

## DIAGNOSTIC TESTS

# Mannitol Challenge Does Not Confirm Bronchial Hyperreactivity in Some Histamine-Responsive Asthmatic Children

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**Objective.** The aim of this study was to compare the usefulness of mannitol provocation test to that of classical histamine challenge in children with PC<sub>20</sub>FEV<sub>1</sub> histamine lower than 4 mg/ml. **Methods.** Twenty-two adolescent patients (mean age of 15.4 ± 4.1 years) with established asthma (PC<sub>20</sub>FEV<sub>1</sub> histamine below 4 mg/ml) were included in this study. Bronchial challenge with mannitol was performed 1–2 days after the test with histamine. **Results.** The fraction of positive results of mannitol test was markedly lower when compared with the histamine challenge (72.7% vs. 100%,  $p = .015$ ). The test was discontinued in one case due to severe coughing after inhalation of 315 mg of mannitol. Coughing during inhalation of dry mannitol powder occurred in most patients, although drinking water after subsequent doses alleviated this symptom in nearly all of them. Of note, triboelectrification of the inhaler and capsules was observed during the administration of consecutive mannitol doses, markedly hindering the delivery of this provoking agent. The relative decrease in FEV<sub>1</sub> resulting from bronchial provocation was significantly lower following mannitol delivery when compared with the histamine test (70.3% vs. 81.6% of resting value,  $p < .001$ ). Significant correlation was not observed between the values of PC<sub>20</sub>FEV<sub>1</sub> histamine and PD<sub>15</sub>FEV<sub>1</sub> mannitol levels. **Conclusions.** Bronchial challenge with mannitol can be used as a screening test in everyday practice, but one cannot exclude bronchial hyperresponsiveness based on its negative results. Moreover, its usefulness is limited by the influence of static on the delivery of sequential mannitol doses and coughing which can be often associated with mannitol inhalation.

**Keywords** asthma, bronchial hyperresponsiveness, provocation, screening, spirometry

## INTRODUCTION

According to the currently established definition, asthma is a chronic inflammatory disorder associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing (1). Spirometric signs of obstruction are usually absent in patients with mild asthma, but can be detected during symptomatic periods in patients with moderate or severe form of this disease (2). Frequently, patients referred to the specialist due to suspected asthma show neither specific symptoms nor spirometric abnormalities. Detecting bronchial hyperresponsiveness strongly supports the diagnosis of asthma; however, it should be remembered that enhanced bronchial reactivity is not asthma specific (3). It has been suggested by several authors that asthma can be excluded based on the negative result of bronchial provocation test, although this claim has been contested by other investigators (4). Methacholine and histamine challenges belong to the classic provocation tests, enabling the detection of bronchial hyperresponsiveness along with quantification of its severity (5, 6). However, they are not used frequently in everyday practice because they require special dosing equipment and experienced personnel to obtain reliable results (5).

In late 1990s, Australian researchers described for the first time a provocation test utilizing dry powder of mannitol. Only spirometer and dry powder inhaler, widely used for the delivery of dry forms of drugs, are needed to perform this test (7). The mannitol test has raised hopes that the simplicity of this procedure will be reflected in widespread distribution of bronchial reactivity assessment, perhaps even in the offices of family doctors (8, 9). Many studies dealing with various aspects of the mannitol challenge were performed thus far (10–16), and as a result, commercially available kits, comprising an inhaler and capsules with various mannitol doses, have received approval from the U.S. Food and Drug Administration (FDA) for bronchial reactivity testing (17).

The aim of this study was to compare the usefulness of mannitol provocation test to that of classical histamine challenge (routinely used at our clinic) in children with PC<sub>20</sub>FEV<sub>1</sub> histamine lower than 4 mg/ml. The following parameters were considered during comparative analysis: result consistency, subjective preferences of patients, their objective tolerance to both provoking agents, and duration of both tests.

## METHODS

This research was conducted within the statute project of the Medical University in Białystok, and both the study protocol and all the procedures were approved by the Local Ethics Committee. The patients and their parents gave

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their informed written consent before the start of any procedure. Forty patients suspected of asthma based on clinical symptoms were screened for non-specific bronchial responsiveness to histamine. Qualification criterion,  $PC_{20}FEV_1$  histamine below 4 mg/ml, was met by 22 individuals. Due to budgetary shortages of our project, the number of available kits for mannitol provocation challenge was limited ( $n = 22$ ). Therefore, qualification to the latter was based on a cutoff value of 4 mg/ml for histamine instead of generally accepted 8 mg/ml. Clinical characteristics of our patients are summarized in Table 1. Bronchial challenge with mannitol was performed 1–2 days after the test with histamine.

