>83%</td>
<td>63%</td>
</tr>
<tr>
<td>Peanut allergy</td>
<td>Peanut extract</td>
<td>Santos (2014)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>≥4.78% CD63+ ND</td>
<td>98%</td>
<td>96%</td>
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<tr>
<td></td>
<td>Ara h 2</td>
<td>Glaumann (2012)&lt;sup&gt;22&lt;/sup&gt;</td>
<td></td>
<td>92%</td>
<td>77%</td>
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<tr>
<td>Hazelnut allergy</td>
<td>Hazelnut extract</td>
<td>Brandstrom (2015)&lt;sup&gt;58&lt;/sup&gt;</td>
<td>CD-sens &gt; 1.7</td>
<td>100%</td>
<td>97%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Erdmann (2003)&lt;sup&gt;65&lt;/sup&gt;</td>
<td></td>
<td>85%</td>
<td>80%</td>
</tr>
<tr>
<td>PFAS to hazelnut</td>
<td></td>
<td></td>
<td>≥6.7% CD63+</td>
<td></td>
<td></td>
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<tr>
<td>Peach allergy</td>
<td>Peach extract</td>
<td>Gamboa (2007)&lt;sup&gt;62&lt;/sup&gt;</td>
<td>&gt;20% CD63 + SI CD63 &gt;2</td>
<td>87%</td>
<td>69%</td>
</tr>
<tr>
<td></td>
<td>Pru p 3</td>
<td></td>
<td></td>
<td>77%</td>
<td>97%</td>
</tr>
<tr>
<td>PFAS to apple</td>
<td>Apple extract</td>
<td>Ebo (2005)&lt;sup&gt;64&lt;/sup&gt;</td>
<td>≥17% CD63+</td>
<td>88%</td>
<td>75%</td>
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<tr>
<td>PFAS to carrot</td>
<td>Carrot</td>
<td>Erdmann (2000)&lt;sup&gt;65&lt;/sup&gt;</td>
<td>≥8.9% CD63+ ≥6.3% CD63+&lt;sup&gt;65&lt;/sup&gt;</td>
<td>85%</td>
<td>85%</td>
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<tr>
<td>PFAS to celery</td>
<td>Celery</td>
<td>Erdmann (2003)&lt;sup&gt;65&lt;/sup&gt;</td>
<td></td>
<td>85%</td>
<td>80%</td>
</tr>
</tbody>
</table>

SI, stimulation index; PFAS, pollen-food syndrome; ND, not determined.
Basophil activation test discriminates between peanut allergy and tolerance

N=104 (12 NR)

*** p<0.001

** p<0.05

Santos AF et al J Allergy Clin Immunol 2014
Basophil activation test discriminates between peanut allergy and tolerance

<table>
<thead>
<tr>
<th>Optimal cut-off</th>
<th>Primary population n=104 (43 PA, 36 PS, 25 NA)</th>
<th>Validation population n=65 (25 PA, 24 PS, 16 NA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>97.6% (87.4; 99.9)</td>
<td>83.3% (74.0; 92.7)</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>96.0% (86.3; 99.5)</td>
<td>100.0% (100.0; 100.0)</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>95.3% (84.2; 99.4)</td>
<td>100.0% (100.0; 100.0)</td>
</tr>
<tr>
<td>NPV (%)</td>
<td>98.0% (89.1; 99.9)</td>
<td>90.2% (82.8; 97.7)</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>96.7% (93.1; 100)</td>
<td>93.4% (87.2; 99.7)</td>
</tr>
</tbody>
</table>

