Methacholine versus Mannitol Challenge in the Evaluation of Asthma

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Conflict of interest

- Dr Sandra Anderson is the Inventor on the patent that cover these applications for mannitol
- The patent is owned by her employer SSWAHS
- The rights to commercialise the intellectual property are licensed to Pharmaxis Ltd
- Dr Anderson purchased her own shares on the open market and holds no options.
- Dr Anderson is a consultant to Pharmaxis Ltd
- Dr Anderson receives a 10% share of the royalties paid to SSWAHS
Q: Why do we use bronchial provocation tests in the evaluation of Asthma?

Performing the appropriate bronchial provocation test (BPT) by using either a direct or an indirect stimulus to identify bronchial hyperresponsiveness (BHR) reduces the possibility of over and under diagnosis of asthma based on history and symptoms.
Q: When do we need to know about bronchial hyperresponsiveness?

- Upon presentation of a person with symptoms suggestive of asthma but with normal lung function and doubt about the diagnosis of asthma.

- In a person entering an occupation or recreational activity where BHR could be a potential problem.

- In a person well-controlled on treatment for a long period in whom it may be useful to back titrate treatment e.g. the dose of steroid.

- Following exposure to an occupational irritant or allergen that has induced symptoms of asthma.
Q: Why do we need to know about bronchial hyperresponsiveness?

• In a person with normal lung function BHR, to an indirectly acting stimulus, is an indirect index of the presence of airway inflammation.

• The inflammation of asthma involves eosinophils and mast cells.

• The number of these cells & concentration of mediators of bronchoconstriction are reduced with treatment with inhaled steroids & BHR is concomitantly reduced.
Q: What are the types of Bronchial Provocation Tests that are used to evaluate asthma?

**INDIRECT challenge test: e.g. mannitol**

"Indirect challenges act by causing the release of endogenous mediators that cause the airway smooth muscle to contract, with or without effect in inducing microvascular leakage.


**DIRECT challenge test: e.g. methacholine**

The agent is administered and acts on a specific receptor on the bronchial smooth muscle causing it to contract and the airways to narrow. Identifies bronchial responsiveness consistent with asthma or with airway injury or airway remodeling.
Q: What is the source of the mediators that cause Bronchial Smooth Muscle to contract?

Indirect means the stimulus comes from cells e.g. the mast cell eosinophil

Direct means the agonist acts on the smooth muscle

Histamine

Prostaglandins

Leukotrienes

Eosinophils

Bronchial smooth muscle
Why perform a bronchial provocation test?

- *Is it asthma?*
  - to exclude asthma?
    - one needs a sensitive test
  - to confirm asthma?
    - one needs a specific test

- *to manage asthma?*
  - one needs a test that correlates with the response of asthma to treatments
Is Methacholine a Rule Out test?

• Methacholine challenge has been promoted for its clinical utility in excluding the diagnosis of asthma based on a negative test result.

• Is a negative methacholine test result still valid as a rule out test?
Is Mannitol a Rule in test?

- In contrast to methacholine, the challenges that act indirectly, via release of mediators such as exercise or mannitol, are promoted as tests that confirm the diagnosis of asthma if the test result is positive but do not rule out asthma if the test result is negative.

- Is it valid to consider mannitol as a rule in test for asthma and if so, what is the significance of a negative test result?
What equipment is required for a Mannitol Test?

Equipment

- Aridol™ Kit (containing Aridol™ capsules, inhaler device and instruction leaflet)
- Spirometer & mouthpiece
- Nose clip
- Timer (which can be set to 60 seconds)
- Calculator
- Bronchodilator (eg albuterol) & volumatic spacer (if using a metered dose inhaler)
- Oxygen and other relevant emergency equipment should be readily available as per standard Bronchial Provocation Testing protocols.

www.mannitoltest.info
Inhaled agent: Dry powder Mannitol
Progressive Protocol: 0, 5, 10, 20, 40, 80, 160, 160, 160 mg
Measurements: FEV$_1$ Pre & 1 min post dose with highest value for FEV$_1$ recorded
Positive Response: Fall FEV$_1$ $\geq$15% or Fall FEV$_1$ $\geq$10% between consecutive doses
Expression of result:
Sensitivity PD$_{15}$
Reactivity Response Dose Ratio
Recovery: Final % fall FEV$_1$ / Cumulative dose
Recovery: Bronchodilator or spontaneous

