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META-ANALYSIS

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Correlation Between *RASSF1A* Gene Promoter Hypermethylation in Serum or Sputum and Non-Small Cell Lung Cancer (NSCLC): A Meta-Analysis

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ADG 1,2,3,4 **Zhen Zhang***
BD 5 **Shaohui Yan***
AF 6 **Haijing Cui**
F 1,2,3,4 **Hui Chen**
ABG 7 **Jianming Liu**

1 Department of Lung Cancer, Tianjin Medical University Cancer Institute and Hospital, Tianjin, P.R. China
2 Tianjin Lung Cancer Center, Tianjin, P.R. China
3 Tianjin Key Laboratory of Cancer Prevention and Therapy, Tianjin's Clinical Research Center for Cancer, Tianjin, P.R. China
4 National Clinical Research Center for Cancer, Tianjin, P.R. China
5 Department of Thoracic Oncology, Fourth Hospital of Qinhuangdao, Qinhuangdao, Hebei, P.R. China
6 Department of Oncology, The First Hospital of Shijiazhuang, Shijiazhuang, Hebei, P.R. China
7 Department of Thoracic Surgery, Tangshan People's Hospital, Tangshan, Hebei, P.R. China

* Zhen Zhang and Shaohui Yan contributed equally to this work

Corresponding Author: Zhen Zhang, e-mail: zzsx@eyou.com
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Background: The aim of this study was to evaluate the efficacy of *RASSF1A* promoter hypermethylation of serum or sputum in diagnosis of non-small cell lung cancer (NSCLC) by pooling open published data.

Material/Methods: Open-published studies relevant to *RASSF1A* promoter hypermethylation and NSCLC diagnosis were screened through Medline, EMBASE, the Cochrane Library, Web of Science, Google Scholar, and CBM. Number of cases of true positive (tp), false positive (fp), false negative (fn), and true negative (tn) by *RASSF1A* gene promoter hypermethylation was extracted from each of the include original studies. The combined diagnostic sensitivity, specificity, and symmetric receiver operating characteristic curve (SROC) were calculated, as was the effect size.

Results: Twelve studies with 826 NSCLC and 598 controls were included in the present work. The combined sensitivity and specificity were 0.45 (95%CI: 0.41–0.48) (random effects) and 0.99(95%CI: 0.98–1.00) (fixed effects) respectively. The pooled positive likelihood ratio (+LR) and negative likelihood ratio (–LR) were 20.27 (9.64–42.61) and 0.53 (0.42–0.66), respectively, through the random effects model. The combined DOR was 46.63 (95%CI: 17.30–125.65) through the fixed effects model. The AUC of the SROC was 0.9989, calculated through Moses's model for *RASSF1A* promoter hypermethylation as a biomarker in diagnosis of NSCLC.

Conclusions: The low diagnostic sensitivity for *RASSF1A* gene promoter hypermethylation indicated that it is not suitable for NSCLC screening. However, the high specificity made it effective for NSCLC confirmation diagnosis, which could be used instead of pathological diagnosis.

MeSH Keywords: Lung Neoplasms • Meta-Analysis • Methylation

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Background

Publications have reported hypermethylation of the promoter region of cancer-suppressor genes is common in peripheral blood in early stage NSCLC or several years before clinical diagnosis. This indicated that detection of methylation status in body fluids such as serum or sputum can be an effective method for NSCLC screening or for early diagnosis. These cancer-suppressor genes include P16 [1], P53, RARβ [2], MGMT [3–5], and *RASSF1A*. *RASSF1A,* also known as ras association domain-containing protein 1 gene, is associated with the pathogenesis of a variety of cancers when there is loss or altered expression of this gene, and hypermethylation of the promoter region is known to cause *RASSF1A* gene loss of expression. RASSF1A is one of the most frequently epigenetically inactivated tumor-suppressor genes in carcinomas. As a component of the Ras/PI3K/AKT, Ras/RAF/MEK/ERK, and Hippo cancer pathways, inactivation of RASSF1A is an important factor contributing to pathogenesis and progression of malignant carcinomas. Hypermethylation of *RASSF1A* gene in serum or sputum has been widely reported [6–8]. However, its diagnostic performance as a biomarker is unclear because of the small sample sizes of previous related publications. Therefore, we performed a meta-analysis by pooling all relevant published studies to assess its performance in clinical diagnosis.

