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Diagnoctic Value of Circulating Cell-Free DNA in Lung Cancer Detection: A Systematic Review

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Abstract: Lung cancer remains a leading cause of cancer-related mortality worldwide, with poor prognosis largely due to late-stage diagnosis. Early detection is essential to improve survival outcomes; however, current screening methods have limitations, including false-positive results and radiation exposure. This study aimed to evaluate the diagnostic value of circulating cell-free DNA as a minimally invasive biomarker for lung cancer detection through liquid biopsy. This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines using the Population, Intervention, Comparison, and Outcome framework. Literature searches were performed up to March 2026 in several databases. A total of 265 records were identified, of which three studies met the inclusion criteria. The findings demonstrated that circulating cell-free DNA-based approaches, including mutation profiling and deoxyribonucleic acid methylation analysis, showed clinically meaningful diagnostic performance, with sensitivity ranging from 67% to 87.8% and specificity from 73% to 96%. Methylation-based methods demonstrated higher sensitivity and the ability to differentiate lung cancer from non-malignant pulmonary diseases. Overall, circulating cell-free DNA analysis shows strong potential as a minimally invasive tool for lung cancer detection.

Keyword: Lung Cancer, Circulating Cell-Free DNA, cfDNA, Liquid Biopsy, DNA Methylation, Diagnostic Accuracy.

INTRODUCTION

Lung cancer remains a major clinical challenge and continues to represent a significant global health burden(Vicidomini, 2023). To enhance early detection rates, numerous screening initiatives have been implemented in recent years, with the aim of improving early diagnosis and ultimately leading to better patient prognosis. Among all malignancies, lung cancer remains the most frequently diagnosed cancer and the leading cause of cancer-related mortality worldwide According to the International Agency for Research on Cancer (IARC), nearly 20 million new cancer cases and 9.7 million cancer-related deaths were reported globally in 2022 (Bray et al., 2024; Li et al., 2022).

Despite advances in therapeutic strategies, lung cancer prognosis remains poor because many patients are diagnosed at advanced stages, where curative treatment options are limited. Early detection therefore plays a crucial role in improving survival outcomes. Currently, low-dose computed tomography (LDCT) is the standard screening modality for high-risk population. However, its clinical utility is limited by false-positive results, overdiagnosis, radiation exposure, and low screening adherence rates. These limitations have prompted the development of minimally invasive diagnostic approaches for earlier and more accurate detection of lung cancer (Smolarz et al., 2025).

Circulating cell-free DNA (cfDNA) has emerged as a promising biomarker in liquid biopsy for cancer detection. cfDNA consists of fragmented DNA released into the bloodstream through apoptosis, necrosis, and active cellular secretion, while the tumor-derived fraction, known as circulating tumor DNA (ctDNA), contains cancer-specific genetic and epigenetic alterations that may serve as diagnostic markers (Wan et al., 2017) .Compared with conventional tissue biopsy, cfDNA analysis offers several advantages, including minimal invasiveness, repeatability, and the ability to provide real-time molecular information. Recent studies have investigated various cfDNA-based biomarkers for lung cancer detection, including mutation profiling, methylation analysis, hydroxymethylation signatures, and fragmentation patterns. However, the reported diagnostic performance remains inconsistent across studies due to variations in methodologies, patient populations, and biomarker targets. (de Koning et al., 2020)

Therefore, this systematic review aims to evaluate the diagnostic value of circulating cell-free DNA in lung cancer detection and summarize current evidence regarding its clinical applicability.

METHOD

This systematic review was carried out following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. In addition, the review was designed using the Population, Intervention, Comparison, and Outcome (PICO) framework.

Search Strategy

A comprehensive literature search was performed through March 2026 across several electronic databases, including PubMed, Springer, Wiley, and Taylor & Francis. To identify relevant studies, a systematic search string was employed using Boolean operators (AND/OR) to combine terms related to lung cancer, cell-free DNA (cfDNA), and diagnostic performance. The specific search architecture was defined as follows (("lung cancer" OR "lung neoplasm" OR "pulmonary cancer" OR "bronchogenic carcinoma" OR NSCLC OR "non-small cell lung cancer" OR SCLC OR "small cell lung cancer")) AND (("cell-free DNA" OR cfDNA OR "circulating DNA" OR ctDNA OR "circulating tumor DNA" OR "liquid biopsy" OR "plasma DNA" OR "serum DNA")) ND (("diagnostic accuracy" OR sensitivity OR specificity OR

