Original Article
A meta-analysis: the efficacy of asthma diagnosis based on exhaled nitric oxide

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Abstract: Objectives: This study aims to evaluate the efficacy of asthma diagnosis based on exhaled nitric oxide (FeNO). Methods: By computer and manual search, all researches about eNO detection for asthma diagnosis were collected. According to the evaluation criteria of QUADAS scale, the data from 14 studies were analyzed by RevMan software, and the sensitivity (Sen), specificity (Spe) were analyzed. Results: A total of 14 articles were included in this study. Quality evaluation, the inclusion of studies, the use of the same reference standards were evaluated independently from the reference standards, All studies were well controlled for bias (? rephrase). The possibility of the disease progression bias is small and the reference standard interpretation may be large (? rephrase). The disease spectrum, the criteria for inclusion in the population, and the quality of the report are not described. Conclusions: Summary of the current FeNO detection and diagnosis of asthma, FeNO detection of asthma has a certain diagnostic value, the high quality research is needed for further analysis.

Keywords: Asthma, FeNO, diagnosis, meta-analysis

Introduction

In asthma, inflammation can be eosinophilic or neutrophilic [1]. Arguably, asthma may be best diagnosed according to the type of airway inflammation [2]. There are several ways to quantify airway inflammation, such as detecting the percentage of eosinophils in the sputum and FeNO (fraction exhaled nitric oxide). FeNO correlates with other markers of asthma such as eosinophilia in sputum [3]. In recent years, exhaled nitric oxide (FeNO) index as a non-invasive determination of airway inflammation has been proposed. In patients with inflammatory airway diseases such as asthma, FeNO levels were significantly increased [4]. Many reports have demonstrated that FeNO can identify asthma and non-asthmatic patients [5], and that the level of FeNO is correlated with the degree of airway responsiveness [4]. These suggest that FeNO is a marker of airway inflammation and has potential value for the diagnosis of asthma. Moreover, Measurement of FeNO can be used as a safe, simple and rapid test for the diagnosis of asthma, and can be repeatedly detected in different periods of the disease,

[6]. However, the diagnostic value of FeNO was not determined. Therefore, the aim of this meta-analysis is to evaluate the efficacy and accuracy of FeNO test in the diagnosis of asthma.

Methods

Search strategies

Refer to "The BayesLibrary of Diagnostic Studies And Reviews" [7] make the search strategy. Electronic search Medline, Cochrane Library, OVID, Baidu Scholar, CNKI. Deadline is December, 2015. Main key words are: nitric oxide, exhaled nitric oxide, asthma, bronchial asthma, asthma sensitivity, diagnostic technique and procedure, reference values, likelihood function diagnostic.

Study selection

Inclusion criteria: (1) the object of study: clinical suspected to suffer from asthma, the number of cases ≥20; (2) for evaluation test: FeNO detection; (3) reference standard: detection of respiratory flow or airway reactivity [inducing

Table 1. The characteristics of studies were included in this meta-analysis

Study	Year	Tample	Reference standard	FeNO determinations	Sen %	Spe %
Berkman [8]	2005	85	Bronchial provocation test	eNO250 >7 ppb	83	89
				eNO250 >4 ppb	95	58
				eNO250 >12 ppb	55	96
Arora [9]	2006	172	Bronchial provocation test/ respiratory flow measurment	eNO50 >10.5	86	21
				eNO50 >46	17	100
Chatkin [10]	1999	38	Bronchial provocation test/ respiratory flow measurment	eNO45 >30	75	87
Miedinger [11]	2007	101	Bronchial provocation test/ respiratory flow measurment	eNO50 >20	64	59
				eNO50 >47	42	96
Deykin [12]	2002	62	Bronchial provocation test	eNO50 >30	71	75
Dupont [13]	2003	155	Bronchial provocation test	eNO50 >13	85	80
Franklin [14]	2003	155	Bronchial provocation test	eNO35 >18.4	81	79
Heffler [15]	2006	48	Bronchial provocation test/ respiratory flow measurment	eNO50 >36	78	60
Malmberg [16]	2003	83	Respiratory flow measurment	eNO50 >9.7	86	92
Smith [17]	2004	47	Respiratory flow measurment	eNO50 >20	88	79
Liu Na [18]	2011	87	Bronchial provocation test	eNO >34.5	71.2	68.6
Chai Jing [19]	2010	118	Bronchial provocation test	eNO >32.5	82.7	87.9
Ren Xubin [20]	2009	101	Bronchial provocation test	eNO >36.5	92.7	83.3
Yang Yanjuan [21]	2013	305	Bronchial provocation test	eNO >50	70.3	84.62

Table 2. The quality evaluation of included studies

Score	Item 1	Item 2	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 11	Item 13	Item 14
Yes	18%	60%	80%	100%	100%	100%	25%	25%	33%	0%	100%
No	0%	5%	20%	0%	0%	0%	0%	0%	0%	100%	0%
Unclear	82%	35%	0%	0%	0%	0%	75%	75%	67%	0%	0%

agent is histamine, acetyl choline (MCH), hypertonic saline, sports, etc.]; (4) measurement results: the sensitivity (Sen) and specificity (Spe). From the original studies we obtain and calculate the relevant data of four grids: true positive values, false positive values, false negative values and true negative values.