Anderson SD et al AJRCCM 1997;156:758-765,
Brannan JD Anderson SD et al Respiratory Research 2005; Dec PHASE 3 trial
Anderson SD et al Respiratory Research 2009; January PHASE 3 trial
Sverrild A et al J Allergy Clin Immunol 2009; 24:928-932
Levels of Bronchial hyperresponsiveness to mannitol

Cumulative dose of mannitol (mg)

% Fall FEV1

Severe BHR Moderate BHR
< 35mg < 155mg

Mild BHR
> 155mg

Normal

Recovery can be Spontaneous or with Bronchodilator Recovery after Dry Mannitol Challenge

Anderson SD et al, 1997; AJRCCM 156:758-65
Q: What Population Studies have been performed using mannitol and methacholine?

- A301 was the 1st Phase 3 study using mannitol as a BPT. It was carried out in well defined groups including 492 asthmatic and healthy subjects both adults and children. (Brannan JD et al Resp Res 2005;6:144)

- A305 was the 2nd Phase 3 study using both mannitol and methacholine and exercise. It was in a group of 275 subjects with symptoms of asthma who entered the study without a definite diagnosis of asthma (Anderson SD et al Respir Res 2009: 10:4)

- Copenhagen Study comprised an unselected sample of 238 young adults and mannitol and methacholine was used to identify those with respiratory physician diagnosed asthma (Sverrild A et al J Allergy Clin Immunol 2010;124:928)

- Athlete Study: 58 young elite skiers with asthma like symptoms had provocation tests using mannitol, methacholine and dry air hyperpnea (Sue-Chu M et al Brit J Sports Med 2010;44:827)
Study in well defined populations of asthmatic and healthy subjects

Respiratory Research

The safety and efficacy of inhaled dry powder mannitol as a bronchial provocation test for airway hyperresponsiveness: a phase 3 comparison study with hypertonic (4.5%) saline

John D Brannan¹, Sandra D Anderson *¹, Clare P Perry¹, Ruth Freed-Martens¹, Anna R Lassig², Brett Charlton² and the Aridol Study Group

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This article is available from: http://respiratory-research.com/content/6/1/144

Brannan JD Anderson SD et al Respiratory Research 2005; 6:144
http://respiratory-research.com/articles/browse.asp
A301 Phase 3 trial Per Protocol - Patients’ characteristics

• 592 subjects (466 adults, 126 children) included 487 (82.3%) asthmatics and 105 non asthmatics.
• Age 6 – 83 mean 34.8 yr
• Majority had mild disease
  - Half the asthmatic cohort had infrequent symptoms
  - Only 11.3% reported symptoms interfering with normal activity.
• Majority had good lung function.
  - 50% of the asthmatics had a FEV1 > 95% of predicted.
  - The mean FEV1 was 3.0 L in the asthmatics and 3.2 L in the non-asthmatics.
• 78.4% taking at least one medication.
  - 228 on combination therapy / 164 on monotherapy with ICS

Brannan JD et al. Respir Res 2005;6:144
<table>
<thead>
<tr>
<th></th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ATS 2005 ADULTS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GeoMean PD_{15}</td>
<td>185 (147, 233)</td>
<td>97 (77, 123)</td>
<td>84 (54, 129)</td>
</tr>
<tr>
<td><strong>CHILDREN</strong></td>
<td>Infrequent Episodic</td>
<td>Frequent Episodic</td>
<td>Persistent</td>
</tr>
<tr>
<td></td>
<td>(n=17)</td>
<td>(n=12)</td>
<td>(n=53)</td>
</tr>
<tr>
<td>GeoMean PD_{15}</td>
<td>144 (92, 223)</td>
<td>120 (69, 208)</td>
<td>73 (50, 108)</td>
</tr>
</tbody>
</table>
Cumulative doses of mannitol to cause $PD_{15}$

- 60% children responded in $\leq 155$ mg or 6 capsules
- 83.5% children responded in $\leq 315$ mg or 10 capsules

