Diagnostic Value of p16 Methylation for Malignant Pleural Effusion
A Meta-analysis

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ABSTRACT

Objective: To evaluate the overall diagnostic performance of the p16 methylation for diagnosing malignant pleural effusion (MPE).

Methods: All published literature in English and Chinese were reviewed. Sensitivity, specificity, likelihood ratio and diagnostic odds ratio (DOR) were pooled by using random-effects model or fixed-effects model. Summary receiver operating characteristic (SROC) curve was used to evaluate the overall diagnostic value.

Results: Six studies were included with a total of 378 cases. The sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR) and DOR of p16 methylation in the diagnosis of MPE were 0.41 [95% confidence interval (CI) 0.35, 0.48], 0.97 [95% CI 0.93, 0.99], 9.57 [95% CI 4.53, 20.20], 0.61 [95% CI 0.45, 0.82] and 19.82 [95% CI 8.35, 47.04], respectively. The area under the curve (AUC) was 0.864.

Conclusion: Pleural p16 methylation test plays a useful role in the diagnosis of MPE.

Keywords: Malignant pleural effusion, meta-analysis, p16 methylation

Valor Diagnóstico de la Metilación p16 en el Derrame Pleural Maligno
Un Meta-análisis

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RESUMEN

Objetivo: Evaluar el rendimiento diagnóstico general de la metilación p16 para el diagnóstico del derrame pleural maligno (DPM).

Métodos: Se revisó toda la literatura publicada en inglés y chino. La sensibilidad, especificidad, razón de verosimilitud, y el odds-ratio diagnóstico (DOR) fueron agrupados mediante el modelo de efectos aleatorios o el modelo de efectos. La curva de las características operativas de resumen del receptor (SROC) fue usada para evaluar el valor diagnóstico general.

Resultados: Se incluyeron seis estudios con un total de 378 casos. La sensibilidad, especificidad, razón de verosimilitud positiva (PLR), razón de verosimilitud negativa (NLR) y el DOR de la metilación p16 en el diagnóstico de DPM, fueron 0.41 [95% intervalo de confianza (IC) 0.35 0.48], 0.97 [95% IC 0.93, 0.99], 9.57 [95% IC 4.53, 20.20], [95% IC 0.45, 0.82] 0.61 y 19.82 [95% IC 8.35, 47.04], respectivamente. El área bajo la curva (AUC) fue 0.864.

Conclusión: La prueba de metilación p16 pleural desempeña un papel útil en el diagnóstico del DPM.

Palabras claves: derrame pleural maligno, meta-análisis, metilación p16

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INTRODUCTION

Pleural effusion is commonly found in patients suffering from different kinds of diseases and sometimes it reveals the malignancy (1). Differentiation of malignant and benign pleural effusion is of great importance because of the significant difference in the treatment and prognosis (malignant pleural effusion (MPE) often means a poor prognosis and more aggressive treatment, such as an chemotherapy). Nevertheless, the establishment of an aetiological diagnosis of pleural effusion is often difficult and challenging. Current methods are far from perfect; they are either invasive or insufficient (2, 3). As a standard method for the diagnosis of MPE, classic cytology findings are positive in only 60% of cases on average, which can be increased slightly by performing closed pleural biopsy (4). As good as the diagnostic accuracy of the thoracoscopy is, this invasive procedure imposes physical and mental stress on the patients and may not be well accepted. Additionally, it is expensive and not available at all facilities (5).

Previous studies have investigated the diagnostic value of many tumour markers but only with limited success (6). Other factors like vascular endothelial growth factor [VEGF] (7) or telomerase have been studied but they are not as good as expected either (8). Therefore, some new effective and efficient pleural markers or methods should be identified to aid in the diagnosis of MPE.

P16 gene, an important tumour suppressor gene, plays an important role in regulating the cell cycle, and mutation in p16 increases the risk of developing a variety of cancers (9). Several epigenetic research has demonstrated that aberrant hypermethylation of p16 gene is highly associated with carcinogenesis (10). Recent publications have also found the presence of promoter hypermethylation of p16 genes in bodily fluids, including pleural fluid (11). It represents a noninvasive alternative or complementary test. Therefore, an increasing number of studies consider p16 gene methylation test to be an effective and efficient way to diagnose MPE.

