


## ORIGINAL ARTICLE

# ***P16*<sup>INK4a</sup> gene promoter methylation as a biomarker for the diagnosis of non-small cell lung cancer: An updated meta-analysis**

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**Abstract**

**Background:** This meta-analysis was conducted to investigate the diagnostic performance of *P16*<sup>INK4a</sup> gene promoter methylation as a biomarker of non-small cell lung cancer (NSCLC).

**Methods:** Two reviewers independently searched the Web of Science, PubMed, Cochrane, Embase, China National Knowledge Infrastructure, and Chinese Biomedical Literature databases. Publications relevant to *P16*<sup>INK4a</sup> gene promoter methylation in serum or bronchoalveolar fluid/sputum were screened and included in this meta-analysis. Pooled diagnostic sensitivity, specificity, and symmetric receiver operating characteristic curve were calculated.

**Results:** Twenty-six publications with 1768 lung cancer cases and 1323 controls were included. The pooled sensitivity, specificity, positive and negative likelihood ratios, and diagnostic odds ratio were 0.46 (95% confidence interval [CI] 0.43–0.48), 0.90 (95% CI 0.88–0.91), 6.33 (95% CI 3.89–10.30), 0.57 (95% CI 0.50–0.65) and 10.72 (95% CI 6.94–16.56), respectively, for *P16*<sup>INK4a</sup> gene promoter methylation as a biomarker for the diagnosis of NSCLC. The area under the symmetric receiver operating characteristic curve was 0.75 with a standard error of 0.004. No publication bias was detected via line regression test ( $t = 0.95$ ;  $P = 0.35$ ) and Begg's funnel plot.

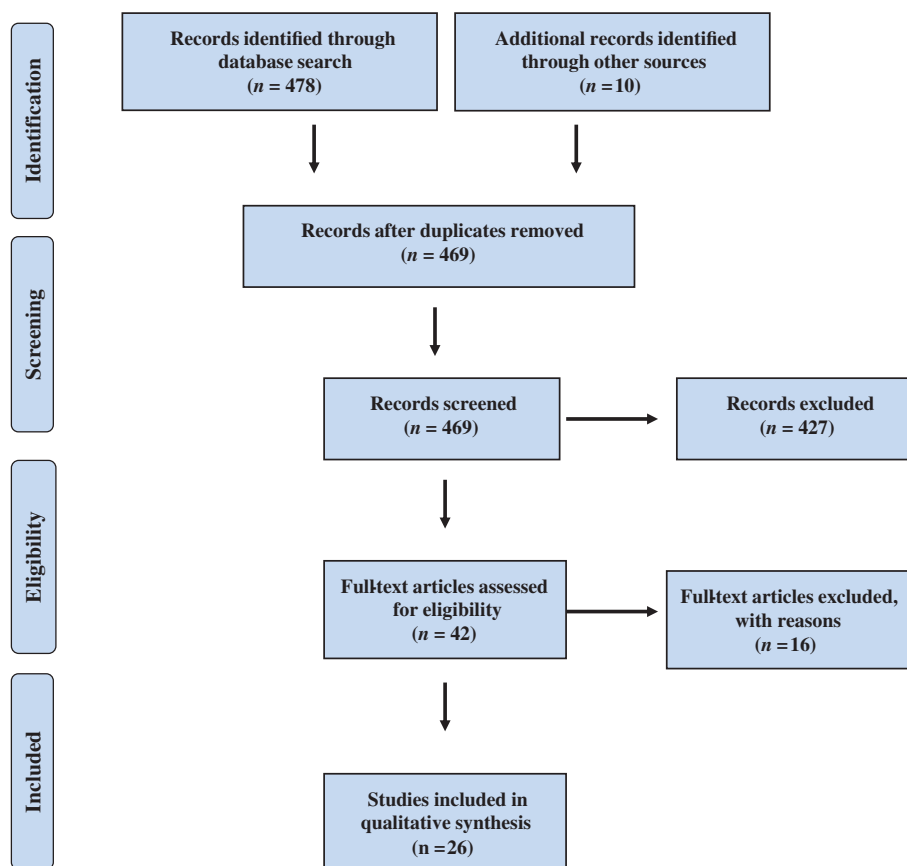
**Conclusion:** *P16*<sup>INK4a</sup> gene promoter methylation detection in serum or bronchoalveolar fluid/sputum may be a potential biomarker for NSCLC diagnosis; however, the sensitivity was relatively low, which is not suitable for NSCLC screening.

