

## Induced sputum eosinophil count for the diagnosis of bronchial asthma

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Bronchial asthma is a chronic inflammatory disorder of the airways, in which many cells, and cellular elements play a role. It is a problem worldwide, with an estimated 300 million individuals affected and 15 million disability-adjusted life years lost annually.<sup>1</sup> Although asthma was regarded as an untreatable disease earlier, the global initiative for asthma<sup>2</sup> (GINA) has emphasized that asthma can be controlled by standard management. Accordingly, accurate diagnosis and appropriate surveillance would be the prerequisite. Currently, the diagnosis of asthma is based on the clinical, or pulmonary function parameters, which cannot effectively guide the therapy.<sup>2</sup> With the unveiling of airway inflammation mechanisms, the methods to monitor airway inflammation have been viewed as complementary to the conventional tools in the diagnostic process. Several ways are now available to collect samples from the lower airways for studying features of airway inflammation. Among them, sputum induction has a great advantage due to its non-invasive manner. In addition, it can provide more direct and additional information on the current inflammatory status of the airways,<sup>3</sup> and guide the treatment with corticosteroids to reduce exacerbation rates. Therefore, induced sputum eosinophil count would become a promising novel diagnostic tool for asthma. It is now necessary to evaluate the accuracy of this technique due to the unsettled value of its diagnostic accuracy. We conducted a systematic review, and meta-analysis of published studies where the induced sputum eosinophil count was adopted for detecting asthma.

This meta-analysis was performed between July 2009 and November 2009 at the West China Hospital, Sichuan, P. R. China. A comprehensive search was carried out using PubMed, Ovid, EMBASE, VIP, CNKI and CBMdisc to identify relevant studies published before November 2009. Some additional published, unpublished, and ongoing studies were also identified by manual review. Inclusion criteria included induced sputum eosinophil count for diagnosing bronchial asthma, comparison of induced sputum result with pulmonary function parameters as reference standard (according to GINA, the diagnosis of asthma should be based on the presence of one of the following items: spirometry, airway responsiveness, and peak expiratory flow [PEF] variability) and the numbers used to calculate sensitivity and specificity were reported. Secondary literature, and animal studies were excluded during the process. Two reviewers

independently completed screening, study selection, and data extraction. Disagreements were resolved by discussion. Study quality was assessed using the quality assessment of diagnostic accuracy studies (QUADAS). Meta-analysis was undertaken with Meta-Disc statistical software.<sup>4</sup> The heterogeneity caused by threshold effect was explored through spearman correlation analysis. Heterogeneity other than threshold effect was tested with chi-square test, and Cochran's Q-test. The effect model was adopted according to the extent of heterogeneity. The pooled sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio were calculated, and summary receiver operating characteristic (SROC) curves were performed. Finally, we conducted sensitivity analysis to assess whether changing the inclusion criteria influenced the results. A *p*-value less than 0.05 was considered significant. All our data analysis was undertaken with Meta-Disc statistical software.

In total, 9 studies including 659 patients were appropriate for the meta-analysis. The statistical results show the spearman correlation coefficient was -0.583 (*p*=0.09). The  $\chi^2$  value of sensitivity, and specificity was 31.18 (*p*=0.0001), and 19.31 (*p*=0.0133). The Cochran's Q value of positive likelihood ratio was 21.97 (*p*=0.005), negative likelihood ratio was 49.99 (*p*=0.0000), and diagnostic odds ratio was 31.45 (*p*=0.0001). Then, random effect model was selected to calculate the diagnostic parameters. The pooled sensitivity was 0.80 (95% confidence interval (CI): 0.75, 0.84), specificity was 0.90 (95% CI: 0.87, 0.93), positive likelihood ratio was 7.03 (95% CI: 3.78, 13.08), negative likelihood ratio was 0.23 (95% CI: 0.14, 0.39), and diagnostic odds ratio was 4.36 (95% CI: 12.59, 93.79). The SROC curve was performed where the summary area under the SROC was 0.9208. The Q index value was 0.8543. At last, the sensitivity analysis did not reveal any significant factor that influenced the stability of the included literature.