"positive predictive value" OR PPV OR "negative predictive value" OR NPV OR "ROC curve" OR AUC))

Study selection

The studies included in this review consisted of original research investigating the use of circulating cell-free DNA (cfDNA)–based liquid biopsy for detecting lung cancer in patients with suspected or confirmed disease. Most analyses utilized plasma-derived cfDNA. Eligible designs included observational and diagnostic accuracy studies that compared cfDNA-based approaches with reference standards such as histopathology or established clinical diagnoses, including comparisons with conventional diagnostic methods and control groups (healthy individuals or benign lung diseases). The outcomes of interest focused on diagnostic performance, particularly sensitivity, specificity, and area under the curve (AUC). Studies were excluded if they were non-English publications, conducted on animal subjects, presented as reviews or editorials, or lacked accessible full-text versions.

Data extraction

Data were extracted on key study characteristics, including the study author and year, population, index test, reference standard, methodology, and reported outcomes.

Quality Assessment

The methodological quality of the included studies was assessed using the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies-2) tool. This tool is specifically designed to evaluate the risk of bias and applicability of diagnostic accuracy studies. The assessment was conducted across four key domains: patient selection, index test, reference standard, and flow and timing. Each domain includes signaling questions to guide judgments regarding potential bias and concerns about applicability, ensuring a structured and transparent evaluation of study quality.

RESULT AND DISCUSSION

Result

A total of 265 records were identified through database searching, including PubMed (n = 62), Taylor and Francis (n = 25), Springer (n = 58), and Wiley (n = 120). After removal of 51 duplicate records, 214 studies remained for title and abstract screening. During the screening process, 208 records were excluded based on irrelevance to the study objectives. A total of 6 reports were subsequently sought for full-text retrieval, of which 2 could not be obtained. Four full-text articles were assessed for eligibility. Among these, one study was excluded due to inclusion of mixed cancer populations without separate lung cancer data. Ultimately, 3 studies met the inclusion criteria and were included in the final systematic review.

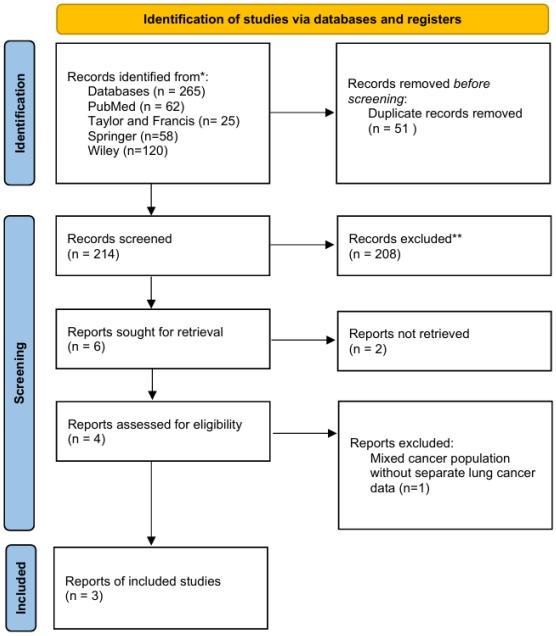

Figure 1. PRISMA Diagram Flowchart

The reviewed studies demonstrate the significant diagnostic potential of cell-free DNA (cfDNA) biomarkers, utilizing both genetic mutations and epigenetic methylation for the non-invasive detection of lung cancer (LC).

Peng *et al.* (2019) reported that ultra-deep sequencing of 65 lung cancer-related genes in plasma ctDNA achieved a sensitivity of 69% and a specificity of 96% in distinguishing malignant lesions from benign ones. This performance was further enhanced to 80% sensitivity and 99% specificity when ctDNA mutations were combined with patient age and a panel of six serum protein biomarkers (NSE, CYFRA 21-1, CEA, ProGRP, CA-125, and SCC) using a linear discriminant analysis (LDA) model (Peng et al., 2019).

Epigenetic profiling also yielded robust results. Weiss *et al.* (2017) validated a two-marker panel of *SHOX2* and *PTGER4* DNA methylation, which achieved an Area Under the Curve (AUC) of 0.88 in a cohort of 172 patients. At a fixed specificity of 90%, this panel reached 67% sensitivity, significantly outperforming traditional protein biomarker panels (AUC 0.91 vs. 0.79, p=0.004) in side by side comparisons.