**Introduction**

Non-small cell lung cancer (NSCLC) is one of the most common clinically diagnosed malignant carcinomas. It is estimated that 234 030 new cases and 154 050 deaths from NSCLC will occur in the United States in 2018.<sup>1</sup> The general prognosis of NSCLC is poor, particularly in advanced-stage patients, with an extremely low five-year survival rate. One of the major reasons for this poor prognosis is the lack of effective lung cancer screening or early diagnostic methods.<sup>2</sup> Several studies have evaluated lung cancer screening methods such as X-ray,<sup>3,4</sup> sputum cytology, and

chest computed tomography (CT);<sup>5</sup> however, such methods yield low sensitivity or specificity and thus are not adequate to diagnose NSCLC at an early stage.

Promoter methylation of tumor suppressor genes is frequently detected in cancer tissue and body fluid in malignant carcinomas such as lung,<sup>6,7</sup> colorectal,<sup>8</sup> and esophageal cancers. Previous studies have reported that methylation of the *P16*<sup>INK4a</sup> gene promoter is common in lung cancer. The methylation frequency of *P16*<sup>INK4a</sup> in serum or bronchoalveolar fluid (BAF)/sputum in lung cancer patients has been widely discussed; however, the exact diagnostic performance of *P16*<sup>INK4a</sup> as a biomarker for

**Figure 1** Publication search process.

NSCLC remains inconclusive. Therefore, we conducted this updated meta-analysis to further evaluate the diagnostic performance of *P16<sup>INK4a</sup>* as a biomarker for NSCLC.

## Methods

### Electronic database search strategy

Two reviewers independently searched the Web of Science, PubMed, Cochrane, Embase, China National Knowledge Infrastructure, and Chinese Biomedical Literature databases for studies relevant to *P16<sup>INK4a</sup>* gene promoter methylation in serum or BAF/sputum. The following keywords were used: non-small cell lung cancer; non-small cell carcinoma, NSCLC, *P16*, *P16<sup>INK4a</sup>*; cyclin-dependent kinase inhibitor 2A, CDKN2A; CDK4 inhibitor; multiple tumor suppressor 1; TP16; methylation; and hypermethylation. Relevant studies were identified and duplicated publications or data were excluded. The title and abstract were then reviewed to locate relevant studies. All potentially suitable studies were reviewed in full-text and all references of included publications were further screened to identify additional relevant publications. The publication search process is demonstrated in Figure 1.

### Inclusion and exclusion criteria

The identified studies were further reviewed to assess whether the inclusion criteria were fulfilled: (i) diagnostic studies relevant to *P16<sup>INK4a</sup>* promoter methylation and NSCLC; (ii) NSCLC diagnosis confirmed by pathology or cytology; (iii) *P16<sup>INK4a</sup>* gene promoter methylation was detected by methylation-specific PCR (MSP), real-time MSP (RT-MSP), or quantitative MSP (q-MSP); (iv) *P16<sup>INK4a</sup>* gene methylation status in serum or BAF/sputum in NSCLC and control subjects was available for each included study. The exclusion criteria were: (i) case reports or literature reviews; (ii) *P16<sup>INK4a</sup>* gene methylation status detected in other specimens, not in serum or BAF/sputum; (iii) studies published in languages other than English or Chinese; and (iv) insufficient data to calculate sensitivity and specificity.

### Statistical analysis

The diagnostic sensitivity, specificity, and symmetric receiver operating characteristic (SROC) curve were pooled by fixed or random effects method according to the statistical heterogeneity across the included studies. Diagnostic sensitivity and specificity were calculated using the following equations: sensitivity = true positive/(true positive +

false negative); specificity = true negative/(true negative + false positive). Publication bias was evaluated by Egger’s line regression test and Begger’s funnel plot. *P* < 0.05 was considered to indicate significant statistical difference.

## Results

### Study characteristics

Initially, 488 relevant publications were identified. After reviewing the title, abstract, and full text, 26 studies relevant to *P16<sup>INK4a</sup>* gene promoter methylation as a biomarker for the diagnosis of NSCLC were included for quantitative analysis.<sup>9–34</sup> Sixteen publications evaluated *P16<sup>INK4a</sup>* gene promoter methylation in serum and 10 in BAF/sputum. The general characteristics of the 26 studies are shown in Table 1.

