



Systematic Review

Diagnostic Value of *CDO1* Promoter Methylation in Lung Cancer via Liquid Biopsy: A Systematic Review and Meta-Analysis

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Abstract

Objective: To evaluate cysteine dioxygenase 1 (*CDO1*) gene promoter methylation in circulating tumor DNA as a biomarker for the early diagnosis of lung cancer. **Methods:** Data up to June 5, 2025, across electronic databases (PubMed, Embase, Web of Science, and the China National Knowledge Infrastructure) were searched. The quality assessment tool for diagnostic accuracy studies-2 (QUADAS-2) checklist was used to assess the risk of bias in the incorporated studies. A random-effects model was employed to generate summary statistics for diagnostic accuracy, which included pooled sensitivity and specificity estimates, the diagnostic odds ratio (DOR), and a summary receiver operating characteristic curve. An exploration of heterogeneity sources was undertaken using meta-regression, followed by a sensitivity analysis to test the consistency of the results. Finally, Deek's funnel plot was generated to estimate publication bias, and the clinical feasibility was evaluated using Fagan's nomogram. **Results:** Seven relevant studies were included in this meta-analysis. No major concerns regarding the quality risk of the included studies were observed. The pooled diagnostic sensitivity, specificity, and DOR values of the *CDO1* promoter methylation for lung cancer were 0.63 (95% CI: 0.60–0.67), 0.78 (95% CI: 0.74–0.82), and 5.96 (95% CI: 4.06–8.74), respectively, and the area under curve was 0.7423. Statistical heterogeneity was observed in sensitivity ($I^2 = 73\%$, $p < 0.1$), specificity ($I^2 = 79.5\%$, $p < 0.1$), and DOR ($I^2 = 42.9\%$, $p < 0.1$); however, variables such as the region, sample source, sample size, and detection method did not significantly affect heterogeneity ($p > 0.05$). The results were robust as the DOR was not overly influenced by the deletion of any single study. No publication bias was observed in this study ($p = 0.74$). Additionally, under a pre-test probability of 20%, the positive post-test probability of *CDO1* promoter methylation in lung cancer was predicted to be 42%. PROSPERO CRD420251131665, <https://www.crd.york.ac.uk/PROSPERO/view/CRD420251131665>. **Conclusion:** The detection of *CDO1* promoter methylation in biofluids represents a promising tool for the early diagnosis of lung cancer. Future studies should focus on improving detection methodologies and investigating combinational strategies with high accuracy.

Keywords: lung cancer; circulating tumor DNA; methylation; cysteine dioxygenase 1

1. Introduction

Despite decades of advancement in treatment, lung and bronchus cancer remains the principal cause of cancer mortality [1–3]. Improving the efficacy of the early detection of lung cancer is critical for improving the clinical outcomes of patients. Although screening with low-dose computed tomography (LDCT) leads to a significant relative reduction (20.0%) in lung cancer-related death (95% CI, 6.8 to 26.7; $p = 0.004$), the failure to accurately distinguish malignant and benign lung nodules through LCDT remains a major challenge [4,5]. Moreover, tissue biopsy continues to be the gold standard for tumor diagnosis owing to its remarkable consistency and high accuracy of results; however, it is limited by its invasive properties, tumor spatial heterogeneity, and inability to achieve continuous monitoring [6]. In recent years, the development of liquid biopsy has revolutionized the early detection, recurrence prediction, and treatment outcome monitoring of lung cancer [6–11]. Compared with tissue biopsy, liquid biopsy presents a non-invasive as well as quick and continuous detection method that focuses on detecting molecular

markers such as circulating tumor cells, circulating tumor DNA (ctDNA), and tumor-derived extracellular vesicles in plasma and other biofluids including urine, bronchial washing fluid, ascites, pleural fluid, and sputum [10,12]. The genetic information carried by ctDNA broadly indicates that in the tumor tissue, including somatic variants, methylation status, and microsatellite instability [13]. Furthermore, owing to the relative short fragment length and short half-life, ctDNA are less affected by intratumor heterogeneity and serve as a real-time tumor biomarker, allowing for dynamic monitoring of tumor progression [6].

