Bronchoprovocation tests in children and adults
Lanny J. Rosenwasser, M.D.
Dee Lyons/Missouri Endowed Chair in Immunology Research

Professor of Pediatrics
Allergy-Immunology Division
Childrens Mercy Hospital
Kansas City, Missouri

Professor of Pediatrics, Medicine and Basic Science
University of Missouri Kansas City School of Medicine
Ömer KALAYCI, MD
Professor of Pediatrics, Allergy and Asthma
Hacettepe University School of Medicine
Ankara, Turkey
Asthma is a disorder of the airways with the following pathophysiological characteristics:

- Chronic inflammation
- Variable airflow obstruction
- Hyperresponsiveness to a variety of “triggers”
“Twitchy” Airways

Bronchial hyperresponsiveness is:

• An abnormal increase in airflow limitation following exposure to a stimulus;

• Alternatively, a threshold response (e.g., ≥20% fall in FEV1) which occurs at a lower point (dose) than in a healthy individual.
Use of provocation tests

- Epidemiological studies
- Clinical studies
- Asthma: diagnosis and differential diagnosis
- Follow-up of asthma treatment
BRONCHIAL PROVOCATION TESTS for ASTHMA

- For diagnosis of asthma in cases where spirometry and reversibility test are not enough.
  - Low specificity %36-54
  - High sensitivity % 84-94
- To rule out asthma diagnosis
  - High negative predictive value
- To determine the severity of asthma
- To determine the response to treatment

ATS 1999
Contraindications

**Absolute**

- Severe airflow limitation
  \[(\text{FEV}_1 < 50\% \text{ pred.}, \text{ or } < 1.0 \text{ L})\]
- Heart attack or stroke in last 3 months
- Uncontrolled hypertension
- Known aortic aneurysm

**Relative**

- Moderate airflow limitation
  \[(\text{FEV}_1 < 60\% \text{ pred.}, \text{ or } < 1.5 \text{ L})\]
- Inability to perform acceptable spirometry
- Pregnancy
- Nursing mothers
Safety of a Low Starting FEV$_1$

- 88 patients with FEV$_1$ <60% predicted (22% - 59%)
- Mean baseline FEV$_1$ 1.39 ± 0.28 L (0.64 – 2.4 L)
- Testing was safe and successful
- 84 patient’s FEV$_1$ returned to 90% of baseline, and 4 required a 2$^{nd}$ treatment

Types of stimuli

- Specific
  - Allergen
  - Aspirin, food
  - Exercise tests

- Nonspecific
  - Metacholine
  - Histamine
  - AMP
  - Cold air
  - Nonisotonic solutions
  - Leukotrienes
  - Serotonin
  - Prostaglandin
  - Tachycinin
Types of Stimuli

- **Direct Stimulus**
  Cause airflow limitation by a direct action on effector cells (e.g., airway smooth muscle cells, mucus producing cells).

- **Indirect Stimulus**
  Cause airflow limitation by an action of cells other than effector cells, which then interact with the effector cells.
Direct stimulus

Effector cells
- Airway smooth muscle cells
- Bronchial endothelial cells
- Mucus producing cells

Indirect stimulus

Intermediary cells
- Inflammatory cells
- Neuronal cells

Airflow limitation
<table>
<thead>
<tr>
<th>Direct Stimuli</th>
<th>Indirect Stimuli</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Acetycholine</td>
<td>• Adenosine</td>
</tr>
<tr>
<td>• Methacholine</td>
<td>• Bradykinin</td>
</tr>
<tr>
<td>• Carbachol</td>
<td>• Metabisulfite / SO&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>• Histamine</td>
<td>• Exercise</td>
</tr>
<tr>
<td>• Prostaglandin D&lt;sub&gt;2&lt;/sub&gt;</td>
<td>• Hyper/hypotonic aerosol</td>
</tr>
<tr>
<td>• Leukotrienes</td>
<td>• Isocap. hyperventilation</td>
</tr>
<tr>
<td></td>
<td>• Mannitol</td>
</tr>
<tr>
<td></td>
<td>• Propanolol (β-blockers)</td>
</tr>
</tbody>
</table>
## FACTORS THAT DECREASE BRONCHIAL RESPONSIVENESS