### Pooled sensitivity and specificity

Because of significant statistical heterogeneity, the diagnostic sensitivity and specificity were pooled using the random effects method. The pooled sensitivity and specificity were

0.46 (95% confidence interval [CI] 0.43–0.48) (Fig 2) and 0.90 (95% CI 0.88–0.91) (Fig 3), respectively, for *P16<sup>INK4a</sup>* gene promoter methylation as a biomarker for the diagnosis of NSCLC.

### Pooled positive and negative likelihood ratios

The diagnostic positive likelihood ratio (+LR) and negative likelihood ratio (–LR) were also pooled by random effect method because of significant heterogeneity. The pooled +LR and –LR were 6.33 (95% CI 3.89–10.30) (Fig 4) and 0.57 (95% CI 0.50–0.65) (Fig 5), respectively, for *P16<sup>INK4a</sup>* gene promoter methylation as a biomarker for the diagnosis of NSCLC.

### Pooled diagnostic odds ratio

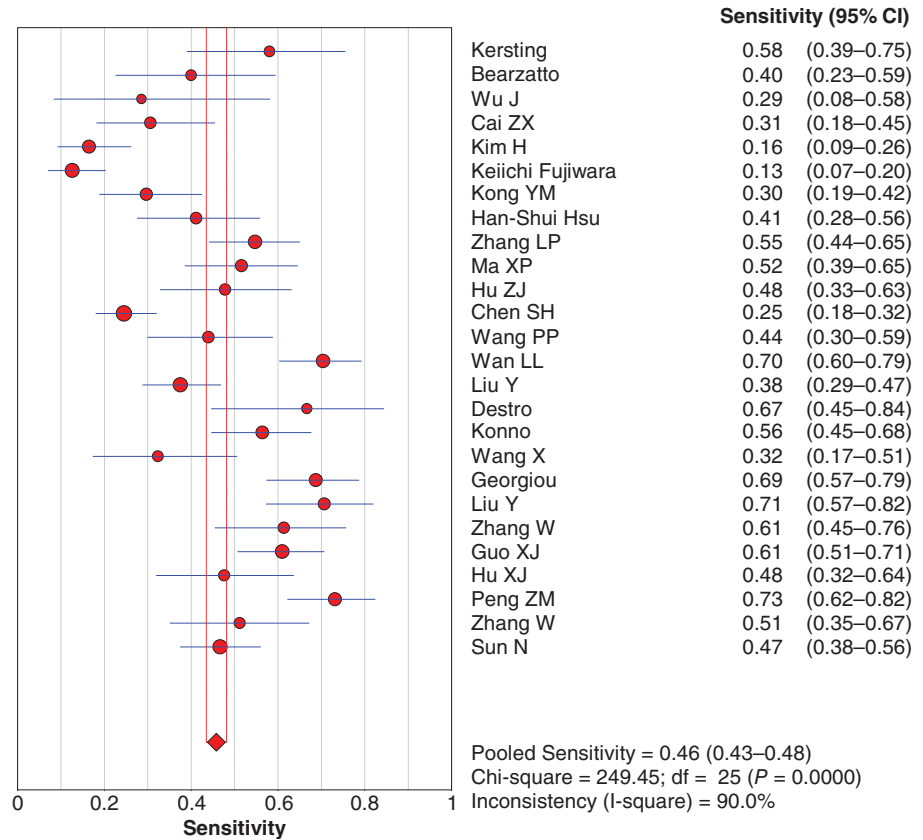
The pooled diagnostic odds ratio (DOR) was 10.72 (95% CI 6.94–16.56) for *P16<sup>INK4a</sup>* gene promoter methylation as a biomarker for the diagnosis of NSCLC (Fig 6).