Aberrant DNA methylation is an early tumorigenic event associated with poor therapeutic outcomes, making it a viable target for both early cancer detection and prognostic assessment [14,15]. For example, the detection of Septin9 DNA methylation in plasma (Epi proColon) is used for the early screening of colorectal cancer, while its sensitivity and specificity still need to be improved [16,17]. Although methylation markers including short stature homeobox gene 2 (SHOX2) and Ras association domain family 1A (RASSF1A) have shown promising diagnostic value for



lung cancer, their accuracy is still under clinical evaluation [18]. Therefore, other methylation markers need to be uncovered. The tumor suppressor gene cysteine dioxygenase type 1 (*CDO1*) has garnered considerable attention in recent years as a potential ctDNA methylation marker across human cancers [19–21]. *CDO1* is a crucial enzyme catalyzing cysteine oxidation, causing the reduction of antioxidant glutathione and synthesis of taurine. Consequently, *CDO1* is fundamentally involved in facilitating both ferroptosis and apoptotic cell death in malignant cells [22]. Genetic and epigenetic alterations, particularly promoter methylation, have been identified across cancers [22]. Early in 2012, *CDO1* was identified as one of the hypermethylated and downregulated genes in human lung squamous cell carcinoma via whole-genome analysis [23]. *CDO1* promoter methylation, either alone or in combination with other targets, demonstrates promising diagnostic prospects in lung cancer [24–26].

In light of the correlation observed for *CDO1* promoter methylation in lung cancer, we aim to explore the feasibility of non-invasive *CDO1* promoter methylation detection for the early diagnosis of lung cancer by systematically reviewing the published data.

2. Data and Methods

2.1 Data Sources and Search Strategy

We conducted this meta-analysis in accordance with preferred reporting items for systematic review and meta-analysis of diagnostic test accuracy studies (PRISMA-DTA) [27]. The research protocol has been registered on PROSPERO with registration number CRD420251131665, <https://www.crd.york.ac.uk/PROSPERO/view/CRD420251131665>. Two researchers independently searched online databases containing data up to June 5, 2025, including PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), Embase (<https://www.embase.com>), Web of Science (<https://www.webofscience.com/>), and the China National Knowledge Infrastructure (CNKI) (<https://www.cnki.net/>). The key terms used in the search strategies were (“*CDO1*” OR “*Cysteine Dioxygenase 1*”) AND (“*Lung Neoplasm*” OR “*Lung Cancer*” OR “*Pulmonary Neoplasm*”) (Supplementary Table 1).

2.2 Inclusion and Exclusion Criteria

The inclusion criteria were as follows:

- (1) Population (P): The subjects of the included studies should be patients diagnosed with lung cancer.
- (2) Intervention (I): The performance of *CDO1* ctDNA methylation for the early detection of lung cancer should have been evaluated.
- (3) Comparison (C) and Outcome (O): Studies should include adequate data to obtain the true positive (TP), false positive (FP), true negative (TN), and false negative (FN) values for analysis of the sensitivity and specificity of *CDO1* promoter methylation in lung cancer screening.

(4) Study design (S): Eligible studies for inclusion incorporate both retrospective and prospective researches.

Studies were excluded based on the following criteria:

- (1) P: Studies based on other types of cancer.
- (2) I: Studies that did not assess *CDO1* promoter methylation for lung cancer detection, along with review articles, meta-analyses, patents, and reports based solely on cell lines or animal models. Studies using samples acquired from tissue were also excluded.
- (3) C and O: Publications lacking sufficient data for analysis.

2.3 Quality Assessment

We employed the Quality Assessment Tool for Diagnostic Accuracy Studies-2 (QUADAS-2) tool to assess the risk of bias in the studies included in our analysis [28]. The evaluation of bias risk was conducted independently by two reviewers following four key points including patient selection, index test, reference standard, and flow and timing. The results were presented as “high risk”, “low risk”, or “unclear risk”. Finally, the two reviewers revised the results and resolved the conflicts. Review Manager 5.3 (The Cochrane Collaboration, Oxford, UK) was utilized for visualization using the assessment.