Furthermore, Wielscher *et al.* (2015) employed a multiplexed methyl-sensitive restriction enzyme (MSRE) enrichment technique, effectively separating LC from non-cancer and control groups with 87.8% sensitivity and 90.2% specificity. Their optimized four-gene model (*HOXD10, PAX9, PTPRN2,* and *STAG3*) demonstrated strong stability, providing an AUC of 0.85 and a sensitivity of 97% in an independent validation set.(Wielscher et al., 2015)

These epigenetic signatures were capable of differentiating LC from complex interrelated pulmonary conditions, such as COPD and Interstitial Lung Disease (ILD), with specificities of approximately 88%. Notably, total cfDNA concentrations were significantly elevated in LC patients, with levels increasing by 2.8 to 35.6 ng/ml compared to healthy controls, even in early stages (TNM I-II). Overall, the integration of cfDNA methylation and mutation profiling offers a reliable, minimally invasive strategy to reduce the high false-positive rates associated with current imaging techniques like LDCT.

Table 1. Data Extraction of Included Studies

Study	Population	Index Test	Reference Standard	Method	Outcome

Peng et al., 2019	Pulmonary lesions (136 malignant, 56 benign; n=192)	Plasma ctDNA (NGS)	Histopathology	Ultra-deep NGS (Sec-Seq, 65 genes)	Sensitivity 69%, Specificity 96%
Weiss et al., 2017	LC (n=50), benign lung disease (n=50), healthy (n=72)	cfDNA methylation (SHOX2/PTGER4, PCR)	Clinical diagnosis	Bisulfite conversion + real-time PCR	Sensitivity 67%, Specificity 73%, AUC 0.88
Wielscher et al., 2015	LC (n=33), COPD (n=42), ILD (n=68), healthy (n=61); validation LC (n=23), healthy (n=23)	cfDNA methylation (MSRE-qPCR)	Clinical diagnosis	MSRE digestion + multiplex qPCR	Sensitivity 87.8%, Specificity 90.2%, AUC 0.91 (validation: Sens 97%, Spec 73%, AUC 0.85)

The methodological quality of the included studies was assessed using the QUADAS-2 tool across four domains: patient selection, index test, reference standard, and flow and timing. Overall, one study (Peng et al., 2019) demonstrated a low risk of bias across all domains, indicating high methodological quality.

In contrast, two studies (Weiss et al., 2016 and Wielscher et al., 2015) showed a high risk of bias in the patient selection domain, primarily due to the use of case-control designs, which may introduce selection bias and limit generalizability. Additionally, Wielscher et al. (2015) also presented a high risk of bias in the index test domain, suggesting potential concerns related to test interpretation or lack of pre-specified thresholds.

The reference standard domain demonstrated consistently low risk of bias across all included studies, indicating that appropriate methods were used to establish the target condition. Similarly, all studies showed low risk of bias in the flow and timing domain, suggesting that patient progression through the study and timing of index and reference tests were appropriate.

Overall, two of the three studies were judged to have a high overall risk of bias, mainly driven by concerns in patient selection and, to a lesser extent, index test methodology. These findings highlight the need for cautious interpretation of diagnostic accuracy results, particularly in studies employing non-representative populations.