**Table 1** Study characteristics

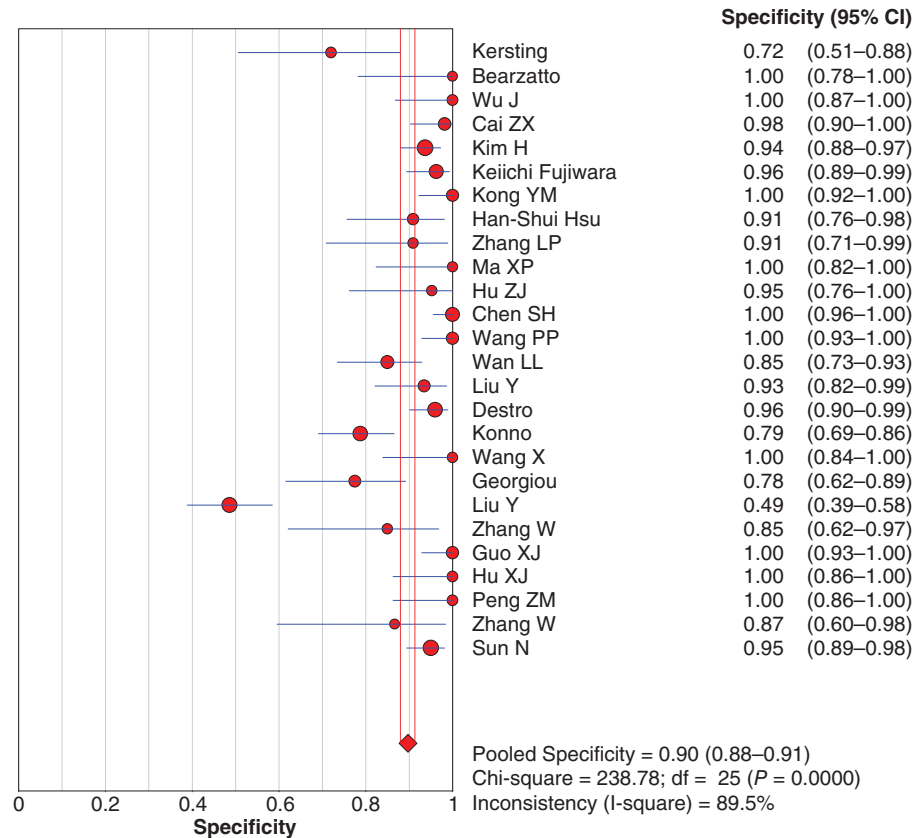
Study	Year	Area	NSCLC	Control	Distribution				Specimen
					Tp	Fp	Fn	Tn	
Kersting <i>et al.</i> <sup>9</sup>	2000	US	31	25	18	7	13	18	Serum
Bearzatto <i>et al.</i> <sup>10</sup>	2002	Italy	30	15	12	0	18	15	Serum
Wu <i>et al.</i> <sup>11</sup>	2002	China	14	26	4	0	10	26	Serum
Cai <i>et al.</i> <sup>12</sup>	2003	China	49	55	15	1	34	54	Serum
Kim <i>et al.</i> <sup>13</sup>	2004	Korea	85	127	14	8	71	119	Serum
Fujiwara <i>et al.</i> <sup>14</sup>	2005	US	111	80	14	3	97	77	Serum
Kong <i>et al.</i> <sup>15</sup>	2007	China	64	46	19	0	45	46	Serum
Hsu <i>et al.</i> <sup>16</sup>	2007		51	33	21	3	30	30	Serum
Zhang <i>et al.</i> <sup>17</sup>	2008	China	95	22	52	2	43	20	Serum
Ma <i>et al.</i> <sup>18</sup>	2009	China	62	19	32	0	30	19	Serum
Hu <i>et al.</i> <sup>19</sup>	2009	China	46	21	22	1	24	20	Serum
Chen <i>et al.</i> <sup>20</sup>	2010	China	159	81	39	0	120	81	Serum
Wang <i>et al.</i> <sup>21</sup>	2016	China	50	50	22	0	28	50	Serum
Wan <i>et al.</i> <sup>22</sup>	2017	China	98	60	69	9	29	51	Serum
Liu <i>et al.</i> <sup>23</sup>	2017	China	120	46	45	3	75	43	Serum
Destro <i>et al.</i> <sup>24</sup>	2004	Italy	24	100	16	4	8	96	BAF/sputum
Konno <i>et al.</i> <sup>25</sup>	2004	Japan	78	94	44	20	34	74	BAF/sputum
Wang <i>et al.</i> <sup>26</sup>	2004	China	34	21	11	0	23	21	BAF/sputum
Georgiou <i>et al.</i> <sup>27</sup>	2007	Greece	80	40	55	9	25	31	BAF/sputum
Liu <i>et al.</i> <sup>28</sup>	2008	China	58	107	41	55	17	52	BAF/sputum
Zhang <i>et al.</i> <sup>29</sup>	2004	China	44	20	27	3	17	17	BAF/sputum
Guo <i>et al.</i> <sup>30</sup>	2008	China	100	50	61	0	39	50	BAF/sputum
Hu <i>et al.</i> <sup>31</sup>	2009	China	42	25	20	0	22	25	BAF/sputum
Peng <i>et al.</i> <sup>32</sup>	2010	China	82	25	60	0	22	25	BAF/sputum
Zhang <i>et al.</i> <sup>33</sup>	2012	China	41	15	21	2	20	13	BAF/sputum
Sun <i>et al.</i> <sup>34</sup>	2012	China	120	120	56	6	64	114	BAF/sputum

