Methacholine challenge — Comparison of an ATS protocol to a new rapid single concentration technique

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Received 6 February 2009; accepted 1 June 2009
Available online 10 July 2009

KEYWORDS
Methacholine challenge; Bronchial hyperreactivity; Five-breath dosimeter method; Aerosol provocation system

Summary
Bronchial methacholine challenge is well established in asthma diagnostics and research. ATS guidelines propose a five step standard dosimeter (SDM) protocol with incremental concentrations of methacholine to calculate the concentration causing a 20% drop in FEV1 in an individual (PC20FEV1). In contrast, the aerosol provocation system (APS) by Viasys automatically determines the administered dose of methacholine by measuring the effective nebulisation time and referring it to drug concentration and nebuliser power. Therefore, it offers a feasible and less time-consuming provocation procedure by applying incremental doses (PD20) of methacholine using a single concentration (16 mg/mL methacholine, APS-SC). In this study we compared these two methods in 52 young adults (25 ± 5.8 years). Following a screening visit, subjects were randomly assigned to undergo either SDM or APS-SC followed by the other method within 1 week. A close correlation between concentration and dosage causing a 20% fall of FEV1 was found (r = 0.69, p < 0.001).

Using the ATS categorisation of bronchial responsiveness we interpreted the results of the APS-SC method as follows: PD20 methacholine < 0.3 mg as “moderate to severe BHR”, 0.3 – 0.6 mg as “mild BHR”, 0.6 – 1.0 mg as “borderline BHR”, and > 1.0 mg as “normal bronchial response”.

We conclude that the five-step APS-SC is a suitable method, providing reliable results. In clinical practice the APS-SC is a timesaving procedure and less prone to errors since only one dilution of methacholine is necessary compared to the SDM.

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Abbreviations: BHR, Bronchial hyperreactivity; MCT, methacholine challenge testing; ATS, American Thoracic Society; APS, aerosol provocation system; SDM, standard dosimeter method; APS-SC, aerosol provocation system-single concentration.

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0954-6111/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2009.06.007
Introduction

Methacholine challenge testing (MCT) is a well-established method of assessing airway responsiveness. The most common clinical indication is to evaluate the likelihood of asthma in patients in whom the diagnosis is suggested by current symptoms but is not obvious. American Thoracic Society (ATS) guidelines suggest two dosing protocols: the 2-min tidal breathing method, and the five-breath dosimeter method. The five-breath dosimeter protocol consists of fewer dilution steps and is therefore more suitable. Both methods have some inherent pitfalls. These include errors in dilution and inaccurate delivery if equipment is not calibrated. Categorisation of bronchial responsiveness PC20 is suggested as follows (PC20 FEV1, mg/mL): 16 normal bronchial responsiveness, 4.0–16 borderline BHR (positive test), and 1.0 moderate to severe BHR. The interpretation of results is not consistent as a positive test is often defined as a PC20 < 8 or < 16 mg/mL. ATS guidelines recommend the use of an intermediate area of “borderline BHR” to improve the specificity of MCT. A negative methacholine challenge result is commonly defined as non-response to the highest concentration (PC20 > 8–25 mg/mL). False-negative methacholine challenges occur much less frequently than false-positive results. Cockcroft et al. found in a random selection of young students in subjects with current symptomatic asthma a negative predictive value of 100% and a positive predictive value of only 29%. The aerosol provocation system (APS, Viasys healthcare) is a flow-controlled nebuliser system which performs reproducible provocation tests and ensures accurate dosage. With each inspiration the patient inhales a specific dose of the applied substance, e.g., methacholine. In contrast to methods with different concentrations, the APS delivers incremental doses of methacholine using a constant concentration. The advantages are prevention of dilution errors and saving the time for preparing the dilutions. Between the different steps the dilution doesn’t need to be replaced. Thus, the APS is user-friendly and suitable for everyday clinical practice, particularly in combination with a short-protocol.

Despite a widespread distribution of the APS, solid data about interpretation of results of MCT with this system is rare. This study compares two short protocols namely the five-breath dosimeter method and a short-protocol using the APS. We provide an assessment of the categories using the APS based on the ATS categorisation of bronchial responsiveness.