#### *Bronchial Provocation Test with Histamine*

The test was performed by the five-breath method in accordance with Rosenthal (18), using a DeVilbiss 646 jet nebulizer connected to a Koko Digidoser dosimeter/spirometer (Ferraris, USA), powered by compressed air. The following concentrations of histamine hydrochloride were used: 0.025, 0.25, 2.5, 10.0, and 25.0 mg/ml. Spirometry was performed with Koko Digidoser dosimeter/spirometer (Ferraris, USA) according to European Respiratory Society/American Thoracic Society (ERS/ATS) guidelines (19, 20). Spirometry was performed 3 minutes after each inhalation until at least a 20% decrease in  $FEV_1$  was determined. In such cases, computer software (KoKo PFT Software v.4.11; Ferraris, USA) calculated  $PC_{20}FEV_1$  value (mg/ml), and the result of the test was considered positive. If 20% decrease in  $FEV_1$  was not observed following inhalation of 25 mg/ml of histamine, the result of the test was considered negative.

#### *Bronchial Provocation Test with Mannitol*

The test was performed with commercially available Aridol® kits (Pharmaxis Ltd, Australia), in accordance with Anderson protocol (21–23), until the patient had a positive response or 635 mg of mannitol had been administered. The result of the test was considered positive whenever at least a 15% decrease in  $FEV_1$  was noted compared with the resting value (determined after inhaling the content of 0 mg capsule) or at least a 10% incremental fall in  $FEV_1$  was noted between consecutive mannitol doses. Computer software (KoKo PFT Software v. 4.11) calculated  $PD_{15}FEV_1$  mannitol value (mg) in such cases. If coughing appeared after inhalation of mannitol dose, the patient was given a swig of water which was usually sufficient to alleviate this symptom. Following the challenge, patients were monitored until their  $FEV_1$  values returned to the resting level.

#### *Statistical Analysis*

The normal distribution of continuous variables was tested using the Shapiro–Wilk test. Mean values between the results of provocation tests with histamine and mannitol were compared using the paired Student's *t*-test, and the distributions of qualitative variables were compared by means of the Pearson's chi-square test. Additionally, Pearson's coefficient of correlation (*r*) was calculated between  $PC_{20}FEV_1$  and  $PD_{15}FEV_1$ . All calculations were

performed using Statistica 8 (StatSoft®, Tulsa OK, USA) software, and statistical significance was defined as  $p \leq .05$ .

## RESULTS

All patients who qualified to bronchial provocation tests with histamine and mannitol had at least moderate bronchial hyperresponsiveness ( $PC_{20}FEV_1$  histamine below 4 mg/ml). As many as 21 (95.5%) out of 22 examined patients required inhalation of only the three lowest histamine concentrations (0.025, 0.25, and 2.5 mg/ml) to obtain at least a 20% decrease in  $FEV_1$ . Inhaling the fourth concentration of histamine (10 mg/ml) was needed in only one patient, and 25 mg/ml was not required in any case. Neither cough nor any other adverse effects were observed during histamine delivery. In the case of mannitol provocation test, administration of all 19 capsules (corresponding to nine series of inhalations) was required in 6 out of 22 patients. In five patients, the result of the challenge was negative, and the test was discontinued in one case due to severe coughing after inhalation of 315 mg of mannitol. Coughing during inhalation of dry powder occurred in most patients, although drinking water after subsequent doses alleviated this symptom in nearly all of them.

Of note, triboelectrification of the inhaler and capsules was observed during the administration of consecutive mannitol doses, markedly hindering the delivery of this provoking agent. Frequently, the capsules were attracted by the static electricity to the surface of puncturing chamber and, as a result, did not fall into spinning chamber during the inhalation. This markedly prolonged the administration of consecutive doses and one can question whether inhaled particles of mannitol were sufficiently atomized. Attraction of the capsules by the inhaler was also clearly visible during the replacement of the emptied capsule. We tried to prevent triboelectrification by attaching a metal object to the drug chamber after each inhalation. Although helpful, this did not eliminate the problem entirely.

We compared mean values of maximal decrease in  $FEV_1$  resulting from bronchial provocations with histamine and mannitol. The relative decrease was significantly lower following mannitol delivery when compared with the histamine test (70.3% vs. 81.6% of resting value,  $p < .001$ ).

Significant correlation was not observed between the values of  $PC_{20}FEV_1$  histamine and  $PD_{15}FEV_1$  mannitol levels ( $r = 0.325$ ,  $p = .140$ ; Figure 1), also when non-responders were excluded ( $r = 0.284$ ,  $p = .287$ ).