Although the accuracy of p16 gene methylation detection for the diagnosis of MPE has been extensively studied, the precise diagnostic value remains unclear. This meta-analysis was performed to establish the overall accuracy of pleural p16 methylation detection in the diagnosis of MPE.

MATERIALS AND METHODS
Search strategy and study selection

Databases including Medline (using PubMed as the search engine), Embase, Ovid, Web of Science and Cochrane Database (up to March 2012) were searched to identify relevant studies. References of articles were also searched manually. The search terms were ''p16'', ''methylation'', ''malignant pleural effusion'', ''sensitivity and specificity'' and ''accuracy''. The languages were limited to English or Chinese. Articles such as conference abstracts, letters to the journal editors and so on were excluded because of the

limited data. A study was incorporated into the meta-analysis when both sensitivity and specificity of p16 for the diagnosis of MPE were provided. Two reviewers (M Li and SJ Guo) independently judged study eligibility when screening the citations. Disagreement was resolved by consensus.

Data extraction and quality assessment

The included literature was evaluated independently by two reviewers (M Li and SJ Guo). The reviewers were blinded to publication details such as the author, the country, the name of the journal, *etc.* Patient characteristics, test method, cut-off value, sensitivity, specificity and methodological quality were retrieved from the included studies. To assess the trial methodology, we used the QUADAS (quality assessment for studies of diagnostic accuracy) tool (12).

Statistical analysis

We used standard methods recommended for the diagnostic accuracy of meta-analyses (13). We computed the following indices of test accuracy for each study: sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR) and diagnostic odds ratio (DOR).

The sensitivity and specificity of each single test threshold identified for each study were used to plot a summary receiver operating characteristic [SROC] (14, 15). Spearman's rank correlation was performed as a test for threshold effect. Heterogeneity was tested using the I^2 with significance set at $p < 0.05$. The average sensitivity, specificity and other related measurements of the studies were calculated by using random-effects model or fixed-effects model (16, 17). If there were enough studies, subgroup analyses would be performed to explore potential between-study heterogeneity (18). Statistical softwares used to perform the analysis were: Stata, version 8.2 (Stata Corporation, College Station, TX), Meta-Test, version 0.6 (New England Medical Center, Boston, MA) and Meta-DiSc for Windows (XI Cochrane Colloquium, Barcelona, Spain).

RESULTS

After a search of the literature, 39 studies concerning p16 methylation and pleural effusion were selected. Among them, 33 research papers were excluded because they went beyond the scope of the present study or due to unrelated, duplicated or inappropriate data. The remaining six studies were available for the meta-analysis, with a total number of 378 patients.

Quality of the literature and study characteristics

In our meta-analysis, the average sample size of the included studies was 65 (range 31 to 81). Diagnosis of MPE was made based on cytological or pathological findings, which are ''gold standard''. Among the six studies, four studies reported blinded interpretation of p16 methylation assays independent of the reference standard and four studies were

designed as prospective study. All included studies had QUADAS scores ≥ 10. The clinical characteristics and other information are outlined in Tables 1 and 2.

0.41, 95% confidence interval (CI) 0.35, 0.48), while specificity ranged from 0.87 to 1.00 (pooled 0.97, 95% CI 0.93, 0.99). The PLR was 9.57 (95% CI 4.53, 20.02), NLR

Table 1: Summary of included studies

| Study/Year | Country | Patients no. | | Test results | | | | QUADAS score |
		MPE	non-MPE	TP	FP	FN	TN	
Malcolm (2005)	USA	24	7	13	0	11	7	12
Benlloch (2006)	Spain	53	34	31	0	22	34	12
Katayama (2007)	Japan	47	34	8	0	39	34	12
Chen (2007)	Chinese Taipei	31	39	8	1	23	38	11
Lee (2008)	South Korea	26	17	5	0	21	17	10
Liu (2010)	China	36	30	25	4	11	36	11

MPE = malignant pleural effusion, QUADAS = quality assessment for studies of diagnostic accuracy, TP/FP = true positive/false positive, FN/TN = false negative/true negative

