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

Johannes Schulze*[,a], Martin Rosewich [a], Carsten Riemer, Melanie Dressler, Markus A. Rose, Stefan Zielen

Department of Pediatric Pulmonology and Allergy, Children's Hospital, Goethe-University, Theodor Stern Kai 7, 60590 Frankfurt am Main, Germany

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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 FEV_1 in an individual ($PC_{20}FEV_1$). 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 (PD_{20}) 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 FEV_1 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: PD_{20} 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.

* Corresponding author. Tel.: +49 6963015381.
E-mail address: johannes.schulze@kgu.de (J. Schulze).
[a] These authors contributed equally to this study.

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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.[1] American Thoracic Society (ATS) guidelines[1] suggest two dosing protocols: the 2-min tidal breathing method,[2] and the five-breath dosimeter method.[3] 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.[4] Categorisation of bronchial responsiveness PC_{20} is suggested as follows (PC_{20} FEV_1, mg/mL): 16 normal bronchial responsiveness, 4.0—16 borderline BHR, 1.0—4.0 mild BHR (positive test), and 1.0 moderate to severe BHR.[1] The interpretation of results is not consistent as a positive test is often defined as a $PC_{20} < 8$ or < 16 mg/ml.[1] 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 ($PC_{20} > 8$—25 mg/ml). False-negative methacholine challenges occur much less frequently than false-positive results.[1] 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%.[5]

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.[7,8] 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
FEV_1 [% predicted]	102.7 ± 13.0
VC [% predicted]	97.9 ± 13.4

Data are presented as mean $\pm$ SD unless otherwise indicated.

Study design

This was an unblinded, randomised, crossover study consisting of two visits. Each visit was at least 48 hours but not more than 7 days apart. Participants attended an initial visit to assess baseline characteristics and lung function. After that, participants were randomised to one of the two methacholine challenge methods by using a random list. On the same visit the first challenge was performed. Primary endpoint was the concentration or dose causing a 20% drop in FEV_1 from baseline. During the provocation oxygen saturation and heart rate were monitored. The duration of each test was documented. After the challenge the participants received one to three puffs of Salbutamol (0.2 mg) until the FEV_1 returned to at least 80% of the baseline value. On the second visit the other methacholine challenge protocol was performed.

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 metchacholine 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.[1] The methacholine dilutions were delivered through a mouthpiece attached to a DeVilbiss 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 FEV_1 was measured. $PC_{20}FEV_1$ was calculated by logarithmic interpolation.[9] 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, FEV_1 was measured, and PD_{20} FEV_1 was calculated by logarithmic interpolation[9] 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 PD_{20} to a given PC_{20}. For these cut-points inter-rater agreement was measured using Cohen's kappa coefficient.

Table 2 Steps and methacholine concentrations during bronchial provocation using the APS-SC.

Step	Dose of methacholine [mg]	Cumulative dose of methacholine [mg]
1	0.01	0.01
2	0.10	0.11
3	0.40	0.51
4	0.80	1.31
5	1.60	2.91

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 FEV_1 values of $> 80\%$ predicted and baseline FEV_1 was similar for both methods (SDM FEV_1 102%, SD 13.4%, APS-SC 103%, SD 13.7%, $p = 0.33$), as well as the maximal post-challenge fall of FEV_1 (SDM FEV1 25.6%, SD 12.7%, APS-SC 28.7%, SD 10.8%, $p = 0.08$). For FEV_1 and FVC manoeuvres, ATS/ERS test criteria for acceptability and repeatability were met in 87.3% of all measurements.[10] 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 $PD_{20}FEV_1$ to APS-SC, and two were nonresponsive to APS-SC but had a positive $PC_{20}FEV_1$ 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[1] (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 PD_{20} 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 PD_{20} cut-points with the highest kappa statistic for each PC_{20} (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 $PC_{20}FEV_1$ and PD_{20} FEV_1. 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.

