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Usefulness of mannitol challenge testing for diagnosing asthma in everyday clinical practice

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ABSTRACT

Background: The mannitol test is widely used for assessment of airway responsiveness in patients with possible asthma, but our knowledge of the value in everyday clinical practice is limited.

Objective: To investigate the diagnostic value of the mannitol test in a clinical setting.

Methods: All patients having a mannitol challenge test as part of their diagnostic work-up for asthma at the respiratory outpatient clinic, Hvidovre Hospital, over a 5-year period were included in the present analysis. Case history, including previous diagnoses, spirometry, exhaled nitric oxide (F_ENO) was obtained. The mannitol challenge was performed according to guidelines, i.e. increasing doses of mannitol (from 5 to 635 mg) until maximum dose or a 15% decline in FEV₁ (PD₁₅) was achieved (positive test defined as a PD₁₅ < 635 mg).

Results: Our cohort comprised 566 patients (210 men). Post-challenge, 247 patients were diagnosed with asthma by a specialist in respiratory medicine, of whom 138 (56%) had a positive mannitol test. A diagnosis of asthma was ruled out by the specialist in 319 individuals, of whom 18 (6%) had a positive mannitol test and 77 (24%) previous doctor-diagnosed asthma (but with no evidence of a confirmatory test). The specificity and sensitivity of the test was 94% and 56%, respectively. Female gender ($p=0.005$), increasing BMI ($p<0.001$), exercise-induced cough ($p=0.002$), night-time respiratory symptoms ($p=0.002$), low FEV₁/FVC ratio ($p=0.008$) and high F_ENO ($p<0.001$) were determinants for a positive mannitol test.

Conclusion: In everyday clinical work-up of patients with possible asthma, the sensitivity and specificity of the mannitol challenge test is comparable to previous controlled trials.

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Introduction

Asthma is characterized by variable, in most cases fully reversible airway obstruction, airway inflammation and airway hyperresponsiveness. Typical symptoms of asthma include shortness of breath, coughing, wheezing and chest tightness, triggered by a variety of stimuli, including allergens and physical activity. According to the Global Initiative for Asthma (GINA) guidelines (1) a diagnosis of asthma should be based on symptoms and a test confirming variable airflow obstruction. However, as many patients with possible asthma present with normal lung function, it may be challenging to confirm a diagnosis of asthma. However, bronchial challenge tests may overcome this, as patients with asthma often have airway hyperresponsiveness irrespective of their level of lung function (2).

The various bronchial challenge tests can be categorized as either direct or indirect. Direct challenge tests trigger a response by direct stimulation of the airway smooth muscle with agents like methacholine or histamine. On the other hand, the indirect challenges, e.g. eucapnic voluntary hyperventilation, exercise and mannitol, triggers bronchoconstriction via inflammatory mediators released in the airways (3). Mannitol works by increasing the osmolarity in the airways, which leads to the release of inflammatory mediators such as histamine, prostaglandins and leukotrienes. These inflammatory mediators act on the airway smooth muscle and cause bronchoconstriction (4).

Previous clinical studies have shown that the indirect tests, compared to the direct tests, often have a higher specificity, i.e. a higher percentage of healthy individuals correctly identified as such, whereas they

have a lower sensitivity, i.e. a lower percentage of patients with the disease who also have a positive test (5,6).

The mannitol challenge test was developed to facilitate more widespread use of bronchial provocation tests, as it is quickly done, requires a minimum of specialized equipment and can be performed by a single health care professional. It is commercially available as a test kit that contains capsules with mannitol and a disposable inhalation device, thus making it possible for smaller clinics to perform mannitol challenge tests (7).

Previous studies of the mannitol challenge test in the diagnostic work-up of patients with possible asthma have primarily been controlled trials in selected cohorts (5,8). However, most of the diagnostic work-up in patients with possible asthma is done in everyday clinical practice, and the purpose of this study was, therefore, to examine the diagnostic value of the mannitol challenge test in everyday clinical practice in a respiratory specialist care setting together with identifying clinical determinants for a positive test.