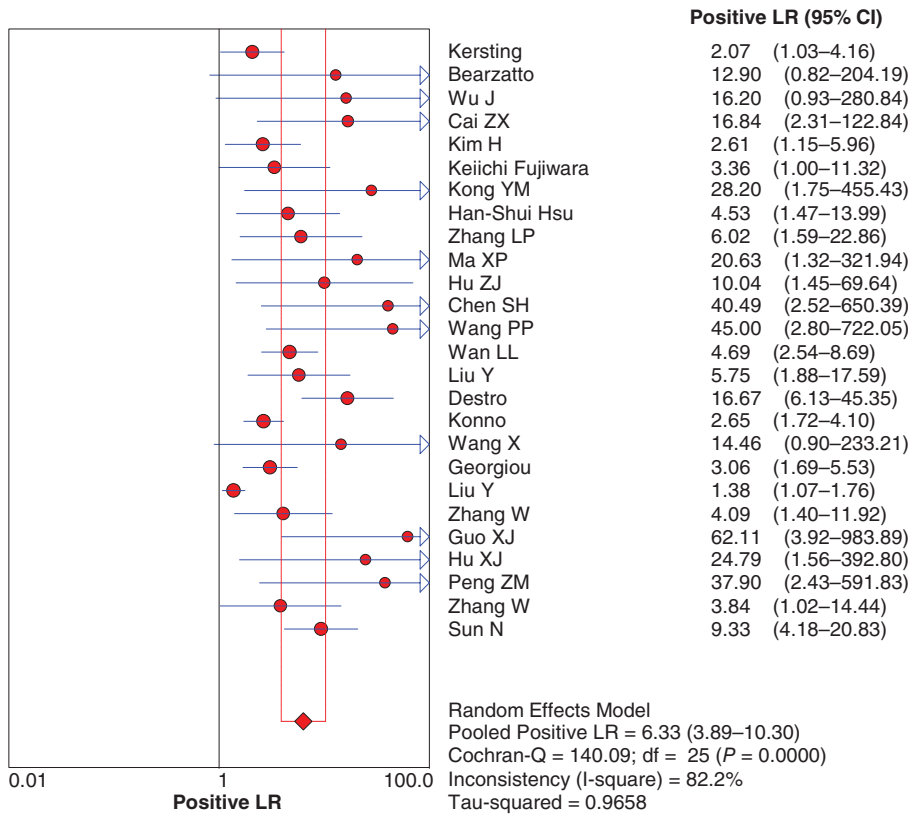
BAF, bronchoalveolar fluid; fn, false negative; fp, false positive; NSCLC, non-small cell lung cancer; tn, true negative; tp, true positive; US, United States.

**Figure 2** Forest plot of the sensitivity of *P16<sup>INK4a</sup>* gene promoter methylation as a biomarker for the diagnosis of non-small cell lung cancer. CI, confidence interval.