Methods

Subjects

Fifty five subjects aged 14–45 years with known BHR, who participated already in former studies, were recruited. Following the first visit they were randomised to one of the two methods. Subjects’ baseline characteristics are summarised in Table 1. Subjects were excluded if they had a therapy with inhaled or oral corticosteroids, long acting beta-agonists or leukotriene receptor antagonists. The study was approved by the Ethics Committee of the Goethe-university, written informed consent was obtained in advance from each subject (in minors additionally from the parents).

Table 1  Subjects’ characteristics.

| Subjects [n] | 52 |
| Female/male [n] | 31/21 |
| Smokers [n/%] | 9/17.3 |
| Age [yr] | 25 ± 5.8 |
| Weight [kg] | 67.3 ± 14.3 |
| Height [cm] | 174.1 ± 9.5 |
| FEV1 [% predicted] | 102.7 ± 13.0 |
| VC [% predicted] | 97.9 ± 13.4 |

Data are presented as mean ± SD unless otherwise indicated.

Test products

Methacholine chloride solutions were prepared from a commercial powder (Fagron Ltd., Barsbüttel, Germany) by a clinical pharmacist. A stock solution of 16 mg/mL metacholine in 50 ml 0.9% saline (pH 4.8) was made. The solution was stored at 4 °C up to three months. For the five-breath dosimeter method, different dilutions containing 0.0625 mg/mL, 0.25 mg/mL, 1.0 mg/mL, 4.0 mg/mL, and 16.0 mg/mL were prepared.

Standard dosimeter method (SDM)

The dosimeter method was performed according to American Thoracic Society guidelines using the above mentioned concentrations. The metacholine dilutions were delivered through a mouthpiece attached to a DeVilbis 646 nebuliser (DeVilbiss Co., Sommerset, USA) driven by a ZAN200 ProvAir II ultrasound dosimeter (nSpire Health Ltd, Oberthulba, Germany). The ZAN200 ProvAir II was calibrated to produce an output of 0.009 mL per 0.6-s actuation. Particle size was 5 μm. During tidal breathing (functional residual capacity) the subject was instructed to inhale slowly and deeply from the nebuliser. Soon after the inhalation began the dosimeter was triggered. Participants...
were encouraged to inhale slowly and deeply over 5 s to total lung capacity and to hold the breath for another 5 s. This was repeated for a total of five inhalations. 120 s after each step FEV₁ was measured. PC₂₀FEV₁ was calculated by logarithmic interpolation. Before and after each inhalation step the nebuliser was weighed using a balance (Kern 440-21A) accurate to 0.001 g, and inhaled doses of methacholine were calculated.

**Single concentration method using the Aerosol Provocation System (APS-SC)**

The APS dosimeter technique (VIASYS Healthcare GmbH) allows computer controlled production of aerosol, using a jet-type nebuliser (Sidestream, Medic Aid) powered by compressed air. Integrated calibration procedures ensure highly constant and reproducible nebuliser output. Major advantages of this system are real-time visualisation of dose administration and breathing pattern.

The system exactly and automatically determines the administered dose of methacholine by measuring the effective nebulisation time at inspiration of any breath and referring it to drug concentration and nebuliser power.

Subjects were instructed to inhale slowly and deeply from the nebuliser with maximal flows below 0.5 L/s. APS was calibrated to produce an output of 160 mg/min, particle size was 3.2 μm. The doses of inhaled methacholine with a concentration of 16 mg/mL were increased according to the following pattern, from step 1 to 5: 0.01, 0.1, 0.4, 0.8, and 1.6 mg. Thus, the entire protocol delivered cumulative doses of 0.01, 0.11, 0.51, 1.31 and 2.91 mg (Table 2). Two minutes after each inhalation, FEV₁ was measured, and PD₂₀ FEV₁ was calculated by logarithmic interpolation using an integrated program.