Material and methods

Study design

In this retrospective study, electronic medical records were reviewed for all patients referred for mannitol challenge testing in 2012–16 (5-year period) as part of their diagnostic work-up by a specialist in respiratory medicine at the respiratory outpatient clinic, Hvidovre Hospital. Patients were referred for mannitol challenge test provided they, as judged by the specialist in respiratory medicine seeing them at the first visit to the out-patient clinic, had symptoms suggesting a diagnosis of asthma and no previous test confirming a diagnosis of asthma. To be included in the present cohort, the patients had to fulfill the following criteria: (1) ≥ 18 years of age and (2) Pre-challenge $FEV_1 > 70\%$ pred.

Material

Case history, including symptoms, current treatment (if any), and previous diagnoses, and baseline characteristics, including sex, age, body mass index, were extracted from the medical records. The patients were classified as having pre-disposition to asthma and/or rhinitis if they had first-degree relatives with asthma and/or rhinitis. The respiratory day-time symptoms were divided into wheeze, cough, dyspnea, including exercise-induced symptoms, chest tightness, recurrent

lower respiratory infections, and likewise, night-time respiratory symptoms were divided into wheeze, cough and dyspnea.

Patients were categorized as never smokers, previous smokers (i.e. nonsmokers for at least 12 months) and current smokers (daily tobacco consumption); and lifetime tobacco exposure for ever smokers was calculated as pack-years (one pack-year = 20 cigarettes a day for one year).

Skin prick test results, if available, were recorded. The skin prick test routinely included birch, grass, mug wort, horse, dog, cat, *Dermatophagoides pteronyssinus*, *D. farinae*, *Aspergillus fumigatus*, *Alternaria iridis* and *Cladosporium herbarum*.

Spirometric measurements, including the pre-challenge spirometry, included forced vital capacity (FVC) and FEV_1 in liters and percent predicted, and FEV_1/FVC ratio were collected from medical records as well. Findings from bronchodilator reversibility tests were included, if available ($n = 284$).

Fractional exhaled nitric oxide (F_{ENO}) was measured in all patients prior to the mannitol challenge test.

Mannitol challenge test

The mannitol challenge test was performed using a commercially available kit (Aridol/Osmohale) with pre-filled capsules of mannitol with different doses (0 mg to 160 mg). Mannitol was administered by inhalation in nine doses up to a cumulative dose of 635 mg (5, 10, 20, 40, 2×40 , 4×40 , 4×40 , 4×40 mg). The first dose is 0 mg of mannitol, i.e. placebo, and the FEV_1 measured post-dose is recorded as baseline FEV_1 and used to calculate target level. A positive test was defined as a 15% decline from baseline in FEV_1 , i.e. the PD_{15} . However, if a 10% fall in FEV_1 was observed between two consecutive doses, the test was terminated and considered positive. Otherwise, the test was stopped when a cumulative dose of 635 mg was reached, i.e. negative test. If the patient had a 10% drop between two consecutive doses of mannitol, the cumulative dose administered was recorded as PD_{15} (7). Patients were told not to take asthma medication, if prescribed any, 12 h before the test and smoking had to be avoided for at least 6 h before the test, if necessary, the test was rescheduled.

Definition of asthma

Asthma was diagnosed (or ruled out) by a board-certified specialist in respiratory medicine based on

symptoms, history and all available test results, including spirometry and the mannitol challenge test.

Statistical analysis and ethics statement

Data were analyzed using SPSS (IBM). The results are expressed as means $\pm$ standard deviations, exact number, percentages and odds ratios with 95% confidence intervals, as appropriate. Multiple regression analyses were applied to identify clinical characteristics associated with a positive mannitol challenge test using backward deletion of non-significant variables. Furthermore, data were also analyzed with multiple logistic regression analysis with dichotomous variables to identify determinants of a positive challenge test. Smoking status was included in the analyses as current smokers vs. nonsmokers (i.e. never smokers and previous smokers). Values of $p < 0.05$ were considered significant. The sensitivity was calculated as the number of true positives divided by true positives plus false negatives, whereas the specificity was true negatives divided by false positives plus true negatives.

This study was approved by the Danish Data Protection Agency. This study was a non-drug and non-interventional study, but the National Committee on Health Research Ethics were given all relevant study information, although this was not mandatory.