Material and Methods

Databases electronic searching

Authors Zhang Zhen and Dong Lixin independently searched PubMed, EMBASE, OVID, Cochrane Library, CNKI, and CBM databases to find published studies on *RASSF1A* gene promoter hypermethylation and NSCLC. The MESH search terms were: non-small cell lung cancer; NSCLC; hypermethylation; *RASSF1A*; and Ras association domain-containing protein 1. The relevant publications identified from the above databases were initially screened by EndNote and excluded duplicate publications and data. The papers were independently reviewed by 2 reviewers for tile, abstract, and full text to find suitable publications. The publications searching and inclusion procedures are shown in Figure 1.

Publication inclusion and data extraction

Publications initially identified through searching the databases were further evaluated and underwent data extraction. The study inclusion criteria were: (1) case-control or cohort studies related to *RASSF1A* gene promoter hypermethylation and NSCLC; (2) All patients included in each individual study were diagnosed as having NSCLC by cytology or pathology confirmation; (3) Methylation of the *RASSF1A* gene promoter region was assessed by MSP; and (4) Studies published in English or Chinese. The publication exclusion criteria were: (1) Studies published in languages other than English or Chinese; (2) Small cell lung cancer; and (3) *RASSF1A* gene promoter hypermethylation detection in cancer tissue not serum or sputum.

Data extraction

Two reviewers independently reviewed the included studies and general information, including names of first and corresponding authors, year published, methylation detection methods, and specimens used for detection. Data on true-positive, false-positive, false-negative, and true-negative for DNA methylation as a biomarker in diagnosis of NSCLC were extracted from all included articles and were crossed-checked by 2 reviewers.

Statistical analysis

Before pooling the effect sizes of sensitivity, specificity, positive likelihood ratio (+LR), negative likelihood ratio (-LR), and diagnostic odds ratio (DOR), the heterogeneity across the 12 publications was first evaluated with the I^2 test. Fixed- (without statistical heterogeneity) or random-effects models (with statistical heterogeneity) were used for pooling the data. Sensitivity was calculated as tp/(tp+fp) and specificity as tn/(tn+fp). $P<0.05$ was regarded as indicating a statistically significant difference.

Results

Characteristics of the 12 included publications

Initially, 93 studies relevant to *RASSF1A* promoter hypermethylation as a biomarker in diagnosis of NSCLC were identified. After further screening, 81 publications were excluded because they were duplicate publications, case reports, or reviews, or if they did not contain sufficient data to calculation the diagnostic performance. Finally, 12 studies with 826 NSCLC cases and 598 controls were included (Figure 1). All of the 12 included studies used MSP assay as the hypermethylation detection method. Five publications examined the *RASSF1A* gene promoter hypermethylation status in sputum and other 7 studies used serum. The hypermethylation rate in NSCLC ranged from 16.7% to 85.7%, with the median of 44.5%. However, the hypermethylation rate of *RASSF1A* gene promoter ranged from 0% to 3.3%, a median of 0% in healthy control groups. The main characteristic of the12 publications are shown in Table 1.