2.4 Data Extraction

Following a full-text review, we extracted the following data: (1) the first author’s last name and year of publication were extracted as study ID; (2) countries where the studies were carried out; (3) the sample size; (4) the TP, FP, FN, and TN were directly extracted or calculated; (5) tumor-node-metastasis (TNM) stages and pathological subtypes of patients; (6) sample sources, such as plasma, urine, and sputum; and (7) detection methods. Two referees reviewed the studies independently, and any disagreements that arose between the referees were settled via a consensus-based discussion.

2.5 Statistical Methods

The data were analyzed using Meta-DiSc 1.4 (Clinical Biostatistics Unit, Hospital Ramón y Cajal, Madrid, Spain) and Stata 17.0 (StataCorp, College Station, TX, USA) statistical software. The diagnostic accuracy of *CDO1* promoter methylation for lung cancer was pooled using sensitivity, specificity, diagnostic odd ratio (DOR), and the summary receiver operating characteristic curve (sROC). We defined high heterogeneity as an I^2 value greater than 50% or a p -value below 0.10, calculating it with Chi-squared and I^2 statistics. Data with high heterogeneity were pooled using the random-effect model; otherwise, the fixed-effects model was used. Meta-regression analysis was performed to explore the sources of heterogeneity and sensitivity analysis was conducted using the leave-one-out method. The assessment for publication bias was performed by utilizing Deek’s funnel plot, and the feasibility of *CDO1* methyla-