**Statistical analysis**

For statistical analysis BiAS for Windows™ (Version 8, epsilon-publisher, Frankfurt, Germany) was used. Relationship between the two methods was examined by determining the Pearson correlation coefficient. Cut-points of APS-SC were primarily defined by using Hahn-prognosis intervals, which is based on a regression analysis. Hahn-prognosis estimates the variability of corresponding residuals to a given value, e.g., the variability of expected PD₂₀ to a given PC₂₀. For these cut-points inter-rater agreement was measured using Cohen’s kappa coefficient.

<table>
<thead>
<tr>
<th>Step</th>
<th>Dose of methacholine [mg]</th>
<th>Cumulative dose of methacholine [mg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td>0.10</td>
<td>0.11</td>
</tr>
<tr>
<td>3</td>
<td>0.40</td>
<td>0.51</td>
</tr>
<tr>
<td>4</td>
<td>0.80</td>
<td>1.31</td>
</tr>
<tr>
<td>5</td>
<td>1.60</td>
<td>2.91</td>
</tr>
</tbody>
</table>

To assess the significance of the difference between both methods McNemar’s test was used. If marginal frequencies, e.g. subjects who react only to one of both methods, in a 2 × 2 classification table are not homogeneous, McNemar is significant ($p > 0.05$). Differences between both methods were measured with student’s t-test.

**Results**

**Subject characteristics**

In total, 31 female and 21 male subjects aged 14–45 years completed the study. One participant started a therapy with inhaled corticosteroids between the visits and was excluded. Two participants didn’t appear for the second provocation and were excluded. All subjects ($n = 52$) had baseline FEV₁ values of > 80% predicted and baseline FEV₁ was similar for both methods (SDM FEV₁ 102%, SD 13.4%, APS-SC 103%, SD 13.7%, $p = 0.33$), as well as the maximal post-challenge fall of FEV₁ (SDM FEV₁ 25.6%, SD 12.7%, APS-SC 28.7%, SD 10.8%, $p = 0.08$). For FEV₁ and FVC manoeuvres, ATS/ERS test criteria for acceptability and repeatability were met in 87.3% of all measurements. All participants performed adequate MCTs. Of these, six were nonresponsive neither to the SDM nor to the APS-SC method. Eight were nonresponsive to the SDM but had a positive PD₂₀FEV₁ to APS-SC, and two were nonresponsive to APS-SC but had a positive PC₂₀FEV₁ to SDM. All subjects were included in the calculations. For those, who were nonresponsive to any of the methods, maximum concentration or dose, 16 mg/mL or 2.91 mg of methacholine respectively, were assumed (Fig. 1 A).

**Comparison of the two methods**

Pearson correlation for the two methods was $r = 0.69$ ($p < 0.001$) (Fig. 1 A). Using Hahn-prognosis test we calculated the corresponding thresholds for different categories of BHR according to ATS-guidelines (Fig. 1 B). A concentration of 8 mg/mL methacholine was considered as the usually accepted cut-point of BHR. Moderate to severe BHR would correspond with a PD₂₀ methacholine < 0.3 mg, mild with 0.3–0.6 mg, borderline with 0.6–1.0 mg, and normal bronchial response > 1.0 mg. Moreover, thresholds were calculated using only subjects with data within the range ($n = 36$). Results were similar to the data involving all subjects ($r = 0.54$, $p < 0.001$). The association between tests was confirmed calculating the PD₂₀ cut-points with the highest kappa statistic for each PC₂₀ (Table 3). All cut-points showed significant agreements. At the 8 mg/mL cut-point both methods would detect 27 of 52 (52%) subjects with BHR and exclude 16 of 52 (31%). Thus, the overall agreement was 83% (McNemar, $p = 0.51$) (Table 4).

