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The sensitivity and specificity of the mannitol bronchial challenge test to identify asthma in different populations: a systematic review

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Summary

OBJECTIVE: Asthma is associated with bronchial hyperresponsiveness, assessed by bronchial provocation tests such as the mannitol test. We aimed to assess the data on sensitivity and specificity of the mannitol test in diagnosing asthma.

DATA SOURCES: We searched electronically the Medline, Embase and Central databases from 1997 to 2019.

STUDY SELECTION: Inclusion criteria were the assessment of the validity of the mannitol test. Risk of bias was assessed using the Quality Assessment of Diagnostic Accuracy Studies tool (QUADAS-2). Data were extracted according to a prespecified list and analysed qualitatively.

RESULTS: A total of 27 studies (4589 individuals, age 6–85 years, cross-sectional [n = 18] and case-controlled [n = 9] study design) were included. Overall sensitivity and specificity ranged from 8% (95% confidence interval [CI] 1–27) to 100% (95% CI 93–100) and 75% (95% CI 67–82) to 100% (95% CI 85–100). Excluding case-controlled design, studies conducted in a clinical setting showed a range from 19% (95% CI 14–27) to 91% (95% CI 59–100) for sensitivity and from 75% (95% CI 67–82) to 100% (95% CI 80–100) for specificity. Heterogeneity was high owing to differences in the populations examined and the methods used.

CONCLUSIONS: Studies on the accuracy of the mannitol test were heterogeneous. Overall specificity was higher than sensitivity and therefore the mannitol test seems to be a suitable diagnostic tool to confirm asthma. However, the high level of heterogeneity among the included studies makes a conclusive statement on the accuracy of the mannitol test difficult and further research is needed. As bronchial provocation tests can be especially useful in patients with an intermediate probability of asthma diagnosis, further studies are needed that include subjects with asthma symptoms but intermediate probability of asthma diagnosis.

Keywords: *mannitol, bronchial challenge test, asthma, bronchial hyperresponsiveness, Aridol, athletes, fire fighters*

Introduction

Asthma is a chronic inflammatory airway disease with an estimated 300 million affected individuals worldwide [1]. The chronic inflammation of the airways is associated with airway hyperresponsiveness with recurrent episodes of wheezing, breathlessness, chest tightness, coughing and provocation by typical triggers [1].

A correct diagnosis of asthma is essential if appropriate drug therapy is to be given. The diagnosis of asthma should not be based on respiratory symptoms alone as the symptoms may be unspecific [1, 2]. According to the Global Initiative for Asthma (GINA) guidelines, the diagnosis of

ABBREVIATIONS:

GINA	Global Initiative for Asthma
BPT	bronchial provocation tests
EVH	eucapnic voluntary hyperpnoea
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
ATS	American Thoracic Society
ERS	European Respiratory Society
HSROC	hierarchical summary receiver operating characteristic
CI	confidence interval
FEV1	forced expiratory volume in 1 second
TP	true positive
FP	false positive
FN	false negative
TN	true negative
ROC	receiver operating characteristic
COPD	chronic obstructive pulmonary disease

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asthma is made by the presence of variable respiratory symptoms and a confirmed variable expiratory airflow limitation with objective pulmonary function tests [1].

Bronchial provocation tests (BPTs) are particularly useful for the detection of airway hyperresponsiveness and diagnosing asthma on occasions where the lung function testing shows normal results. Two different methods for bronchial provocation tests exist, the ´direct´ and the "indirect" method. "Direct" bronchial provocation tests cause airway narrowing by acting "directly" on their respective receptors on bronchial smooth muscle, causing contraction [1, 3, 4]. The "direct" tests are very sensitive for identifying airway hyperresponsiveness. A limitation of direct tests is that they act directly on the smooth muscle, and can show airway hyperresponsiveness even without any active airway inflammation. This direct effect may cause false positive test results and thus reduce specificity [1, 3, 5].

In contrast to this, "indirect" challenge tests such as the mannitol test cause airway narrowing by releasing a wide variety of mediators of bronchoconstriction from inflammatory cells within the walls of the airways [3]. Because of this mechanism indirect tests are more specific for identifying asthma that is currently active [1, 3, 5, 6]. The mannitol test has the advantage of a standardised protocol, ease of administration, shortness of procedure and good safety profile due to a progressive dose-response challenge. The test can be stopped before severe falls of FEV1 occur, making it an attractive alternative to the "direct" test methods where different protocols exist [3, 6, 7]. In 2007, mannitol was included as a bronchoprovocation test in the GINA guidelines [1].

Several studies have investigated the accuracy of the mannitol test to identify asthma. However, the results from these studies differ substantially [8–13]. This systematic review aims to clarify this point. Our objective was to investigate the sensitivity and specificity of the mannitol test to diagnose asthma compared to accepted reference standards (GINA) in children and adults with and without asthma symptoms. We included cross-sectional, cohort and case-controlled studies. Characteristics (reference standard, different settings, populations) were recorded. Methodological quality of the studies was assessed with QUADAS-2 [14].

Methods

Protocol

The methods of data extraction and inclusion criteria were specified in advance and documented in a protocol, which is available upon request.

Search strategy and data sources

We performed a systematic search of three electronic databases to identify studies evaluating the accuracy of the mannitol BPT for the diagnosis of asthma. A research librarian experienced in literature searches for systematic reviews developed a search strategy in collaboration with the investigators (see appendix 1). We systematically searched Medline (through Ovid or PubMed), Embase (through Ovid) and Central databases from January 1997, as the mannitol BPT was originally described by Anderson et al. in 1997 [15], to February 2019. Participants of any age were considered. No publication status restrictions were imposed. We checked the reference lists of the identified studies as well as the reference lists of identified narrative reviews, published on diagnostic tests for asthma after 1997, and visited the Aridol website (accessed February 2019) to identify other relevant studies. A hand-search for conference proceedings of the American Thoracic Society (ATS), European Respiratory Society (ERS), and Chest and World Allergy Congress was performed to search for possible additional studies.

Study selection

To be eligible for inclusion in the systematic review, studies had to fulfil the following criteria:

1. Population: Patients with suspected or diagnosed asthma, healthy participants of population studies, or participants of studies investigating asthma in the workplace.
2. Index test: The index test was the mannitol bronchial provocation test using the protocol originally described by Anderson et al. or the Aridol package leaflet [7].
3. Reference standard: We accepted the following reference standards to diagnose asthma: "clinical diagnosis of asthma" (physician makes diagnosis based on respiratory symptoms of asthma in conjunction with the results of the clinical examination and a bronchial provocation test); or "physician diagnosed asthma" (physician had diagnosed asthma but it was unclear how he did it). Another accepted reference standard was a test result in an exercise challenge, eucapnic voluntary hyperpnoea or specific inhalation test, performed in patients who were included if they had respiratory symptoms. A positive bronchial provocation or exercise test in subjects without respiratory symptoms was not considered sufficient to diagnose asthma.
4. Study types: We included cross-sectional, cohort and case-controlled studies.
5. Outcome measure: We included studies that reported the diagnostic accuracy (sensitivity and specificity) of the mannitol BPT.

We excluded animal studies and "dose-finding studies", as well as studies in which a two-by-two table could not be established even after contacting the relevant investigators. We took care to exclude duplicate studies.