<table>
<thead>
<tr>
<th>Factor</th>
<th>Minimum Time Interval from Last Dose to Study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medications</strong></td>
<td></td>
</tr>
<tr>
<td>Short-acting inhaled bronchodilators, such as isoproterenol,</td>
<td>8 h</td>
</tr>
<tr>
<td>isetharine, metaproterenol, albuterol, or terbutaline</td>
<td></td>
</tr>
<tr>
<td>Medium-acting bronchodilators such as ipratropium</td>
<td>24 h</td>
</tr>
<tr>
<td>Long-acting inhaled bronchodilators, such as salmeterol, formoterol,</td>
<td>48 h</td>
</tr>
<tr>
<td>tiotropium</td>
<td>(perhaps 1 wk for tiotropium)</td>
</tr>
<tr>
<td><strong>Oral bronchodilators</strong></td>
<td></td>
</tr>
<tr>
<td>Liquid theophylline</td>
<td>12 h</td>
</tr>
<tr>
<td>Intermediate-acting theophyllines</td>
<td>24 h</td>
</tr>
<tr>
<td>Long-acting theophyllines</td>
<td>48 h</td>
</tr>
<tr>
<td>Standard β₂-agonist tablets</td>
<td>12 h</td>
</tr>
<tr>
<td>Long-acting β₂-agonist tablets</td>
<td>24 h</td>
</tr>
<tr>
<td>Cromolyn sodium</td>
<td>8 h</td>
</tr>
<tr>
<td>Nedocromil</td>
<td>48 h</td>
</tr>
<tr>
<td>Hydroxyzine, cetirizine</td>
<td>3 d</td>
</tr>
<tr>
<td>Leukotriene modifiers</td>
<td>24 h</td>
</tr>
<tr>
<td><strong>Foods</strong></td>
<td></td>
</tr>
<tr>
<td>Coffee, tea, cola drinks, chocolate</td>
<td>Day of study</td>
</tr>
</tbody>
</table>
## Factors that increase Bronchial Hyperresponsiveness

<table>
<thead>
<tr>
<th>Factor</th>
<th>Duration of effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure to environmental antigens</td>
<td>1-3 weeks</td>
</tr>
<tr>
<td>Occupational sensitizers</td>
<td>Months</td>
</tr>
<tr>
<td>Respiratory infections</td>
<td>3-6 weeks</td>
</tr>
<tr>
<td>Air pollutants</td>
<td>1 week</td>
</tr>
<tr>
<td>Cigarette smoke</td>
<td>Uncertain, ind. variation</td>
</tr>
<tr>
<td>Chemical irritants</td>
<td>Days to months</td>
</tr>
</tbody>
</table>
What do most people use to evaluate airway hyperreactivity?
Questionnaire to prominent and active investigators using bronchial provocation techniques.

- 44 of 94 responses
- Methacholine (63%)
  - Histamine (17%)
  - Exercise (8%)
  - Specific antigens (5%)

Scott GC, Braun SR.
Direct Stimuli

Methacholine
- Most widely used
- Well standardized
- Easy to obtain today
- Better differentiates reactive/nonreactive airways

Histamine
- Good correlation with methacholine
- More side effects
- Development of tachyphylaxis
Metacholine

Synthetic acetylcholine derivative
Metabolized by choline esterase
Effects blocked by atropin and other anticholinergics

pH < 6
Cons > 0.3 mg/ml
Stable at least 3 months
at 4°C

Mch should be diluted with saline and NOT with buffered solutions
Patient Preparation

• Withhold medications that will interfere

• Explain the test, but don’t over do it
  • They aren’t going to have an asthma attack!!
  • Avoid the impact of suggestion.

• Consent form

• Pre-test questionnaire

• Withhold coffee, tea, cola drinks, chocolate for day of study
<table>
<thead>
<tr>
<th>Medication</th>
<th>Withholding Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-acting inhaled bronchodilators</td>
<td>8 hrs</td>
</tr>
<tr>
<td>Med.-acting bronchodilators (e.g., ipratropium)</td>
<td>24 hrs</td>
</tr>
<tr>
<td>Long-acting bronchodilators</td>
<td>48 hrs</td>
</tr>
<tr>
<td>Oral bronchodilators</td>
<td>12-48 hrs</td>
</tr>
<tr>
<td>Cromolyn sodium</td>
<td>8 hrs</td>
</tr>
<tr>
<td>Nedocromil</td>
<td>48 hrs</td>
</tr>
<tr>
<td>Leukotriene modifiers</td>
<td>24 hrs</td>
</tr>
</tbody>
</table>
2 min tidal breathing