For those subjects who had a positive reaction within the range ($n = 36$), we measured the inhaled methacholine doses and length of time at the point of PC₂₀FEV₁ and PD₂₀ FEV₁. Mean dose for SDM was 0.89 mg (SD 0.84) methacholine, and for APS-SC 0.51 mg (SD 0.50) methacholine ($p < 0.005$). Mean duration of SDM was 23.4 min (SD 6.08) and of APS-SC 15.3 min (SD 6.04) ($p < 0.001$), respectively.
Discussion

In subjects with a history of bronchial hyperreactivity we investigated the relationship between the ATS recommended short five-breath dosimeter protocol and our own short protocol. We demonstrated the correlation and agreement between both methods to be significant. Data about the use of APS in MCTs are rare and only a few studies compare the APS to standard methods. Hagmolen often Have et al. investigated the relationship between SDM and APS-SC challenge in 22 children with asthma. They found an intra class correlation (ICC) of 0.80, showing a strong agreement. Praml and colleagues used the MCT protocol of the European Community Respiratory Health Survey (ECRHS). For a more reliable handling, they replaced the Mefar MB3 nebuliser and adapted the protocol to the APS. They found an overall agreement of 74% with significantly different results for both methods (McNemar, \( p < 0.005 \)). This was due to a larger number of PD20-positive subjects in the APS method and the variation between the two methods was not at random.

There is a major difference in the protocols. The study in children and our study used a single concentration of 39.2 mg/mL or 16 mg/mL methacholine, respectively. In the adult study, concentration of methacholine was switched in the middle of the protocol from 12.5 to 66.7 mg/mL. Both methods (PC and PD) contain a part of the other method, but changes in concentrations and doses would influence the outcome.

However, using a single concentration, the concentration of the starting dose will be higher. This could influence the bronchial reaction and generate false-positive methacholine challenges. We saw a trend that in concentrations above 4 mg/mL methacholine subjects tend to react more to the APS-SC than to the SDM. However, this was not of statistical significance.

![Figure 1](image-url) Distribution of provocative concentration of methacholine versus provocative dose of methacholine causing a 20% fall of FEV1. (A) The regression was calculated using all subjects (n = 52) including those 16 outside the range of 16 mg/mL and 2.91 mg methacholine, respectively (\( r = 0.69 \)). (B) Hahn 95% prognosis interval. The bold line shows the regression, whereas the outer lines represent the band of expected PD20 values corresponding to a given PC20 value.

### Table 3

Comparison between optimum cut-points for agreement between the dosimeter and APS method.

<table>
<thead>
<tr>
<th>PC20 cut point [mg/mL]</th>
<th>PD20 cut point [mg]</th>
<th>Δ PD20</th>
<th>Agreement [%]</th>
<th>Cohen’s kappa (linear)</th>
<th>( p )-value (of kappa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.3</td>
<td>0.1</td>
<td>76.9</td>
<td>0.46</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>2.0</td>
<td>0.4</td>
<td>0.2</td>
<td>76.9</td>
<td>0.43</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>4.0</td>
<td>0.6</td>
<td>0.4</td>
<td>78.9</td>
<td>0.54</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>8.0</td>
<td>1.0</td>
<td>0.9</td>
<td>82.7</td>
<td>0.59</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>16.0</td>
<td>1.9</td>
<td></td>
<td>78.9</td>
<td>0.56</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

### Table 4

Distribution of positive and negative results comparing dosimeter and APS method.

<table>
<thead>
<tr>
<th>PC20 &lt;8.0 mg mL(^{-1})</th>
<th>PD20 &lt;1.0 mg</th>
<th>≥1.0 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td>33</td>
<td>19</td>
<td>52</td>
</tr>
</tbody>
</table>

McNemar test (\( p = 0.51 \)).
A purpose of this study was to generate categories of BHR. MCT is most often considered when asthma is a serious possibility and traditional methods, most notably spirometry performed before and after administration of a bronchodilator, have not established or eliminated the diagnosis. Therefore, a grading system comprising BHR, borderline, and normal limits in this clinical setting is adequate. Looking to the future, use of MCT in asthma may have a more prominent role in helping in decision making about treatment, such as when to change doses of anti-inflammatory treatment, or even stop treatment if airway responsiveness has been normalised. Interestingly, there is one study which favours the use of MCT as guide to long term treatment and its clinical and histopathologic outcome. Since asthma therapy gets more diverse, we believe that therapy monitoring with a more distinct system of severity is helpful. However, its clinical use has to be proven in future studies.