Results

Patient characteristics

A total of 566 patients were included in the study, 356 (63%) women and 210 (37%) men, mean age 43 years (range 18 to 84 years), more baseline characteristics are given in Table 1. Pre-test clinical characteristics, including lung function and $F_{E}NO$, are given in Table 2. The distribution of respiratory symptoms is given in Figure 1.

Diagnostic yield of the mannitol challenge

Out of the 566 mannitol challenge tests performed, 410 (72%) were negative and 156 (28%) were positive (Table 3). Of these patients, 247 were diagnosed with asthma by the specialist in respiratory medicine after the mannitol challenge, although 109 (44%) of them had a negative mannitol test. This corresponds to a sensitivity of 56%. Eighteen patients had a positive mannitol challenge test but were not classified as having asthma owing to a lack of symptoms consistent with an asthma diagnosis. This corresponds to a specificity of 94%.

Table 1. General characteristics of the 566 patients with possible asthma who had a mannitol challenge test as part of their diagnostic work-up.

Characteristics	Total (n = 566)
Female*	63% (356)
Age (years)	42.7 $\pm$ 15.1
Body mass index	26.2 $\pm$ 5.8
Tobacco exposure (pack-years)	16.8 $\pm$ 112.7 (144)
Never smokers	67.7% (383)
Pre-test asthma diagnosis Rhinitis*	33.2% (188) 18.7% (106)
Predisposition to asthma*	23.5% (133)

The results are given in means, $\pm$ standard deviations and number of patients.

*Percentages and number of patients.

Table 2. Clinical characteristics, i.e. spirometry and exhaled nitric oxide, according to sex (n = 566).

	Males (n = 210)	Females (n = 356)
FEV ₁ (liter)	3.64 $\pm$ 0.79	2.78 $\pm$ 0.64
FEV ₁ (%pred.)	95.3 $\pm$ 24.8	93.1 $\pm$ 14.0
FVC (liter)	4.69 $\pm$ 0.97	3.47 $\pm$ 0.71
FVC (%pred.)	96.4 $\pm$ 17.7	107.6 $\pm$ 15.3
FEV ₁ /FVC	0.77 $\pm$ 0.08	0.80 $\pm$ 0.07
FeNO (ppb)	28.2 $\pm$ 25.3	19.7 $\pm$ 16.9

The results are given in means $\pm$ standard deviations.

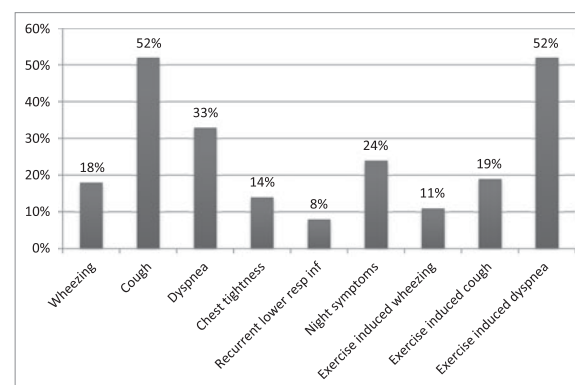


Figure 1. Distribution of respiratory symptoms among 566 patients with possible asthma.

Table 3. Mannitol challenge test result vs. post-challenge specialist diagnosis of asthma in 566 unselected patients having mannitol challenge test performed as part of their diagnostic work-up in a specialist care setting.

		Mannitol challenge test		
		Negative	Positive	Total
Asthma diagnosis after mannitol challenge	Negative	301	18	319
	Positive	109	138	247
	Total	410	156	566

The 156 patients with a positive mannitol challenge tests had a mean PD15 of 279 mg ($\pm$ 201, further details are given in Table 4). Of the 188 patients with a pre-challenge diagnosis of asthma, the diagnosis was confirmed in 111 patients (59%; 49 with a negative

Table 4. Level of hyperresponsiveness to mannitol in 153 patients (49 males) with a positive test according to sex.

	Male (<i>n</i> = 49)	Female (<i>n</i> = 104)
PD15 (mg)	307 ± 188	265 ± 206

mannitol challenge test), whereas the remaining 77 patients (41%; three with a positive mannitol challenge test) were classified as non-asthmatic by the specialist in respiratory medicine (Table 5).