Data taken from: Santos AF et al J Allergy Clin Immunol 2014
BAT to tree nuts

Hazelnut

Cashew nut

Sesame

***p<0.001

Santos AF et al, unpublished
<table>
<thead>
<tr>
<th>Extract or allergen</th>
<th>Author, year</th>
<th>Cut-offs</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
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</thead>
<tbody>
<tr>
<td><strong>Cow’s milk</strong></td>
<td>Sato 2010</td>
<td>SI CD203c ≥1.9</td>
<td>89%</td>
<td>83%</td>
<td>86%</td>
<td>86%</td>
</tr>
<tr>
<td></td>
<td>Rubio 2011</td>
<td>&gt;6% CD63+ (resolution)</td>
<td>91%</td>
<td>90%</td>
<td>81%</td>
<td>96%</td>
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<tr>
<td><strong>Casein</strong></td>
<td>Sato 2010</td>
<td>SI CD203c ≥1.3</td>
<td>67%</td>
<td>71%</td>
<td>74%</td>
<td>63%</td>
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<tr>
<td><strong>Egg white</strong></td>
<td>Sato 2010</td>
<td>SI CD203c ≥2.4 (baked egg allergy)</td>
<td>74%</td>
<td>62%</td>
<td>85%</td>
<td>44%</td>
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<tr>
<td></td>
<td>Sato 2010</td>
<td>SI CD203c ≥1.7 (raw egg allergy)</td>
<td>77%</td>
<td>63%</td>
<td>92%</td>
<td>33%</td>
</tr>
<tr>
<td><strong>Ovomucoid</strong></td>
<td>Sato 2010</td>
<td>SI CD203c ≥1.7 (baked egg allergy)</td>
<td>80%</td>
<td>73%</td>
<td>90%</td>
<td>53%</td>
</tr>
<tr>
<td></td>
<td>Sato 2010</td>
<td>SI CD203c ≥1.6 (raw egg allergy)</td>
<td>83%</td>
<td>83%</td>
<td>97%</td>
<td>42%</td>
</tr>
<tr>
<td><strong>Ovalbumin</strong></td>
<td>Ocmant 2009</td>
<td>≥5% CD63+ or SI CD203c ≥1.6 (egg allergy)</td>
<td>77% (CD63)</td>
<td>100% (CD63)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>63% (CD203c)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Wheat</strong></td>
<td>Tokuda 2009</td>
<td>&gt;11.1% CD203c+ (wheat allergy)</td>
<td>86%</td>
<td>58%</td>
<td>77%</td>
<td>71%</td>
</tr>
<tr>
<td><strong>Omega-5 gliadin</strong></td>
<td>Tokuda 2009</td>
<td>nTri a 19: &gt;14.4% CD203c+ (wheat allergy)</td>
<td>86%</td>
<td>58%</td>
<td>77%</td>
<td>71%</td>
</tr>
<tr>
<td></td>
<td>Tokuda 2009</td>
<td>rTri a 19: &gt;7.9% CD203c+ (wheat allergy)</td>
<td>83%</td>
<td>63%</td>
<td>81%</td>
<td>67%</td>
</tr>
<tr>
<td><strong>Peach</strong></td>
<td>Gamboa 2007</td>
<td>&gt;20% CD63+ and SI CD63 &gt;2</td>
<td>87%</td>
<td>69%</td>
<td>-</td>
<td>-</td>
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<tr>
<td><strong>Pru p 3</strong></td>
<td>Gamboa 2007</td>
<td>&gt;20% CD63+ and SI CD63 &gt;2</td>
<td>77%</td>
<td>97%</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
BAT using single allergens

**Ara h 1**

**Ara h 2**

**Ara h 3**

**Ara h 6**

**Ara h 9**

Mayorga C et al, Allergy 2014
BAT using single peanut allergens

Allergic vs Non allergic

**p<0.001

Santos AF et al, unpublished
<table>
<thead>
<tr>
<th>Likelihood of clinical allergy from history</th>
<th>Low (&lt;0.35 KU/L or &lt;3 mm)</th>
<th>Intermediate (0.35 to &lt;15 KU/L or 3 to &lt;8 mm)</th>
<th>High (≥15 KU/L or ≥8 mm)</th>
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</thead>
<tbody>
<tr>
<td>High</td>
<td>Possible allergy</td>
<td>Probable allergy</td>
<td>Allergy</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Possible allergy</td>
<td>Possible allergy</td>
<td>Probable allergy</td>
</tr>
<tr>
<td>Low</td>
<td>No allergy</td>
<td>Possible allergy</td>
<td>Possible allergy</td>
</tr>
</tbody>
</table>

Stiefel G & Roberts G. Arch Dis Child Educ Pract Ed 2012
Sensitised patients with equivocal allergy test results

<table>
<thead>
<tr>
<th></th>
<th>Peanut allergic</th>
<th>Peanut sensitized but tolerant</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>5 (2-6)</td>
<td>4 (0.5-13)</td>
<td>0.964</td>
</tr>
<tr>
<td>Oral exposure to peanut - n (%)</td>
<td>0 (0%)</td>
<td>7 (19.5%)</td>
<td>0.618</td>
</tr>
<tr>
<td>SPT to peanut (mm)</td>
<td>7 (2-9)</td>
<td>2 (0-12)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Peanut-sIgE (KU/A/L)</td>
<td>0.94 (0.14, 14.5)</td>
<td>0.81 (0.01, 35.7)</td>
<td>0.964</td>
</tr>
<tr>
<td>Ara h 1-sIgE (KU/A/L)</td>
<td>0.03 (0.01, 8.67)</td>
<td>0.06 (0, 3.79)</td>
<td>0.622</td>
</tr>
<tr>
<td>Ara h 2-sIgE (KU/A/L)</td>
<td>0.15 (0.05, 8.95)</td>
<td>0.06 (0.01, 1.84)</td>
<td>0.023</td>
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<tr>
<td>Ara h 3-sIgE (KU/A/L)</td>
<td>0.01 (0.01, 1.62)</td>
<td>0.05 (0, 1.36)</td>
<td>0.189</td>
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<tr>
<td>Ara h 8-sIgE (KU/A/L)</td>
<td>0.01 (0.01, 4.66)</td>
<td>0.01 (0, 35.8)</td>
<td>0.893</td>
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<tr>
<td>Ara h 9-sIgE (KU/A/L)</td>
<td>0.01 (0.01, 0.28)</td>
<td>0.02 (0, 11.0)</td>
<td>0.823</td>
</tr>
</tbody>
</table>

N=8                               N=36

Santos AF et al, J Allergy Clin Immunol 2014
BAT discriminates between peanut allergy and tolerance in peanut-sensitised patients with equivocal allergy tests

