Methacholine Challenge*
Test-Shortening Procedures

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Study objectives: Validation of test-shortening procedures for the 2-min tidal breathing methacholine challenge method.

Design: Retrospective chart review.

Setting: Tertiary-care university clinical pulmonary function laboratory.

Patients: One thousand subjects aged 10 to 85 years (mean ± SD, 44.5 ± 16.0 years), 44.5% male, referred for methacholine challenge.

Intervention: Two-minute tidal breathing methacholine challenge was performed, with both physician and technician access to published test-shortening procedures.

Measurements and results: There were 315 positive test results (provocative concentration of methacholine causing a 20% fall in FEV₁ [PC20] < 8 mg/mL) and 685 negative test results. The subjects with positive test results were less likely to be male (39.1 vs 47.5%; p < 0.02) and had lower FEV₁ (91.8 ± 14.9% predicted vs 97.2 ± 13.9% predicted; p < 0.001). The average starting PC20 was between 0.5 mg/mL and 1.0 mg/mL; the most common PC20 was 1 mg/mL (67%). There were 431 skipped concentrations in 380 subjects. The mean number of methacholine inhalations was 3.7 ± 1.1 (3.9 ± 0.1 for negative test results vs 3.3 ± 1.2 for positive test results; p < 0.001).

Eighteen subjects had a > 20% FEV₁ fall on the first inhalation, and 11 subjects had a > 20% FEV₁ fall after a skipped concentration. In only one case (0.1%) an FEV₁ fall > 40% on the first concentration was reported, compared with no cases after a skipped concentration and seven cases with a > 40% FEV₁ fall after a routine doubling dose step-up.

Conclusions: The 2-min tidal breathing methacholine test in clinical practice can be safely shortened to an average of less than four inhalations using starting concentrations based on FEV₁, asthma medication, and clinical features, and by occasionally omitting concentrations.

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Key words: methacholine tests; safety; standardization; test shortening

Abbreviation: PC20 = provocative concentration of methacholine causing a 20% fall in FEV₁

The 2-min tidal breathing methacholine challenge test¹–³ was developed by Dr. F. E. Hargreave, by modification of the Dutch 30-s tidal breathing method.⁴ The full protocol of doubling methacholine concentrations from 0.03 to 8 mg/mL (including saline solution) will take > 50 min. For many years now, we have routinely practiced test-shortening procedures, including selected higher starting concentrations and occasional skipped concentrations, as outlined in our test methodology booklet³ and as reproduced in the European Respiratory Society guidelines.⁵ However, these test-shortening guidelines are not widely appreciated, and there are limited data to validate them other than by many years of successful practical use in several laboratories.

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The current investigation examines 1,000 randomly selected test results of clinical methacholine challenges from our clinical lung function laboratory. The goals of this retrospective evaluation were threefold: (1) to assess how our clinical bronchoprovocation laboratory was performing vis-à-vis these guidelines; (2) to provide retrospective validation for the safety of these test-shortening guidelines; and (3) to make more widely known the test-shortening guidelines.
Materials and Methods

Test Selection

One thousand randomly selected methacholine challenge tests performed between 1995 and 2001 were extracted from the files at the Royal University Hospital Pulmonary Function Laboratory. This represents approximately 50% of the tests performed during the 6-year period. Methacholine challenges were identified in two ways. First, challenge test reports in excess of 5 years were identified before shredding. Second, more recent methacholine challenges were identified by the provincial government billing code for physician interpretation of these studies. For subjects who had more than one test, the first test identified was used. There was no obvious selection bias. Tests were collected until the study reached the arbitrary total of 1,000. The remaining (approximately) 1,000 tests were not examined. Methacholine challenges in our clinical laboratory are performed in subjects with symptoms consistent with asthma and nondiagnostic flow rates. Methacholine challenges are not performed in subjects with known asthma and are not performed to monitor treatment. Because this was a retrospective chart review, neither informed consent nor ethics approval was required.

Methacholine Challenge

The 2-min tidal breathing methacholine challenge was performed as described.1-5 Aerosols were generated with a Bennett-Twin jet nebulizer (Puritan Bennett; Carlsbad, CA) calibrated to have an output (mass loss) of 0.13 mL/min. The aerosol was delivered to the patient via a loosely applied facemask, the nose was clipped, and the aerosol was inhaled for 2 min of tidal breathing. Saline solution was used as a diluent, and this was inhaled first, followed at 5-min intervals (between the start of one inhalation and the start of the next inhalation) by doubling concentrations of methacholine. The available concentrations of methacholine in the clinical laboratory were from 0.03 to 16 mg/mL. Tests were routinely performed only as far as concentrations of 8 mg/mL. The FEV1 was initially measured from a full spirogram, in triplicate, before the start of the test. Single determinations of FEV1 without a full spirogram (ie, without a vital capacity) were then performed 30 s and 90 s after each inhalation. Technically inadequate spirograms were discarded and were immediately repeated. The percent change in FEV1 was calculated from the lowest post-saline solution FEV1 to the lowest post-methacholine FEV1. The test was continued until the FEV1 had fallen by 20% or more; however, occasionally, in the clinical laboratory, at the discretion of the technician, the test was stopped at levels of 17%, 18%, or 19% fall in FEV1 (PC20). The provocative concentration of methacholine causing a 20% fall in FEV1 (PC20) was then interpolated from the log-dose vs concentration response curve using an algebraic formula7 and, in subjects with a fall of slightly <20% in FEV1, the values were extrapolated using a single point formula.8