Determinants of positive mannitol challenge test

Significant predictors for the female sex ($p = 0.005$), increasing BMI ($p < 0.001$), night-time respiratory symptoms ($p = 0.02$), exercise-induced cough ($p = 0.002$), low FEV₁/FVC ratio ($p = 0.008$) and high fraction of exhaled nitric-oxide (FeNO) ($p < 0.001$) (Table 6). Further analyses, revealed that FeNO > 50 ppb was associated with a 3.1 higher likelihood of a positive challenge test (95% CI 1.6–6.2, $p < 0.001$).

No significant associations were found between age, a predisposition for asthma, daytime respiratory symptoms, FEV₁, FVC, a pre-test diagnosis of asthma or any medication prescribed and a positive mannitol challenge test.

Discussion

This study showed that in everyday clinical practice with unselected patients the mannitol challenge had a sensitivity and specificity of 56% and 94%, respectively. Of the patients with a pre-challenge diagnosis of asthma (188 patients) 59% were also classified as having asthma by the specialist in respiratory medicine after the mannitol challenge test.

The sensitivity and specificity found in this study are in accordance with earlier controlled studies like the one carried out by Sverrild et al. (9) where the sensitivity and specificity were found to be 59% and 99%, respectively. Another study by Anderson et al. (6) found similar results with a sensitivity of 56%, but a specificity of 73%. A large occupational study by Menezes et al. performed in the workplace also found a lower sensitivity ranging from 35.4–61.9% and a high specificity 90.2–94.9% (10).

The mannitol challenge failed to identify airway hyperresponsiveness in 19% (109 patients) of patients that were diagnosed with asthma by the specialist in respiratory medicine but had a negative mannitol challenge test. It has been suggested in earlier studies (11,12) that the mannitol challenge test might not be as sensitive for mild asthma. Another possible

Table 5. Pre-challenge doctor-diagnosed asthma vs. asthma diagnosed by a specialist in respiratory medicine after the mannitol challenge test.

		Asthma diagnosed after mannitol challenge		
		Negative	Positive	Total
Asthma diagnosis before mannitol challenge	Negative	342	136	378
	Positive	77	111	188
	Total	419	247	566

explanation could be that a small number of the patients were already taking inhaled corticosteroids (ICS, budesonide and fluticasone) before the mannitol challenge test. Although patients refrained from taking their medications 12 h prior to the test, the medication is likely to have had impact on the test results (13).

The present study found several predictors of positive mannitol challenges. Sex was a predictor where females were more likely to have a positive mannitol challenge. It should be noted that the majority of the participants in this study were women (63%). Several studies have found that both the female sex and rhinitis is associated with airway hyperresponsiveness (14,15).

Except for night symptoms and exercise-induced cough none of the other symptoms were significant for predicting a positive mannitol challenge test. The most probable explanation is that patients with night-time symptoms and exercise-induced cough may at the time of referral, and by that diagnostic evaluation, have higher degree of asthma activity than their underlying asthma severity than patients with other respiratory symptoms.

Of the clinical characteristics the only significant predictor was a low FEV₁/FVC ratio, which in patients indicates airflow limitation and is therefore not unexpectedly correlated with a positive mannitol test. The airflow limitation in patients with asthma is likely to reflect long-standing inflammation in the airways which produces mucus and hypertrophy and hyperplasia of the smooth muscles in the bronchial wall, i.e. airway remodeling (16). Similarly, in the study by Romberg et al. (17) both FEV₁, expressed as a percentage of predicted value, and the FEV₁/FVC ratio were found to positively predict a positive mannitol test.

A high FeNO were found to be significantly correlated with a positive mannitol challenge. FeNO is known to be associated with eosinophilic asthma but less so with other phenotypes such as neutrophilic asthma (18). A high FeNO indicates a high likelihood of eosinophilic asthma (19) and is therefore associated

Table 6. Predictors of a positive mannitol challenge test in 566 patients with possible asthma.

