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Diagnostic comparison of methacholine and mannitol bronchial challenge tests for identifying bronchial hyperresponsiveness in asthma: a systematic review and meta-analysis

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ABSTRACT

Objective: Bronchial hyperresponsiveness (BHR) is a representative feature of asthma. Although methacholine and mannitol are commonly used for bronchial challenge tests, the optimal roles of the two agents for assessing BHR remain unclear. We compared the diagnostic performance of methacholine and mannitol in bronchial challenge tests.

Methods: A systematic literature search was performed using MEDLINE, EMBASE, and the Cochrane Central Register. The sensitivity, specificity, diagnostic odds ratio (DOR), and a hierarchical summary of the receiver-operating characteristic curve (HSROC) of the two agents for detecting BHR in asthma were pooled using meta-analysis. A meta-regression analysis was used to identify potential sources of heterogeneity within the selected studies.

Results: We identified six studies comprising 565 patients. The pooled sensitivity, specificity, and DOR of methacholine were 0.61 (95%CI, 0.44–0.76), 0.93 (95%CI, 0.70–0.99), and 23.47 (95% CI, 2.51–219.89), respectively. The pooled sensitivity, specificity, and diagnostic odds ratio of mannitol were 0.50 (95%CI, 0.28–0.73), 0.97 (95% CI, 0.94–0.99), and 35.22 (95% CI, 8.82–140.62), respectively. The area under the HSROC for mannitol was higher than that for methacholine (0.97 vs. 0.81, $p < 0.01$). Considerable between-study heterogeneity was present for sensitivity and specificity in studies of both index tests. Univariate meta-regression analysis revealed that age and sex of the study participants were probable sources of heterogeneity for specificity in studies of methacholine.

Conclusion: Although mannitol showed better diagnostic performance than methacholine for identifying BHR in asthma, substantial between-study heterogeneity necessitates caution when interpreting the data.

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Introduction

Bronchial hyperresponsiveness (BHR) is airway hyper-sensitivity resulting in excessive narrowing in response to stimuli and is a common feature of asthma (1). A variety of bronchial challenge tests (BCTs) has been developed to measure BHR (1). The tests are classified as direct and indirect according to mechanism of action (2,3). Direct BCTs directly stimulate airway smooth muscle receptors using agents such as muscarinic analogs, histamine, leukotrienes, or prostaglandins (2). In comparison, indirect stimulators, such as mannitol, hypertonic saline, adenosine monophosphate, or exercise, indirectly induce bronchoconstriction by

releasing inflammatory mediators after mast cell stimulation (3).

Methacholine and mannitol are representative direct and indirect BCTs, respectively. However, because the two agents have different mechanisms of action, they might have different diagnostic properties. Methacholine is considered the traditional and standard agent for detecting BHR. It has been reported to be highly sensitive for identifying BHR, and negative methacholine BCT reliably excludes diagnosis of asthma (2). Mannitol BCT can be easily and rapidly performed using pre-filled mannitol capsules and a handheld powder device (4). Because mannitol utilizes an indirect pathway of bronchoconstriction,

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it has high specificity and can be used to confirm asthma (4).

Although several studies have compared the diagnostic accuracy of the two index tests for BHR, no meta-analysis has compared these agents in a systematic manner with respect to BHR in asthma. The aim of this study was to compare the diagnostic properties of methacholine and mannitol BCT for identifying BHR for asthma based on a systematic review and meta-analysis of clinical trial data.

Materials and methods

Data sources and search strategy

This meta-analysis is in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses of Diagnostic Test Accuracy Studies Statement (5). The study protocol was registered with the PROSPERO International Prospective Register of Systematic Reviews (http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42019119784). We conducted a comprehensive search of three electronic databases (MEDLINE, EMBASE, and the Cochrane Central Register) up to March 2019, to identify potentially relevant articles using the following search terms: asthma, airway or bronchial hyperresponsiveness, bronchoconstriction, methacholine, and mannitol. As this study was a systematic review of published articles, neither informed consent nor ethics approval was required. We also performed a manual search of the references listed in relevant review articles.