Combined diagnostic sensitivity and specificity

The chi-square test and I^2 test demonstrated significant heterogeneity in sensitivity (I^2=92.6%), (Figure 2A). The pooled sensitivity was 0.45 (95%CI: 0.41–0.48) in the random-effects

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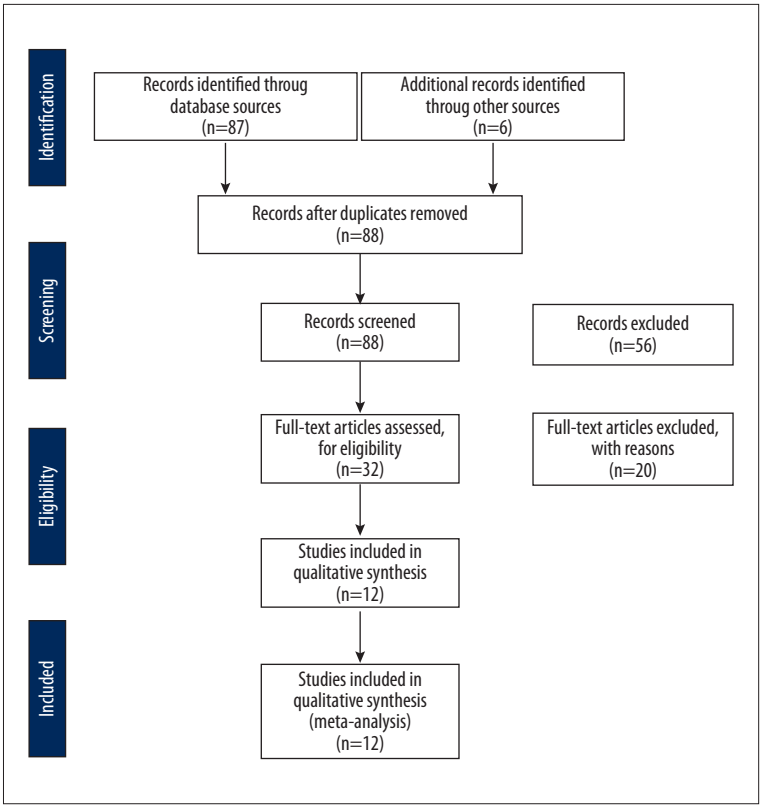

Figure 1. Relevant publications electronic searching process.

Table 1. General characteristics of the 12 included publications.

First author	Year	Case		Control		Methods	Specimen
		+	–	+	–		
Kang C [6]	2011	8	29	0	24	MSP	Sputum
Ye G [7]	2005	5	25	0	27	MSP	Sputum
Zhou F [8]	2014	56	74	1	129	MSP	Serum
Sun N [9]	2012	20	100	4	116	MSP	Sputum
Shan C [10]	2008	16	19	0	15	MSP	Sputum
Zhai X [11]	2014	22	20	0	40	MSP	Serum
Zhao P [12]	2014	28	34	0	46	MSP	Serum
Wen L [2]	2012	45	11	0	52	MSP	Serum
Peng Z [13]	2010	52	30	0	25	MSP	Sputum
Liu G [14]	2007	42	54	0	32	MSP	Serum
Li W [15]	2012	48	8	0	52	MSP	Serum
Wang Y [16]	2007	27	53	0	35	MSP	Serum

model and the combined diagnostic specificity was 0.99 (95%CI: 0.98–1.00) in the fixed-effects mode (Figure 2B).

Combined diagnostic +LR and –LR

Because of significant heterogeneity, the +LR and –LR were pooled in the random-effects model. The combined +LR and –LR were 20.27 (9.64–42.61) and 0.53 (0.42–0.66), respectively (Figure 3).

