Basophil activation test in drug hypersensitivity

Suspicion of drug hypersensitivity

- Urticaria Anaphylaxis
- Basophil Activation Test – BAT
- Lymphocyte transformation test – LTT
- Cytokine secretion
- Cytotoxicity

Immediate Type

Delayed Type

Eczema Exanthem DRESS Hepatitis
When/Why use BAT for the diagnosis of immediate type DHR?

<table>
<thead>
<tr>
<th>When?</th>
<th>Why?</th>
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<tr>
<td>→ Identification of culprit drug in anaphylaxis or urticaria</td>
<td>→ Differentiation between 1. irrate toxic reactions → BAT - 2. pseudoallergy → BAT - 3. IgE-mediated reactions → BAT+</td>
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<tr>
<td>→ Skin test is not available</td>
<td>→ No risk for the patient</td>
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<tr>
<td>→ Skin test is too risky</td>
<td>→ Safe alternative</td>
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<td>→ Cross-reactivity</td>
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Test characteristics

- Ex-vivo functional test
- Minimal amount of blood
- Rapid (3h)

- Basophil activation is expressed by % of CD63 and CD203c upregulation
- SI>2 and clear CD63 populations is considered as positive
- Sensitivity of 50-90% and Specificity around 80-95%
- A negative test does not rule out drug hypersensitivity
Case 1: intraoperative anaphylaxis

Case description:
Anaphylactic shock during anesthesia 09/16
Skin test: rocuronium (+), mivacurium (+), chlorhexidine (+)
sIgE to chlorhexidine 13.6kU/l

Findings:
Sensitization to the antiseptic agent chlorhexidine
No sensitization to muscle relaxants detectable by BAT

Hypothesis:
IgE BAT+ rocuronium MRGPRX2 BAT-
MRGPRX2 (pseudoallergy): dose dependend
IgE-mediated: less dose dependend

Conclusion:
Avoidance of chlorhexidine and rocuronium
mivacurium is probably compatible
Case description:
Anaphylactic shock during anesthesia 12/15 with cefuroxime tryptase elevation (5.2 → 29.9 mg/l) during i.d. skin testing with β-lactams
Findings and conclusion:

• Sensibilization detectable to all β-lactam antibiotics
• No-Sensibilization to ciprofloxacin (Ciproxin) and rocuronium (Esmeron) detectable

Suggestion: Avoidance of all β-lactam antibiotics
Case 3: Cross-reactivity analysis with BAT (example PPI)

Case description:
Urticaria after two doses pantoprazol
Change to esomeprazol → Anaphylaxis one hour after first dose

Findings:
Sensitization to the pantoprazol, esomeprazol and rabeprazol detectable

Conclusion:
Because of cross-reactivity, avoid all PPIs if possible.
List of commercially offered drugs for BAT

- **B-Lactams:**
  
  Penicillin G, Tazobactam, Meropenem, Amoxicillin, Clavulanic acid,
  Cefuroxime, Ceftriaxone, Cefaclor, Cefazolin

- **Fluoroquinolones:**
  
  Ciprofloxacin, Moxifloxacin, Levofloxacin

- **Muscle relaxant:**
  
  Rocuronium, Suxamethonium, Mivacurium, Atracurium

- **Protein Pump Inhibitors (PPIs)**
  
  Lanzoprazol, Esomeprazol, Pantoprazol, Omeprazol

Others: Carboxymethylcellulose, Metamizol, Chlorhexidine, Neomycine

We offer to test other drugs with...

- well documented clinic (severe reaction e. g. anaphylaxis)
- positive skin test results or skin test not possible/not available

... on experimental basis
Is BAT useful to detect ciprofloxacin allergy?

...yes, for the detection of IgE-mediated reaction!

Keep in mind that:

Muscle relaxants: rocuronium, mivacurium, ...

Fluoroquinolones: Ciprofloxacin, Moxifloxacin, ...

...can induce IgE-mediated allergy and pseudoallergy (MRGPRX2)

Skin test + → MRGPRX2 or IgE

BAT + → IgE-mediated
Serum based BAT (indirect BAT): principle

1. **Isolation**: PBMCs from a well-characterized basophil donor were isolated by Ficoll gradient.

2. **Stripping**: PBMCs were incubated for 5 min with lactic acid (pH 3.9) to disrupt the high affinity binding of IgE to its receptor (FcεRI) followed by neutralization.

3. **Resensitization**: Patient serum was added to the "stripped" basophils and incubated for 2 h at 37°C, 5% CO₂. IgE within the serum will bind with to its receptor.

4. **BAT**: Basophils were afterwards stimulated with serial dilutions of appropriate drug or medium (negative control), anti-IgE and anti-FcεRI (positive control) and incubated for 30 min at 37°C, 5% CO₂.

Future application for the diagnosis of immediate type ADR?
Serum based BAT: results

Findings:

• Basophil activation detectable with divalent drugs (e.g. chlorhexidine)

• more challenging with monovalent drugs such β-lactams

• cephalosporins worked better than amoxicillin

• other monovalent drugs need to be tested in detail

Requires a better understanding of drug-protein interaction and conditions for IgE crosslinking
## Indirect BAT vs. Direct BAT

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<tr>
<th>Indirect BAT</th>
<th>Direct BAT</th>
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<tr>
<td><strong>Pro:</strong></td>
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<tr>
<td>• usage of patient sera</td>
<td>• already established for many drugs</td>
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<tr>
<td>• no time limitation after blood withdrawal</td>
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<tr>
<td>• easy storage at 4°C</td>
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<tr>
<td>• international analysis possible</td>
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<tr>
<td>• batch analysis</td>
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<tr>
<td>• compatible with IgE non-responders</td>
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<tr>
<td><strong>Contra:</strong></td>
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<tr>
<td>• not yet functional with all drugs</td>
<td>• 20h time window between blood withdrawal and test</td>
</tr>
<tr>
<td>• well characterized donors needed</td>
<td>• Patients’ blood needed</td>
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T cell assays
- Lymphocyte transformation test — LTT
- Cytokine secretion
- Cytotoxicity
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