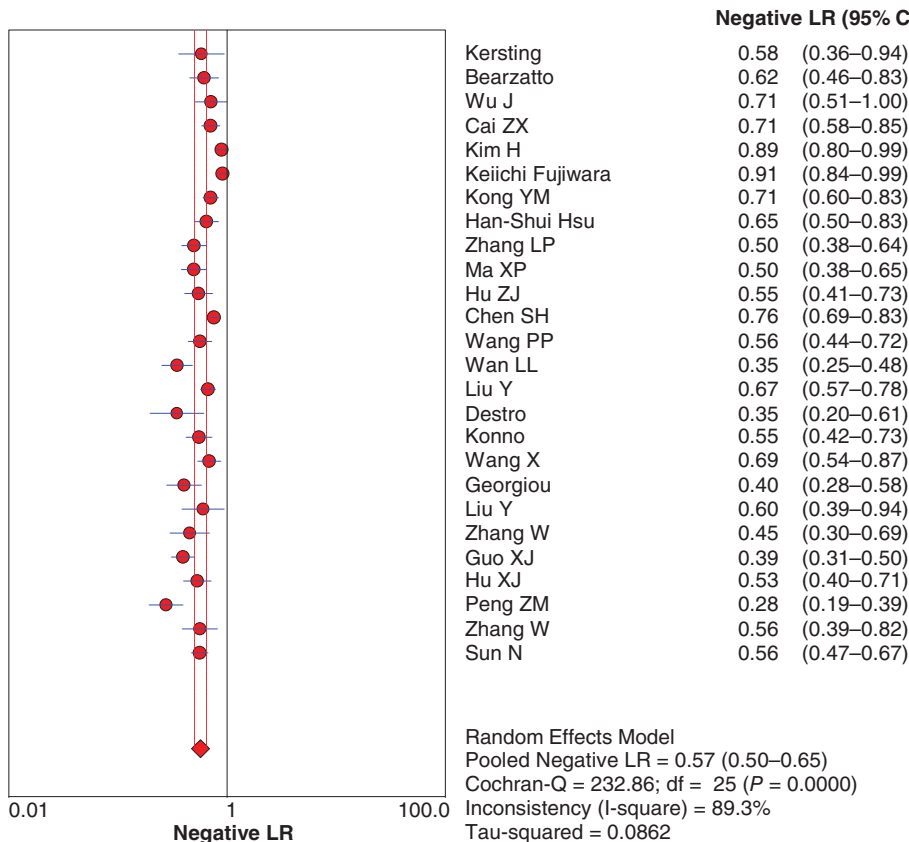


**Figure 3** Forest plot for specificity of *P16<sup>INK4a</sup>* gene promoter methylation as a biomarker for the diagnosis of non-small cell lung cancer. CI, confidence interval.