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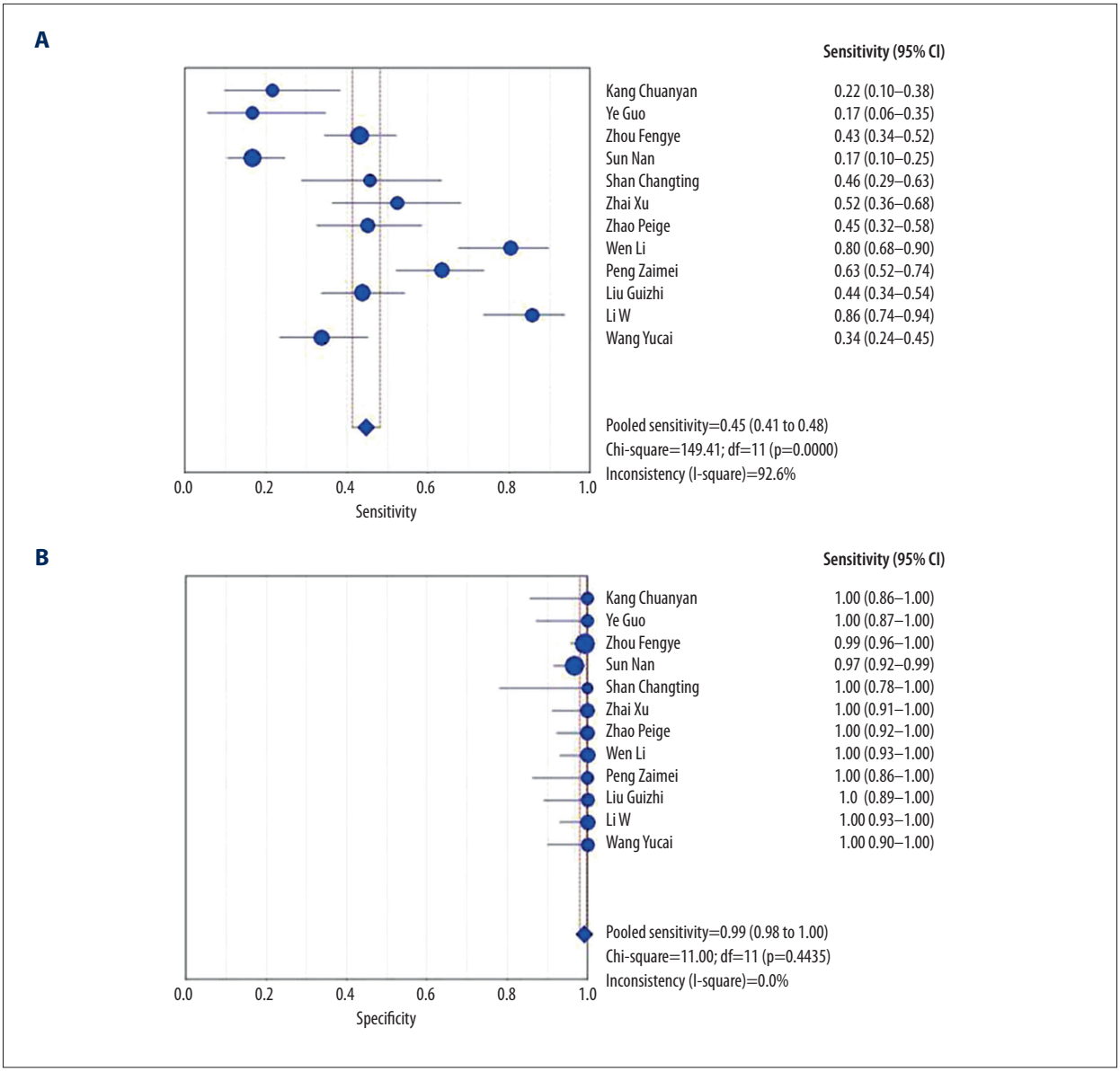

Figure 2. Forest plot of diagnostic sensitivity and specificity (**A:** diagnostic sensitivity; **B:** diagnostic specificity).

Combined diagnostic odds ratio

The combined DOR was 46.63 (95%CI: 17.30–125.65) in the fixed-effects model (Figure 4).

Combined ROC curve and AUC

The AUC was 0.9989 calculated through Moses's model for *RASSF1A* gene promoter hypermethylation as a biomarker in diagnosis of NSCLC (Figure 5).

Publication bias evaluation

Publication bias was evaluated by Egger's line regression, test and no significant publication bias was found (p>0.05).