Inclusion and exclusion criteria

Reviewed studies had to meet the following inclusion criteria to be included in this analysis: (1) full-length reports published in peer-reviewed English language journals; (2) directly compared methacholine and mannitol challenge tests; (3) comprised patients with assessment of BHR; (4) compared results between asthmatics and controls, and (5) provided sufficient data to calculate absolute numbers of true-positive, false-positive, false-negative, and true-negative results. Review articles, case reports, commentaries, and studies reporting outcomes without raw data were excluded.

Asthma was defined as (1) previous diagnosis of asthma or (2) identification of both respiratory symptoms such as wheezing, shortness of breath, chest tightness, or cough and evidence of variable expiratory airflow limitation using bronchodilator response, BCTs, or peak expiratory flow meter, according to the 2019 Global Initiative for Asthma Global Strategy for

Asthma Management and Prevention (6). A positive methacholine BCT was defined as a 20% decrease in forced expiratory volume in one second (FEV1) after inhalation of methacholine (7). A positive mannitol BCT was defined as a 15% decrease from baseline FEV1 in response to a cumulative total dose of 635 mg or a 10% decrease in FEV1 between two consecutive mannitol doses (8).

Data extraction and quality assessment

Two authors independently conducted an extraction of potentially relevant studies and reviewed each study according to predefined eligibility criteria, after which data were extracted. Any disagreements that arose during study selection or data extraction were resolved by discussion. A predefined form was used to extract data from each study. The information extracted from selected studies consisted of patient demographics, study design, and objectives. As recommended by the Cochrane Collaboration, we used the Quality Assessment of Diagnostic Accuracy Studies (QUADAS)–2 Tool to assess the risk of bias in diagnostic test accuracy (9). The included studies were qualitatively assessed for risk of bias and applicability concerns arising from each of the four domains: patient selection, index test, reference standard, and flow/timing of patient selection, in accordance with the QUADAS-2 Tool system (9). Discrepancies were resolved by consensus between the two authors (J-U.S. and J.L.).

Data synthesis and statistical analysis

The data are presented as mean values for continuous variables and as frequencies (%) for categorical variables. We extracted the numbers of patients with true-positive, false-positive, false-negative, and true-negative test results either directly or through recalculation based on the reported measures of accuracy in combination with the prevalence and sample size of the included study. The pooled sensitivity and specificity were the main measures of diagnostic accuracy for the two tests. Sensitivity was calculated as true asthmatic cases with a positive test/all asthmatic cases, and specificity was calculated as true non-asthmatic cases with a negative test/all non-asthmatic cases. We also calculated the pooled positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and area under the receiver-operating characteristic curve (AUC) as point estimates with a 95% confidence interval (CI) (10). We constructed