Table 2: Characteristics of included studies

Study/Year	Country	Assay method	Reference standard	Cross-sectional design	Blinded design	Prospective
Malcolm (2005)	USA	MSP	Histology	Yes	Yes	Yes
Benlloch (2006)	Spain	MSP	Histology	Yes	Yes	Unknown
Katayama (2007)	Japan	MSP	Histology	Yes	Yes	Yes
Chen (2007)	Chinese Taipei	MSP	Histology	Unknown	Yes	Yes
Lee (2008)	South Korea	MSP	Histology	No	Unknown	No
Liu (2010)	China	MSP	Histology	Unknown	Unknown	Yes

MSP = methylation-specific polymerase chain reaction

Diagnostic accuracy

The forest plots of sensitivity and specificity of p16 methylation assays for the diagnosis of MPE are shown in Fig. 1. The sensitivity varied between 0.17 and 0.69 (pooled

was 0.6 (95% CI 0.45, 0.82) and DOR was 19.82 (95% CI 8.35, 47.04). The I^2 values of sensitivity, specificity, PLR, NLR, and DOR were 87.9%, 57.3%, 0.0%, 87.4% and 0.0%, respectively. In the pooled analysis of sensitivity, specificity and PLR, random-effects model was used because the heterogeneity across studies showed significant difference ($p < 0.05$, $I^2 > 50$%); when analysing the pooled DOR and NLP, fixed-effects model was performed because there was no significant heterogeneity between the studies ($p > 0.05$, $I^2 < 50$%).

As is shown in Fig. 2, SROC curve was used to summarize overall test performance, which shows the trade-off between sensitivity and specificity. Q-value is used as a global measure of test efficacy. It is the intersection point of the SROC curve with a diagonal line from the left upper corner to the right lower corner of the ROC space and corresponds to the highest common value of sensitivity and specificity for the test. In the meta-analysis, the maximum joint sensitivity and specificity of our study was 0.799 (the Q-value). The area under the curve (AUC) was 0.869, suggesting that the overall diagnostic value was not as high as expected.
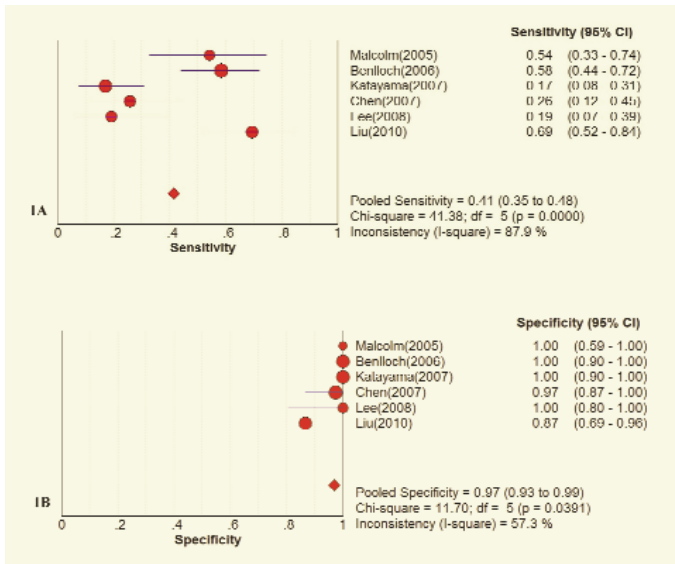

Fig. 1: Forest plots of sensitivity (A) and specificity (B) for p16 methylation assay for the diagnosis of malignant pleural effusion. The point estimates of sensitivity and specificity from each study are shown as solid circles. Error bars indicate 95% CI.
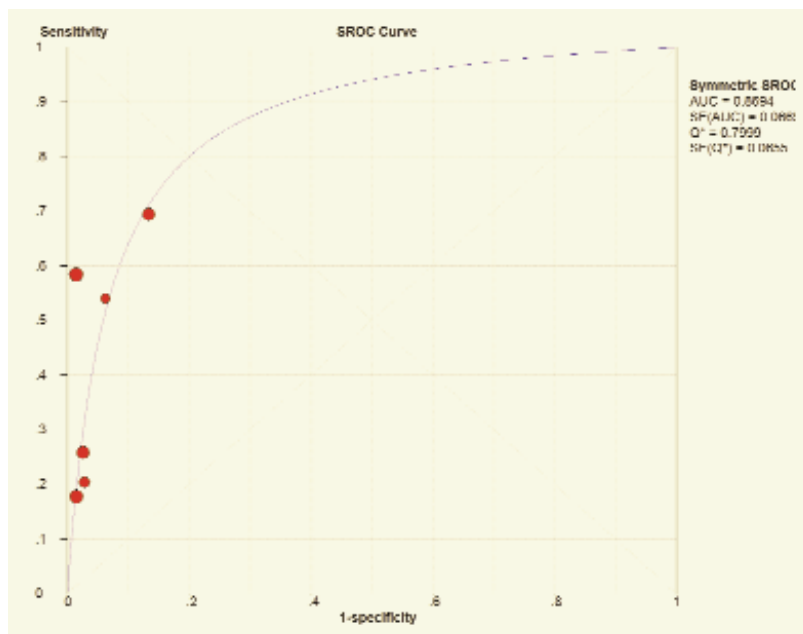
Fig. 2: Summary receiver operating characteristic curve (SROC) of pleural p16 methylation for the diagnosis of malignant pleural effusion. The size of each solid circle represents the sample size of each study. The regression SROC curve indicates the overall diagnostic accuracy.