Discussion

In China, lung cancer is the leading cause of malignant carcinoma-related mortality for males and the second leading cause of cancer deaths for females [17,18]. It was reported that in 2018, more than 150 000 lung cancer-related deaths occurred in China [19]. Lung cancer has become a serious threaten to health worldwide. Non-small cell lung cancer (NSCLC), accounting for

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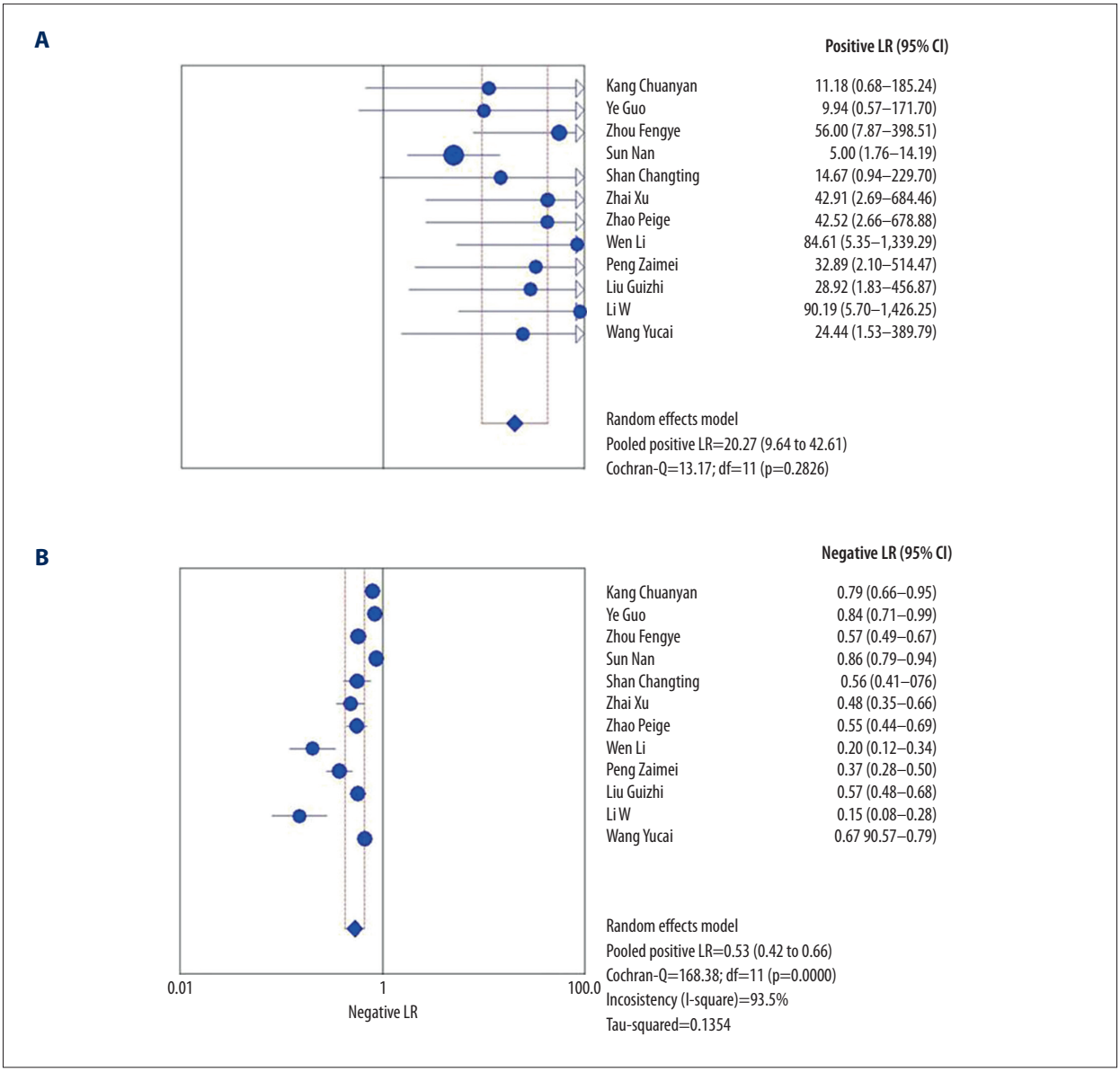

Figure 3. Forest plot of diagnostic +LR and –LR for RASSF1A gene promoter hypermethylation as biomarker in diagnosis of NSCLC (**A:** +LR; **B:** –LR).