In a first step, two reviewers independently screened titles and abstracts. Any articles that were deemed to be potentially relevant by one of the reviewers were marked. Studies that were judged to be ineligible by both reviewers based on the title and abstract were not assessed further. In a second step, the full texts of all the potentially eligible articles were retrieved so that they could be screened, again independently and in duplicate by two reviewers. Study eligibility was evaluated using pre-piloted forms with the above mentioned inclusion and exclusion criteria. Any disagreement was resolved by consensus. If consensus was not achieved, a third reviewer had the decisive vote.

Assessment of risk of bias of studies

The methodological quality and risk of bias of the selected studies was assessed independently and in duplicate by two

reviewers with the Quality Assessment of Diagnostic Accuracy Studies tool (QUADAS-2; see appendix 2), which assessed common sources of bias in diagnostic studies.

Data extraction

From all the eligible studies after full text screening, two reviewers extracted predefined data independently and in duplicate using an extraction form (see appendix 3). Further data were extracted post hoc, including FEV1, atopy status and stopping of medication prior to testing. In cases of missing information from the articles, we contacted the authors of the respective studies to provide further details. If it was not possible to construct two-by-two tables, the studies were excluded from the final analysis. Controversies were resolved by discussion. If consensus was not achieved, a third reviewer had the decisive vote.

Data synthesis and analysis

For each included study, we constructed two-by-two tables by comparing the results of the mannitol BPT with the respective reference standard. Sensitivity and specificity were the main measures of accuracy of the mannitol test, which we calculated using the data of the two-by-two tables. The true and false negative and positive rates were recorded. Sensitivity and specificity were plotted in receiver operating characteristic space. To explore the different populations, we grouped studies according to study design, study setting and age group in a forest plot.

Results

Study selection

The literature search provided a total of 836 citations after removing duplicates. After those in which the subject of this review was not addressed were excluded, 221 remained potentially relevant and were retrieved for full text screening (fig. 1). Finally, 27 studies [8–10, 12, 13, 15–36] met the inclusion criteria for our systematic review, all of them published in English between 1997 and 2018.

Study and population characteristics

Eighteen of the included studies were cross-sectional studies [8–10, 12, 16–20, 26–30, 32, 34–36], nine studies used a case-controlled design [13, 15, 16, 25–29, 35], including asthmatics and a healthy control group.

The included studies involved a total of 4589 participants. The age range was from 6 to 85 years, and the percentage of males ranged from 25 to 100%. Most studies included only adults [8, 12, 15, 16, 21, 23–28, 30–33], eight studies included adults and children [9, 10, 13, 17, 18, 21, 23, 33], three studies included only children [19, 24, 26]. Twelve studies were conducted in a clinical setting [10, 13, 15, 17, 21–24, 28, 29, 32, 36], with participants attending any kind of clinical institution. The other studies used a non-clinical setting [8, 9, 12, 16, 18–20, 25–27, 30, 31, 33–35]; most of these included elite athletes as their study population, and two studies specifically investigated military conscripts and fire fighters [8, 12].

In most of the studies, having symptoms consistent with asthma was an inclusion criterion. However, in some studies, all of them in a nonclinical setting, having asthma symptoms was not required. Most of the case-controlled studies included a population that already had a diagnosis of asthma and a healthy control group.

In eight studies, it was mentioned that smokers were excluded [10, 15, 18, 26–29, 34]. Nine studies showed the numbers of current smokers included in the study [8, 9, 12, 16, 25, 31, 32, 35, 36], and in ten studies, no information was given on the smoking status of the participants [13, 17, 19–24, 30, 33].

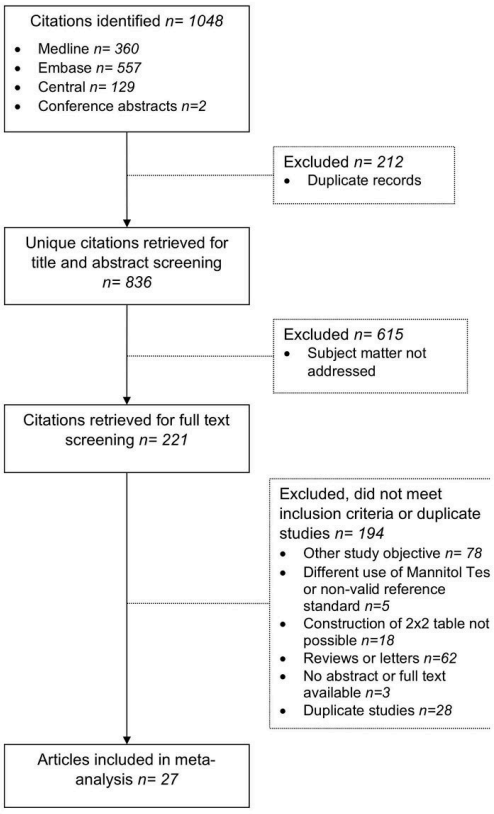
In all the included studies, the mannitol challenge test was conducted according to the protocol by Anderson et al. or by the Aridol package leaflet.

The following reference standards were used. Twelve studies used “clinical diagnosis” as a reference standard [8–10, 12, 13, 17, 21, 23, 24, 32, 35, 36], eight studies used “test result” [15, 17, 20, 23, 24, 26, 31, 33], and seven studies used “physician diagnosed asthma” [16, 22, 29, 30, 32, 34, 35]. In eight studies, anti-asthmatic therapy was appropriately stopped prior to the tests [10, 20, 23, 25, 27, 33–35]. The individual characteristics of the included studies and their population are summarised in tables 1 and 2, including information about atopy status and FEV1.

Risk of bias assessment of studies

Overall, the quality of the included studies was good with a low risk of bias from the procedure and interpretation of the mannitol test and the patient flow. Only nine studies mentioned that the results of the mannitol test were interpreted without knowing the results of the reference standard [8, 10, 12, 13, 17, 20, 21, 24, 34]. The domains “patient selection” and “reference standard” showed heterogeneous results concerning methodological quality (see

Figure 1: Identification of studies.



appendix 4). The risk of bias in the patient selection was high in all the studies that used a case-controlled design [13, 15, 16, 25–29, 35]. Applicability was judged to be limited for studies that included only a special population such as elite athletes, young male military conscripts or fire fighters [8, 12, 16, 18, 19, 26, 30, 31, 33–35], and studies, that excluded smokers [10, 15, 18, 26–29, 34]. Concerning the reference standard, the risk of bias was usually rated low in all studies that used a "clinical diagnosis of asthma" as a reference standard, and remained unclear in studies using "physician diagnosed asthma" as a reference standard. In seven studies, we observed a high risk of bias as the mannitol test was part of the reference standard and blinding of the test results was not done [8, 12, 18–20, 31, 33].

Diagnostic accuracy of the mannitol test

Overall, sensitivity and specificity were very heterogeneous, with values ranging from 8% (95% CI 1–27%) to 100% (95% CI 93–100%) for sensitivity and 75% (95% CI 67–82%) to 100% (95% CI 85–100%) for specificity. [8–10, 12, 13, 15–36,] We graphically presented the high level of between-study heterogeneity in the ROC space plot in figure 2. To explore the different populations, we grouped studies according to the populations (clinical vs nonclinical and children vs adults and mixed; fig. 3). As case-controlled studies represent the highest risk for bias [37], we tabulated them in a separate forest plot in figure 3.