Exclusion criteria: no full text documents, no original literature, animal test and basic research.

Data extraction and management

By reading topic and abstract of study, eliminate those studies which do not obviously meet with inclusion criteria and the document of a draft cast more, merge duplicates; Further search for the full text of the first screen to be included; Looking for early screening fitting into the full text; A reading of the original

text, according to the inclusion and exclusion criteria to determine the inclusion of the literature.

Refer to the data extraction table in The Bayes Library of Diagnostic Studies and Reviews and design data extraction table which suit this study. Data extraction table includes four parts (PICO): Patients, Index test, Comparison, Reference standard, Outcome. Two reviewers extracted independently data. We will resolve disagreements by consensus or by involving a third person.

Study quality evaluation

This scale is a tool for evaluating the accuracy of diagnostic tests. The quality of study was evaluated from three aspects: bias, variance and report quality. Every item was scored by using "yes", "no", "unclear". "Yes" means that

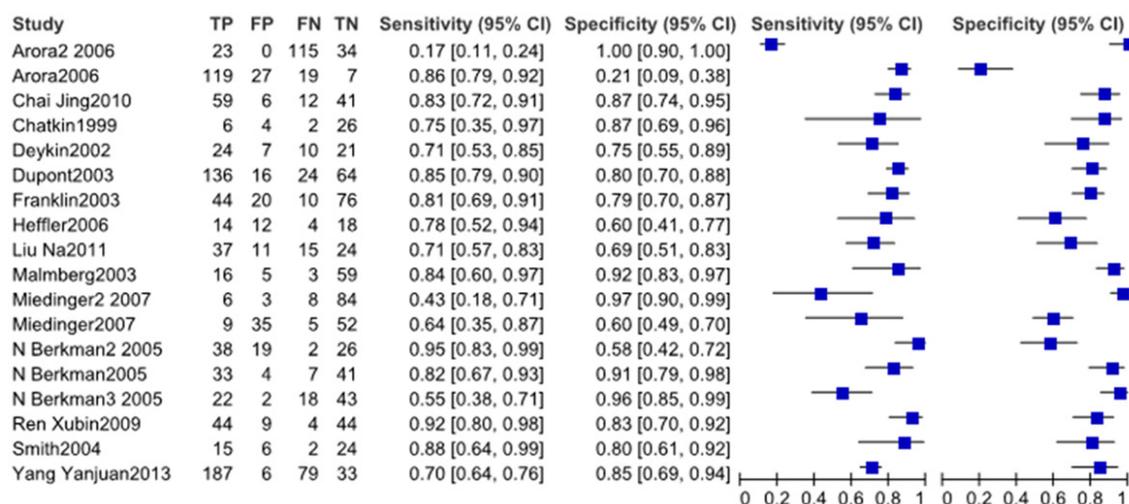| Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Arora2 2006	23	0	115	34	0.17 [0.11, 0.24]	1.00 [0.90, 1.00]		
Arora2006	119	27	19	7	0.86 [0.79, 0.92]	0.21 [0.09, 0.38]		
Chai Jing2010	59	6	12	41	0.83 [0.72, 0.91]	0.87 [0.74, 0.95]		
Chatkin1999	6	4	2	26	0.75 [0.35, 0.97]	0.87 [0.69, 0.96]		
Deykin2002	24	7	10	21	0.71 [0.53, 0.85]	0.75 [0.55, 0.89]		
Dupont2003	136	16	24	64	0.85 [0.79, 0.90]	0.80 [0.70, 0.88]		
Franklin2003	44	20	10	76	0.81 [0.69, 0.91]	0.79 [0.70, 0.87]		
Heffler2006	14	12	4	18	0.78 [0.52, 0.94]	0.60 [0.41, 0.77]		
Liu Na2011	37	11	15	24	0.71 [0.57, 0.83]	0.69 [0.51, 0.83]		
Malmberg2003	16	5	3	59	0.84 [0.60, 0.97]	0.92 [0.83, 0.97]		
Miedinger2 2007	6	3	8	84	0.43 [0.18, 0.71]	0.97 [0.90, 0.99]		
Miedinger2007	9	35	5	52	0.64 [0.35, 0.87]	0.60 [0.49, 0.70]		
N Berkman2 2005	38	19	2	26	0.95 [0.83, 0.99]	0.58 [0.42, 0.72]		
N Berkman2005	33	4	7	41	0.82 [0.67, 0.93]	0.91 [0.79, 0.98]		
N Berkman3 2005	22	2	18	43	0.55 [0.38, 0.71]	0.96 [0.85, 0.99]		
Ren Xubin2009	44	9	4	44	0.92 [0.80, 0.98]	0.83 [0.70, 0.92]		
Smith2004	15	6	2	24	0.88 [0.64, 0.99]	0.80 [0.61, 0.92]		
Yang Yanjuan2013	187	6	79	33	0.70 [0.64, 0.76]	0.85 [0.69, 0.94]		