$n = 85$

Frequency

Total dose for mannitol (mg)
<table>
<thead>
<tr>
<th>“Gold Standard” Clinicians Diagnosis</th>
<th>Clin Dx</th>
<th>Clin Dx Adjusted For ICS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannitol n = 492</td>
<td>Sensitivity 59.8 Specificity 94.5</td>
<td></td>
</tr>
<tr>
<td>Mannitol n = 333</td>
<td>Sensitivity 89.0 Specificity 95.0</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity increased from 60 to 89% when the data were adjusted for the confounding effect of benefit from treatment with inhaled corticosteroids (ICS) and mannitol negative subjects taking ICS were removed.

Age 6 - 83 yrs (Mean 34.8 yr)

Brannan JD Anderson SD et al 2005 Respiratory Research 6: 144
What did we learn from A301?

• Mannitol had a high specificity and there were very few false positive tests in the healthy population.

• The sensitivity for mannitol to identify clinically recognised asthma improved from 60 to 89% when the benefit of current treatment with inhaled corticosteroids was taken into account.
COPENHAGEN STUDY was in 14–24 yr olds in a general population with asthmatics identified by a respiratory physician.

Original article

Airway hyperresponsiveness to mannitol and methacholine and exhaled nitric oxide: A random-sample population study

Asger Sverrild, MD, Celeste Porsbjerg, MD, PhD, Simon Francis Thomsen, MD, PhD, and Vibeke Backer, MD, DMSc

Copenhagen, Denmark

Background: Studies of selected patient groups have shown that airway hyperresponsiveness (AHR) to mannitol is more specific than methacholine for the diagnosis of asthma, as well as more closely associated with markers of airway inflammation in asthma.

Objective: We sought to compare AHR to mannitol and methacholine and exhaled nitric oxide (eNO) levels in a nonselected population sample.

Methods: In 238 young adults randomly drawn from the nationwide civil registration list in Copenhagen, Denmark,

Abbreviations used

AHR: Airway hyperresponsiveness
BPT: Bronchial provocation test
eNO: Exhaled nitric oxide
IQR: Interquartile range
RDR: Response-dose ratio
ROC: Receiver operating characteristic

COPENHAGEN STUDY Subject Characteristics


One respiratory physician only made the diagnosis based on symptoms in last 1yr, in combination with an FeNO >30 ppb, a history of allergic rhino-conjunctivitis, dermatitis, a positive skin test response, a familial predisposition to atopic disease, non-allergic rhino-conjunctivitis or an FEV<sub>1</sub> /FVC ratio <75%.
TABLE II. Results of 238 randomly selected adolescents tested with inhaled mannitol and methacholine: 51 asthmatic subjects and 187 nonasthmatic subjects

<table>
<thead>
<tr>
<th></th>
<th>Asthma (n=51)</th>
<th>No asthma (n=187)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methacholine</td>
<td>Methacholine</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>曼尼托尔</td>
<td>+</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>9</td>
</tr>
</tbody>
</table>

TABLE III. Diagnostic properties of inhaled mannitol and methacholine in 238 randomly selected subjects

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methacholine</td>
<td>69 (57-78)</td>
<td>80 (77-83)</td>
<td>49 (40-56)</td>
<td>90 (87-93)</td>
</tr>
<tr>
<td>Mannitol</td>
<td>59 (51-63)</td>
<td>98 (96-99)</td>
<td>91 (78-97)</td>
<td>90 (88-91)</td>
</tr>
</tbody>
</table>

What did we learn from the Copenhagen study?

- Mannitol PD$_{15}$ had a high specificity (98%) compared with methacholine PC$_{20}$ 80% (e.g. there were more false positive tests in non-asthmatics using methacholine than there were using mannitol).