- Dilutions of Mch are prepared and kept at RT for 30 min before use

### DILUTION SCHEMES FOR THE TWO RECOMMENDED METHACHOLINE DOSING SCHEDULES

<table>
<thead>
<tr>
<th>Label Strength</th>
<th>Take</th>
<th>Add NaCl (0.9%)</th>
<th>Obtain Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Dilution schedule</strong> using 100-mg vial of methacholine chloride and the 2-min tidal breathing protocol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mg</td>
<td>100 mg</td>
<td>6.25 ml</td>
<td>A: 16 mg/ml</td>
</tr>
<tr>
<td>3 ml of dilution A</td>
<td>3 ml</td>
<td>B: 8 mg/ml</td>
<td></td>
</tr>
<tr>
<td>3 ml of dilution B</td>
<td>3 ml</td>
<td>C: 4 mg/ml</td>
<td></td>
</tr>
<tr>
<td>3 ml of dilution C</td>
<td>3 ml</td>
<td>D: 2 mg/ml</td>
<td></td>
</tr>
<tr>
<td>3 ml of dilution D</td>
<td>3 ml</td>
<td>E: 1 mg/ml</td>
<td></td>
</tr>
<tr>
<td>3 ml of dilution E</td>
<td>3 ml</td>
<td>F: 0.5 mg/ml</td>
<td></td>
</tr>
<tr>
<td>3 ml of dilution F</td>
<td>3 ml</td>
<td>G: 0.25 mg/ml</td>
<td></td>
</tr>
<tr>
<td>3 ml of dilution G</td>
<td>3 ml</td>
<td>H: 0.125 mg/ml</td>
<td></td>
</tr>
<tr>
<td>3 ml of dilution H</td>
<td>3 ml</td>
<td>I: 0.0625 mg/ml</td>
<td></td>
</tr>
<tr>
<td>3 ml of dilution I</td>
<td>3 ml</td>
<td>J: 0.031 mg/ml</td>
<td></td>
</tr>
<tr>
<td><strong>B. Optional dilution schedule using 100-mg vial of methacholine chloride and five-breath dosimeter protocol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mg</td>
<td>100 mg</td>
<td>6.25 ml</td>
<td>A: 16 mg/ml</td>
</tr>
<tr>
<td>3 ml of dilution A</td>
<td>9 ml</td>
<td>B: 4 mg/ml</td>
<td></td>
</tr>
<tr>
<td>3 ml of dilution B</td>
<td>9 ml</td>
<td>C: 1 mg/ml</td>
<td></td>
</tr>
<tr>
<td>3 ml of dilution C</td>
<td>9 ml</td>
<td>D: 0.25 mg/ml</td>
<td></td>
</tr>
<tr>
<td>3 ml of dilution D</td>
<td>9 ml</td>
<td>E: 0.0625 mg/ml</td>
<td></td>
</tr>
</tbody>
</table>
2 min tidal breathing

1. Basal (diluent) spirometry
2. Nose clip
3. 2 min tidal breathing
4. FEV1 within 30-90 sn
5. Max 3 min and 3-4 manoeuvres for FEV1; best FEV1
6. 5 min between two nebulizations
7. Salbutamol at the ned of the test followed by spirometry in 10 min
5 breath dosimeter

- 0.0625, 0.25, 1, 4, 16 mg/ml

- During tidal breathing

- At the end of exhalation (at functional residual capacity), slow and deep inhalation

- Start the dosimeter right after the start of inhalation

- Inhalation time: 5 sec

- Breath holding at TLC: 5 sec

- Five repeats of the same procedure

- Total duration: 2 min
Perform Baseline Spirometry

* FEV1 >70% Predicted?
  Yes
  Administer diluent or first dose of methacholine, and perform spirometry after the appropriate delay
  FEV1 decline >20%
    Yes
    Record signs and symptoms, Give albuterol, wait 10 minutes, and perform spirometry
    No
    Administer next dose of methacholine, and perform spirometry after the appropriate delay
    FEV1 decline >20%
      Yes
      Record signs and symptoms, Give albuterol, wait 10 minutes, and perform spirometry
      No
      16 mg/ml dose given?
        No
        Study completed
        Yes
        FEV1 decline >10%
          Yes
          Record signs and symptoms, Give albuterol, wait 10 minutes, and perform spirometry
          No
          Study completed
  No
  FEV1 decline >20%
    Yes
    Record signs and symptoms, Give albuterol, wait 10 minutes, and perform spirometry
    No
    Study completed
Technical Factors and Aerosols