DISCUSSION

The diagnosis of malignancy in pleural effusions continues to be a challenging clinical problem and traditional methods are limited (4). To find a new and effective diagnostic method for MPE will be of great importance. Detection of p16 methylation has been proposed as an alternative non-invasive way (10).

Our meta-analysis investigated the overall diagnostic performance of pleural effusion p16 methylation assay in the MPE with a specificity of 0.97 (95% CI 0.93, 0.99), indicating a promising role in confirming MPE. In contrast to the higher specificity, the sensitivity was only 0.41 (95% CI 0.35, 0.48) which is not sufficient to exclude non-MPE. Therefore, negative tests do not mean the absence of MPE, and patients with negative p16 methylation results still have a fairly high chance of having MPE. This trade-off has significant clinical implications.

To present a global summary of the test performance, the SROC curve was applied, which indicates the trade-off between sensitivity and specificity (25). The present meta-analysis based on SROC curve has shown the maximum joint sensitivity and specificity (Q value) was 0.799, and the AUC was 0.869, indicating that the overall accuracy was not as high as expected.

Diagnostic odds ratio, the ratio of the odds of positive test results in the diseased relative to the odds of positive test results in the non-diseased, is another indicator of test accuracy. The higher the DOR value, the better the discriminatory tests will perform (26). In our meta-analysis, we have found that the mean DOR was 19.82 (95% CI 8.35,

47.04), suggesting that the test seems to be a useful tool to aid in the diagnosis of MPE.

At the same time, both PLR and NLR are also presented as measures of diagnostic accuracy in our study (26) because likelihood ratios are considered to be more clinically meaningful (27, 28). A PLR value of 9.57 suggests that patients with MPE have an approximately 10-fold higher chance of being assay positive compared with patients without MPE, which is helpful in the clinical practices. On the other hand, a NLR value of 0.61 means that if the assay result was negative, the probability that this patient has MPE is approximately 61%, which is too high to rule out the MPE. In some cases of suspected malignancy and inconclusive initial findings, p16 methylation should be determined prior to the performance of invasive procedures, thereby optimizing the cost-benefit ratio. For patients, especially those who have clinical data suggesting an MPE but negative cytology on analysis of the pleural fluid, it is better to test their level of p16 methylation in pleural effusions. In this situation, a positive result of p16 methylation may indicate the need for biopsy.

It should be emphasized that our meta-analysis still has some limitations although comprehensive search strategy and data extraction were performed. Firstly, we did not include studies published in languages other than English or Chinese, nor did we include unpublished studies or abstracts from conferences, which may have led to publication bias. Secondly, only six studies with 378 cases were included, and the limited number of patients may have influenced the results of the meta-analysis. Because of that, we cannot use QUADAS

scores to perform the meta-regression to analyse the effect of study quality on the relative DOR of p16 methylation in the diagnosis of MPE. Effects of other covariates on DOR (cross-sectional design, consecutive or random sampling of patients, blind design, prospective data collection and assay method) were also not analysed for the same reason.

In conclusion, the current evidence suggests that p16 methylation assay in pleural effusion may improve the ability to get the diagnosis of malignancy right although it falls short of perfection. It is helpful to guide the inclusion of patients who may benefit from further invasive pathologic examination.

Conflict of interest
The authors declare that we have no conflict of interest.

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