80% of all lung cancer, is the most common malignant tumor according to clinical diagnosis. Generally, the prognosis of NSCLC is poor, with a total 5-year survival rate of 15.8%. Disease recurrence and remote metastasis are the main causes of treatment failure and death for NSCLC [20]. Therefore, early diagnosis or screening of high-risk subjects are key for improving the general prognosis of NSCLC. At present, the most commonly used lung cancer screening or early diagnosis methods are chest radiograph examination, sputum cytology, and chest CT. However, the results of previously studies were not satisfactory because of low sensitivity or low specificity.

Hypermethylation of its CpG-island promoter region of tumor-suppressor genes has been widely discussed as a biomarker for NSCLC diagnosis or screening [21–23]. Konecny [24] evaluated the value of SHOX2 methylation in serum samples for diagnosis of lung cancer. They found that methylation of the SHOX2 gene is a reliable marker of lung malignancies. Gu et al. performed a meta-analysis evaluating the correlation between P16 gene promoter methylation and lung cancer risk, reporting that the P16 gene promoter hypermethylation rate was significantly different between cancer and autologous normal control tissues, indicating P16 gene promoter hypermethylation is potential biomarker for non-small cell lung cancer diagnosis.

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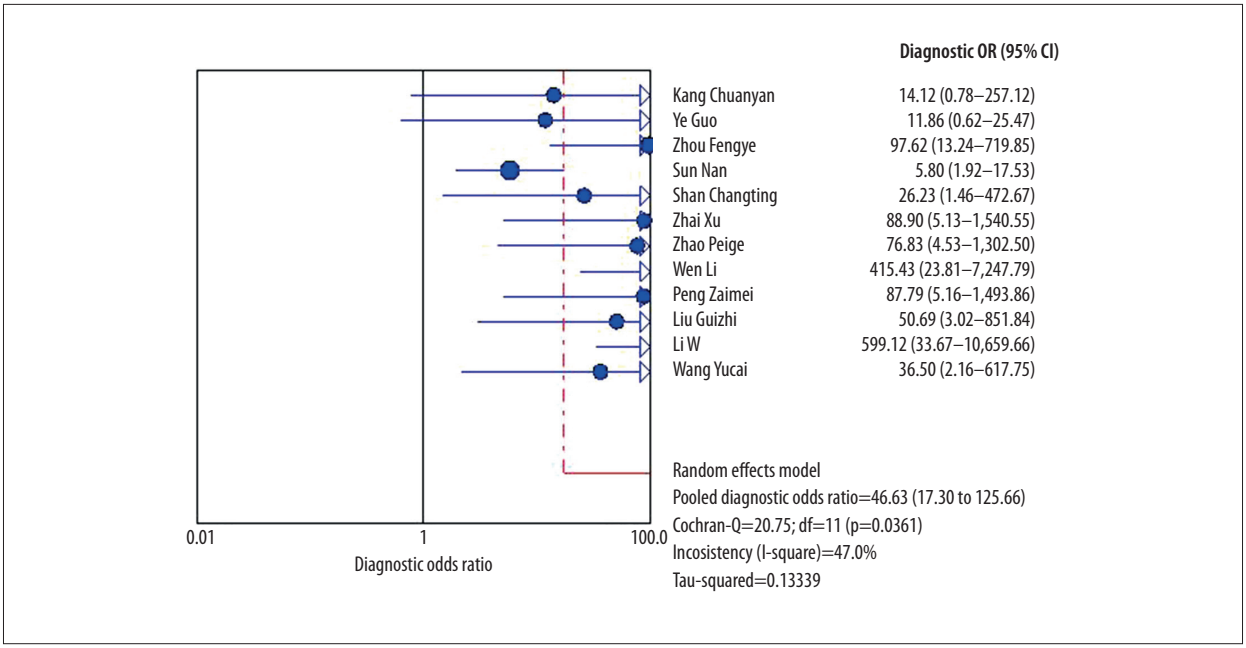

Figure 4. Forest plot of diagnostic odds ratio for RASSF1A gene promoter hypermethylation as biomarker in diagnosis of NSCLC.