- Nebulizer output
- Aerosol particle size
- Tubing
- Lung volume
- Inspiratory flow rate
- Breathhold time
Spirometry

• Change in FEV$_1$ is the primary outcome measure
• Spirometry should meet ATS guidelines for acceptability
• The quality of the spirogram should be examined after each maneuver
• Full FVC efforts lasting $\geq$ 6 sec should be performed at baseline and after diluent
• If the FEV$_1$ is the only outcome measure, the expiratory maneuver can be shortened to about 2 sec at other stages
• If shortened maneuver is used, assure inspiration is complete
Provocative Concentration (PC)

The exact concentration that causes a specific fall in a PFT parameter:

\( PC_{20\text{FEV}_1} \)  
Concentration that causes a 20% fall in \( \text{FEV}_1 \)

\( PC_{40\text{SG}_{aw}} \)  
Concentration that causes a 40% fall in specific conductance
FEV1 değeri düşüşü

Metakolin konsantrasyonu

salin  0.031  0.0625  0.125  0.25  Mch PC20
• Asthma probability = % 30–70
  PC20 = 16 mg/ml
  Patient does not have asthma

• Asthma prob % 30–70
  PC20 = 1.0 mg/ml,
  Asthma

• Astım olasılığı % 30–70
  1.0 mg/ml < PC20 < 16 mg/ml
  Asthma (??)
1. Mild intermittent asthma but the patient is not well aware of symptoms

2. Patient does not exercise or confront stimuli that can cause bronchoconstriction

3. Mild BHR is due to another reason such as a recent UTI or smoke exposure

4. Asymptomatic asthma which will clinically be overt in a few years (% 15-45)
Quality Control

- **Nebulizer output**
  - Verify output initially & after every 20 uses, until an appropriate testing schedule is established for lab.
  - Output for 2-min. TB neb. = 0.13 to 0.15 mL/min ± 10%
  - Output for DeVilbiss neb. = 0.009 mL/actuation ± 10%

- Verify concentrations of solutions

- Verify challenge procedure

- Keep records of QC procedures
Safety

Precautions for Patient Safety

• Trained staff close enough to respond quickly to an emergency

• Medications to treat bronchospasm must be present in testing area

• A stethoscope, sphygmomanometer, and pulse oximeter should be available
Precautions for Technician Safety

- Try to minimize technician exposure
- Testing room should have adequate ventilation (> 2 AC/hr)
- Use of exhalation filters useful in TB method
- Those with asthma are at increased risk and should take extra precautions to minimize their exposure
<table>
<thead>
<tr>
<th>PC$_{20}$ (mg/mL)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 16</td>
<td>Normal BHR</td>
</tr>
<tr>
<td>4.0 - 16</td>
<td>Borderline BHR</td>
</tr>
<tr>
<td>1.0 - 4.0</td>
<td>Mild BHR (positive test)</td>
</tr>
<tr>
<td>&lt; 1.0</td>
<td>Moderate to severe BHR</td>
</tr>
</tbody>
</table>
Exercise-induced Bronchoconstriction (EIB)

Exercise-induced Asthma (EIA)
EIB Factors

- Exercise needs to be continuous
- Type of exercise matters
- Intensity: 60-80% max causes greatest severity
- Duration
- Air temperature and humidity
Exercise induced bronchoconstriction
Exercise provocation

Water loss

- Thermal effect
- Osmolar effect

- Warming rate > cooling

Mediator release

Histamine, PG, LT, tachykinin etc
Vascular hypothesis

- Cooling of the airways
- Vasoconstriction in bronchial vessels
- End of exercise
- Sudden and significant increase in the blood volume in peribronchial vascular plexus
- Reactive hyperemia and edema in airway walls.
Osmolar hypotnesis

- Water loss due to evaporation
- Increase in ion concentration in the periciliary fluid
- Hyperosmolarity
- Mediator release
- Bronchoconstriction
Diagnosis of EIA

• History and Physical exam
• Free running test
• Exercise provocation
Inhalation

- Nose clips
  - Decreases water loss from the nasal airway.