This is the first attempt using a standard method to define the categories for APS-SC. Sierstedt et al. had a similar approach to assess cut-points of BHR comparing the tidal breathing PC20 cut-point was associated with a doubling of PC20 cut-points. However, the ECRHS protocol contains incremental dosages and concentrations. Thus, it would be closer to the tidal breathing method. In our study, Hahn-prognosis and Cohen’s kappa test gave major cut-off-points with a significant agreement. Each doubling dose in the SDM was associated with a doubling of the difference of two consecutive doses in APS-SC (Table 3). Cut-points revealed highest Cohen’s kappa and percent agreements, suggesting a linear relationship between both methods.

Studies comparing different methods of bronchial challenges are not conclusive. Yan et al. found in histamine provocations obtained by a hand held DeVilbiss nebuliser not significant different values from those obtained by a DeVilbiss 646 nebuliser. In children, Sears et al. showed with a modified MCT protocol from Chai et al. in comparison to a longer protocol of Hargrave et al. a good agreement between the two techniques.

Although previous studies have compared both recommended ATS protocols, results are not consistent. Ryan et al. found no difference between the methods. This was confirmed by Wubbel et al., the PC20 methacholine obtained by either method showed no significant difference. In contrast, in the study of Cockcroft et al. the five-breath dosimeter PC20 was almost twofold greater than the 2-min tidal breathing PC20. Potential reasons for these observations are that a double dose is administered by the tidal-breathing method, and that inhalations to TLC are recognised to be bronchoprotective, particularly in subjects with airway responsiveness in the normal, borderline, or mild range.

This data was confirmed by the study of Allen et al. The dosimeter PC20 was larger than the tidal breathing method and the difference was greater in subjects with mild BHR having a PC20 > 2 mg/mL. The authors suggest that subjects with mild asthma and mild BHR behave more like normal subjects with regard to the bronchoprotective effect of a deep inhalation. They conclude that SDM results in a potentially marked reduction in the sensitivity of the challenge leading to potential for misdiagnosis.

In studies comparing SDM to APS, measurement of the inhaled dose of methacholine (PD20) revealed different values. Analysis of 18 subjects showed a small, but significant difference in PD20. In children, using a concentration of 39.2 mg/mL methacholine, PD20 for APS-SC was significantly higher than the PD20 for SDM. The difference of PD20 is consistent with our results. However, PD20 in our trial was lower for APS-SC than for SDM. Approximately 70% of the aerosol produced by the DeVilbiss 646 nebuliser is within the ideal size range (< 5 μm) for optimal delivery to the small airways. The fine particle fraction (< 5 μm) of the APS nebuliser was on average 49.7%. Thus, particle size is not an explanation for the difference in PD20. Our results for PD20 are consistent with the data of Siersted et al., showing that cumulative dosages up to 2.9 mg methacholine lead to positive reactions. These results suggest that 16 mg/mL methacholine is a suitable concentration. Higher concentrations (e.g., 32 mg/mL) would result in a greater amount of inhaled methacholine, lower concentrations (e.g., 8 mg/mL) would double inhalation time and extend the test.

Without doubt the APS is a time-saving method and the handling is easy. In the first trial, mean duration of SDM was 30.5 and of APS 16.8 min. Compared to our data, SDM lasted 23.4 min and APS was similar. However, in the calculation we included only the measurement, not the preparation of a large number of methacholine solutions as required in the SDM method.

In conclusion, methacholine challenge using the APS and a short protocol is a timesaving and reliable method. A single concentration of 16 mg/mL methacholine is a good compromise between elevated concentrations and an acceptable inhalation time. There is a close relationship between categories of BHR compared to the ATS five-breath dosimeter method. Considering BHR, defined as provocative dose of methacholine causing a 20% fall of FEV1, the cut-point of 8 mg/mL methacholine agrees with a dose of 1 mg methacholine. These results are helpful for the daily use of APS in research and clinical practice.

Acknowledgements

This study was supported by a grant of the Dr. Marschner foundation.

We would like to thank Dr. Hanns Ackermann, institute for biomathematics, Goethe-University, Frankfurt Germany, and Hans-Jürgen Smith, Viasys Healthcare Germany, for their support.

Conflict of interest

There is no conflict of interest for any author.

References