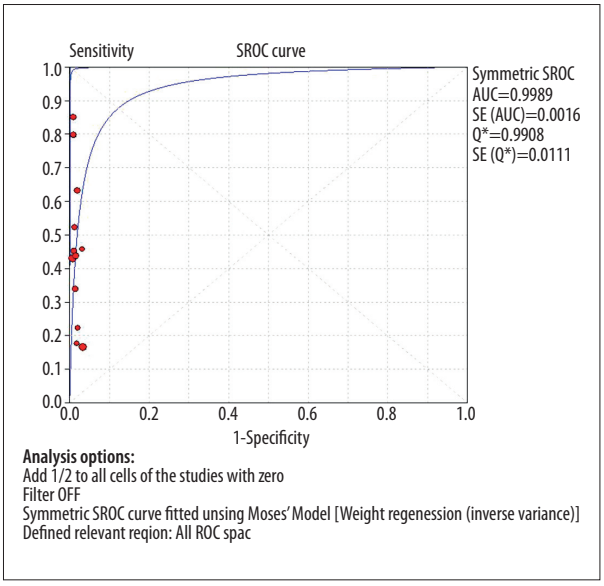

Figure 5. ROC curve of RASSF1A gene promoter hypermethylation as a biomarker in diagnosis of NSCLC.

The *RASSF1A* gene is located on chromosome 3 in humans, encoding a protein similar to RAS. *RASSF1A,* also known as ras association domain-containing protein 1 gene, is associated with the pathogenesis of a variety of cancers when there is loss or altered expression of this gene. Promoter region hypermethylation is a known cause of *RASSF1A* gene loss of expression. It has been demonstrated that loss or altered expression of *RASSF1A* is associated with pathogenesis of cervical cancer and lung cancer. It was reported [25] that *RASSF1A* was silenced in cancer cells when the promoter region was hypermethylated. The methylation promoter region of *RASSF1A* gene was also widely evaluated in body fluid of NSCLC patients. Huang [26] and Wang [27] performed 2 meta-analyses relevant to *RASSF1A* hypermethylation and lung cancer, respectively. Huang evaluated the hypermethylation status of cancer tissue versus normal lung tissue, showing a correlation between *RASSF1A* hypermethylation and lung cancer risk. Wang work evaluated the prognostic value of RASSF1A promoter hypermethylation in non-small cell lung carcinoma, showing that hypermethylation of RASSF1A is an independent prognostic factor for poor prognosis of NSCLC. However, there has been no previous meta-analysis of its diagnostic performance as a biomarker for NSCLC diagnosis. Therefore, we searched the relevant databases and pooled the relevant studies in order to evaluate the clinical efficacy of *RASSF1A* gene promoter hypermethylation as a biomarker for NSCLC diagnosis. We found that the low diagnostic sensitivity of *RASSF1A* gene promoter hypermethylation indicated it is not suitable for use in NSCLC screening. However, the high specificity made it effective for NSCLC confirmation diagnosis, which can be used instead of pathological diagnosis to some extent.

Conclusions

The low diagnostic sensitivity of *RASSF1A* gene promoter hypermethylation indicates it was not suitable for NSCLC screening. Our study has certain limitations. Firstly, significant heterogeneity was found in the effect size of sensitivity, +LR, –LR, and DOR. Secondly, the general quality of the include studies was

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poor, especially for Chinese publications. Therefore, large-scale prospective diagnostic trials are needed to further investigate the clinical performance of *RASSF1A* promoter hypermethylation biomarker for NSCLC diagnosis.

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Conflict of interest

None.

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