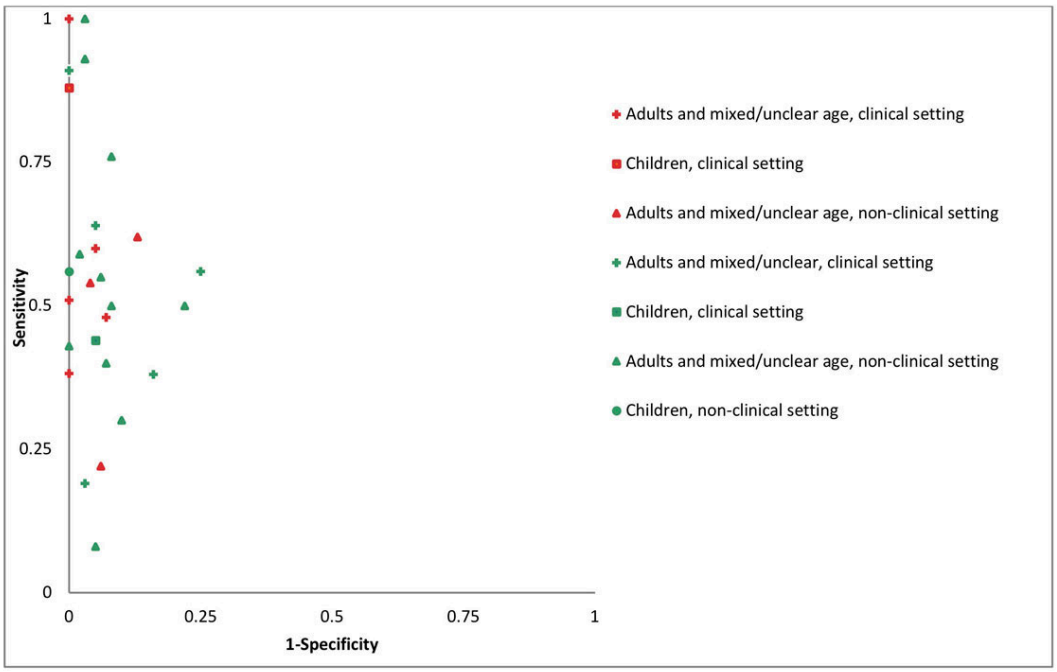
When all case-control studies were excluded, cross sectional and cohort studies conducted in a clinical setting showed a range from 19% (95% CI 14–27%) to 91% (95% CI 59–100%) for sensitivity and from 75% (95% CI 67–82%) to 100% (95% CI 80–100%) for specificity [10, 19, 21, 32, 33, 36]. Cross-sectional and cohort studies conducted in a nonclinical setting showed a range from 8% (95% CI 1–27%) to 100% (95% CI 95–100%) for sensitivity and from 78% (95% CI 68–86%) to 100% (95% CI 85–100%) for specificity [8, 9, 12, 16, 18–20, 26, 27, 30, 34, 35]. In all studies that considered patients who had stopped asthma medication appropriately prior to testing, sensitivity and specificity ranged from 22% (95% CI 12–35%) to 91% (95% CI 59–100%) and from 75% (95% CI 67–82%) to 100% (95% CI 80–100%), respectively [10, 20, 23, 25, 27, 33–35]. In the studies that did not stop asthma medication appropriately, sensitivity and specificity ranged from 8% (95% CI 1–27%) to 100% (95% CI 95–100%) and from 78% (95% CI 68–86%) to 100% (95% CI 85–100%). [9, 13, 15, 17, 18, 22, 24–28, 32–36]

Discussion

We found a high level of heterogeneity among the included studies and explored reasons for this by assessment of the different study designs and methods used, population characteristics such as atopy status and smoking, and risk of bias. We explored populations further by dividing them into subgroups, showing forest plots as well as giving the range of accuracy. There is no evidence that accuracy of the mannitol tests differs according to the populations examined.

Reasons for the heterogeneity in sensitivity and specificity of the mannitol test have previously been discussed and

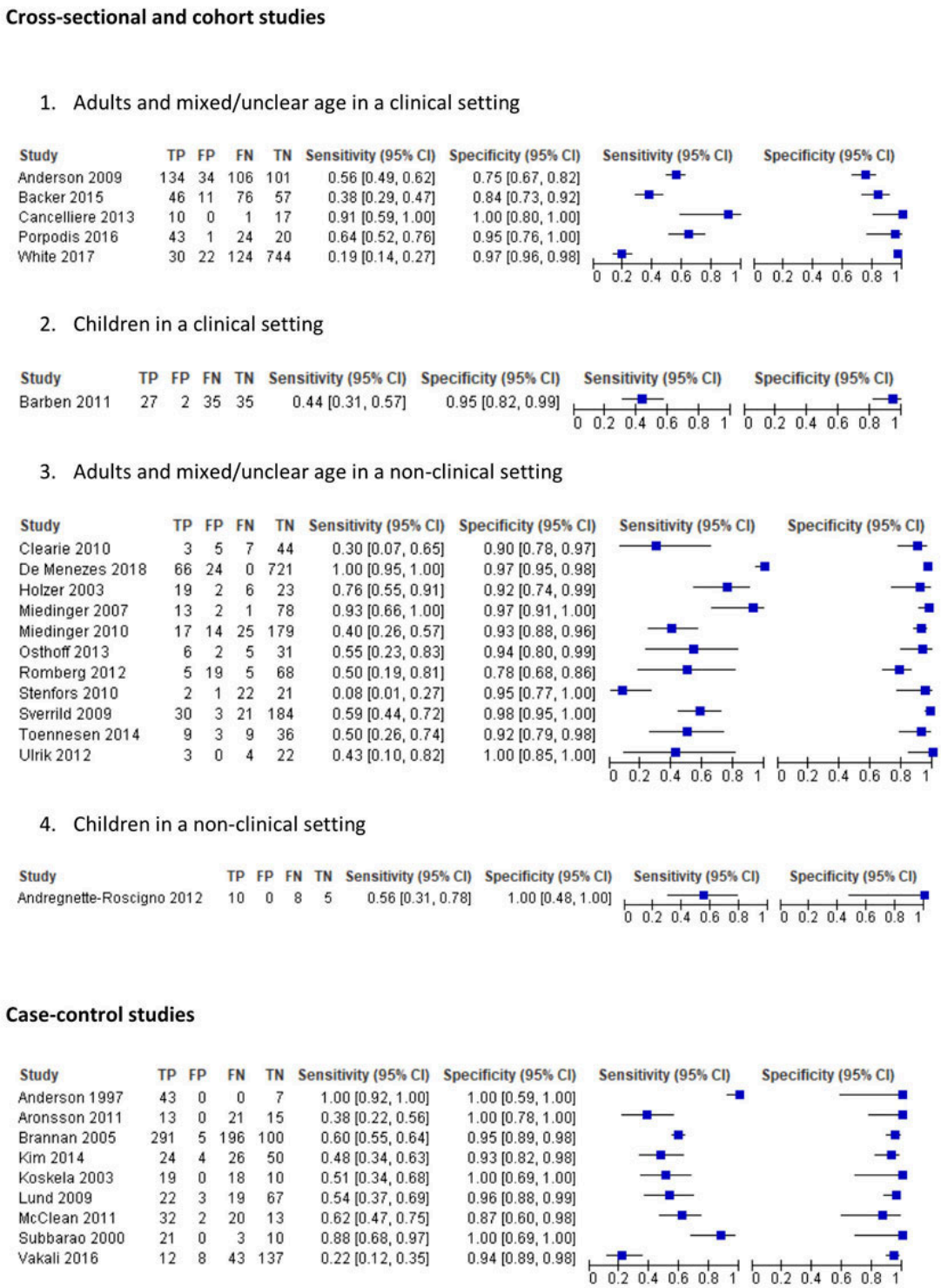
Figure 2: ROC space plot of all included studies (n = 27)