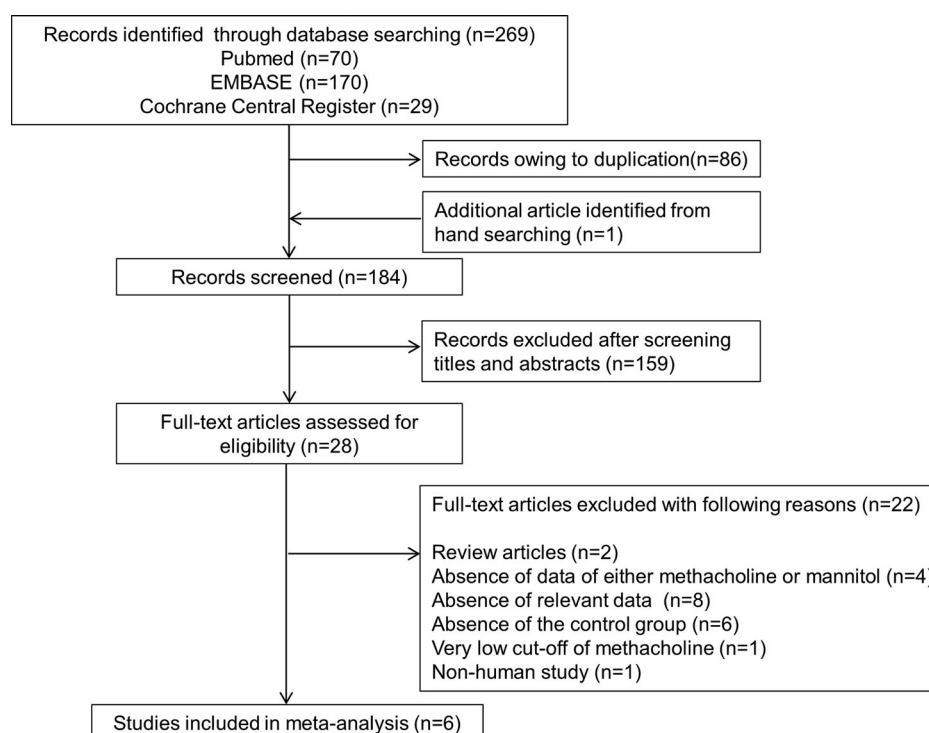


Figure 1. Flow diagram for identification of eligible studies.

hierarchical summary receiver-operating characteristic curves (HSROCs). Between-study statistical heterogeneity was assessed using I^2 and the Cochrane Q test (11). Heterogeneity was assessed using I^2 statistics on a scale of 0–100%, and $I^2 > 50\%$ was chosen as a substantial level of between-study heterogeneity (11). Analysis via meta-regression was performed in cases of substantial heterogeneity to identify potential sources of bias (12). A p values < 0.05 was considered statistically significant. Statistical analyses were performed with Stata statistical software (Version 14.2, Stata Corp LP, College Station, TX, USA) and Review Manager (Version 5.3, Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark).

Results

Study search parameters and data inclusion

The literature search process is illustrated in Figure 1. We initially identified 70 articles from PubMed, 170 articles from EMBASE, 29 articles from the Cochrane library, and 1 article from manual searching. After removing duplicate articles, we screened 184 potentially eligible articles. After reviewing the title and abstracts, 159 search records were removed, and the remaining 28 articles were further evaluated by reading the full text. Twenty-two articles were excluded

for the reasons shown in Figure 1. Finally, six studies were included in our analysis (13–18).

Table 1 summarizes the features of the included studies. Six studies involving 565 subjects met the defined inclusion criteria, and the number of patients in each trial ranged from 28 to 238. All studies were published between 2010 and 2017. Two studies included participants who used ICS prior to testing (17,18). Three studies used a cutoff of 16 mg/mL as the provocative concentration of methacholine causing a 20% decrease in FEV1 (PC20) to indicate positive results (14–16). The remaining three studies used the provocative delivered dose of methacholine causing a 20% decrease in FEV1 (PD20) (13,17,18). The rate of atopy was presented in four studies (13,15,16,18), and smoking histories were presented in one study (18).

All studies reported a low risk of concern with regard to “risk of bias” and “applicability concerns” domains. The quality of the included studies was deemed satisfactory for QUADAS assessment. However, the QUADAS tool identified unclear blinding in result interpretation and reporting of uninterpretable results as potential sources of bias. Publication bias could not be assessed because the number of studies was less than 10.

Diagnostic accuracy for identifying BHR

Pooled estimates of sensitivity, specificity, and DORs of the two index tests are presented in Figures 2 and

Table 1. Characteristics of the studies included in the meta-analysis.