Quality analysis was shown in Table 1. One hundred percent of the studies well controlled the bias in reference standard, differential verification, incorporation, and withdrawal. Only 2 studies produced partial verification bias. The probability of bias in index test interpretation, reference standard interpretation, and disease progression was increased due to the poor reporting. The inclusion criteria in all the studies were clearly described, which prevented the selection criteria variation. Although all the articles reported the age, genders, setting, and severity in detail, the patients in one study were relatively younger and the disease status of patients was severer in another study, which may slightly influence the generalization of this review. All the literature well reported the sample

**Table 1** - Quality of included studies.

Item	Yes	No	Unclear
1. Was the spectrum of patients representative of the patients who will receive the test in practice? (spectrum composition)	78	22	0
2. Were selection criteria clearly described? (selection criteria)	100	0	0
3. Is the reference standard likely to correctly classify the target condition? (reference standard)	100	0	0
4. Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the 2 tests? (disease progression bias)	56	11	33
5. Did the whole sample or a random selection of the sample receive verification using a reference standard of diagnosis? (partial verification)	78	22	0
6. Did patients receive the same reference standard regardless of the index test result? (differential verification)	100	0	0
7. Was the reference standard independent of the index test? (incorporation bias)	100	0	0
8. Was the execution of the index test described in sufficient detail to permit replication of the test? (index test execution)	89	0	11
9. Was the execution of the reference standard described in sufficient detail to permit its replication? (reference standard execution)	78	0	22
10. Were the index test results interpreted without knowledge of the results of the reference standard? (test review bias)	44	0	56
11. Were the reference standard results interpreted without knowledge of the results of the index test? (reference standard review bias)	56	0	44
12. Were the same clinical data available when test results were interpreted as would be available when the test is used in practice? (clinical review bias)	100	0	0
13. Were uninterpretable/intermediate test results reported? (un interpretable test results)	100	0	0
14. Were withdrawals from the study explained? (withdrawals)	100	0	0

Data are express as percentage

size, inducing material, and selection of sputum while only one article poorly described the quality criteria of the sputum sample, and the processing procedure. The test performance, and positive standard were not clearly described in 2 studies, which may affect the implementation of this test. No obvious concealing of uninterpretable/intermediate results was found.

The data show us the high-pooled sensitivity (0.80), specificity (0.90), and accuracy (AUC: 0.9208). However, the heterogeneity among studies was significant. The sample size was too small to undertake meta-regression for identifying the causes of heterogeneity, but some aspects may be suggested: (1) Threshold effect: the Spearman rank correlation indicated that the threshold effect in our meta-analysis was almost significant. In this situation, the best summary of the results of the studies may be a summary ROC curve rather than a single point. (2) Reference standard: the accuracy of reference standard can influence the accuracy of index test verification. In this review, we adopted the generally recognized reference standards, which included spirometry, airway responsiveness, and PEF variability. There is a slight difference in accuracy among the 3 methods. Moreover, the use of hypertonic saline in airway responsiveness test is less sensitive than methacholine as reported. (3) Index test: There is a possibility of diversity in the sputum processing procedure in different laboratories due to the lack of universal standard criteria for this technique, which may interfere with the comparability of various

studies. It was reported that the inhalation duration, the size of inhaled particles; the method of subject preparation and the processing method might influence the cell count in sputum. Most of the sputum induction procedures referred to the pin's design,<sup>5</sup> which allowed the comparability among different studies. However, as some studies did not give detailed descriptions, there may be some factors resulting in heterogeneity. (4) Spectrum of disease: there were some variations in patient age, and disease severity among different studies, which might have caused the heterogeneity. Finally, Study limitations follows: (1) We used the widely recognized QUADAS to conduct quality assessment, which has also been reported problems with coverage, ease of use, clarity of instructions and validity.<sup>6</sup> (2) We did not perform sub-group analysis, or meta-regression as there was not sufficient literature, so considerable heterogeneity remained unexplained. Then, the descriptive analysis of the heterogeneity was conducted instead. (3) The induced sputum technique also has some disadvantages that need to be noted: Some individuals may fail to produce enough sputum for the unpleasant feelings when inhaling hypertonic saline. Moreover, the handling and analysis of the sputum sample was time-consuming, which may also set obstacles for this method being used as a routine tool.

In summary, induced sputum eosinophil count has a certain value for the diagnosis of asthma, which indicates this non-invasive, and direct airway inflammation

monitoring method may play a significant role in the diagnostic study of asthma in the future. However, due to the high heterogeneity in the measures of diagnostic accuracy, this method alone cannot be currently recommended to replace conventional tests. Further high quality and larger sample size trials are required to confirm its accuracy.

Received 28th December 2009. Accepted 5th May 2010.

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