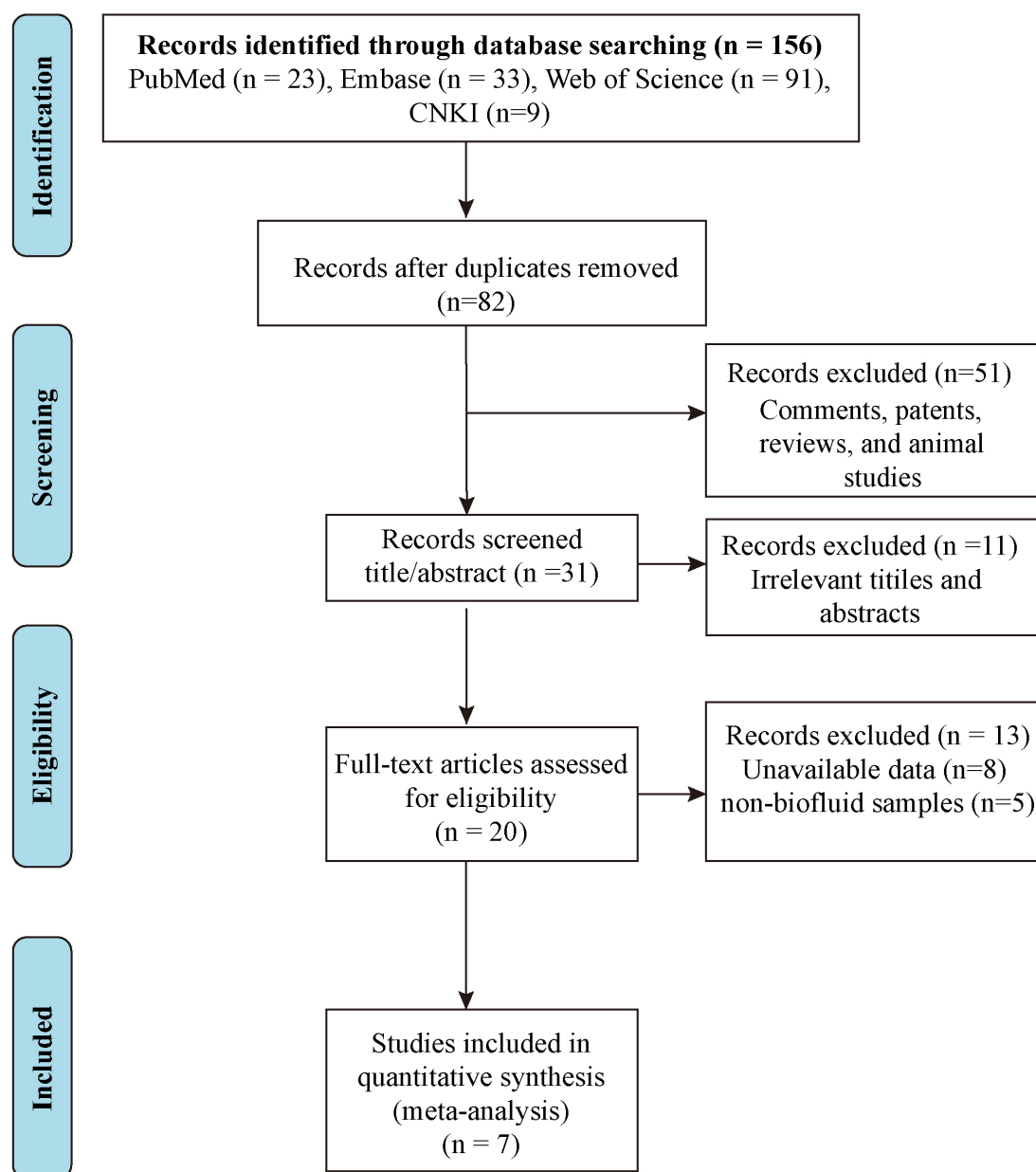


Fig. 1. Flow chart of literature retrieval according to inclusion and exclusion criteria. CNKI, China National Knowledge Infrastructure.

tion clinical application was evaluated using Fagan's nomogram. We defined a p -value of less than 0.05 as indicative of statistical significance.

3. Results

3.1 Study Selection and Characteristics of the Included Studies

In total, 156 studies pertaining to *CDO1* gene methylation in the context of lung cancer were initially identified from the electronic database. Following the exclusion of 74 duplicate records and 51 other types of publications (including comments, patents, reviews, and animal studies), the titles and abstracts of 31 publications were reviewed.

Subsequently, 11 studies were excluded for failing to satisfy the predetermined inclusion criteria and the full texts of 20 articles were assessed for eligibility. Finally, after eliminating 13 studies for which data were unavailable or which were based on other sample sources, 7 relevant publications were selected for meta-analysis [26,29–34] (Fig. 1). The main characteristics of the seven studies are presented in Table 1 (Ref. [26,29–34]). In five studies, plasma was used for the detection of *CDO1* promoter methylation, and urine and sputum were applied in the other three studies. With the exception of the investigations by Wang *et al.* [34] and Chen *et al.* [33], which employed quantitative polymerase chain reaction (qPCR), the remaining five studies

	Risk of Bias				Applicability Concerns		
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard
Chen 2017	+	−	+	+	+	+	+
Chen 2020	+	+	+	+	+	+	+
Hulbert 2017	?	+	+	+	+	+	+
Liu B 2020	?	?	+	+	+	+	+
Park 2024	+	+	+	+	+	+	+
Wang 2020	+	−	+	+	+	+	+
Wever 2022	?	+	+	+	+	+	+

− High	? Unclear	+ Low
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Fig. 2. Risk of bias and applicability concerns of included studies (QUADAS-2). QUADAS-2, Quality Assessment Tool for Diagnostic Accuracy Studies-2.

utilized quantitative methylation-specific polymerase chain reaction (qMSP) to assess *CDO1* promoter methylation status in biofluids [26,29–32].

3.2 Quality Assessment and Statistical Heterogeneity

Quality assessment was performed using QUADAS-2 tool and the results are shown in Fig. 2. The risk of bias of the included studies was evaluated through patient selection, index test, reference standard, and flow and timing. Regarding the patient selection, three studies were assessed as “unclear” owing to lack of information on whether they enrolled consecutive patients and whether they avoided a case-control design. In the assessment of the index test, two studies received a “high risk” rating because their results were interpreted with prior knowledge of the reference standard’s outcomes. The risk of bias was deemed “unclear” for one study, as the publication omitted details on whether the index test was interpreted independently of the reference standard. For the reference standard and flow and timing, all studies were assessed as “low risk”. In summary, no major concerns regarding quality risk of the included studies were observed.

Chi-squared and I-squared statistic algorithms showed significant statistical heterogeneity in sensitivity ($I^2 = 73\%$, $p < 0.1$), specificity ($I^2 = 79.5\%$, $p < 0.1$), and DOR ($I^2 = 42.9\%$, $p < 0.1$) (Fig. 3). The heterogeneity may be due to the differences in the TNM stages, ethnic backgrounds, and pathological types of patients. The heterogeneity may also result from variation in the sample sources, sample size, and detection methods. Therefore, we utilized a random-effects model for all statistical analyses to obtain reliable results.