- The sensitivity of mannitol PD$_{15}$ to identify those with clinically recognised asthma was lower than methacholine (59% vs 69%) although small improvements may have occurred when adjusting for treatment.
A 305 was a population of subjects with symptoms but without a definite diagnosis of asthma at study entry.
A305 Rationale

• No previous large study has reported the utility of bronchial provocation testing in patients without a clear diagnosis of asthma

• Mannitol is a bronchial provocation test approved in the USA
### Patient demographics (enrolled subjects)

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
</table>
| **Age**    | • Mean (SD) = 24.9 (10.6) yrs  
             | • range = 6 - 50 yrs            |
| **BMI**    | • Mean (SD) = 24.5 (4.7)        
             | • Range = 14.4 - 39.9           |
| **Gender** | • 46.4% male  
             | • 53.6% female                  |
| **Ethnicity** | • Caucasian – 74%  
                 |     | Hispanic – 9%                   
                 | • Black – 9%                    
                 |     | Asian – 5%                      
                 | • Other – 2%                    

A-305
Patient characteristics at baseline (enrolled)

- **FEV$_1$**
  - Mean (SD) = 91 (8.7)% of predicted at baseline

- **Atopy**
  - 78% of subjects

- **β$_2$ reversibility**
  - 10.8% of all screened subjects
  - 7.5% of PP subjects reversed

- **NAEPPPII asthma severity rating**
  - Mean (SD) = 1.2 (0.6)*
  - * 92.2% graded at Step 1 or 2 by clinician at Visit 1, on scale 0 - 4

This was a group of patients with normal spirometry, mild symptoms with an unclear diagnosis.
NAEPP II Asthma Severity Grading?
From Guidelines for Dx and Mx of Asthma NIH 1997 NHLBI

94.2% of 375 subjects graded at Step 1 or 2 at visit 1

0 = None 1.3%  1 = Mild intermittent 81.6%, 2 = Mild Persistent 12.6%, 3 = Moderate persistent 4.6 %, 4 = Severe persistent 0%

<table>
<thead>
<tr>
<th>STEP 2</th>
<th>Symptoms ≥ 2 times a week but &lt; 1 time a day</th>
<th>&gt; 2 times a month</th>
<th>FEV₁ or PEF ≥ 80% predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild Persistent</td>
<td>Exacerbations may affect activity</td>
<td></td>
<td>PEF variability 20 – 30%</td>
</tr>
<tr>
<td>STEP 1</td>
<td>Symptoms ≤ 2 times a week</td>
<td>≤ 2 times a month</td>
<td>FEV₁ or PEF ≥ 80% predicted</td>
</tr>
<tr>
<td>Mild Intermittent</td>
<td>Asymptomatic and normal PEF between exacerbations</td>
<td></td>
<td>PEF variability &lt; 20%</td>
</tr>
<tr>
<td></td>
<td>Exacerbations brief (from a few hours to a few days); intensity may vary</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
A305 Study Design

Visit 1
- Medical history
- Physical exam
- Skin testing
- FEV<sub>1</sub> reversibility
- NAEPP
- Likelihood of having asthma

Visit 2
- Exercise challenge #1

Visit 3
- Exercise challenge #2

Visit 4
- Mannitol or methacholine challenge

Visit 5
- Mannitol or methacholine challenge
  - Clinical diagnosis

Randomization
- All visits separated from next visit by ~ 1 to 4 days
- Order for mannitol and methacholine challenges was randomized within the exercise negative and exercise positives arms

Blinding
- Mannitol and methacholine challenges performed by staff different from those performing the rest of the procedures
- There was no access to results across groups
- Clinician doing diagnosis at Visit 5 had access to all data except mannitol and the methacholine challenge results
Methacholine and mannitol were equally sensitive for identifying bronchial hyperresponsiveness in the population:

- **Methacholine** (5 breath dosimeter method)
  \[ PC_{20} < 16 \text{ mg/ml} \text{ (fall in FEV}\textsubscript{1} \text{ of } 20\% \text{ from baseline)} \]
  \[ n=156 \text{ were positive} \]

- **Mannitol**
  \[ PD_{15} < 635 \text{ mg} \text{ (fall in FEV}\textsubscript{1} \text{ of } 15\% \text{ from baseline)} \text{ (or subject experienced a } 10\% \text{ between dose fall in FEV}\textsubscript{1} \text{)} \]
  \[ 168 \text{ were positive} \]