Roc space plot for the mannitol bronchial challenge test in different age groups and presentations
Orange data points are derived from case-control studies
Green data points are derived from cohort or cross-sectional studies

may be false negative and false positive mannitol tests. One reason for the heterogeneity in sensitivity and specificity may be current asthma treatment and lack of current asthma inflammation, which may lead to false negative results as the mannitol test just shows how many participants have active asthma at the time of assessment. A positive result for mannitol indicates the presence of inflammatory cells and a sufficient concentration of mediators to cause bronchoconstriction. A negative test result indicates that one of these elements is missing, such as is the case in treated asthmatic patients with inhaled corticosteroids (ICS), β2 agonists or leukotriene inhibitors [38, 39]. An

Figure 3: Forest plot of the sensitivity and specificity of the mannitol test showing several subgroups of the included cross-sectional studies (n = 18) and case-controlled studies (n = 9).TP = true positive; FP = false positive; FN = false negative; TN = true negative; CI = confidence interval



example for this circumstance is the study of Brannan et al., who assessed that the sensitivity of the mannitol test to identify asthma was 59.8%, which rose to 88.9% when those asthmatics taking ICS, who were negative to mannitol challenge, were excluded [13]. In a later analysis of the adult data from this study, Brannan et al. reported that 49% of the asthmatic patients taking ICS daily were negative to mannitol [40]. This is confirmed by our analysis in which sensitivity was higher in studies in which asthma medication was stopped for an appropriately long time according to Anderson et al. than in those studies in which this was not the case [10].

Another case in which a mannitol test can be negative is when a trigger for asthma is taken away and no asthma inflammation is present, which has been seen for reduced exposure to house dust mites in house dust mite allergic patients [41]. Overall, the fact that the specificity was high demonstrates that there are few false positive tests. A reason for false positive results could be current smoking, which has been previously examined by Stolz et al. [42] Current smokers were excluded in several of the examined studies, a possible reason for the few false positive tests.

To a lesser degree, different reference standards may have caused heterogeneity. Even though the GINA guidelines give advice on the best diagnostic approach in asthma, a single gold-standard test does not exist [1]. We therefore accepted various reference standards.

The ROC space plot indicated that there are some studies that are clearly separate from the main group and which we would like to discuss in more detail.

In this context we need to discuss those studies in which the mannitol test was not only the index test but was also included in the reference standard, as this may lead to an overestimation of sensitivity and specificity. Looking at the studies by Miedinger [12], De Menezes [31] and Cancelliere [33], we find support for this assumption as all three show exceptionally high sensitivity. As we only included studies that combined clinical symptoms with the mannitol test result to make a diagnosis, we still assume that these are truly asthmatic patients. However, incorporation bias is of concern and we did acknowledge the risk of bias to be high (see appendix 4). An exclusion of the studies including mannitol in their reference standard may have caused an inclusion bias, as a a certain phenotype of asthma may have been selected.

In contrast, the studies by Clearie [22], Stenfors [30], Vakali [35] and White [32] showed lower sensitivity than the other studies. The studies by Clearie, Stenfors and Vakali were all conducted in nonclinical settings. They included athletes. In the study by Clearie, the fact that the elite swimmers continued to take inhaled corticosteroids before testing could be another reason for the sensitivity of only 30%, as corticosteroid treatment has been shown to inhibit indirect bronchial hyperresponsiveness, as mentioned previously [13]. In the study by Stenfors [30], the slection of asthmatic athletes were probably biased towards subjects with mild and/or well-controlled asthma, as only 21% of them had bronchial hyperresponsiveness, 29% had experienced shortness of breath post-exercise and 15% had an asthma attack in the last 12 months. Additionally, 37% were taking anti-inflammatory medicine, including steroids. As the mannitol protocol is fixed, it is not possible to administrate additional doses to elucidate a response in those with mild asthma, which may cause false negative results and thus low sensitivity in this situation [38]. In the study by White [32], the use of ICS might be a reason for the low sensitivity of 19%. However, even after participants with a negative mannitol test who were using ICS were excluded, there was no real change in sensitivity. A reason for the low sensitivity in this study might be the fact that all participants seem to have only mild asthma, showing normal pulmonary function and only a few reporting the use of ICS in the past 12 months.

The mannitol test showed high specificity diagnosing asthma in all studied populations independent of age group or study setting.

Due to its heterogeneity, we cannot postulate a certain phenotype of asthma in which mannitol is specifically useful. Previous studies reported a higher specificity and correlation with eosinophilic asthma [38, 43–46], which we cannot confirm because of the present heterogeneity of the studies. What we do see is the strength of the mannitol test to confirm asthma owing to the high specificity showed in our systematic review. This, as well as the high practicability of the mannitol test, could make it a useful diagnostic test in certain population groups such as athletes in a nonclinical setting.

As our systematic review focused on the mannitol test and we did not include a review on methacholine test, a direct comparison with a direct test such as methacholine is therefore not possible. From the literature it is known that the methacholine test shows a high sensitivity and a high negative predictive value and therefore seems to be a good test to exclude asthma. It therefore, also due to the low sensitivity of the mannitol test emphasised in our review, remains the test of choice to exclude asthma in patients with symptoms that suggest asthma, but are caused by another condition [38].

This was the first systematic review assessing the accuracy of the mannitol test in diagnosing asthma. In 2011, a systematic review assessed the accuracy of the mannitol test, but the diagnosis differed from our systematic review as this review concentrated on the diagnosis of exercise-induced bronchoconstriction, and the objective differed as it focussed on comparing eucapnic voluntary hyperpnoea and mannitol with standard exercise challenge testing [47]. Three studies were detected of which only one was also included in our systematic review [10]. Comparable to our results they found that there are only a few studies that assessed the accuracy of the mannitol test, and that heterogeneity was high [47]. Several reviews have discussed the advantages and disadvantages of indirect challenge tests, including mannitol, but no systematic review has been performed with an assessment of the risk of bias of publications [38, 39, 48, 49].

We conducted this systematic review according to a prespecified protocol, using a comprehensive literature search strategy and multiple reviewers, which strengthened the analysis by avoiding publication bias and selection bias. Another strength was the application of the Quality Assessment of Diagnostic Accuracy Studies instrument [14].

Our systematic review has limitations. There is a possibility of publication bias as we may have missed some

studies despite systematic screening. There is also the risk of study selection bias, which we aimed to overcome by using two independent reviewers. Another limitation was that not all studies were designed as accuracy studies and methodological issues limited the generalisability of the results. Even though the general quality of the included studies was good, one concern was the blinding of the assessors, which was poorly reported. Studies followed a strict, predefined protocol of the mannitol test, and it was therefore deemed unlikely that a lack of blinding would have caused bias. In the studies where the methacholine test was part of the reference standard, the lack of blinding was not an important concern, as the methacholine test is an objective test. Because of selection bias, we plotted case-controlled studies separately. Our main concern about the case-controlled design was selection bias, as patients who have difficult-to-diagnose asthma are usually not included. This may lead to an overestimation of sensitivity and specificity.

Conclusions

The 27 studies included in the systematic review showed very heterogeneous results concerning the accuracy of the mannitol test in the diagnosis of asthma. This may have been caused by different study methods that resulted in false negative or false positive tests, as well as different study settings, populations and reference standards. This between-study heterogeneity hindered the formation of a conclusive statement on the accuracy of the mannitol test and there needs to be further research. In future studies, factors that may influence sensitivity and specificity such as smoking, stopping of asthma medications and current asthma symptoms, FEV1 and atopy status (information about seasonal allergies and time of assessment) should be considered and clearly stated.