Study, year	Design	Country	No. of total patients	Age, years (mean or median)	Male, %	FEV ₁ , % (mean)	Atopy, %	Cutoff value for methacholine BCT	Population
Aronsson, 2011 (13)	Single-center study	Sweden	49	34.6	46.9	94.4	82% in asthmatics and 0% in controls	2,000 µg of PD20	Mild persistent asthmatics and controls
Cancelliere, 2013 (14)	Single-center study	Spain	28	32	25	NA	NA	16 mg/dl of PC20	Participants with persistent asthma-like symptoms
Kim, 2014 (15)	Multi-center study	South Korea	104	43.8	28.8	93.2	18% in asthmatics and 0% in controls	16 mg/ml of PC20	Patients diagnosed with asthma by specialist physicians; and controls
Porpodis, 2017 (16)	Single-center study	Greece	88	38.6	46.6	88.5	40.1% in asthmatics and 52.4% in controls	16 mg/dl of PC20	Participants with asthma related symptoms in the previous month but without previous diagnosis of asthma and without initiation of treatment
Sue-Chu, 2010 (17)	Single-center study	Norway	58	18.1	62.1	NA	NA	1814 µg of PD20	Non-smoking cross-country and biathlon ski athletes
Sverrild, 2010 (18)	Single-center study	Denmark	238	18.8	60.5	93.6	77% in asthmatics and 32% in controls	8 µmol of PD20	Young adults randomly drawn from the nationwide civil registration list.

BCT, Bronchial Challenge test; FEV₁, Forced expiratory volume in 1 s; NA, not available; PC20, provocative concentration of methacholine causing a 20% fall in FEV₁; PD20, provocative delivered dose of methacholine causing a 20% fall in FEV₁.

3. The pooled sensitivity of methacholine across studies was 0.61 (95% CI, 0.44–0.76; $I^2 = 81.73\%$, $p < 0.01$), and pooled specificity was 0.93 (95% CI, 0.70–0.99; $I^2 = 90.88\%$, $p < 0.01$). The pooled PLR and NLR were 9.57 (95% CI, 1.49–61.47; $I^2 = 78.8\%$, $p < 0.01$) and 0.41 (95% CI, 0.25–0.66; $I^2 = 86.53\%$, $p < 0.01$), respectively. The DOR for methacholine was 23.47 (95% CI, 2.51–219.89; $I^2 = 100\%$, $p < 0.01$).

The pooled sensitivity for mannitol across studies was 0.50 (95% CI, 0.28–0.73; $I^2 = 85.21\%$, $p < 0.01$), and pooled specificity was 0.97 (95% CI, 0.94–0.99; $I^2 = 35.14\%$, $p = 0.17$). The pooled PLR and NLR were 18.07 (95% CI, 6.71–48.61; $I^2 = 5.27\%$, $p = 0.07$) and 0.51 (95% CI, 0.31–0.84; $I^2 = 95.13\%$, $p < 0.01$), respectively. The DOR for mannitol was 35.22 (95% CI, 8.82–140.62; $I^2 = 98.22\%$, $p < 0.01$). Comparison of the diagnostic properties of the two agents revealed similar pooled sensitivity and specificity ($p = 0.50$ for sensitivity and $p = 0.11$ for specificity). Figure 4 shows HSROCs for the index tests and indicates that the AUC of mannitol was statistically greater than that of methacholine (0.97 vs. 0.81, $p < 0.01$).

Univariate meta-regression analysis for identifying BHR in asthma

A substantial degree of between-study heterogeneity was observed in the sensitivity and specificity among studies of the two tests. Table 2 shows the results of univariate meta-regression analyses for identifying potential sources of heterogeneity with respect to diagnostic performance of both index tests. For studies that evaluated methacholine, age (≥ 30 vs. < 30 years) and sex of the study participants (male $\geq 50\%$ vs. $< 50\%$) were probable sources of the heterogeneity of specificity. For studies that evaluated mannitol, plausible sources of heterogeneity were found for neither sensitivity nor specificity.