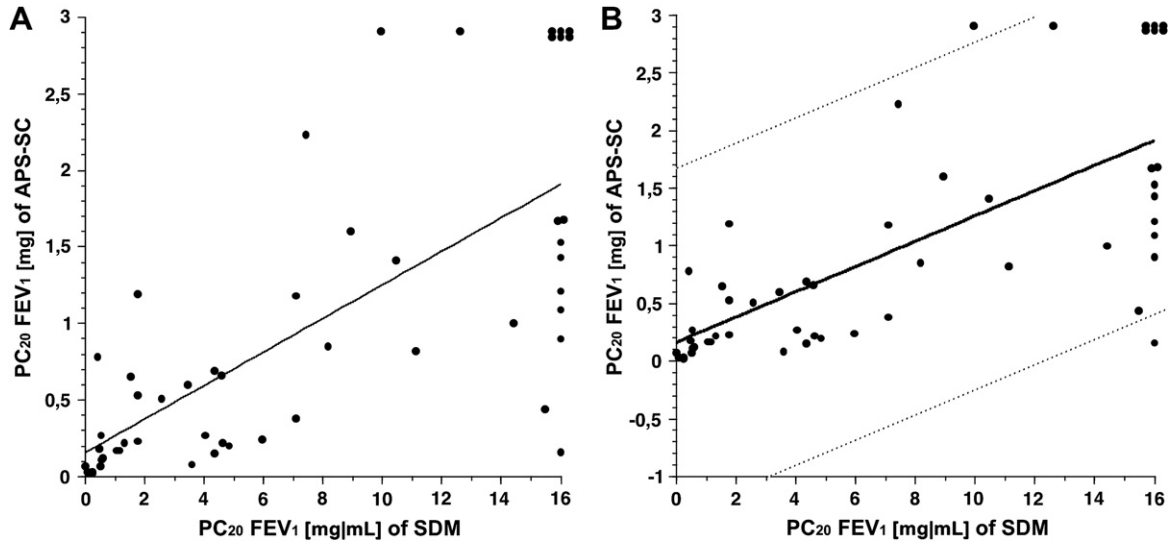

Fig. 1 Distribution of provocative concentration of methacholine versus provocative dose of methacholine causing a 20% fall of FEV_1. (A) The regression was calculated using all subjects (n = 52) including those 16 outside the range of 16 mg/mL and 2.91 mg methacholine, respectively (r = 0.69). (B) Hahn 95 % prognosis interval. The bold line shows the regression, whereas the outer lines represent the band of expected PD_{20} values corresponding to a given PC_{20} value.

Table 3 Comparison between optimum cut-points for agreement between the dosimeter and APS method.

PC_{20}- cut point [mg/mL]	PD_{20}- cut point [mg]	Δ PD_{20}	Agreement [%]	Cohen's kappa (linear)	*p*-value (of kappa)
1.0	0.3	0.1	76.9	0.46	< 0.005
2.0	0.4	0.2	76.9	0.43	< 0.005
4.0	0.6	0.4	78.9	0.54	<0.001
8.0	**1.0**	**0.9**	**82.7**	**0.59**	**< 0.001**
16.0	1.9	–	78.9	0.56	<0.001

Discussion

In subjects with a history of bronchial hyperreactivity we investigated the relationship between the ATS recommended short five-breath dosimeter protocol[1] 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.[7,8,11] Hagmolen often Have et al.[8] 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[11] 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, $p = 0.004$). This was due to a larger number of PD_{20}-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[8] and our study used a single concentration of 39.2 mg/mL or 16 mg/mL methacholine, respectively. In the adult study,[11] 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.

Table 4 Distribution of positive and negative results comparing dosimeter and APS method.

		PD_{20}		Σ
		<1.0 mg	≥1.0 mg	
PC_{20}	<8.0 mg mL^{-1}	27	3	30
	≥8.0 mg mL^{-1}	6	16	22
Σ		33	19	52

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.[1] 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.[12] Interestingly, there is one study[13] 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.[14] had a similar approach to assess cut-points of BHR comparing the tidal breathing method to ECRHS protocol. In this study, each doubling PC_{20} cut-point was associated with a doubling of PD_{20} 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.[15] 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.[16] showed with a modified MCT protocol from Chai et al.[17] in comparison to a longer protocol of Hargreave et al.[18] a good agreement between the two techniques.

Although previous studies have compared both recommended ATS protocols, results are not consistent. Ryan et al.[19] found no difference between the methods. This was confirmed by Wubbel et al.,[4] the PC_{20} methacholine obtained by either method showed no significant difference. In contrast, in the study of Cockcroft et al.[6] the five-breath dosimeter PC_{20} was almost twofold greater than the 2-min tidal breathing PC_{20}. Potential reasons for these observations are that a double dose is administered by the tidal-breathing method,[6] and that inhalations to TLC are recognised to be bronchoprotective, particularly in subjects with airway responsiveness in the normal, borderline, or mild range.[20] This data was confirmed by the study of Allen et al.[21] The dosimeter PC_{20} was larger than the tidal breathing method and the difference was greater in subjects with mild BHR having a $PC_{20} > 2$ 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 (PD_{20}) revealed different values. Analysis of 18 subjects showed a small, but significant difference in PD_{20}.[7] In children, using a concentration of 39.2 mg/mL methacholine, PD_{20} for APS-SC was significantly higher than the PD_{20} for SDM.[8] The difference of PD_{20} is consistent with our results. However, PD_{20} 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.[6] The fine particle fraction (< 5 μm) of the APS nebuliser was on average 49.7%.[11] Thus, particle size is not an explanation for the difference in PD_{20}. Our results for PD_{20} are consistent with the data of Siersted et al.,[14] 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.[7] 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 FEV_1, 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.

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Conflict of interest

There is no conflict of interest for any author.

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