Because of the high specificity the mannitol test showed in our systematic review, it seems to be a good test to confirm a diagnosis of asthma. This, and the advantage of a standardised protocol with an easy and safe test procedure, can make it a good diagnostic tool also in a nonclinical setting. To exclude asthma, however, methacholine seems to remain the test of choice as the literature shows high sensitivity, whereas in our review we could often only show low sensitivity and heterogeneous results for the mannitol test.

As bronchial provocation tests can be especially useful in patients with an intermediate probability of asthma diagnosis, further studies are needed that include subjects with asthma symptoms but an intermediate probability of asthma diagnosis. In these studies, a longitudinal follow-up would be useful in order to verify the diagnosis and establish an appropriate reference standard.

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Potential competing interests
Prior to this study, David Miedinger and Jörg Leuppi received free mannitol bronchial provocation test kits from Pharmaxis Ltd. to perform clinical studies investigating patients with asthma/COPD or individuals in the workforce.

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Table 1: Study and population characteristics of all studies included in the systematic review – I.

Study author, year of publication	Country	Study design	Population setting	No. of participants analysed	Male, n (%)	Mean age, years	Age range, years	Health inclusion criteria	Mannitol test: protocol, definition of positive test	Reference standard	Sensitivity, % (95% CI)	Specificity, % (95% CI)	TP	FP	FN	TN
Anderson, 1997 [15**]**	Australia	Case-control	Clinical: local community	50	Asthmatics: 11 (26%), non-asthmatics: not reported	24	18–39	Asthma diagnosis with current response to hypertonic saline and a healthy control group	Protocol by Anderson, FEV1 fall >20% (PD15 calculated)	Test result: 4.5% NaCl*	99.0 (88.0–100.0)	99.0 (52.0–100.0)	43	0	0	7
Anderson, 2009 [10**]**	USA	Cross-sectional	Clinical: university hospital	375	182 (49%)	24.3	6–50	Asthma symptoms	Package leaflet, FEV1 fall >15% or dFEV1>10% between consecutive doses	Clinical diagnosis	55.8 (49.3–62.2)	74.8 (66.6–81.9)	134	34	106	101
Brannan, 2005 [13**]**	Australia	Case-control	Clinical: general population and from pulmonary function clinics	592	272 (46%)	34.7	6–83	With or without Asthma symptoms	Protocol by Anderson, FEV1 fall >15%	Clinical diagnosis	59.8 (55.2–64.1)	95.2 (89.2–98.4)	291	5	196	100
Clearie, 2010 [22**]**	Scotland, UK	Cross-sectional	Nonclinical: elite swimmers	59	Not reported	15.2	Not reported	Athletes with and without physician diagnosed asthma	Package leaflet, FEV1 fall >15% or dFEV1>10% between consecutive doses	Physician diagnosed	30.0 (6.7–65.2)	89.8 (77.8–96.6)	3	5	7	44
Holzer, 2003 [23**]**	Australia	Cross-sectional	Nonclinical: elite athletes	50	15 (30%)	21	16–42	Asthma symptoms or doctors diagnosis of asthma	protocol by Anderson, FEV1 fall >15%	Test result: eucapnic voluntary hyperpnoea challenge test*	76.0 (55.0–91.0)	92.0 (74.0–99.0)	19	2	6	23
Koskela, 2003 [25**]**	Finland	Case-control	Clinical: patients from outpatient clinic and healthy volunteers	47	26 (55%)	Asthmatics: 49, non-asthmatics: 41	19–85	Patients with recently diagnosed asthma and healthy control group	Protocol by Anderson, FEV1 fall >15%	Clinical diagnosis	51.4 (34.4–68.1)	100 (69.2–100)	19	0	18	10

Lund, 2009 [16]	Denmark	Case-control	Nonclinical: elite athletes and general population	111	64 (58%)	Asthmatics: 24–27.8, non-asthmatics 20.4–25.1	18–35	Elite athletes with and without asthma and non-athletes with and without asthma	Protocol by Anderson, FEV1 fall >15%	Physician diagnosed	53.7 (37.4–69.3)	95.7 (88–99.1)	22	3	19	67
Miedinger, 2007 [12]	Switzerland	Cross-sectional	Nonclinical: full time fire fighters	94	94 (100%)	41	23–64	Fire fighters with and without physician diagnosed asthma	Protocol by Anderson, FEV1 fall >15%	Clinical diagnosis	92.9 (66.1–99.8)	97.5 (91.3–99.7)	13	2	1	78
Miedinger, 2010 [8]	Switzerland	Cross-sectional	Nonclinical: military conscripts	235	235 (100%)	Not reported	18–20	Conscripts with and without physician diagnosed asthma	Package leaflet, FEV1 fall >15% or dFEV1>10% between consecutive doses	Clinical diagnosis	40.5 (25.6–56.7)	92.7 (88.1–96.0)	17	14	25	179
Sverrild, 2009 [9]	Denmark	Cross-sectional	Nonclinical: general population	238	96 (40%)	18.9	14–24	None specified	Protocol by Anderson, FEV1 fall >15%	Clinical diagnosis	58.8 (44.2–72.4)	98.4 (95.4–99.7)	30	3	21	184
Aronsson, 2011 [28]	Sweden	Case-control	Clinical: outpatient department at a university hospital and control group	49	23 (47%)	35	21–65	Asthma diagnosis and healthy control group	Package leaflet, FEV1 fall >15% or dFEV1>10% between consecutive doses	Clinical diagnosis	38.2 (22.2–56.4)	100 (78.2–100)	13	0	21	15
Subbarao, 2000 [26]	Canada	Case-control	Clinical: general population	34	21 (60%)	10	6–13	Asthma diagnosis and healthy control group	Protocol by Anderson, FEV1 fall >20% (PD15 calculated)	Test result: Methacholine test*	87.5 (67.6–97.3)	100 (69.2–100)	21	0	3	10
Barben, 2011 [19]	Switzerland	Cross-sectional	Clinical: outpatient clinic	99	63 (64%)	12	6–17	Asthma symptoms	Package leaflet, FEV1 fall >15% or dFEV1>10% between consecutive doses	Clinical diagnosis	43.5 (31–56.7)	94.6 (81.8–99.3)	27	2	35	35
Stenfors, 2010 [30]	Sweden	Cross-sectional	Nonclinical: cross-country skiing or biathlon athletes	46	24 (52%)	21	19–31	None specified	Protocol by Anderson, FEV1 fall >15%	Physician diagnosed	8.3 (1.0–27.0)	95.5 (77.2–99.9)	2	1	22	21