3.3 Meta-Analysis

The pooled diagnostic performance of *CDO1* promoter methylation for the early identification of lung cancer demonstrated a sensitivity of 0.63 (95% CI: 0.60–0.67) and a specificity of 0.78 (95% CI: 0.74–0.82) (Fig. 3). The pooled DOR was 5.96 (95% CI: 4.06–8.74) (Fig. 3), suggesting that a population bearing *CDO1* promoter methylation had 5.96 times higher detection risk than the general population. In addition, the area under curve (AUC) was 0.7423 (Fig. 3), indicating that *CDO1* promoter methylation in biofluids had prediction potential for lung cancer.

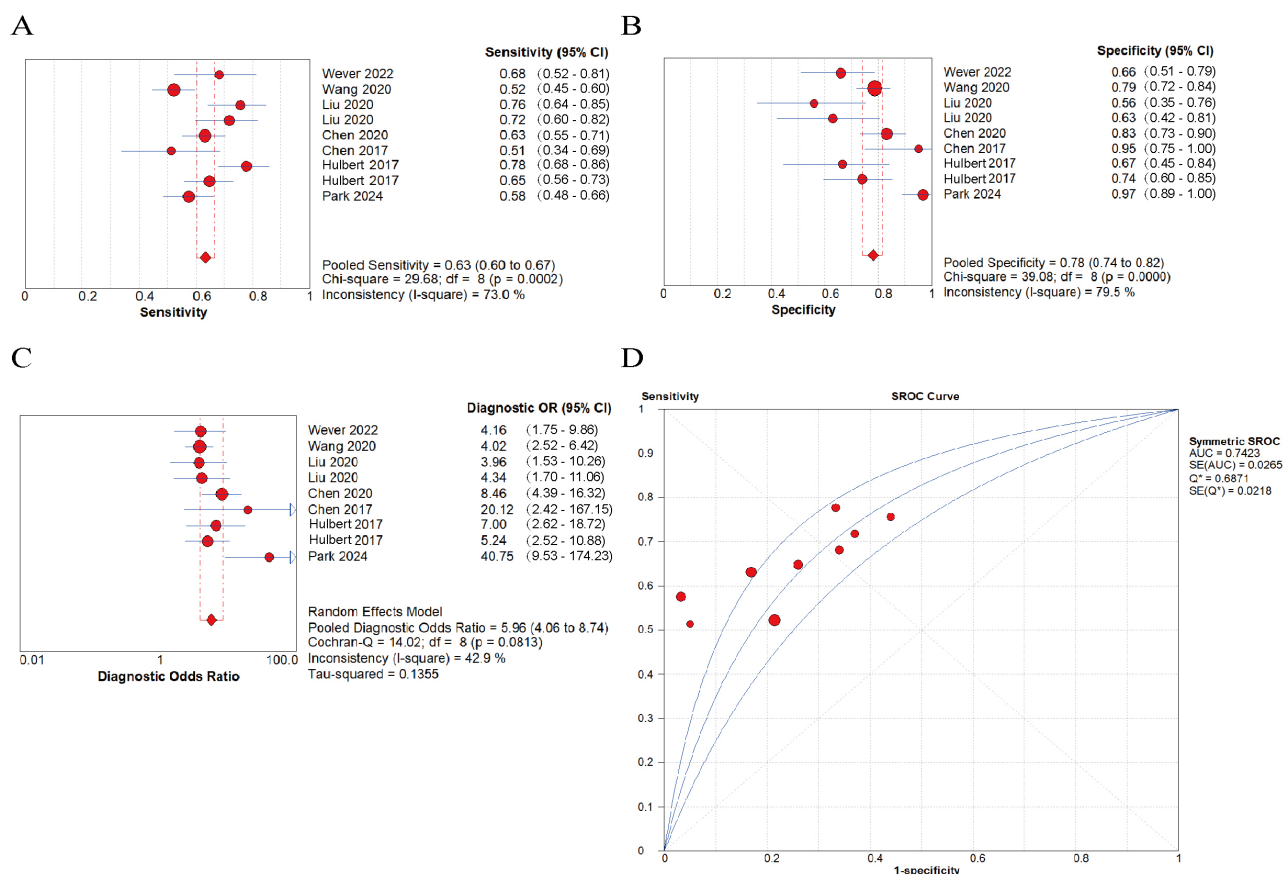


Fig. 3. Diagnostic performance of cysteine dioxygenase 1 (*CDO1*) promoter methylation using biofluids for the early detection of lung cancer. (A) pooled sensitivity; (B) pooled specificity; (C) pooled diagnostic odds ratio (DOR); (D) summary receiver operating characteristic curve (sROC) with the Q*-index.