	Odds ratio (95% confidence interval)	<i>p</i> values
BMI	1.08 (1.04; 1.12)	0.001
Female sex	2.02 (1.24; 3.28)	0.005
Nocturnal respiratory symptoms	1.82 (1.08; 2.40)	0.02
Exercise-induced cough	1.10 (1.04; 1.18)	0.002
FEV ₁ /FVC < 0.70	1.25 (1.05; 1.46)	0.008
FeNO > 25 ppb	1.04 (1.01; 1.07)	0.001

with a positive mannitol challenge (20). Several studies have found that FeNO may predict a positive mannitol challenge. Both studies by Sverrild et al. (19) and Backer et al. (21) found a high correlation between the two. In the controlled study by Sverrild et al. proposed that FeNO could be used exclusively instead of the mannitol challenge since the correlation was so high in their study. However, as observed in the present study, other studies have found association between FeNO and responsiveness to mannitol challenge, but not to an extent that the challenge did not provide further diagnostic information (22). For the present study this, however, was not found. The population study by Backer et al. found a lower correlation as well which they hypothesized was because of the inclusion of both smokers and patients on ICS treatment.

The best way to use the mannitol challenge as part of the diagnostic work-up in patients with possible asthma is still being discussed (8,16,23), but with a high specificity it is useful to exclude asthma as a diagnosis and a positive test indicates a very high likelihood of asthma. With the sensitivity being somewhat lower and with frequent false negatives, a negative mannitol challenge cannot be used alone to rule out asthma. However the mannitol challenge provides the respiratory specialist with diagnostic information that otherwise would not be available (8).

The mannitol challenge test has several advantages. It has been proposed that besides aiding in diagnosing asthma patients the mannitol challenge can provide information on a patient's response to ICS treatment because of its reflection on the on-going inflammation (17). Porsbjerg et al. (24) found an association between inflammation and the response to the mannitol challenge test. In a study done by Brannan et al. (13) the relationship between budesonide treatment and the mannitol challenge test were examined. They found that besides improving lung function the treatment also lowered the response to the mannitol challenge test. This suggests that the mannitol test could predict how well patients will respond to treatment with inhaled corticosteroids. For patients already on inhaled corticosteroids, the mannitol

challenge test can help the physician to determine if the treatment is sufficient. Lipworth et al. (25) used the mannitol challenge test to assess how well controlled patients were, and by up-titrating the treatment with inhaled corticosteroids, they reduced the degree of hyperresponsiveness, symptoms and exacerbations in the patients with asthma.

Study limitations

An important limitation of the present study is the use of data extracted from medical records, as the enrolled patients were seen by different specialist in respiratory medicine leading to differences in available information from case histories and diagnostic work-up.

Some patients were prescribed inhaled corticosteroids by the respiratory specialist before the mannitol challenge test was performed, which provided the study with a slightly higher percentage of patients on inhaled corticosteroids compared to other studies, as inhaled corticosteroids are known to reduce airway hyperresponsiveness and therefore affects the response to the mannitol challenge (13). In a study comparing the diagnostic validity of mannitol and methacholine in patients not treated with inhaled corticosteroids, a slightly higher specificity (97%) was reported, which they ascribed to no prior use of inhaled corticosteroids (22).

Since many patients were referred from their general practitioner to the respiratory outpatient clinic for further diagnostic work-up, they might be more difficult to diagnose, e.g. patients with cough-variant asthma, or have phenotypes of asthma that are less responsive to standard therapy, e.g. non-eosinophilic asthma. Patients were not excluded from the study if they had comorbidities, were current smokers, or were already on maintenance therapy, nor were high age an exclusion criterion. Many of the patients were current smokers or former smokers, and by that may have had some component of COPD in their disease, in spite of having a spirometry with a pre-bronchodilator FEV₁ > 70%pred., as patients with COPD may also present with positive mannitol challenges (26).

The lack of a gold standard to diagnose asthma makes it hard to assess the validity of the mannitol challenge test. In the present study, the diagnosis of asthma was an overall assessment made by a respiratory specialist guided by case history and available tests and it might, therefore, not be fully reproducible if the patient had been seen by another specialist (5). However, our findings are comparable to other studies, which suggests the limitations did not considerably affect the results.

In conclusion, our study of patients having a mannitol challenge test as part of their diagnostic work-up in every day clinical practice revealed a similar specificity and sensitivity for the mannitol challenge as found controlled studies.

Disclosure statement

No potential conflict of interest was reported by the authors.

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