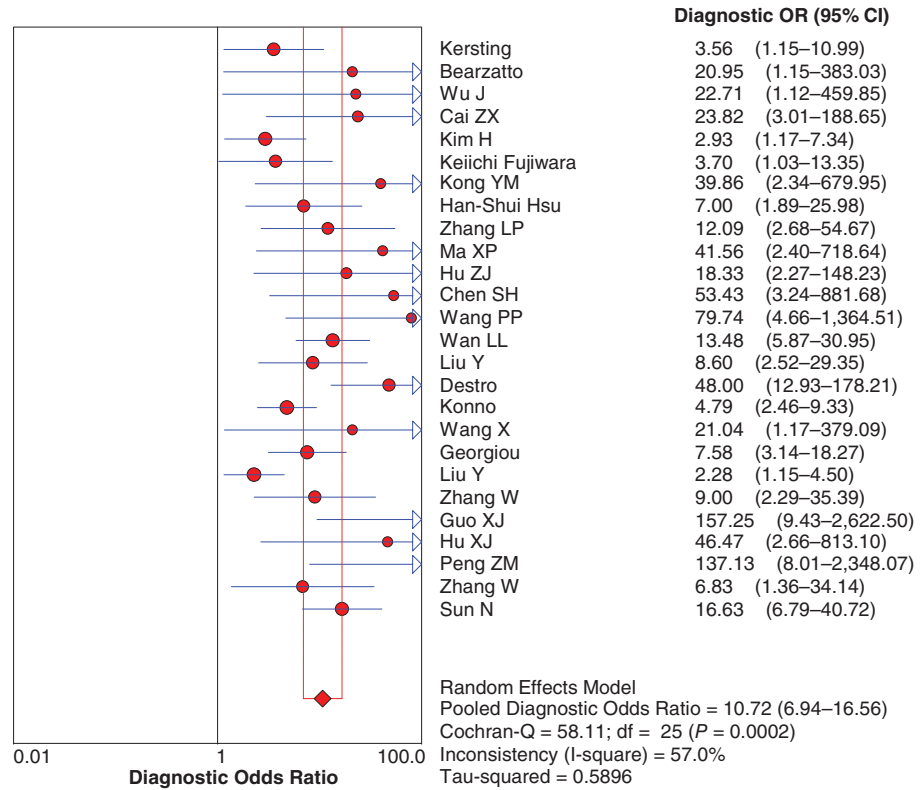


**Figure 4** Forest plot of the negative likelihood ratio (LR). CI, confidence interval.

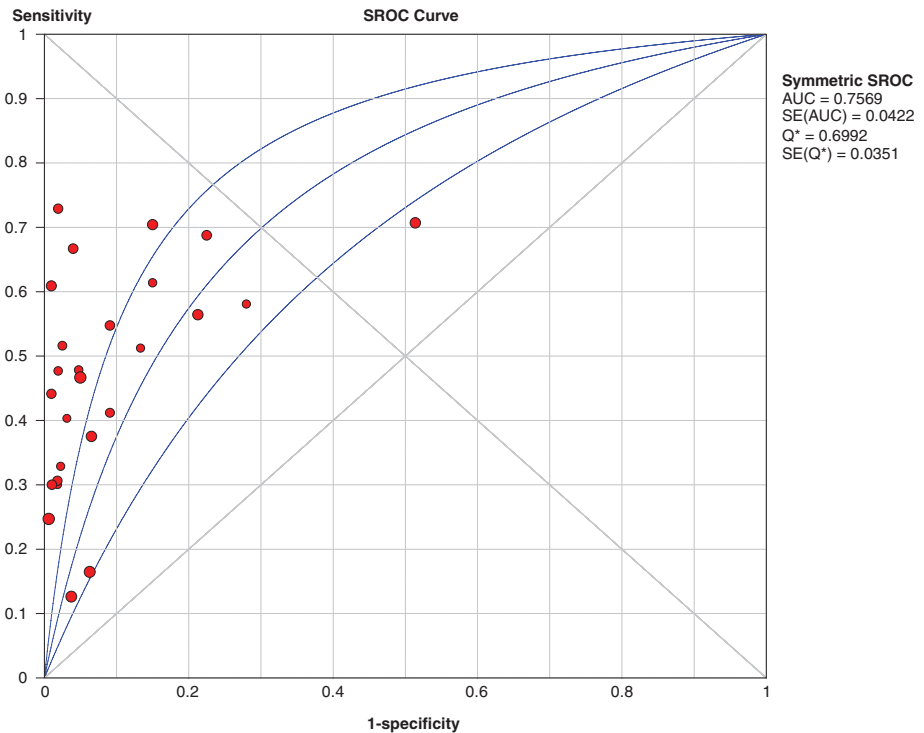


**Figure 5** Forest plot of the positive likelihood ratio (LR). CI, confidence interval.

**Figure 6** Forest plot of the diagnostic odds ratio (OR).



**Figure 7** The pooled symmetric receiver operating characteristic (SROC) curve for *P16* gene promoter methylation for the diagnosis of non-small cell lung cancer. AUC, area under the curve; SE, standard error.



**Table 2** Diagnostic performance in subgroup analysis

Diagnostic index	Serum			BAF/sputum		
	Point estimate	95% CI	I <sup>2</sup>	Point estimate	95% CI	I <sup>2</sup>
Sensitivity	0.37	0.34–0.40	89.8%	0.59	0.55–0.62	71.7%
Specificity	0.95	0.93–0.96	72.5%	0.84	0.81–0.87	93.1%
+LR	5.46	3.43–8.69	39.6%	6.15	2.81–13.46	89.6%
–LR	0.64	0.57–0.73	86.9%	0.49	0.51–0.57	64.2%
DOR	9.41	5.67–15.62	32.5%	12.32	5.86–25.94	73.0%
AUC	0.78	0.74–0.82	—	0.71	0.69–0.77	—

+LR, positive likelihood ratio; –LR, negative likelihood ratio; AUC, area under the curve; BAF, bronchoalveolar fluid; CI, confidence interval; DOR, diagnostic odds ratio.

**Symmetric receiver operating characteristic curve**

The area under the SROC curve was 0.75 with a standard error of 0.004 for *P16<sup>INK4a</sup>* gene promoter methylation as a biomarker for the diagnosis of lung cancer (Fig 7).