- **Exercise** (8 minute treadmill challenge)
  \[ \text{FEV}\textsubscript{1} \text{ fall } \geq 10\% \text{ from baseline on at least } 1 \text{ of } 2 \text{ exercise tests} \]
  \[ n=163 \text{ were positive} \]
Percentiles and Geometric mean values for bronchial provocation tests and the maximum% fall in FEV\textsubscript{1} recorded after the two exercise tests

<table>
<thead>
<tr>
<th></th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
<th>Geomean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mann\textsuperscript{+} PD\textsubscript{15} (mg)</td>
<td>72</td>
<td>234</td>
<td>374</td>
<td>158 (129,193)</td>
</tr>
<tr>
<td>Mech\textsuperscript{+} PC\textsubscript{20} (mg/ml)</td>
<td>0.84</td>
<td>2.98</td>
<td>6.53</td>
<td>2.12 (1.7,2.64)</td>
</tr>
<tr>
<td>Exc\textsuperscript{+} % Fall</td>
<td>23.6%</td>
<td>15.5%</td>
<td>12.4%</td>
<td>19.1% (9.25)*</td>
</tr>
</tbody>
</table>

*Mean (SD)

There was 69% concordance in the mannitol and methacholine results
### Gold Standard

Methacholine was not more sensitive than mannitol for identifying a clinical diagnosis of asthma at study-end and was less specific than mannitol in children.

<table>
<thead>
<tr>
<th>“Gold Standard”</th>
<th>Whole Group Clin Dx 5+</th>
<th>Children&lt;18yr Clin Dx 5+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 375</td>
<td>n=115</td>
</tr>
<tr>
<td>Mannitol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>56%</td>
<td>63.2%</td>
</tr>
<tr>
<td>Specificity</td>
<td>73%</td>
<td>81.4%</td>
</tr>
<tr>
<td>Methacholine 16 mg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>51%</td>
<td>66.2%</td>
</tr>
<tr>
<td>Specificity</td>
<td>75%</td>
<td>62.9%</td>
</tr>
</tbody>
</table>

Clinical diagnosis (Clin Dx) of asthma at Visit 5 made on history, examination, FEV1 reversibility, exercise challenge results, skin tests and questionnaire **but NOT** the mannitol and methacholine test results.

Anderson SD et al Respiratory Research 2009, 10:4
Methacholine was less sensitive than mannitol for a clinical diagnosis of asthma at NAEPP II Grade 2 PP population

methacholine $PC_{20}$ 16 mg/mL

<table>
<thead>
<tr>
<th>Agent</th>
<th>NAEPP II step 1 n=305</th>
<th>NAEPP II step 2 n=47</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mannitol</td>
<td>0.56</td>
<td>0.75</td>
</tr>
<tr>
<td>methacholine</td>
<td>0.55</td>
<td>0.62</td>
</tr>
</tbody>
</table>
A 305 Mannitol was not more sensitive than methacholine in identifying EIB

<table>
<thead>
<tr>
<th>“Gold Standard”</th>
<th>Whole Group Clin Dx 5+</th>
<th>Whole Group EIB+ 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 375</td>
<td>N = 375</td>
</tr>
<tr>
<td>Mannitol</td>
<td>Sensitivity</td>
<td>56% 73%**</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>73%</td>
</tr>
<tr>
<td>Methacholine 16 mg/ml</td>
<td>Sensitivity</td>
<td>51% 72%**</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>75%</td>
</tr>
</tbody>
</table>

Clinical diagnosis (Clin Dx) of asthma at the end of the study was made on history, examination, FEV₁ reversibility, exercise challenge results, skin tests and questionnaire but NOT the mannitol and methacholine test results

** When both exercise tests ≥10% fall in FEV₁

Anderson SD et al Respiratory Research 2009, 10:4
Sensitivity of mannitol was improved when those with a prolonged challenge time were excluded.