Discussion

To the best of our knowledge, this is the first meta-analysis to compare methacholine and mannitol as bronchial provocative agents for identifying BHR in asthma. We compared the diagnostic properties of methacholine and mannitol BCTs for detecting BHR in asthma using a systematic review and meta-analysis approach. We calculated the pooled sensitivity and specificity of methacholine and mannitol, and there were no significant differences between the two agents. Although the AUC value for mannitol was higher than that for methacholine, substantial

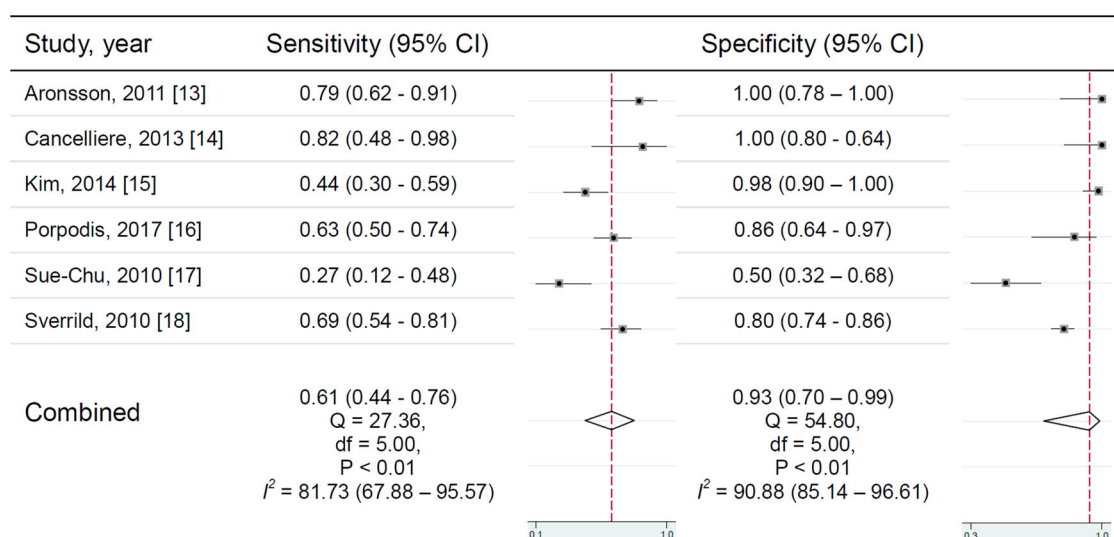


Figure 2. Paired forest plots of sensitivity and specificity of the methacholine bronchial challenge test for identifying bronchial hyperresponsiveness in asthma.