Moreover, we conducted a comparative analysis of *CDO1* and other gene methylation tests including tachykinin-1 (*TAC1*), sex determining region Y-box 17 (*SOX17*), and homeobox A9 (*HOXA9*) to detect lung cancer. The results showed that the pooled sensitivity of *CDO1* promoter methylation was lower than those of *TAC1* (0.69 vs 0.74), *SOX17* (0.67 vs 0.72), *HOXA9* (0.69 vs 0.71), and multi-gene panels (0.67 vs 0.91) (**Supplementary Fig. 1**). However, the pooled specificity of *CDO1* promoter methylation was higher than those of *TAC1* (0.72 vs 0.71), *HOXA9* (0.73 vs 0.52), and multi-gene panels (0.74 vs 0.57), but lower than that of *SOX17* (0.75 vs 0.86) (**Supplementary Fig. 2**).

3.4 Heterogeneity Analysis

To analyze the heterogeneity sources, a meta-regression was conducted and variables included region (Asian countries/Western countries), sample source (plasma/other biofluids), sample size (≥ 100 / <100), and detection method (qMSP/qPCR) were included (Table 2). This analysis revealed that no significant variables affecting the outcomes were revealed in the meta-regression analyses ($p > 0.05$).

3.5 Sensitivity Analysis

We conducted a sensitivity analysis with the leave-one-out approach, whereby each study was iteratively removed, to assess the impact of individual studies on the meta-analytic results. As shown in Table 3 (Ref. [26,29–34]), the pooled DOR and 95% CI were recalculated after each exclusion. The results demonstrated that the pooled DOR remained largely unchanged regardless of the study being omitted, indicating that the overall findings were not overly influenced by any single study and that the conclusions are robust.

3.6 Publication Bias and Feasibility of Clinical Application

The results from Deek's funnel plot revealed an absence of significant publication bias in the present meta-analysis ($p = 0.74$) (Fig. 4A).

Additionally, to evaluate the clinical feasibility of *CDO1* promoter methylation, we performed Fagan's nomogram. The result indicated that under a pre-test probability of 20% (according to the incidence rate of lung cancer among malignancies in China [35]), the positive post-test probability of lung cancer was 42% and the negative post-

Table 1. Major Characteristics of the included studies.

Author (year)	Country	TNM stage	Pathological subtype				Sample source	Sample size (Case/Control)	Cancer	Control	Detection method
			LUSC	LUAD	SCLC	Others			(M+/M-)	(M+/M-)	
Wever <i>et al.</i> (2022) [32]	The Netherlands	I–III	16	27	/	1	Urine	44/50	30/14	17/33	qMSP
Wang <i>et al.</i> (2020) [34]	China	I–IV	29	112	14	23	Plasma	178/173	93/85	37/136	qPCR
Liu <i>et al.</i> (2020) [30]	USA	I–IV	9	65	/	/	Plasma	74/25	56/18	11/14	qMSP
							Urine	71/27	51/20	10/17	
Chen <i>et al.</i> (2020) [29]	China	I	22	139	/	2	Plasma	163/83	103/60	14/69	qMSP
Chen <i>et al.</i> (2017) [33]	China	I	35	/	/	/	Plasma	35/20	18/17	1/19	qPCR
Hulbert <i>et al.</i> (2017) [26]	USA	I–II	26	121	/	3	Sputum	90/24	70/20	20/16	qMSP
							Plasma	125/50	81/37	13/37	
Park <i>et al.</i> (2024) [31]	Korea	I–IV	42	53	26	4	Bronchial washing fluid	125/62	72/53	2/60	qMSP

LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; SCLC, small cell lung cancer; qMSP, quantitative methylation-specific polymerase chain reaction; qPCR, quantitative polymerase chain reaction.