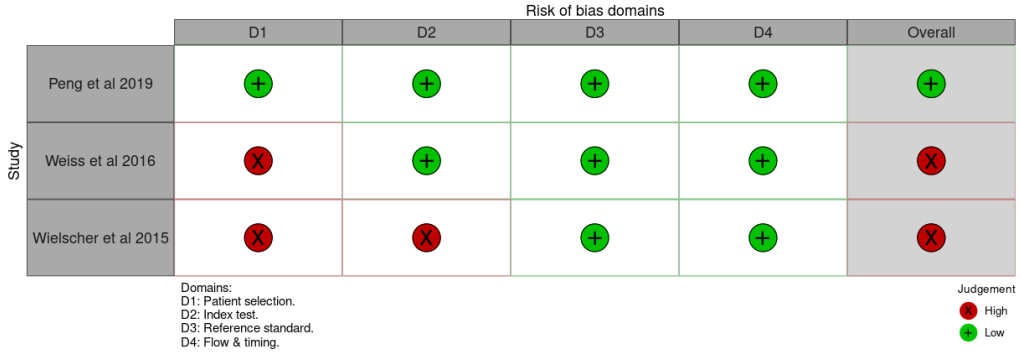

Figure 2. Risk of Bias QUADAS-2

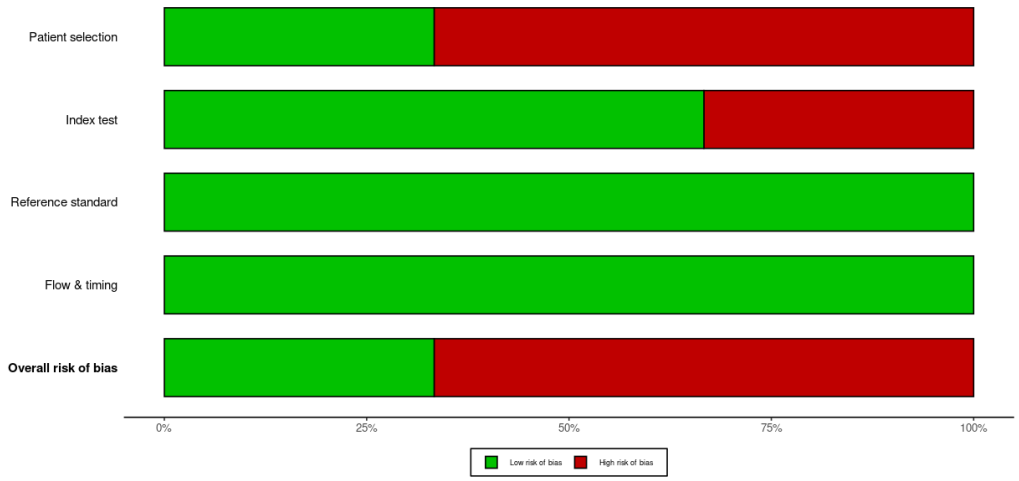

Figure 3. Summary of Risk of Bias Across Included Studies (QUADAS-2)

Discussion

To our knowledge, no systematic review has specifically summarized and compared plasma cell-free DNA (cfDNA)-based liquid biopsy approaches encompassing somatic mutation profiling and DNA methylation analysis for lung cancer detection in populations that include non-malignant pulmonary disease controls. This review included three articles that met the predefined eligibility criteria. A meta-analysis was not conducted due to the limited number of included studies and substantial heterogeneity in study design, analytical methodology, and reported outcomes. All included studies were clinical or translational investigations evaluating the diagnostic performance of plasma cfDNA analysis for lung cancer detection using liquid biopsy specimens.

The analysis of plasma ctDNA through ultra deep next generation sequencing (NGS) represents a technically feasible approach for malignancy assessment of pulmonary lesions (Peng et al., 2019). Using a proprietary Sec-Seq technique targeting the coding regions of 65 cancer-associated genes at an average sequencing depth of 35,000×, Peng et al, demonstrated an overall sensitivity of 69% and specificity of 96% for ctDNA-based lung cancer detection across 192 patients with resectable pulmonary occupying lesions. Notably, detection sensitivity increased progressively with advancing disease stage, reaching 63% at stage I, 83% at stage II, and 94% at stage III, a pattern consistent with the established relationship between tumor burden and the quantity of tumor-derived DNA shed into circulation. These findings underscore a fundamental limitation of mutation-based liquid biopsy, namely its reduced sensitivity in early-stage disease where tumor-derived DNA constitutes only a minor fraction of total cfDNA. To address this limitation, Peng et al. further demonstrated that integrating ctDNA mutation data with patient age and a six-protein serum biomarker panel through linear discriminant analysis (LDA) improved overall sensitivity to 80% at a specificity of 99%, reinforcing the notion that multimodal approaches combining genomic and clinical parameters are likely necessary for reliable diagnostic performance (Peng et al., 2019). This finding is consistent with prior evidence demonstrating that cfDNA-guided therapy selection in advanced-stage lung cancer significantly increased the positivity rate of actionable mutations and improved treatment outcomes compared to tissue biopsy alone (Aggarwal et al., 2019).