McClean, 2011 [29]	Australia	Case-control	Nonclinical: workers at a research institute, hospital, university and volunteers	67	31 (46%)	Asthmatics: 39.4, non-asthmatics: 34	18–66	Asthma diagnosis and healthy control group	Protocol by Anderson, FEV1 fall >15%	Physician diagnosed	61.5 (47.0–74.7)	86.7 (59.5–98.3)	32	2	20	13
Romberg, 2012 [17]	Sweden	Cross-sectional	Nonclinical: elite swimmers	97	55 (54%)	16	13–17	None specified	Protocol by Anderson, FEV1 fall >15%	Test result: exercise test*	50.0 (18.7–81.3)	78.2 (68.0–86.3)	5	19	5	68
Andregnette-Roscigno, 2012 [24]	Spain	Cross-sectional	Nonclinical	23	14 (61%)	12.9	7–17	Asthma symptoms	Protocol by Anderson, FEV1fall >15%	Test result: methacholine test*	55.6 (30.8–78.5)	100 (47.8–100)	10	0	8	5
Ulrik, 2012 [18]	Denmark	Cross-sectional	Nonclinical: elite canoe and kayak athletes	29	24 (83%)	25.1	17–43	None specified	Protocol by Anderson, FEV1 fall >15%	Clinical diagnosis	42.9 (9.9–81.6)	100 (84.6–100)	3	0	4	22
Kim, 2014 [27]	Korea	Case-control	Clinical: university hospital and control group	104	30 (29%)	43.8	18–70	Asthma diagnosis and healthy control group	Protocol by Anderson, FEV1 fall >15%	Clinical diagnosis	48.0 (33.7–62.6)	92.6 (82.1–98.0)	24	4	26	50
Toennesen, 2014 [20]	Denmark	Cross-sectional	Nonclinical: elite athletes	57	42 (74%)	27.5	not reported	Elite athletes participating at the Olympic Games 2008 with or without asthma symptoms	Protocol by Anderson, FEV1 fall >15%	Test result: mannitol or methacholine test*	50.0 (26.0–74.0)	92.3 (79.0–98.4)	9	3	9	36
De Menezes, 2018 [31]	Brazil	Cross-sectional	Nonclinical: workers at a university	811	326 (40%)	32.4	not reported	Contact with laboratory animals	Protocol by Anderson, FEV1 fall >15%	Test result: Mannitol test*	99.9 (93.1–99.9)	96.7 (95.1–97.8)	66	24	0	721
White, 2017 [32]	Australia	Cross-sectional	Clinical: wheezing population and general population	920	Not reported	22	21–23	Wheezing population: wheezing, general population: no wheezing	Protocol by Anderson, FEV1fall >15%	Physician diagnosis	19.5 (13.6–26.6)	97.1 (95.7–98.2)	30	22	124	744
Cancelliere, 2013 [33]	Spain	Cross-sectional	Clinical: university hospital	28	7 (25%)	32	15–54	Asthma-like symptoms (shortness of breath, wheezing, cough)	Protocol by Anderson, FEV1 fall >15%	Test result: Methacholine test and/or Mannitol test*	87.5 (51.6–97.9)	99.7 (75.0–100.0)	10	0	1	17
Osthoff, 2013 [34]	Switzerland	Cross-sectional	Nonclinical: Elite athletes (Swiss	44	30 (68%)	34.4	not reported	non specified (Swiss	Protocol by Anderson,	Physician diagnosed	55.0 (23.4–83.3)	94.0 (79.8–99.3)	6	2	5	31

			paralympic team)					paralympic team)	FEV1 fall >10%							
Backer, 2015 [21]	Denmark	Cross-sectional	Clinical: university hospital	190	82 (43%)	32.1	15–not reported	Symptoms suggesting asthma	Protocol by Anderson, FEV1 fall >15%	Clinical diagnosis	38.0 (34.0–44.0)	82.0 (71.0–89.0)	46	11	76	57
Porpodis, 2016 [36]	Greece	Cross-sectional	Clinical: university hospital	88	41 (47%)	38.6	not reported	Asthma-like symptoms (shortness of breath, wheezing, cough)	Protocol by Anderson, FEV1 fall >15%	Clinical diagnosis	64.0 (51.5–75.5)	95.0 (76.2–99.9)	43	1	24	20
Vakali 2016 [35]	Greece, UK	Case-control	Nonclinical: elite athletes	200	100 (50%)	Asthmatics: 20.4, non-asthmatics: 22.1	20.7–22.5	Athletes with and without physician diagnosed asthma	Protocol by Anderson, FEV1 fall >15%	Physician diagnosed	21.8 (12.0–35.0)	95.0 (89.4–97.6)	12	8	43	137

CI = confidence interval; FEV1 = forced expiratory volume in 1 second; TP = true positive; FP = false positive; FN = false negative; TN = true negative

* A positive test result was only accepted as a reference standard when the included subjects had respiratory symptoms

Table 2: Study and population characteristics of all included studies in the systematic review – II.

Study author, year of publication	Smoking status	Appropriate stop of antiasthmatic therapy prior to test*	Asthma related symptoms	Time between asthma diagnosis and mannitol test	FEV1 (L) asthma	FEV1(% pred) asthma	FEV1 (L) control	FEV1 (% pred) control	FEV1 (L) all	FEV1 (% pred) all	Atopy in asthmatics	Atopy in controls	Atopy in all	Publication status
Anderson, 1997 [15]	All non-smokers	No	Asthmatics: yes, non-asthmatics: no symptoms	Not reported	Not reported	82.9 (SD 12.9)	Normal values	Normal values	Not reported	Not reported	100%	43%	92%	Peer-reviewed
Anderson, 2009 [10]	All non-smokers	Yes	Yes, current	Couple of weeks	Not reported	Not reported	Not reported	Not reported	3.32 (SD 0.82)	93.6 (SD 10)	Not reported	Not reported	16% to 50%	Peer-reviewed
Brannan, 2005 [13]	Not reported	No	Asthmatics: yes, current, non-asthmatics: no symptoms	Simultaneously	Not reported	Not reported	Not reported	Not reported	3.0 (SD 0.9)	95.0 (SD 14.5)	Not reported	Not reported	Not reported	Peer-reviewed
Clearie, 2010 [22]	Not reported	No	Yes, 26 (43%) with exercise induced symptoms	3 days	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	14	24%	Peer-reviewed
Holzer, 2003 [23]	Not reported	Yes	Yes, current	1 week	Not reported	Not reported	Not reported	Not reported	Within normal limits (>80% of predicted)	103.6 (SD 10.8)	Not reported	Not reported	Not reported	Peer-reviewed
Koskela, 2003 [25]	Current smokers: 6; former smokers: 14; never smoked: 27	Yes	Asthmatics: yes, current, non-asthmatics: no symptoms	2 weeks	2.9 (95% CI 2.6–3.1)	81 (95% CI 76–86)	3.9 (95% CI 3.1–4.7)	99 (95% CI 95–104)	Not reported	Not reported	38%	10%	Not reported	Peer-reviewed
Lund, 2009 [16]	Current smokers: 7	Elite athletes: no, non-athletes: yes	All athletes (54): yes	Simultaneously	4.37 (SD 0.19) for elite athletes; 3.73 (SD 0.14) for non-elite athletes	98.2 (SD 1.94) for elite athletes; 82.6 (SD 2.63) for non-elite athletes	4.91 (SD 0.15) for elite athletes, 3.87 (SD 0.13) for non-elite athletes	Elite: 105.4 (SD 2.10); non-elite: 96.9 (SD 1.82)	Not reported	Not reported	Elite athletes: 21%; non-elite athletes: 100%	Elite athletes: 51%; non-elite athletes: 29%	Not reported	Peer-reviewed