Santos AF et al. J Allergy Clin Immunol 2014

N=44

*** p<0.001

** p<0.01:

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>Accuracy</th>
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</thead>
<tbody>
<tr>
<td>BAT</td>
<td>0.95</td>
<td>92%</td>
</tr>
<tr>
<td>SPT</td>
<td>0.83</td>
<td>82%</td>
</tr>
<tr>
<td>PsLgE</td>
<td>0.49</td>
<td>63%</td>
</tr>
<tr>
<td>Ara h 2</td>
<td>0.73</td>
<td>76%</td>
</tr>
</tbody>
</table>

Santos AF et al. J Allergy Clin Immunol 2014
Sequential use of allergy tests to diagnose food allergy

<table>
<thead>
<tr>
<th>BAT as a second step in the diagnostic process</th>
<th>Correct Diagnoses</th>
<th>False positives</th>
<th>False negatives</th>
<th>Number of BAT</th>
<th>Change in OFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPT + Specific IgE</td>
<td>67 (64%)</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>SPT → BAT</td>
<td>98 (94%)</td>
<td>3 (3%)</td>
<td>2 (2%)</td>
<td>24 (23%)</td>
<td>-35 (-97%)</td>
</tr>
<tr>
<td>Specific IgE → BAT</td>
<td>93 (89%)</td>
<td>5 (5%)</td>
<td>3 (3%)</td>
<td>41 (39%)</td>
<td>-33 (-92%)</td>
</tr>
<tr>
<td>Ara h 2 → BAT</td>
<td>99 (95%)</td>
<td>2 (2%)</td>
<td>2 (2%)</td>
<td>19 (18%)</td>
<td>-35 (-97%)</td>
</tr>
</tbody>
</table>

Santos AF et al. J Allergy Clin Immunol 2014
Suggested approach for using BAT to diagnose food allergy

- Clinical History
- SPT Specific IgE
- BAT
- Oral food challenge

Santos AF & Lack G, Clin Transl Allergy 2016
BAT reflects the severity and the threshold of allergic reactions

N=52 (3 NR)

***p<0.001  **p<0.01  * p<0.05

Santos AF et al, J Allergy Clin Immunol 2015
BAT reflects the severity and the threshold of allergic reactions

Song Y et al, Ann Allergy Asthma Immunol 2015
BAT reflects the severity and the threshold of allergic reactions

Severity

Threshold

Rubio A et al, Allergy 2011
Monitoring natural resolution of food allergy

%CD63 positive

Concentration (μg/mL)

Allergic Control HM tolerant Outgrown

0 3 × 10^{-4} 0 3 × 10^{-4} 0 3 × 10^{-4} 0 3 × 10^{-4}
Basophil suppression with allergen immunotherapy

...is observed to culprit and bystander allergen and anti-IgE during OIT

...is more marked with OIT than with SLIT and is often transient

Thyagarajan A et al, Clin Exp Allergy 2012

Gorelik M et al, J Allergy Clin Immunol 2015
Outline

• Diagnosis of food allergy
• Utility of BAT in food allergy
• Bringing BAT to clinical practice
Equivocal cases after SPT sIgE
Factors influencing the cut-offs and results of BAT

**Study population**
- Prevalence of food allergy
- Origin: general population versus specialised clinic
- Geographical location
- Associated respiratory and food allergies

**Study design**
- Inclusion criteria (e.g. sensitised/non-sensitised patients)
- Reference standard
- Criteria to refer patients for oral food challenges
- Oral food challenge protocol

**BAT Procedure**
- Allergen extracts: quality, concentration, stability, standardisation
- Interval between blood collection and performance of BAT
- Pre-incubation with IL-3
- Markers and antibodies used for staining ID and activation markers

**Flow cytometry data analyses**
- Cytometer and application settings
- Gating strategy
- Parameters used as outcomes of the test
- Definition of negative gate
- Whether results were corrected for background

Santos AF et al, Clin Transl Allergy 2016
Road map to bring BAT to clinical practice

- **Laboratory procedure**
  - Standardization of:
    - Reagents
    - Allergen extracts
    - Protocol
    - Flow cytometry equipment and settings

- **Clinical performance**
  - Diagnostic studies:
    - For different food allergens
    - In different clinical populations

- **Flow cytometry data analyses**
  - Standardization of:
    - Flow cytometry data analyses
    - Parameters selected as the outcome of BAT

- **Clinical utility**
  - Impact on health outcomes:
    - Cost-effectiveness
    - Social benefits

Santos AF & Shreffler WG. Clin Exp Allergy 2017
Take-home messages:

• BAT has high specificity and sensitivity to diagnose food allergy and can be used to monitor clinical response to treatment and possible resolution of food allergy.

• A positive BAT confirms the diagnosis of food allergy and averts OFC (mainly positive OFC). Patients with a negative BAT or non-responder basophils still require OFC.

• In order to make the transition to clinical practice, standardisation of the laboratory procedure, flow cytometry and data analyses is required and rigorous clinical validation and an assessment of the impact of BAT on health and social outcomes and its cost-effectiveness are warranted.
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