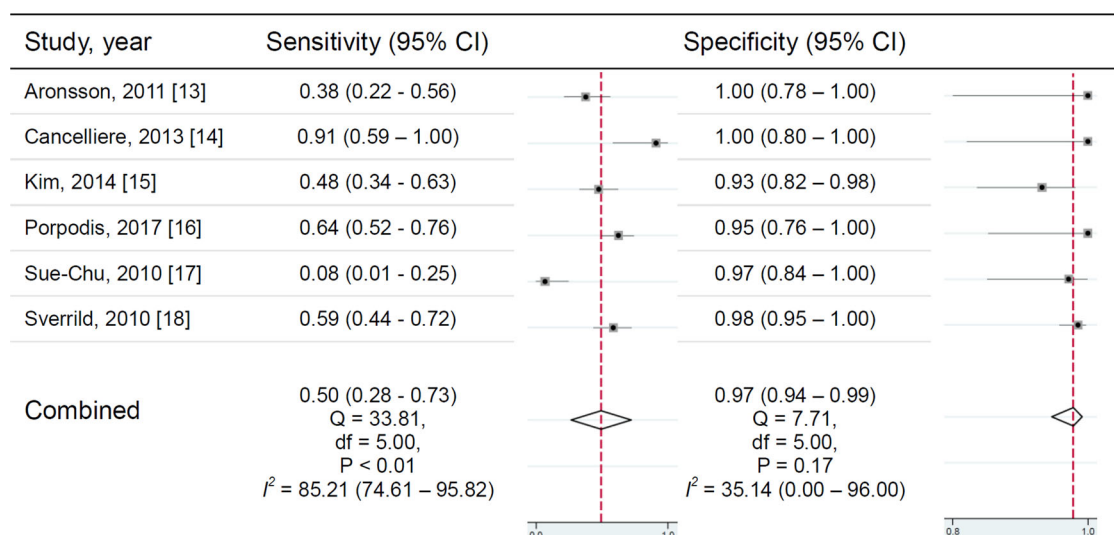


Figure 3. Paired forest plots of sensitivity and specificity of the mannitol bronchial challenge test for identifying bronchial hyperresponsiveness in asthma.

heterogeneity was identified in the pooled estimates for the two agents. Meta-regression analysis showed that age and sex of study participants were probable sources of between-study heterogeneity for studies that evaluated methacholine.

A recent systematic review focusing on the mannitol test revealed that overall sensitivity and specificity ranged from 0.08 (95% CI, 0.01–0.27) to 1.00 (95% CI, 0.93–1.00) and 0.75 (95% CI 0.67–0.82) to 1.00 (95% CI, 0.85–1.00) (19), respectively. A systematic review on the diagnostic accuracy of the methacholine test has not yet been reported. Our research is valuable in that there are no previous systematic studies

comparing the mannitol test with a direct test such as methacholine.

Methacholine BCT has relatively high sensitivity for identifying BHR (7). For this reason, a negative methacholine BCT has been primarily used to exclude a diagnosis of asthma (2,20). However, the sensitivity of methacholine was 0.61 in our pooled estimates, which was not as sensitive for identifying BHR as is currently widely recognized. Previous clinical studies have reported that the sensitivity of the methacholine BCT for detecting asthma had a wide range of values (21). The sensitivity of the test has varied depending on study population, race, and presence of atopy (21).