## DISCUSSION

Spector et al. (24) asked in their 2009 report whether the mannitol test can replace the bronchial provocation challenges with methacholine and histamine. Their article concluded with the notion that the mannitol test can replace all other bronchial challenges since none of them is neither asthma specific nor sufficiently explains the pathogenesis of this disease (24).

TABLE 1.—Detailed characteristics of study participants ( $n = 22$ ).

| Id    | Gender         | Age (years)             | Height (cm)               | Weight (kg)              | FEV <sub>1</sub> (%)     | Total IgE (kU/l)           | Specific IgE (class)   | PC <sub>20</sub> FEV <sub>1</sub> histamine (mg/ml) | PD <sub>15</sub> FEV <sub>1</sub> (mg) |
|-------|----------------|-------------------------|---------------------------|--------------------------|--------------------------|----------------------------|------------------------|-----------------------------------------------------|----------------------------------------|
| 1     | M              | 13                      | 165.0                     | 54.0                     | 104                      | 382                        | 5                      | 3.564                                               | >635.0                                 |
| 2     | F              | 16                      | 160.0                     | 57.0                     | 101                      | 3045                       | 6                      | 0.214                                               | 60.0                                   |
| 3     | M              | 10                      | 146.5                     | 35.6                     | 110                      | 814                        | 5                      | 0.021                                               | >635.0                                 |
| 4     | F              | 17                      | 164.0                     | 61.4                     | 85                       | 903                        | 2                      | 1.756                                               | >635.0                                 |
| 5     | M              | 14                      | 174.0                     | 52.7                     | 85                       | 11                         | 0                      | 1.220                                               | 40.0                                   |
| 6     | F              | 19                      | 160.0                     | 50.2                     | 76                       | 528                        | 0                      | 2.492                                               | 475.0                                  |
| 7     | M              | 18                      | 187.0                     | 78.8                     | 62                       | 263                        | 3                      | 0.045                                               | 31.0                                   |
| 8     | M              | 12                      | 157.5                     | 41.0                     | 78                       | 282                        | 3                      | 0.010                                               | 24.0                                   |
| 9     | M              | 14                      | 178.5                     | 70.3                     | 78                       | 877                        | 5                      | 0.020                                               | 105.0                                  |
| 10    | M              | 10                      | 133.5                     | 31.7                     | 75                       | 392                        | 4                      | 0.019                                               | >635.0                                 |
| 11    | F              | 39                      | 167.5                     | 81.0                     | 123                      | 370                        | 4                      | 0.661                                               | 62.0                                   |
| 12    | M              | 15                      | 167.0                     | 50.4                     | 79                       | 280                        | 5                      | 0.300                                               | 81.0                                   |
| 13    | M              | 13                      | 169.0                     | 61.0                     | 68                       | 500                        | 3                      | 0.353                                               | 178.0                                  |
| 14    | F              | 11                      | 159.5                     | 39.0                     | 76                       | 380                        | 5                      | 1.110                                               | >635.0                                 |
| 15    | M              | 9                       | 140.0                     | 36.0                     | 76                       | 1050                       | 6                      | 0.273                                               | 9.6                                    |
| 16    | F              | 18                      | 168.0                     | 60.0                     | 82                       | 362                        | 3                      | 0.010                                               | 9.5                                    |
| 17    | F              | 18                      | 156.5                     | 56.9                     | 102                      | 108                        | 3                      | 0.557                                               | 85.0                                   |
| 18    | M              | 20                      | 173.5                     | 87.5                     | 88                       | 1189                       | 4                      | 0.341                                               | >635.0                                 |
| 19    | F              | 18                      | 159.5                     | 92.0                     | 113                      | 749                        | 3                      | 0.019                                               | 580.0                                  |
| 20    | F              | 11                      | 146.5                     | 33.0                     | 97                       | 338                        | 4                      | 0.022                                               | 260.0                                  |
| 21    | F              | 15                      | 155.0                     | 56.5                     | 96                       | 263                        | 0                      | 1.075                                               | 46.7                                   |
| 22    | M              | 8                       | 125.0                     | 24.0                     | 119                      | 103                        | 0                      | 1.658                                               | 144.0                                  |
| Total | M = 12, F = 10 | 15.4 ± 6.3 <sup>a</sup> | 159.7 ± 14.6 <sup>a</sup> | 55.0 ± 18.6 <sup>a</sup> | 89.7 ± 16.9 <sup>a</sup> | 381 (280–814) <sup>b</sup> | 3.5 (3–5) <sup>b</sup> | 0.200 (0.299–1.132) <sup>c</sup>                    | 72.8 (47.8–226.1) <sup>c</sup>         |

<sup>a</sup>Arithmetic mean ± SD, <sup>b</sup>Median (interquartile range), <sup>c</sup>Geometric mean (95%CI).