<table>
<thead>
<tr>
<th>Exercise Positive Cut-Points - % fall from baseline</th>
<th>10%</th>
<th>15%</th>
<th>20%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mannitol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 372</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>58.6</td>
<td>69.4</td>
<td>78.6</td>
</tr>
<tr>
<td>Specificity</td>
<td>65.2</td>
<td>62.0</td>
<td>60.8</td>
</tr>
<tr>
<td><strong>Excluding those with challenge &gt;35 min</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 319</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>64.1</td>
<td>75.3</td>
<td>82.7</td>
</tr>
<tr>
<td>Specificity</td>
<td>59.9</td>
<td>57.0</td>
<td>55.4</td>
</tr>
<tr>
<td><strong>Methacholine 16 mg/ml</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 375</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>55.2</td>
<td>67.4</td>
<td>80.3</td>
</tr>
<tr>
<td>Specificity</td>
<td>68.9</td>
<td>66.1</td>
<td>65.2</td>
</tr>
</tbody>
</table>
There was a high rate of negative methacholine tests in those given a clinical diagnosis of asthma

If methacholine negative had been adhered in order to rule out a diagnosis of asthma then: 118 (49%) fewer subjects would have been diagnosed

<table>
<thead>
<tr>
<th></th>
<th>Clin Dx +ve n=240</th>
<th>Clin Dx –ve n=135</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methacholine +ve</td>
<td>122 GM 2.14 mg/ml</td>
<td>34 2.04 mg/ml</td>
</tr>
<tr>
<td>Methacholine -ve</td>
<td>118 &gt; 16 mg/ml</td>
<td>101 &gt; 16 mg/ml</td>
</tr>
</tbody>
</table>

Diagnosis by a Respiratory Physician on basis of comprehensive information only such as History, Examination, FEV₁, Reversibility two exercise tests, Skin tests and Questionnaires

Anderson SD et al Respir Res 2009:
What did we learn about methacholine from A 305?

• That Methacholine was no more sensitive for identifying BHR than mannitol or exercise and no more sensitive than mannitol for identifying a clinical diagnosis of asthma.

• There was a high rate of methacholine negative test results (49%) in those given a clinical diagnosis of asthma by a respiratory physician at the end of the study.

• Based on comprehensive information that included History, \( \text{FEV}_1 \) reversibility, two exercise tests, skin tests, questionnaire (the results of the methacholine or mannitol test results were withheld) suggests that:

• A negative methacholine test result should not be relied upon to rule out a diagnosis of asthma in subjects with symptoms of asthma but without a definite diagnosis.

• The importance of these observations is that it is subjects with these characteristics who are most likely to be referred for testing.
When put to the rule out test?

- The long-held belief that the clinical utility of methacholine is in a negative test result excluding a diagnosis of asthma was not upheld when the respiratory physician did not have access to the methacholine test result.

- 54% (118) of all those negative to methacholine were given an asthma diagnosis at Visit 5 on the basis of other information.

- Would the Doctors have reversed their decision had they known the methacholine test was negative?

- If a methacholine negative test had been used to Rule out Asthma then 73 subjects with EIB (mean % fall FEV\textsubscript{1} of 15.6%) and 63 subjects positive to mannitol would have been missed.
What did we learn about Mannitol as a rule in test?

- Mannitol was as sensitive as methacholine for identifying BHR.

- Mannitol was never less sensitive than methacholine to identify a clinical Dx of asthma when cut points of 8, 12 or 16 mg/ml were used.

- The concept that mannitol would identify all those with EIB was not supported by A305 with 42% of those with EIB on 1 of 2 tests being mannitol negative whilst 28% of those with 2 positive tests were missed.

- The clinical utility of mannitol positive test result was upheld by the low number of false positive tests in A301 and Copenhagen studies & the high concordance (78.5%) between a clinical Dx at Visit 5 & a positive mannitol in A305.
If mannitol positive had been adhered to in order to rule in a diagnosis of asthma then 36 more subjects would have been diagnosed.

<table>
<thead>
<tr>
<th></th>
<th>Clin Dx +ve n=240</th>
<th>Clin Dx –ve n=135</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannitol +ve</td>
<td>134</td>
<td>36</td>
</tr>
<tr>
<td>Mannitol -ve</td>
<td>106</td>
<td>99</td>
</tr>
</tbody>
</table>

Diagnosis by a Respiratory Physician on basis of History, Examination, FEV₁, Reversibility 2 exercise tests, Skin tests, Questionnaires

Anderson SD et al. Respir Res 2009:
Mannitol positive subjects compared with mannitol negative subjects