Figure 1. Forest plot of meta-analysis for efficacy of asthma diagnosis based on FeNO.

the study meet with the standard. "No" means that the study don't meet with the standard. "Unclear" is that partially meet with the standard or unable to get enough information from the study.

Statistical analysis method

Assessment of heterogeneity: Any heterogeneity between study results will be described and tested, and assess statistical significance of selected studies using a chi-squared test. The 95% confidence interval was estimated using a random effect model.

Meta-analysis: The results of studies that meet the inclusion criteria will be included in the subsequent meta-analyses. The summary weighted risk ratio and 95% confidence interval will be calculated using Review Manager (RevMan).

Sensitivity analysis: Sensitivity analysis excluding studies with a high risk of bias based on the 'Risk of bias' assessment. Studies that do not have adequate allocation concealment and sequence generation will be removed. Analysis used random effects model.

Results

Data extraction and quality evaluation

Included in the study of basic situation: A total of 167 articles were identified and included in the standard and exclusion criteria, which were included in the 14 studies, 1557 case subjects. The results are shown in **Table 1**.

Study quality evaluation: There are 14 items. According to the characteristics of this study, item 3 (reference criteria), 10 (to be evaluated), and 12 (clinical judgment bias) were removed. The reason for deleting item 3 is that the inclusion criteria of the study have provided the reference standards for the diagnosis of asthma. Deleting item 10 and 12 of the grounds are that the FeNO was detected by automated instrumentation, and no subjective, and FeNO testing was performed before respiratory flow measurement or airway responsiveness was measured, and the results were not explained by the reference standard results and clinical data. So 11 items are finally selected for the quality evaluation of inclusion studies. The quality evaluation results of the study were shown in **Table 2**.

Statistical analysis results

Assessment of heterogeneity: Among the results of all studies there is heterogeneity. So use random effects model combined effect.

Draw SROC curve: Meta-analysis was performed on the same diagnostic criteria, according to the weight of OR, the SROC curve was synthesized. SROC curve is shown in **Figure 2**. The results of meta-analysis were shown in **Figure 1**.

Sensitivity analysis: After meta-analysis of each study, the summary of the sensitivity and specificity has not been changed significantly. It indicates that the stability of inclusion studies is well.
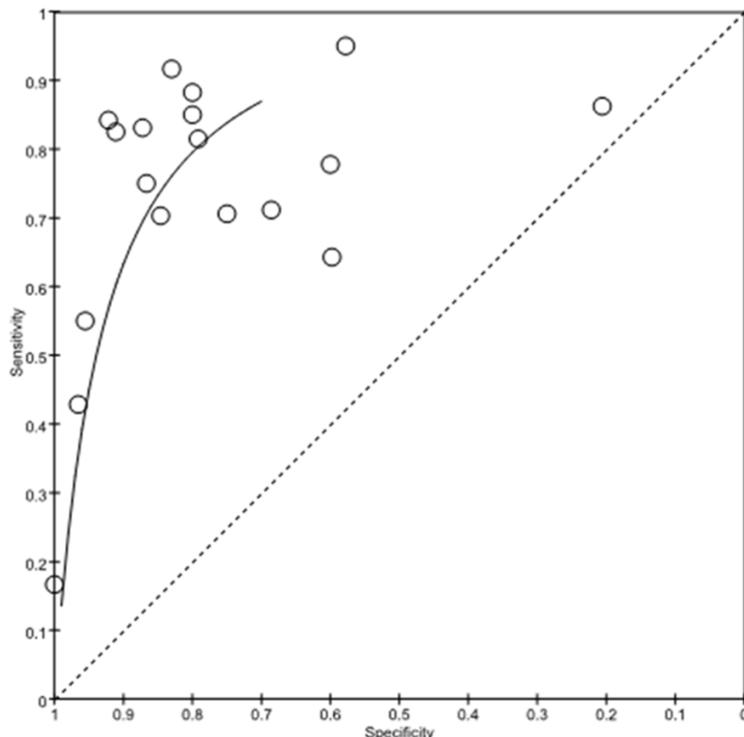
Figure 2. SROC curve.