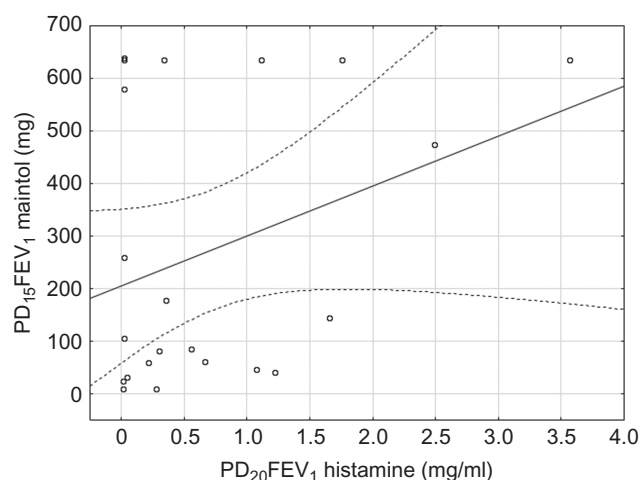


FIGURE 1.—Relationship between  $PD_{20}FEV_1$  histamine and  $PD_{15}FEV_1$  mannitol in asthmatic children ( $n = 22$ ).

In our study, the result of the bronchial challenge with mannitol was negative in the fraction of patients with evident bronchial responsiveness to histamine. In this group of patients with established asthma, the fraction of positive results of mannitol test was markedly lower when compared with the histamine challenge (72.7% vs. 100%). Anderson et al. (25) demonstrated that more than 35-minute duration of mannitol test can affect its sensitivity due to changes in airway osmolality. Although we did not document the exact time of mannitol test in this study, it took more than 30 minutes in most cases. This can be a possible reason behind negative challenges noted in some patients.

Many authors consider methacholine challenge as equal to histamine challenge, although exposure to these two provoking agents is reflected by dyspnea of different severity (9, 26). Searching through available literature, we found no previous studies directly comparing bronchial challenges with histamine and mannitol.

Coughing after mannitol inhalation was revealed as a serious limitation of provocation test with this agent performed in our participants. Coughing hindered markedly the delivery of consecutive mannitol doses and limited proper performance of spirometric measurements. Mannitol is an indirect stimuli that can act on sensory nerves. Significant cough was previously reported in some children subjected to mannitol challenge; similar to our study, it prevented the completion of the test at larger doses (27). However, the mannitol-induced cough has been observed to be short in duration and resolved even in absence of drinking water (27).

Even more serious technical complication that was observed during the delivery of mannitol capsules was the triboelectrification of the inhaler. This phenomenon resulted in attracting mannitol capsules to the surface of puncturing chamber and markedly hindered quick administration of this provoking agent in a required dose. Therefore, inhalators designed for dry, powdered drug delivery, otherwise perfect for administering several

capsules per day, seems suboptimal whenever up to 19 capsules must be given in a short period of time. Consequently, there is a need for technical improvement of inhalers used routinely during bronchial provocation tests with mannitol.

Furthermore, classical bronchial challenges with histamine and methacholine allow quantifying of bronchial hyperresponsiveness in contrast to the test with mannitol which is still considered a qualitative one. Despite important findings, our study also has one potential limitation, namely the way the participants were selected. Apart from being a convenience sample, the limited availability of mannitol test kits resulted in a way that mannitol challenge could only perform equally or underperform compared to histamine challenge. A more ideal way of sampling would have included all participants in both the histamine and mannitol challenge, but this was impossible due to budgetary shortages of our project.

Another potential reason that the histamine challenge test outperformed mannitol challenge in our group is variable mannitol responsiveness of different asthma phenotypes. Several authors observed that the mannitol test is more useful in predicting the inflammatory (eosinophilic) phenotype of asthma (14, 15, 28). However, this latter question requires further research since Leuppi et al. (29) observed that airway hyperresponsiveness to both histamine and mannitol in clinically well-controlled asthmatics appears to be independent of eosinophilic airway inflammation.

## CONCLUSIONS/KEY FINDINGS

Bronchial challenge with mannitol can be used as a screening test in everyday practice, but one cannot exclude bronchial hyperresponsiveness based on the negative results of this test. Moreover, its usefulness is limited by the influence of static on the delivery of sequential mannitol doses and coughing which can be often associated with mannitol inhalation.

## DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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