Test-Shortening Procedure

The published test-shortening procedure included the following steps.3 If spirometry findings are normal (FEV1/vital capacity >80%, FEV1 >70% predicted) and symptoms are well controlled, suggested starting concentrations for methacholine challenge are outlined in Table 1 based on the amount of asthma medication the subject is receiving. The recommended range of starting concentrations is between 0.125 mg/mL and 2 mg/mL; most methacholine challenges in our clinical laboratory are started at 1 mg/mL in subjects with normal lung function. In subjects with reduced lung function, subjects who respond >10% to diluent, or subjects in whom there is a particular worry, the recommended starting concentration is lower (Table 1). The physicians ordering the tests are expected to provide starting concentrations on the test requisitions; however, with increasing familiarity with the guidelines in Table 1, technician judgments about starting concentrations have become standard for the time covered by this review.

The test can be further shortened by omitting a concentration (ie, increasing the dose fourfold) when there has been a fall of <5% FEV1, and no symptoms have been provoked by the most recent methacholine concentration. The decision regarding skipping a concentration is exclusively that of the technician. The most commonly skipped concentrations are 0.5 mg/mL and 2 mg/mL.

Study Design

This is a retrospective evaluation of 1,000 methacholine challenge tests. The challenges were separated into positive test results (PC20 ≤8 mg/mL) and negative test results (PC20 >8 mg/mL). We recorded age, sex, height, baseline FEV1, starting concentration, total number of concentrations inhaled, skipped concentrations, and percent fall in FEV1 at the final concentration. In addition, we specifically looked for how many individuals had a fall of 20% or greater FEV1, either at the first concentration of methacholine inhaled or after a skipped concentration.

Results

The results are summarized in Table 2. There were 685 negative challenge results and 315 positive challenge results. The age and height of the subjects with positive challenge results were not significantly different than those with the negative challenge results. However, within the positive challenge results, there was a significantly lower proportion of men (39.1% vs 47.5% male, p <0.02). The FEV1 of subjects with positive challenge results was lower both in absolute
terms and as a percent predicted (91.8 vs 99.2; p < 0.001). The geometric mean starting concentration was one half a concentration lower in the positive challenge results (0.52 mg/mL vs 0.76 mg/mL; p < 0.001), and the number of concentrations inhaled was significantly less (3.3 vs 3.9; p < 0.001).

The range of starting concentrations is outlined in Table 3. More than two thirds of the tests were started at 1 mg/mL. Only 15 subjects were started with an inhalation outside the range of 0.125 to 1.0 mg/mL; this included 2 subjects at 0.06 mg/mL and 13 subjects at 2 mg/mL. In the 1,000 subjects, the 0.03-mg/mL concentration was not required.

The average number of concentrations inhaled was 3.7 for the total group, 3.9 for the negative test results, and 3.3 for the positive test results. This ranged from one concentration to seven concentrations. There were 431 instances of skipped concentrations in 380 patients (310 in negative test results and 70 in positive test results). Eleven subjects (1% of the total population or 2.9% of the group with a skipped concentration) had a response ≥ 20% after a skipped concentration (26 ± 5.3% FEV₁ fall), and 18 subjects (< 2% of the total population or 5.7% of the positive challenge results) had a fall in FEV₁ ≥ 20% from the first inhalation (24.6 ± 9.0% FEV₁ fall).

There have never been any severe adverse events (ie, cardiac events, bronchoconstriction requiring emergency department treatment, or any medical prescription other than β₂-agonist metered-dose inhaler) in the methacholine challenge laboratory. From these 1,000 challenge test results, there were eight subjects whose FEV₁ fell by ≥ 40%, including seven subjects after a regular doubling step-up (range 40 to 62%), no subjects after a skipped concentration, and one subject on the first concentration (55%).

**Table 2—Results**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total</th>
<th>PC₂₀ &gt; 8 mg/mL</th>
<th>PC₂₀ ≤ 8 mg/mL</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, No.</td>
<td>1,000</td>
<td>685</td>
<td>315</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>44.5 (16)</td>
<td>44.3 (15)</td>
<td>43.0 (17)</td>
<td>NS</td>
</tr>
<tr>
<td>Male gender, %</td>
<td>44.8</td>
<td>47.5</td>
<td>39.1</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>Height, cm</td>
<td>168 (10)</td>
<td>168 (11)</td>
<td>167 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>FEV₁, L</td>
<td>3.18 (0.8)</td>
<td>3.27 (0.8)</td>
<td>2.99 (0.8)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>% predicted</td>
<td>96.7 (15)</td>
<td>99.2 (14)</td>
<td>91.8 (15)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Starting concentration</td>
<td>0.68</td>
<td>0.76</td>
<td>0.52</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>No. of concentrations</td>
<td>3.7 (1.1)</td>
<td>3.9 (0.9)</td>
<td>3.3 (1.2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ΔFEV₁, %</td>
<td>13 (9.7)</td>
<td>7.7 (5.2)</td>
<td>24.7 (6.6)</td>
<td></td>
</tr>
<tr>
<td>n ≥ 40%</td>
<td>8</td>
<td>8</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>PC₂₀†</td>
<td>&gt; 8</td>
<td>&gt; 8</td>
<td>2.6</td>
<td></td>
</tr>
</tbody>
</table>

*Data are presented as mean (SD) unless otherwise indicated. NS = not significant.
†Geometric mean.