- In mannitol +ve subjects a PC_{20} Methacholine is more frequent (66% vs 25%) and the PC_{20} significantly lower 1.64 vs 3.54 mg/ml $p<0.001$ (Anderson SD et al Respir Res 2009)

- In mannitol +ve subjects EIB is more common (57% vs 33%) and more severe 21±10% vs 16±7% $p<0.001$) (Anderson SD et al Respir Res 2009;10:4)

- In mannitol +ve subjects FeNO is significantly higher 47 vs 19 ppb (Sverrild S et al JACI 2010)

- In those with eosinophilic asthma 82% are +ve to mannitol (Porsbjerg et al J Asthma 2009:46)
Summary Rule in Rule out:

• The profile of those with a positive mannitol test result was consistent with ruling in a diagnosis of asthma in that there was a low rate of false positive tests in A301 and Copenhagen Study and 78% of subjects in A305 were given the diagnosis of asthma by a respiratory physician. In all studies the physician was blinded to the mannitol test result.

• The profile of those with a negative methacholine test result was less consistent with ruling out a diagnosis of asthma in that 49% of subjects in A305 were given the diagnosis of asthma by a respiratory physician when they were blinded to the methacholine test result.
What about the subjects *without* a Clinical Diagnosis at Visit 5?

$n=135$

- 25% were positive to mannitol
- 9% had EIB >10% fall in $FEV_1$ Mean fall 13% ± 3
- 1.0% had EIB with 2 tests >10% fall
- 22% positive to methacholine ≤16 mg/ml
- 8% were positive to methacholine and mannitol
- 2% positive to mannitol and exercise
- 1% were positive to exercise & mannitol & methacholine

NB clinician giving the diagnosis of asthma had Hx, Qx, reversibility, skin tests and exercise results ONLY
Mannitol Responsiveness occurred at all NAEPPII Steps of Asthma Severity

Grading made at visit 1

0 = None 1.3% 1 = Mild intermittent 81.6%, 2 = Mild Persistent 12.6%, 3 = Moderate persistent 4.6%, 4 = Severe persistent 0%

n = 375 P Males 182 Females 193 A305
In subjects negative to mannitol, BHR to methacholine was less common & \( \text{PC}_{20} \) was significantly higher compared with mannitol positive subjects. This difference in \( \text{PC}_{20} \) is not evident for a positive or negative clinical diagnosis

% of subjects +ve methacholine: 
- Mannitol: 62% +ve, 25% -ve 
- Clin Dx: 51% +ve, 25% -ve 
- Meth: 100% +ve

p < 0.001
NS
Mannitol positive test associated with significantly higher FeNO compared with mannitol negative test

<table>
<thead>
<tr>
<th></th>
<th>Current asthma (n = 51)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mannitol</td>
<td>Methacholine</td>
<td>Mannitol</td>
<td>Methacholine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative (n = 21)</td>
<td>Negative (n = 16)</td>
<td>Positive (n = 30)</td>
<td>Positive (n = 35)</td>
<td></td>
</tr>
<tr>
<td>eNO (ppb), median (IQR)</td>
<td>19 (13-30)</td>
<td>24 (15-39)</td>
<td>47 (35-62)</td>
<td>37 (26-51)</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>.001</td>
<td>.13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eNO &gt;26 ppb, % (n)</td>
<td>29 (6)</td>
<td>44 (7)</td>
<td>70 (21)</td>
<td>57 (20)</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>&lt;.0001</td>
<td>.07</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|                | No asthma (n = 187)    |                  |                      |                  |
|                | Mannitol                | Methacholine     | Mannitol              | Methacholine     |
|                | Negative (n = 184)     | Negative (n = 150)| Positive (n = 3)     | Positive (n = 37)|
| eNO (ppb), median (IQR) | 14 (13-15)          | 13 (12-41)       | 46 (10-214)        | 17 (13-21)       |
| P value        | <.0001                  | .03              |                      |                  |
| eNO >26 ppb, % (n) | 11 (21)               | 11 (16)          | 100 (3)              | 22 (8)           |
| P value        | .01                     | .07              |                      |                  |