Miedinger, 2007 [12]	Current smokers: 33	Not reported	Asthmatics: yes, in the past 12 months, non-asthmatics: unclear	1 week	Not reported	Range of 42–105	Not reported	Not reported	Not reported	103 (SD 12)	86%	Not reported	51%	Peer-reviewed
Miedinger, 2010 [8]	Current smokers: 77	Not reported	Asthmatics: yes, in the past 12 months, non-asthmatics: unclear	48 hours	Not reported	95 (IQR 88;102)	Not reported	98 (IQR 91;105)	Not reported	Not reported	74%	36%	42%	Peer-reviewed
Sverrild, 2009 [9]	Current smokers: 52	No	Asthmatics: yes, in the past 12 months, non-asthmatics: unclear	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	96.92 (SD 10.60)	Not reported	Not reported	42%	Peer-reviewed
Aronsson, 2011 [28]	All non-smokers	No	Asthmatics: current exercise induced symptoms 27 (79%), non-asthmatics: no symptoms	A couple of weeks	3.7 (SD 1.0)	95.5 (SD 14.2)	3.6 (SD 0.8)	98.6 (SD 6.8)	Not reported	Not reported	82%	0%	Not reported	Peer-reviewed
Subbarao, 2000 [26]	All non-smokers	No	Asthmatics: yes, current, non-asthmatics: no symptoms	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	87%	0%	Not reported	Peer-reviewed
Barben, 2011 [19]	Not reported	No	Yes, current	A few days	Not reported	Not reported	Not reported	Not reported	Not reported	97 (IQR 88;104)	Not reported	Not reported	89.0%	Peer-reviewed
Stenfors, 2010 [30]	Not reported	No	In 17% classical Symptoms of exercise induced asthma	Not reported	4.7 (SD 1.1)	97.7 (SD 14.0)	4.5 (SD 0.5)	101.4 (SD 7.5)	Not reported	Not reported	55%	50%	Not reported	Peer-reviewed
McClean, 2011 [29]	All non-smokers	No	Asthmatics: were well controlled	Not reported	Not reported	87 (SD 13.0)	Not reported	104 (SD 14.4)	Not reported	Not reported	83%	53%	Not reported	Peer-reviewed
Romberg, 2012 [17]	Not reported	No	Exercise induced symptoms 75 (77.3%), current asthma symptoms 60 (62.0%), current asthma symptoms with exercise induced symptoms 54 (55.7%): past 12 months	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	112 (IQR 104;118)	Not reported	Not reported	54%	Peer-reviewed
Andreg-nette-Roscigno, 2012 [24]	Not reported	No	Yes	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	70%	Peer-reviewed

Ulrik, 2012 [18]	All non-smokers	No	Asthmatics with previous diagnosis of asthma: yes, asthmatics with no previous diagnosis of asthma: no, non-asthmatics: not reported	Simulta-neously	4.5 (SD 0.6)	103.3 (SD 13.3)	4.8 (SD 0.9)	109.1 (SD 14.8)	Not re-ported	Not re-ported	57%	18%	Not re-ported	Peer-reviewed
Kim, 2014 [27]	All non-smokers	Yes	Asthmatics: yes, in the past 6 months, non-asthmatic: no	Not re-ported	Not reported	91.2 (SD 12.2)	Not reported	95.0 (SD 19.4)	Not re-ported	Not re-ported	Allergic rhinitis 66%, ato-pic der-matitis 18%, al-lergic conjunc-tivitis 22%	allergic rhinitis 15%, ato-pic der-matitis 0%, aller-gic con-junctivitis 2%	allergic rhi-nitis 39%, atopic der-matitis 9%, allergic conjunctivi-tis 12%	Peer-reviewed
Toen-nesen, 2014 [20]	Not re-ported	Yes	Asthmatics: yes (unclear if current or in the past), non-asthmatics: no	Not re-ported	Not reported	117 (SD 15)	Not reported	117.3 (SD 11.8)	Not re-ported	117.2 (12.7)	17%	18%	18.00%	Peer-reviewed
De Menezes, 2018 [31]	Current smokers: 69 (8.5%)	No	Yes, in the past 12 months	Not re-ported	3.35 (SD 0.64)	91.8 (SD 11.5)	3.54 (SD 0.75)	97.4 (SD 11.3)	Not re-ported	Not re-ported	Not re-ported	Not re-ported	47%	Peer-reviewed
White, 2017 [32]	Current smokers: 158	No	General popula-tion: unclear, wheezing popula-tion: 148 (100%) in the past 12 months	Not re-ported	Not reported	General pop-ulation: 95 (SD 11.60), wheezing population: 95 (SD 11.58)	Not reported	General popu-lation: 98 (SD 10.76); wheez-ing popula-tiond: 98 (SD 10.23)	Not re-ported	Not re-ported	General popula-tion: 75%, wheezing popula-tion 75%	General popula-tion: 55%; wheezing popula-tion: 62%	Not re-ported	Peer-reviewed
Cancel-liere N, 2013 [33]	Not re-ported	Yes	Yes, current	Not re-ported	Not reported	Not reported	Not reported	Not reported	Not re-ported	Not re-ported	Not re-ported	Not re-ported	Not re-ported	Peer-reviewed
Osthoff, 2013 [34]	All non-smokers	Yes	Asthmatics: yes, current, non-asth-matics: unclear	Not re-ported	Not reported	Not reported	Not reported	Not reported	3.63	Not re-ported	32%	Not re-ported	Not re-ported	Peer-reviewed
Backer, 2015 [21]	Only re-ported that indi-viduals older than 40 years with > 10 pack-years were ex-cluded	No	Yes, current	2–3 weeks	3.7 (SD 0.9)	97 (SD 17)	3.8 (0.9)	97 (SD 17)	3.8 (SD 0.9)	95 (SD 17)	63%	44%	56%	Peer-reviewed

Porpodis, 2016 [36**]**	Current smokers: 17, former smokers: 16, never smoked: 55	No	Yes, in the last month	14–20 days	Not reported	Not reported	Not reported	Not reported	3.2 (SD 0.9)	88.5 (SD 12.6)	Not reported	Not reported	43%	Peer-reviewed
Vakali, 2016 [35**]**	Smokers: 8%	Yes (but refer to ATS Crapo criteria)	Yes	Not reported	4.07 (95% CI 3.9–0.2)	Not reported	4.29 (95%CI 4.1–4.5)	Not reported	4.1 (95%CI 4.1–4.2)	Not reported	62%	44%	49%	Peer-reviewed

CI = confidence interval; FEV1 = forced expiratory volume in 1 second; IQR = interquartile range; SD = standard deviation

* according to Anderson et al. [10]

Appendix 1: Search strategy

The search strategy included the term (mannitol) combined with (asthma OR bronchial* OR bronchoconstrict*). In Embase the terms were: 'mannitol'/exp OR 'mannitol'/syn AND ('asthma'/exp OR 'asthma'/syn OR bronchial* OR 'bronchoconstriction'/exp OR 'bronchoconstriction'/syn), in PubMed/Medline they were: mannitol AND (asthma OR bronchial* OR bronchoconstrict*). For our search there was no language restriction imposed.

Appendix 2: Assessment of methodological quality

The methodological quality of the selected studies was graded independently and in duplicate by two reviewers with the Quality Assessment of Diagnostic Accuracy Studies tool (QUADAS-2, an improved, redesigned tool since the original QUADAS tool), a validated tool for the quality assessment of diagnostic accuracy studies [14]. The QUADAS-2 tool includes 4 domains (patient selection, index test, reference standard, and flow and timing). Each domain is assessed in terms of risk of bias, and the first 3 domains also in terms of concerns regarding applicability. Some signalling questions are included to help judge the risk of bias. We added some signalling questions to the predefined QUADAS-2 form, which seemed to be important to us judging our included studies. Disagreements were resolved by consensus. If consensus was not achieved, a third reviewer had the decisive vote.