Table 2. Meta-regression analysis.

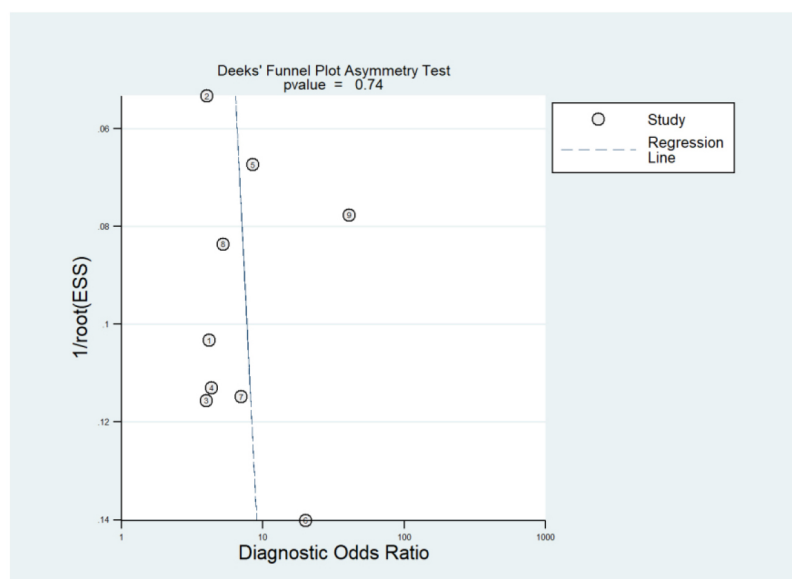
Meta-Regression (Inverse Variance weights)					
Variables	Coeff.	Std. Err.	<i>p</i> -value	RDOR	[95% CI]
Region	0.804	0.7287	0.3505	2.23	(0.22; 22.71)
Sample source	–0.471	0.5512	0.4556	0.62	(0.11; 3.61)
Sample size	–0.043	0.6757	0.9538	0.96	(0.11; 8.23)
Detection method	1.028	0.6871	0.2314	2.80	(0.31; 24.91)

Tau-squared estimate = 0.1738 (Convergence is achieved after 14 iterations), restricted maximum likelihood (REML) estimation. RDOR, relative diagnostic odds ratio.

Table 3. Sensitivity analysis using leave-one-out method.

Author (year)	DOR	95% CI
Wever <i>et al.</i> (2022) [32]	7.588	4.410–13.056
Wang <i>et al.</i> (2020) [34]	7.885	4.502–13.811
Liu <i>et al.</i> (2020) [30]	8.424	4.585–15.481
Chen <i>et al.</i> (2020) [29]	7.121	4.017–12.623
Chen <i>et al.</i> (2017) [33]	6.612	4.054–10.782
Hulbert <i>et al.</i> (2017) [26]	5.964	3.660–9.720
Park <i>et al.</i> (2024) [31]	5.800	3.855–8.725
Combined	6.930	4.280–11.210

A



B

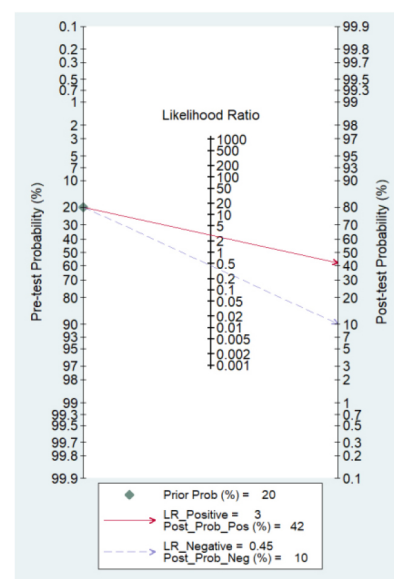


Fig. 4. Potential clinical application of *CDO1* promoter methylation. (A) Deek's funnel plot in evaluation the publication bias. (B) Fagan's nomogram of *CDO1* gene methylation as a biomarker for lung cancer diagnosis.

test yielded a 10% probability (Fig. 4B). It could be illustrated that with a preset predicted incidence rate of 20%, the probability of lung cancer reached 42% when *CDO1* promoter methylation was detected. Conversely, in cases where *CDO1* promoter methylation was not observed, the posttest probability was only 10% (Fig. 4B). The findings suggested that *CDO1* promoter methylation had limited clinical applicability, as its detection positivity was associated with only a modest increase in the positive probability of lung cancer.