Sverrild A et al JACI 2010
AHR, FeNO & inflammatory phenotype

Eosinophilic phenotype have greater AHR and higher FeNO compared to neutrophilic

<table>
<thead>
<tr>
<th></th>
<th>Eosinophilic</th>
<th>Non-eosinophilic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eosinophilic asthma (n=27)</td>
<td>Mixed granulocytic asthma (n=8)</td>
</tr>
<tr>
<td>AHR to mannitol</td>
<td>82%</td>
<td>75%</td>
</tr>
<tr>
<td>PD_{15}(mg) (median (IQR))</td>
<td>107 mg ‡ (185mg)</td>
<td>186mg (379mg)</td>
</tr>
<tr>
<td>eNO (ppb) (median (IQR))</td>
<td>77ppb ‡ (86ppb)</td>
<td>47ppb§§ (79ppb)</td>
</tr>
</tbody>
</table>

So what does BHR to mannitol indicate?

- That there are a sufficient number of cells with a sufficient concentration of mediators
- AND a responsive bronchial smooth muscle to those endogenously released mediators at the time of testing
- Likely but not necessarily to have
- Exercise induced bronchoconstriction
- Eosinophils in sputum
- High FeNO
- Positive methacholine test
- And will respond to inhaled steroids and other drugs used in treatment of asthma
Are we over diagnosing asthma in elite athletes using methacholine?
What about methacholine as Rule in test?

- Many clinicians use a positive methacholine test result as diagnostic of asthma at a $PC_{20}<16 \text{ mg/ml}$.

- Many factors however can contribute to BHR to methacholine.

- BHR at $PC_{20}<16 \text{ mg/ml}$ can reflect airway injury from breathing large volumes of unconditioned air (e.g. cross country skiers or skaters) cigarette smoking or inhalation of pollutants (e.g. swimmers) or remodelling of the airways in response to childhood asthma.

- The findings of positive methacholine responses in elite athletes both with and without symptoms of asthma suggest that we should question if a positive methacholine test result is always consistent with a diagnosis of asthma.
Airway hyperresponsiveness to methacholine, adenosine 5-monophosphate, mannitol, eucapnic voluntary hyperpnoea and field exercise challenge in elite cross-country skiers

Malcolm Sue-Chu,1,2 John D Brannan,3 Sandra D Anderson,3 Nora Chew,4 Leif Bjermer5

Methods  Exhaled nitric oxide concentration ($F_{E\text{NO}}$), spirometry and bronchial challenge in random order with methacholine, AMP and mannitol were consecutively performed on three study days in the autumn. Specific IgE to eight aeroallergens and a self-completed questionnaire about respiratory symptoms, allergy and asthmatic medication were also performed on day 1. Eucapnic voluntary hyperventilation (EVH) and field exercise tests were randomly performed in 33 of the skiers on two study days in the following winter.
High Prevalence of airway hyperresponsiveness to methacholine, and low prevalence of response to AMP and Mannitol in Young skiers with and without symptoms of asthma.

Sue Chu et al Brit J Sports Med 2010
Airway hyperresponsiveness to methacholine, adenosine 5-monophosphate, mannitol, eucapnic voluntary hyperpnoea and field exercise challenge in elite cross-country skiers

Malcolm Sue-Chu,¹,² John D Brannan,³ Sandra D Anderson,³ Nora Chew,⁴ Leif Bjermer⁵

Conclusions  Methacholine hyperresponsiveness is more common in asymptomatic skiers and is a poor predictor of hyperresponsiveness to mannitol and hyperpnoea. The low prevalence of hyperresponsiveness to indirect stimuli may suggest differences in the pathogenesis of methacholine hyperresponsiveness in elite skiers and non-athletes.
A positive methacholine test in an athlete may indicate airway injury rather than asthma.

Thus methacholine is not a useful rule in test in athletes.
Why challenge with Mannitol?

Advantages:
- Dose-response curve obtained
- Median time taken for +ve test 17 min, -ve test 26 min
- More than one mediator involved PGD$_2$, LT’s, Hist
- Predicts potential for exercise-induced asthma
- Negative test in asthmatic = good control of asthma
- Response dose ratio guide to back titration of steroid
- Positive test = currently active airway inflammation
- Identifies those who would benefit from Rx with ICS

Disadvantage:
- Some cough during challenge