The Application of Data-Driven Approaches to Flow Cytometric Analysis of Basophil Activation Testing

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The basophil activation test (BAT)

- Basophil activation testing
  - Ex vivo allergen stimulation
  - Upregulation of activation markers
  - Data acquisition by flow cytometry
- IgE-mediated allergies
  - Allergens: Environmental, Venom, Drug, Food
  - Clinical applications: Diagnosis, Immunotherapy

Variability in BAT

Biological

Technical

Protocol standardization

Analytical

Rare basophil population
Labor intensive
Flow based assay
Dose-response curve
Programmatic flow cytometry data analysis

<table>
<thead>
<tr>
<th>Manual Gating</th>
<th>Autogating</th>
</tr>
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<tbody>
<tr>
<td>Gold standard</td>
<td>Reproducible</td>
</tr>
<tr>
<td>Subjective</td>
<td>Transparent</td>
</tr>
<tr>
<td>Permissive</td>
<td>Quality control</td>
</tr>
<tr>
<td>Labor intensive</td>
<td>High-throughput</td>
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</tbody>
</table>
Clinical Dataset: Peanut oral immunotherapy

- Single center trial (N=30)
  - Pediatric (7-21 years old)
  - Peanut allergic (+SPT, +sIgE)
  - Increasing doses of peanut flour
Basophil activation testing (BAT) analysis

15 minute stimulation at 37°C

Antigen stimulation (Ara h 1, Ara h 2, Ara h 6, whole peanut extracts)

Flow Cytometry
FCS files

Analysis
Compensation
Transformation
Removal of boundary events

Statistical Analysis
Dose-response curves
Area under the curve

Quality Control
Expert Analysis
Outlier Identification
DEVELOPMENT OF THE PROGRAMMATIC APPROACH
• Development

• Application

Patil, et.al.
2017. Flow
Cyto B.
AutoBAT

flowCore

flowFrame

flowFrame

flowFrame

phenoData

flowSet

R/Bioconductor

https://github.com/saritaupatil/AutoBAT
Programmatic application: BATgate

- BATgate() - Function designed for basophil gating and identification of activated basophils (%CD63hi)
- Step-wise gating strategy
Step-wise gating approach

1. Identification of activated basophils
2. CD63 gating
3. Selection of CCR3+ cells
4. Transformation/Removal Boundary Events
5. Selection of Side-scatter low cells

kmeans 2 or 3
Gating on basophils in BAT

Manual

Autogating

Programmatic application: BATgate

- **BATgate()**
  - Function designed for basophil gating and identification of activated basophils (%CD63hi)
  - Step-wise gating strategy
  - Input
    - Workingdir
    - Fluorophores
    - SSno
    - Medcontroltube
  - Outputs
    - Csv file with data statistics for each stimulation condition
    - Pdf file of gating graphics for each condition for visualization
Programmatic application: BATmeasures

- **BATmeasures()**
  - Function designed to derive dose-response measures of antigen stimulated basophils
  - **Measures:**
    - ED50
    - Area-under-the-curve by using log of the stimulation concentration
  - **Input**
    - data: the data.frame being used for measurement of BAT
      - Needs to have removal of any non-dose response data
    - groupvariables: the grouping variables to identify each dose-response series
      - Ie: Patient, visit, stimulation condition
    - output.file: name of the output file
  - **Output**
    - An csv file with the name of the output.file, with ED50 and AUC
52 • Development

294 • Application

269 • Successful

92%
7,166 individual BAT

Patil, et.al. 2017. Flow Cyto B.
GATING CD63 UPREGULATION
CD63 gating

- Comparison of gating strategies for CD63 upregulation
  - Medium-based
  - Anti-IgE based

Patil, et.al. 2017. Flow Cyto B.
Gating on resting basophils

Gating based on anti-FcERI activated basophils

R=0.96 vs.
R=0.84,
p<0.001

Patil, et.al.
2017. Flow Cyto B.
Measures of BAT

MacGlashan, D. (2013) JACI.

### CDmax and ED50

<table>
<thead>
<tr>
<th>CD63hi Basophils</th>
<th>An+gen</th>
<th>S+mula+on</th>
<th>Concentra+on (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basophils</td>
<td></td>
<td></td>
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**Before Aner Patil, et al. 2017.**

**Flow Cyto B.**
LESSONS FROM QUALITY CONTROL MEASURES
Basophil count

CCR3 MRI

Acquisition Date

Patil, et al.
2017. Flow Cyto B.
Influence of basophil sampling on measurement of degranulated basophils

Patil, et.al. 2017. Flow Cyto B.
Influence of basophil sampling on AUC measurement of dose-response curves

Patil, et.al. 2017. Flow Cyto B.
The future of programmatic approaches

• Open-access to analytic method
• Cloud-based analytical platform
• Applications: Multicenter data
• Setting standards for flow cytometry gating
• Machine learning approaches
• Quality control
  – Longitudinal
  – Internal
• Broadening applications
  – Use with other allergens
  – Use with multi-marker basophil experiments
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– Food Allergy Research Team
– Harvard Catalyst, MGH CRC
– Patients and families

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