**Subgroup analysis**

We also conducted subgroup analysis, detecting *P16<sup>INK4a</sup>* gene promoter methylation in serum or BAF/sputum. The pooled diagnostic performances in serum and BAF/sputum are shown in Table 2.

**Evaluation of publication bias**

Publication bias was evaluated by Egger’s line regression test and Begg’s funnel plot. No publication bias was detected by line regression test ( $t = 0.95$ ;  $P = 0.35$ ) or Begg’s funnel plot (Fig 8).

**Discussion**

In China, lung cancer is the most commonly diagnosed malignant carcinoma and the leading cause of cancer mortality in both men and women, particularly in men aged  $\geq 75$  years. As most NSCLC patients are only diagnosed at locally advanced-stage or after remote metastasis has occurred, they are ineligible for surgery. Prognosis is poor, with an extremely low five-year survival rate, because of the lack of effective methods for lung cancer screening or early diagnosis.

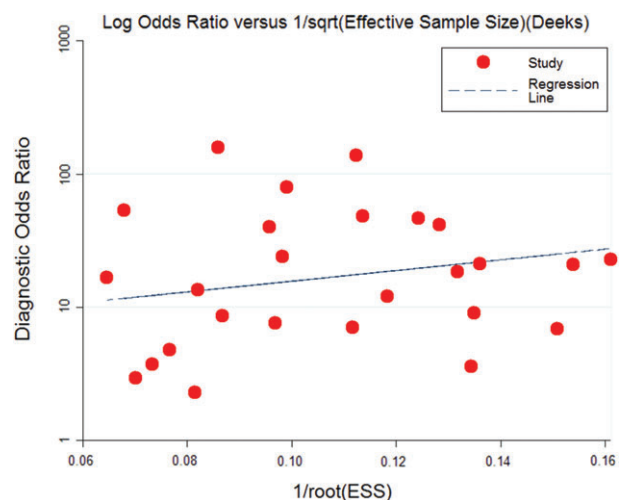
Promoter methylation of tumor suppressor genes is common in body fluid and can be used as a lung cancer diagnosis method or biomarker. Several studies have evaluated its clinical application with acceptable diagnostic performance and high specificity.<sup>35–38</sup>

The *P16<sup>INK4a</sup>* gene, also known as the *CDKN2A* gene, is located on chromosome 9 (9p21.3) and plays an important role in regulating the cell cycle.<sup>39</sup> The promoter region of *P16<sup>ink4a</sup>* is usually hypermethylated in cancer cells of

NSCLC patients. Studies have found that *P16<sup>INK4a</sup>* methylation can be detected in body fluid, such as serum and sputum,<sup>23,24</sup> indicating that detection of *P16<sup>INK4a</sup>* methylation status may be used as an important tool for lung cancer diagnosis, screening, or the monitoring of recurrence.

Two previous meta-analyses evaluated *P16<sup>INK4a</sup>* methylation in serum and sputum as a biomarker for lung cancer diagnosis and concluded that detection of *P16<sup>INK4a</sup>* promoter methylation via these methods was a useful tool for lung cancer diagnosis.<sup>40,41</sup> However, several recently published relevant studies were not included in these meta-analyses. Therefore, we performed an updated meta-analysis, including recently published relevant publications and further evaluated the clinical value of *P16<sup>INK4a</sup>* methylation as a biomarker for NSCLC diagnosis. We confirmed that *P16<sup>INK4a</sup>* gene promoter methylation detection in serum or BAF/sputum may be a potential biomarker for NSCLC diagnosis; however, the sensitivity was relatively low and was thus not suitable for NSCLC screening.

Although our results indicate that *P16<sup>INK4a</sup>* gene promoter methylation represents a promising method for



**Figure 8** A funnel plot of publication evaluation. ESS, effective sample size. (●) Study and (---) Regression lines.

NSCLC diagnosis, there was significant statistical heterogeneity in the process of data merging, which inevitably affected our results. Furthermore, the sample sizes of the included studies were relatively small, which can reduce the statistical power of each included study. Large-scale prospective diagnostic tests should be conducted by multiple health centers to further evaluate the clinical application value of P16<sup>INK4a</sup> gene promoter methylation as an NSCLC diagnostic method.

## Disclosure

No authors report any conflict of interest.

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