**Table 3—Actual Starting Concentrations**

<table>
<thead>
<tr>
<th>Concentrations, mg/mL</th>
<th>Patients, No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03</td>
<td>0</td>
</tr>
<tr>
<td>0.06</td>
<td>2</td>
</tr>
<tr>
<td>0.125</td>
<td>53</td>
</tr>
<tr>
<td>0.25</td>
<td>148</td>
</tr>
<tr>
<td>0.5</td>
<td>113</td>
</tr>
<tr>
<td>1.0</td>
<td>671</td>
</tr>
<tr>
<td>2.0</td>
<td>13</td>
</tr>
</tbody>
</table>

**Discussion**

These results document that in 1,000 randomly chosen methacholine challenges, the most common starting concentration was 1 mg/mL, and the average number of concentrations inhaled was less than four (range, one to seven). This indicates that the average methacholine challenge test, including saline solution, required < 30 min for completion. There were no significant adverse events. Only 29 subjects or 3% of the total population had a fall of ≥ 20% FEV₁ after a skipped methacholine concentration or on the first methacholine concentration, and all but one of these subjects were within the range of 20 to 30% FEV₁ fall; only one subject had a fall of ≥ 40% FEV₁ on a first inhalation, compared with seven subjects with a regular full step-up test.

Albeit a retrospective and post hoc study, these results provide validation of the usefulness and safety of a methacholine challenge shortening protocol. In most cases of methacholine challenge in subjects who are not known to have asthma and who have healthy lung function while not receiving asthma medications, a starting concentration of 1 mg/mL is safe. More than two thirds of our subjects had a starting concentration of 1 mg/mL. The most common single methacholine
regimen was 1, 4, and 8 mg/mL (skipping 2 mg/mL). The second most common methacholine challenge regimen was 0.25 to 1 mg/mL through 4 to 8 mg/mL (skipping 0.5 mg/mL and often 2 mg/mL).

Troyanov et al 9 recently examined the prevalence and determinants of exaggerated bronchoconstriction using similar guidelines for shortening the methacholine challenge in 711 epidemiologic challenges and 408 clinical challenges. Exaggerated bronchoconstriction was defined as $\geq 20\%$ change in $FEV_1$ after saline solution or $\geq 30\%$ change in $FEV_1$ after methacholine. They reported a 10% prevalence of exaggerated bronchoconstriction by their definition; there was a 3.1% prevalence of change in $FEV_1$ $\geq 40\%$. Other aspects of test shortening were not reported. Their clinical cohort does not seem comparable with ours because almost 50% of their subjects were already using inhaled corticosteroids. The median starting concentration was much lower than in our study (0.125 mg/mL), and $>50\%$ of their subjects had a positive test result; these features suggest a trend toward greater prevalence and greater severity of asthma in their cohort. The data of Troyanov et al9 are thus not inconsistent with our data.

These data also revealed interesting differences between the subjects with positive methacholine challenge results and those with negative methacholine challenges. As expected, subjects with a positive methacholine challenge had a lower baseline $FEV_1$ than those with negative challenge results. Also not surprising, the subjects with positive methacholine challenge results inhaled, on average, almost one concentration less than those with negative challenge results. The starting concentration in subjects who proved to have positive methacholine challenge results was significantly lower, by 0.5 concentrations. This is likely either because there was a larger proportion of these subjects who had a low $FEV_1$, or because there was a greater index of suspicion of asthma/airway hyperresponsiveness.

The other significant difference was the smaller proportion of men in the group with positive methacholine challenge results than in the group with the negative methacholine challenge results. Although asthma and atopy are both recognized to be more common in young men,10 airway hyperresponsiveness has been noted to be more common in women.11 The latter has been attributed to smaller lungs in women receiving a proportionately larger dose of methacholine.11 Although this would be expected to be true for dosimeter methods (breath-activated solenoid dosimeters or hand-held dosimeters), one would expect the tidal breathing method would either avoid or minimize this difference. However, a group of referred subjects is not equivalent to a random population; therefore, these data cannot be used to infer that airway responsiveness by the 2-min tidal breathing technique is more common in women than in men. An alternate explanation would be that asthma (undiagnosed) might be more common in women in this age group, or indeed, that there might be a referral bias toward referring more women for assessment. In summary, this report provides a practical guide for safely shortening the 2-min tidal breathing methacholine challenge test procedure.

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