Appendix 3: Data extraction

The two reviewers extracted data on characteristics of studies and patients, the index test and the reference standard as well as test results. Wherever possible, we recorded for each study: Title, author, year of publication, country and journal, conflict of interest and project funding, study aim, study design (cohort study, case-control study, cross-sectional or later follow-up, prospective or retrospective), study population (age, prevalence of asthma, severity of symptoms, co morbidity, smoking status, gender, professional sportsmen, history of asthma, atopy), Stop of anti-asthmatic therapy prior to the index test, Patient selection (consecutive, non-consecutive, random- sample, inclusion- and exclusion criteria), technical details of Mannitol bronchial provocation and reference tests regarding standardisation (protocol, definition for positive/negative test result), performance of the index test (sensitivity and specificity), number of individuals eligible and no of individuals who underwent the tests, number of individuals undergoing either the index and the reference test missing one or the other, time interval between the index and the reference test, side effects from undergoing either the Mannitol bronchial provocation test or reference standard, number of individuals in whom the test was terminated prematurely or was not analysable, reasons for exclusion from test or analysis, inter-observer variability and test reproducibility, reported results (Sensitivity, Specificity, true positive, true negative, false positive, false negative, positive predictive value, negative predictive value), Data for two-by-two table.

Appendix 4: Methodological quality of included studies

	Anderson, 1987[15]	Anderson, 2009[10]	Brannan, 2005[13]	Clearie, 2010[22]	Holzer, 2003[23]	Kostela, 2003[25]	Lund, 2009[16]	Miedinger, 2007[12]	Miedinger, 2010[8]	Sverrild, 2008[9]	Arousson, 2011[28]	Subbarao, 2000[26]	Barben, 2011[19]	Stanfors, 2010[30]	McClean, 2011[29]	Romberg, 2012[17]	Andreguetto-Bocsipoa, 2012[24]	Ulrik, 2012[10]	Kim, 2014[27]	Toennesen, 2014[20]	De Menezes, 2016[31]	White, 2017[32]	Cancelliere 2013[33]	Osthoff, 2013[34]	Backer, 2015[21]	Porpodis, 2016[36]	Vakali, 2016[35]
PATIENT SELECTION																											
Consecutive or random sample enrolment?	unclear	unclear	unclear	unclear	unclear	yes	unclear	no	yes	yes	unclear	unclear	unclear	unclear	unclear	unclear	unclear	unclear	unclear	unclear	no	no	yes	yes	yes	yes	unclear
Case-control design avoided?	no	yes	no	yes	yes	no	no	yes	yes	yes	no	no	yes	yes	no	yes	yes	yes	no	yes	yes	yes	yes	yes	yes	yes	no
Inappropriate exclusions avoided?	no	no	yes	yes	yes	yes	yes	yes	yes	yes	no	no	yes	yes	no	unclear	unclear	no	no	yes	no	yes	yes	no	no	unclear	no
Risk of Bias	**HIGH**	**LOW**	**HIGH**	**LOW**	**LOW**	**HIGH**	**HIGH**	**LOW**	**LOW**	**LOW**	**HIGH**	**HIGH**	**LOW**	**LOW**	**HIGH**	**UNCLEAR**	**LOW**	**LOW**	**HIGH**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**HIGH**
Applicability concerns	**HIGH**	**HIGH**	**LOW**	**HIGH**	**HIGH**	**LOW**	**HIGH**	**HIGH**	**HIGH**	**LOW**	**HIGH**	**HIGH**	**LOW**	**HIGH**	**HIGH**	**HIGH**	**LOW**	**HIGH**	**HIGH**	**HIGH**	**HIGH**	**LOW**	**LOW**	**HIGH**	**LOW**	**LOW**	**HIGH**
INDEX TEST (MANNITOL)																											
Description of Index Test	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Results interpreted without knowing results of the reference standard (Blinding)	no	yes	yes	unclear	unclear	unclear	unclear	unclear	yes	yes	no	unclear	no	unclear	unclear	yes	yes	unclear	unclear	yes	unclear	unclear	no	yes	yes	unclear	unclear
If a threshold was used, was it pre-specified?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Risk of Bias	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**
Applicability concerns	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**
REFERENCE STANDARD																											
Description of Reference standard	yes	yes	yes	no	no	yes	no	yes	yes	yes	yes	yes	yes	no	no	yes	yes	yes	yes	yes	yes	no	yes	no	yes	yes	no
Results interpreted without knowing results of the Mannitol test (Blinding)	yes	yes	yes	yes	yes	yes	yes	no	no	yes	yes	yes	no	yes	yes	unclear	unclear	no	yes	no	no	unclear	no	unclear	yes	yes	yes
Adequate Reference standard	yes	yes	yes	unclear	yes	yes	unclear	yes	yes	yes	yes	yes	yes	unclear	unclear	yes	yes	yes	yes	yes	yes	unclear	yes	unclear	yes	yes	unclear
Mannitol Test was not part of the Reference standard (no incorporation bias)	yes	yes	yes	yes	yes	yes	yes	no	no	yes	yes	yes	no	unclear	unclear	yes	yes	no	yes	no	no	yes	no	unclear	yes	yes	unclear
Risk of Bias	**LOW**	**LOW**	**LOW**	**UNCLEAR**	**LOW**	**LOW**	**UNCLEAR**	**HIGH**	**HIGH**	**LOW**	**LOW**	**LOW**	**HIGH**	**UNCLEAR**	**UNCLEAR**	**LOW**	**LOW**	**HIGH**	**LOW**	**HIGH**	**HIGH**	**UNCLEAR**	**HIGH**	**UNCLEAR**	**LOW**	**LOW**	**UNCLEAR**
Applicability concerns	**LOW**	**LOW**	**LOW**	**UNCLEAR**	**LOW**	**LOW**	**UNCLEAR**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**UNCLEAR**	**UNCLEAR**	**HIGH**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**UNCLEAR**	**LOW**	**UNCLEAR**	**LOW**	**LOW**	**UNCLEAR**
FLOW AND TIMING																											
Appropriate time interval between Mannitol test and reference standard (>24h and <1week)	unclear	unclear	yes	unclear	unclear	no	unclear	yes	unclear	unclear	unclear	yes	yes	unclear	unclear	unclear	yes	unclear	unclear	unclear	no	unclear	no	yes	unclear	no	unclear

Therapeutic intervention avoided between Index test and reference standard	yes	yes	no	unclear	unclear	yes	unclear	yes	unclear	yes	unclear	yes	yes	unclear	no	yes	unclear	no	unclear	yes	no	no	no	yes	unclear	unclear	unclear
Did all patients receive a reference standard	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	no	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Did all patients receive the same reference standard	yes	yes	yes	unclear	unclear	yes	unclear	yes	unclear	yes	unclear	yes	no	unclear	unclear	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Were all patients included in the analysis	yes	unclear	no	no	yes	no	yes	no	no	no	no	no	no	yes	yes	no	yes	yes	yes	yes	no	yes	yes	yes	yes	yes	yes
Were reasons reported for stopping a test	not applicable	yes	yes	yes	not applicable	not applicable	not applicable	yes	yes	yes	yes	yes	yes	not applicable	not applicable	not applicable	not applicable	not applicable	not applicable	not applicable	not applicable	yes	not applicable	not applicable	not applicable	not applicable	not applicable
Risk of Bias	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**LOW**	**UNCLEAR**	**UNCLEAR**	**LOW**	**LOW**	**LOW**	**LOW**	**HIGH**	**HIGH**	**HIGH**	**LOW**	**LOW**	**LOW**	**LOW**