- 25°C dry air
  - AC 20-25 °C and < 50 % relative humidity
  - Filled balloons with two way valves
Exercise provocation

- Treadmill
- Bicycle ergometry
Exercise provocation

- Total duration
  » < 12 years = 6 min
  » > 12 years = 8 min

- By adjusting the speed and inclination, reach 80-90% of max heart rate within the first 2-3 min
  » Max heart rate = 220 - yaş

- By adjusting the speed and inclination, reach 40-60% of max voluntary ventilatio within the first 2-3 min
  » MVV = FEV1 x 35
Exercise provocation

– 4 min running at max heart rate
  – 4.5 km/s ve % 15 eğim

– 1, 5,10,15,20,25,30 min FEV1

– > %10 düşme
Exercise Test Graphics

FEV₁

exercise
ADENOSIN MONOPHOSPHATE
AMP
Adenosine

- Adenosine 5’ – monophosphate (AMP)
- Indirect stimulant
- Releases histamine & other mediators from mast cells
- Action is blocked by antihistamines
- May reflect extent of airway inflammation better than methacholine
AMP

- AMP sodium salt (Sigma-Aldrich, A1752)
- Saline solubility > adenosin
- > 3.125 mg/ml 4°C stability > 25 weeks
- 5 breath dozimeter or 2 min tidal method
  3.125, 6.25, 12.5, 25, 50, 100, 200, 400 mg/ml
- PC20
Stephen L. Tilley and Richard C. Boucher,
The Journal of Clinical Investigation Volume 115 Number 1 January 2005
Specific Antigen

- Performed when proof of sensitivity, avoidance, or immunotherapy required
- Most commonly used in research
- Immediate and late responses
- Strong and lasting responses
Adenosine

- Inhalation of aerosol
- Diluent usually 0.9% saline
- Dosing scheme range 0.04 to 320 mg/mL
- Quadrupling doses reported to be safe and efficient - DeMeer et al., Thorax 2001;56:362-365
Hypertonic saline provocation

- Devilbiss Ultraneb 2000
  - 2 way valve (Laerdal valve No 560 200/850 500, Devilbiss)
  - 60 cm tube (Devilbiss no. 8885)

- Hypertonic sterile saline (4.5 %)
  - 30 sec, 1 min, 2 min, 4 min, 8 min
  - Total 14.5 min

- FEV1 measurement 1 min after the end of inhalation

- > %15 fall: test positive
Saline provocation evaluation

- Positive / negative
  - $> \% 15$ positive
  - $< \% 15$ negative

- Response / dose ratio (RDR)
  - Fall in FEV1 % / quantity of inhaled saline
Mannitol

- Naturally occurring sugar, isomer of sorbitol
- Indirect stimulant
- Dry powder
- Osmotic stimulant for the airway mucosa

Proposed doses:
- 0, 5, 10, 20, 40, 80, 160, 160, 160
- Cumulative dose 0-63

Endpoint: 15% fall in FEV1
Mannitol

- Proposed doses: 0, 5, 10, 20, 40, 80, 160, 160, 160
- Cumulative dose 0-63

- Endpoint: 15% fall in FEV1 measured 1 min after each dose

- Interval between doses: 2 min

- PD!5> 636 Normal
<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannitol vs Hypertonic Saline</td>
<td>80.7 (76.4, 85.1)</td>
<td>86.7 (82.6, 90.7)</td>
</tr>
<tr>
<td>Mannitol vs Clinical Dx</td>
<td>59.8 (55.4, 64.2)</td>
<td>94.5 (89.9, 99.2)</td>
</tr>
<tr>
<td>Hypertonic Saline vs Clinical Dx</td>
<td>65.1 (60.9, 69.3)</td>
<td>95.2 (91.1, 99.3)</td>
</tr>
</tbody>
</table>

*Excluding all taking ICS*

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannitol vs Clinical Dx</td>
<td>70 (62.1, 78.2)</td>
<td>95 (90.7, 99.3)</td>
</tr>
</tbody>
</table>

*Excluding M-ve taking ICS*

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<th>Specificity (95% CI)</th>
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<tr>
<td>Mannitol vs Clinical Dx</td>
<td>89 (85.3, 92.1)</td>
<td>95 (90.7, 99.3)</td>
</tr>
</tbody>
</table>

Oral Challenges

• Performed when proof of sensitivity needed

• Common agents and prevalence
  • Metabisulfite: 5 – 10% in adults
  • Tartrazine: <5%
  • ASA: 4 to 20%

• Time for reaction varies
Occupational Challenges

• Specific challenges considered the gold standard for dx of occupational asthma

• Agents
  • Natural organic (flour, wood dust)
  • Pharmaceuticals (cimetidine)
  • Organic chemicals (isocyanates)
  • Inorganic chemicals (nickel salts)

• Immediate and late responses

• Need for controls (placebo)
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