Beyond mutation profiling, DNA methylation-based approaches have demonstrated complementary diagnostic value for lung cancer detection from plasma. Weiss et al. (2017) evaluated a two-marker methylation panel comprising SHOX2 and PTGER4 in 172 plasma samples using bisulfite conversion followed by real-time PCR. The validation study yielded an area under the curve (AUC) of 0.88, with a sensitivity of 67% at a fixed specificity of 90%, and a specificity of 73% at a fixed sensitivity of 90% (Weiss et al., 2017). Importantly, the

panel maintained meaningful discriminatory performance even when patients with non-malignant lung diseases including chronic obstructive pulmonary disease (COPD), asthma, and pneumonia were incorporated into the control group, demonstrating its clinical utility in a realistic diagnostic setting where benign pulmonary conditions must be distinguished from malignancy. This distinction is particularly relevant given that these conditions are prevalent among high-risk lung cancer screening populations and frequently present with overlapping clinical features (Weiss et al., 2017). The epigenetic events occurring at early stages of development of cancer represent a biologically rational source of cfDNA biomarkers, as aberrant DNA methylation is among the earliest and most consistent molecular alterations detectable in plasma during the development of lung carcinoma (Sharma et al., 2010). A notable methodological strength of this study was its structured development across three sequential training studies prior to validation, which provided a robust framework for marker optimization and assay refinement before final performance assessment.

The diagnostic challenge of differentiating lung cancer from co-existing and clinically overlapping pulmonary pathologies was further addressed by Wielscher et al. (2015), who employed a highly multiplexed methyl-sensitive restriction enzyme (MSRE) qPCR approach targeting 96 methylation loci simultaneously from 400 µL of serum or plasma. This study achieved a sensitivity of 87.8% and specificity of 90.2% for separating cancer from non-cancer cases, and additionally demonstrated the capacity to distinguish lung cancer from interstitial lung disease (ILD) with a specificity of 88% and from COPD with a specificity of 88%. These results represent a level of differential diagnostic resolution not achieved by the other included studies (Wielscher et al., 2015). Independent validation using only four top markers, namely HOXD10, PAX9, PTPRN2, and STAG3, on a separate sample set of 46 patients yielded an AUC of 0.85, confirming both the robustness and translational potential of this minimal marker model. he deliberate inclusion of ILD and COPD as comparator groups constitutes a meaningful design strength, as both conditions carry a significantly elevated risk of lung cancer development, with ILD patients reported to have a two- to eightfold increased risk of lung cancer compared to the general population (Tomassetti et al., 2015).

Taken together, the evidence from this systematic review supports the feasibility and diagnostic potential of plasma cfDNA analysis as a non-invasive complement to existing imaging-based methods for lung cancer detection. DNA methylation-based approaches appear to offer advantages in both absolute sensitivity and differential diagnostic capability, while multimodal strategies that integrate cfDNA data with clinical variables demonstrate the most promise for optimizing overall test performance. Larger prospective trials with standardized methodologies, well-defined high-risk populations, and uniform reference standards are warranted to validate these findings and establish the clinical utility of plasma cfDNA-based liquid biopsy prior to routine implementation.

CONCLUSION

This systematic review demonstrates that plasma cfDNA-based liquid biopsy represents a diagnostically viable and minimally invasive approach for lung cancer detection. Across the three included studies, both somatic mutation profiling and DNA methylation analysis yielded clinically meaningful diagnostic performance, with sensitivities ranging from 67% to 87.8% and specificities from 73% to 96% depending on the analytical platform and study population. DNA methylation-based approaches, particularly multiplexed MSRE-qPCR, demonstrated superior sensitivity and the unique capacity to differentiate lung cancer from co-existing pulmonary conditions such as ILD and COPD, which represent a clinically significant diagnostic challenge. Mutation-based ctDNA profiling via ultra-deep NGS, while highly specific, exhibited stage-dependent sensitivity that limits its utility in early-stage detection as a standalone test. Notably, multimodal strategies integrating cfDNA data with clinical and

proteomic parameters consistently improved overall diagnostic accuracy, suggesting that no single biomarker modality is sufficient in isolation. These findings collectively support the potential role of plasma cfDNA analysis as a complementary tool to low-dose computed tomography in lung cancer screening and diagnostic workup. However, the limited number of included studies, heterogeneity in methodological approaches, and absence of large-scale prospective validation remain important constraints on the generalizability of current evidence. Future studies employing standardized pre-analytical protocols, uniform reference standards, and well-characterized high-risk populations are essential to establish the clinical validity and readiness of cfDNA-based liquid biopsy for routine implementation in lung cancer care.

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