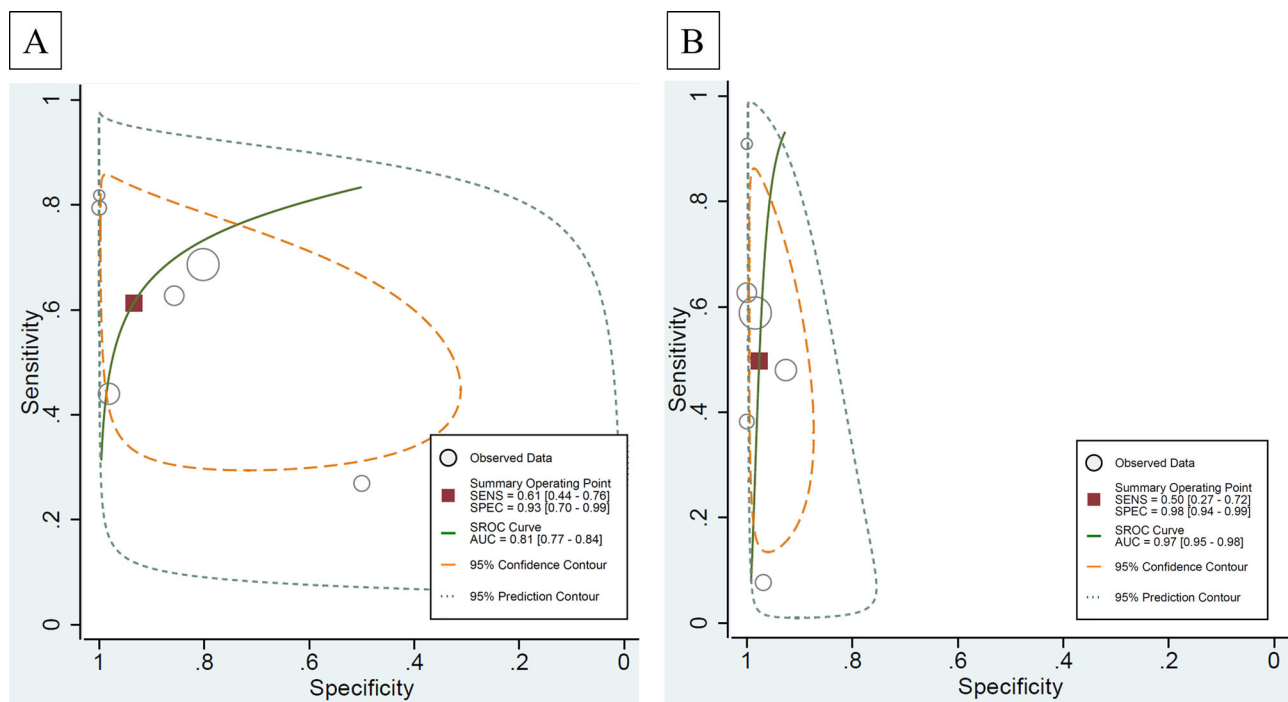


Figure 4. Hierarchical summary of the receiver operating characteristic curves of (A) methacholine and (B) mannitol for identifying bronchial hyperresponsiveness in asthma.

Table 2. Univariate meta-regression analysis to identify heterogeneity in diagnostic properties of methacholine and mannitol bronchial challenge tests for identifying bronchial hyperresponsiveness in asthma.

Variable	No. of studies	No. of patients	Sensitivity		Specificity	
			Adjusted (95% CI)	P value	Adjusted (95% CI)	P value
Studies that evaluated methacholine BCT						
Age, years						
≥30	4	269	0.67 (0.50-0.84)	0.49	0.97 (0.94-1.00)	0.01
<30	2	296	0.50 (0.24-0.77)		0.68 (0.49-0.87)	
Male						
≥50%	2	296	0.50 (0.24-0.77)	0.30	0.68 (0.49-0.87)	<0.01
<50%	4	269	0.67 (0.50-0.84)		0.97 (0.94-1.00)	
No. of patients						
≥100	2	342	0.57 (0.31-0.82)	0.52	0.92 (0.74-1.00)	0.32
<100	4	223	0.65 (0.46-0.84)		0.94 (0.80-1.00)	
Studies that evaluated mannitol BCT						
Age, years						
≥30	4	269	0.60 (0.35-0.85)	0.18	0.97 (0.93-1.00)	0.27
<30	2	296	0.30 (0.02-0.61)		0.98 (0.95-1.00)	
Male						
≥50%	2	296	0.30 (0.02-0.61)	0.19	0.98 (0.95-1.00)	0.77
<50%	4	269	0.60 (0.35-0.85)		0.97 (0.93-1.00)	
No. of patients						
≥100	2	342	0.54 (0.15-0.92)	0.81	0.97 (0.89-1.00)	0.32
<100	4	223	0.47 (0.18-0.77)		1.00 (0.99-1.00)	

The reasons for the relatively low pooled sensitivity of methacholine BCT in our study may be the heterogeneity of the study population and different definitions of a positive test. While three studies used a PC20 cutoff of 16 mg/mL of (14–16), the remaining studies applied different cutoff values (13,17,18). The pooled sensitivity of methacholine was 0.57 on subgroup analysis of a cutoff of 16 mg/mL methacholine (14–16), and it was not substantially different

compared with the overall analysis (Supplementary material Table 1). Due to direct stimulation of the airway by methacholine, the BHR to methacholine may be affected by smoking status, which can cause airway remodeling, a feature of chronic obstructive pulmonary disease (22). Although we investigated smoking histories in our enrolled studies, only one reported participants with smoking history (18). Unlike previous hypotheses, that cross-sectional population study

reported that comparison of exhaled nitric oxide level as a marker of airway inflammation between the two agents was not influenced by smoking status (18). In addition, atopy is known to be associated with increased BHR (23,24). Although four included studies presented rate of atopy (13,15,16,18), we could not calculate the diagnostic differences of BCTs based on presence of atopy.