4. Discussion

Recent investigative efforts have increasingly demonstrated that investigating efficient ctDNA methylation biomarkers for cancer detection have been carried out [14, 36,37]. Methylation biomarkers such as *RASSF1A* and *SHOX2* [38], adenomatous polyposis coli (*APC*) [39], O-6-methylguanine-DNA methyltransferase (*MGMT*) [40], and *CDO1* [24–26] were demonstrated to be effective in the early detection of lung cancer; however, further verification of their accuracy is needed.

In our study, seven relevant studies were finally included for analysis of the diagnostic value of *CDO1* promoter methylation in lung cancer detection. The quality assessment indicated that no major concerns regarding quality risk of the included studies were observed. We observed significant statistical heterogeneity in sensitivity ($I^2 = 73\%$, $p < 0.1$), specificity ($I^2 = 79.5\%$, $p < 0.1$), and DOR ($I^2 = 42.9\%$, $p < 0.1$). Therefore, a random effect model and subsequent subgroup analysis were performed. The pooled diagnostic sensitivity, specificity, DOR, and AUC of *CDO1*

promoter methylation for lung cancer was 0.63 (95% CI: 0.60–0.67), 0.78 (95% CI: 0.74–0.82), 5.96 (95% CI: 4.06–8.74), and 0.7423, respectively. The results were robust as we conducted sensitivity analysis and found that overall findings were not overly influenced by the deletion of any single study. Additionally, the results of this meta-analysis showed no indication of publication bias. Although a meta-regression was conducted, variables including region, sample source, sample size, and detection method did not significantly affect the heterogeneity ($p > 0.05$), which may be attributed to other variables such as TNM stages, pathological subtypes that lack adequate data for analysis.

We also performed a comparative analysis of *CDO1* and other gene methylation tests that were detected in the included studies. The pooled sensitivity of *CDO1* promoter methylation was lower than those of *TAC1* (0.69 vs 0.74), *SOX17* (0.67 vs 0.72) and *HOXA9* (0.69 vs 0.71), while the pooled specificity of *CDO1* was higher than those of *TAC1* (0.72 vs 0.71) and *HOXA9* (0.73 vs 0.52). As multi-gene panels showed significantly higher detection efficiency than individual genes [26,29,30], we also compared the diagnostic efficacy between *CDO1* and multi-gene panels. The results suggested that the pooled sensitivity of multi-gene panels was higher than that of *CDO1* alone (0.91 vs 0.67), while the pooled specificity of multi-gene panels was lower than that of *CDO1* alone (0.57 vs 0.74). The results indicated that DNA methylation sites and multi-gene panels with high diagnostic efficacy and consistency still need to be investigated.

Several limitations should be considered when interpreting the findings of this meta-analysis. (1) The included

publications exhibited significant statistical heterogeneity. The included studies exhibited substantial heterogeneity in patient cohorts, specimen types, and methylation detection methods. Although a random-effects model was applied for statistical adjustment, variations in thresholds and protocols may still compromise clinical applicability. (2) The result may be uncertain owing to the small sample size. (3) The number of available publications was limited; further investigations are urgently needed. (4) Only English and Chinese publications were included, and the failure to cover regional databases and unpublished studies may introduce selection bias.

5. Conclusion

In conclusion, *CDOI* promoter methylation using biofluids showed diagnostic potential for lung cancer diagnosis as a non-invasive method. Future studies should address improving detection methodologies and investigating combinational strategies with high accuracy.

Availability of Data and Materials

The datasets generated or analyzed during this study are available from the corresponding author upon reasonable request.

Author Contributions

XY and QC proposed the idea. YY and ZX extracted the data, performed quality assessment and statistical analysis. YY, ZX, and FL organized the tables and figures, wrote, and revised the manuscript. YJ provided methodology and revised the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/FBL43987>.

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