the total, and the probability of the occurrence of this type of bias (disease progression bias) was small. Item 11 is a reference to the implementation of the blind method (reference standard interpretation bias). Because of the strong subjectivity, the blind method is not implemented, and it can be applied to the result interpretation. In summary, in the 6 evaluation of the bias, the bias of the literature is mainly derived from implementation of blind method when reference standard interpret. The disease spectrum and the inclusion criteria in the study may affect the sensitivity and specificity of the test to be evaluated, and it also affects its popularization. Some diseases may be worse, complications may be more, so the sensitivity of diagnostic tests may be higher, and specificity is lower; some diseases may be lighter, complications may be less, so the sensitivity of diagnostic tests may be lower, but the specificity is higher. The description of disease spectrum should be as detailed as possible, including the prevalence of the disease, the extent of the disease, and the age, gender, ethnicity, medical environment, etc. In the meta-analysis inclusion studies, some diseases are more serious, so the results show that the bias impact seriously on the experimental results of clinical application. In the analysis of results of heterogeneity, all results have heterogeneity. The reasons of heterogeneity: (1) the first consider the effect of diagnosis of boundary values. In this meta-analysis, FeNO detect the boundary value is 4~47 PPB in the diagnosis of asthma. In different experiments, the cut-off point of positive (or negative) test results is different, have a threshold effect. Diagnostic threshold value change, its sensitivity and specific degrees is change too, and SROC curve is not affected by the threshold effect [22]. The SROC curve obtained by this study not only considers the sensitivity and specificity, but also makes the results more intuitive through graphics. (2) Selection of reference standards. The

Discussion

The most important quality of diagnostic tests is a series of patients which were included in the study, the use of reference standards, the implementation of the evaluation test, the results of the evaluation tests and reference standards, blind etc. In addition, the quality of the diagnostic test report is a very important. Diagnostic tests are conducted from the aspects of quality evaluation, the size and source of bias and heterogeneity, to guide the clinical selection of the best diagnostic program, and to make a proper interpretation of the test results. The quality evaluation results of 14 studies showed that the 6 items (item 4, 6, 7, 11, 5, 14) were about bias assessment, and item 5, 6, 7 and 14 meet with all inclusion studies. "Yes" study accounted for 100%. In the study object, the test was accepted and the reference standard was used to confirm the status of the target disease (the different bias), the test was independent from the reference standard (the combined bias), and all the research subjects completed the study (exit). These four aspects better control the bias. Item 4 was evaluated as "yes", which accounted for 80% of

evaluation of clinical diagnostic tests is the first to determine the gold standard [23]. Although there are many detection technologies which have no gold standard, can use the current best method as a reference standard. The sensitivity and specificity of the diagnostic tests were significantly different from the reference standards. The reference standards used in this study were determined by respiratory flow measurement or airway responsiveness. In the measurement of airway responsiveness, the sensitivity of hypertonic saline was lower than that of histamine or MCH, and the specificity was higher [24]. The study showed that the correlation between the test of acetyl choline and histamine was very bad. It suggests a lack of consistency between these closely related diagnostic tests [25]. Moreover, the negative and the exercise induced asthma can't be excluded by the stimulation of acetyl choline, histamine or hypertonic saline [26]. As a result, the FeNO has a different sensitivity and specificity compared with the reference standards. (3) Different laboratory test system. Detection system includes a project detection equipment, operating procedures, quality control, operation personnel, etc. The detection system can guarantee the quality of laboratory test results, and any changes may affect the accuracy of the results of the experiment. There are many factors affecting the FeNO detection, NO level, device, air flow velocity, analyzer, infection, smoking history, etc., which has a great influence on the FeNO results. The ATS/ERS recommended by the online guideline is 50 mL/s, and the flow rate of offline is 350 mL/s [27], but the data used in this study are different about flow rate. In addition, different research design and implementation can also be caused by the difference between the results.

The shortage of this study are: first, the quality of the evaluation, due to the use of the QUADAS scale, lack evaluation for design of the test, so we can't carried out the trial design of the bias caused by the evaluation. It is proved that design of case control study can exaggerate accuracy of the test. Second, the result of combined analysis, didn't do a stratified analysis according to different reference standards and FeNO detection of different expiratory flow rate, detection device (online and offline). Because, the study on this aspect

of the report is unknown, and if the stratification of the inclusion of the study is very small, can't be analyzed.

Disclosure of conflict of interest

None.

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