Mannitol BCT that acts indirectly is traditionally considered more specific for identifying BHR because it provokes bronchoconstriction by activating inflammatory pathways (8). Because mannitol BCT reflects aspects of the pathophysiology of asthma, it is regarded as an established test for clinical asthma diagnosis (8). BHR to mannitol has been reported to have a stronger association with elevated markers of eosinophilic airway inflammation such as sputum eosinophils and exhaled nitric oxide compared with methacholine (25). Our pooled estimates also demonstrated a very high specificity of 0.97 for mannitol. This finding was consistent with those of a recent long-term follow-up cohort (26). In the wheezing subset, BHR to mannitol had high specificity of 0.94 for identifying asthma (26).

The low sensitivity of mannitol can limit diagnostic testing in asthma. Two recent studies reported that the sensitivity of the mannitol test was low in clinical practice (27,28). A systematic review focusing on the mannitol test also reported low sensitivity and heterogeneous results for this test (19). It has been suggested that methacholine is more reliable than mannitol in identifying BHR in a pediatric population presenting with asthma symptoms (29,30). In the present study, the pooled sensitivity of mannitol was not statistically lower than that of methacholine (0.50 vs. 0.61; $p = 0.50$).

There were some limitations to this study that should be considered when interpreting our results. First, since our meta-analysis was based on a relatively small number of trials, our results should be interpreted with caution due to limited statistical power. Although great heterogeneity is generally seen in systematic reviews of studies on diagnostic test accuracy, this study also found substantial between-study heterogeneity among the enrolled studies (31). Second, although participants should preferably withhold ICSs prior to testing, two trials in this study included those treated with ICSs (17,18). BHR to mannitol was more highly associated with eosinophilic airway inflammation and could improve rapidly with ICS therapy (32). This may have introduced bias by causing a negative BCT due to attenuation of BHR. Third, diverse phenotypes of asthma have been widely recognized using

statistical methods such as cluster analysis (33,34). This objective means of categorization would be useful in understanding asthma pathophysiology and selecting specific treatments for individual phenotypes. Because our enrolled studies did not describe asthma phenotypes, we could not calculate the diagnostic differences in BCTs based on specific phenotype of asthma. Finally, although the 2017 International Task Force recommended reporting the results of the methacholine test as PD20 rather than PC20 [7], three trials in our study analyzed outcomes using PD20 (13,17,18).

Conclusion

We found that mannitol had an overall higher diagnostic performance than methacholine in detecting BHR in asthma. However, considering the substantial between-study heterogeneity, our findings do not suggest mannitol as a better differentiating agent compared to methacholine.

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Conflicts of interests

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

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Notes on contributors

J-U.S contributed to data acquisition, data interpretation, and statistical analysis and drafted the manuscript. J.L. contributed to the study design, data acquisition, data interpretation, statistical analysis, writing of the manuscript, and critical revision of the manuscript.

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Supplementary Material

Supplementary Table 1. The pooled sensitivity and specificity of the methacholine bronchial challenge test for identifying bronchial hyperresponsiveness in asthma - the subgroup analysis for a cut-off of 16 mg/mL of methacholine

Study, year	Sensitivity (95% CI)	Specificity (95% CI)
Cancelliere, 2013 [15]	0.82 (0.48 - 0.98)	1.00 (0.80 - 0.64)
Kim, 2014 [16]	0.44 (0.30 - 0.59)	0.98 (0.90 – 1.00)
Porpodis, 2017 [17]	0.63 (0.50 - 0.74)	0.86 (0.64 - 0.97)
	0.57 (0.48 - 0.66)	0.96 (0.89 – 1.99)
	Q = 7.36,	Q = 5.72,
	df = 2.00,	df = 2.00,
Combined	$I^2 = 72.8$	$I^2 = 65.1$