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Efficacy of liquid biopsy for genetic mutations determination in non-small cell lung cancer: a systematic review on literatures

Mohammad Mehdi Jahani[1], Parisa Mashayekhi[2*], Mir Davood Omrani[1,5*] and Azita Azimi Meibody[3,4]

Abstract

Background Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related mortality worldwide and is often diagnosed at advanced stages, limiting treatment options. This systematic review aims to evaluate the efficacy of liquid biopsy in detecting genetic mutations in NSCLC, focusing on its sensitivity, specificity, clinical utility, and potential to guide personalized treatment strategies.

Methods A systematic search was conducted in PubMed, Scopus, Embase, Web of Science, and Cochrane Library to identify relevant studies published between 1990 and September 2024. Eligible studies included comparative analyses of liquid biopsy techniques for NSCLC diagnosis, prognosis, progression, or treatment response. This review adheres to the PRISMA 2020 guidelines and assesses study quality using the QUADAS framework.

Results A total of 30 studies were included. Digital droplet PCR (ddPCR) demonstrated the highest sensitivity, detecting low-frequency mutations such as EGFR T790M at levels as low as 0.01%. Next-generation sequencing (NGS) provided comprehensive mutational profiling, revealing tumor heterogeneity and co-existing mutations but required high-quality plasma samples. The cobas EGFR Mutation Test, while less sensitive than ddPCR, remains widely used due to its clinical accessibility. Emerging methods, including extracellular vesicle analysis, show promise but require further validation. Additionally, liquid biopsy effectively identified non-EGFR mutations, such as KRAS, ALK, and MET amplifications, expanding its clinical applicability.

Conclusion Liquid biopsy is a transformative tool in NSCLC management, offering a minimally invasive approach for mutation detection, disease monitoring, and treatment guidance. Future research should focus on multicenter trials and emerging technologies to enhance clinical integration and broaden applicability across different cancer types.

Keywords Non-small cell lung cancer, Liquid biopsy, Digital droplet PCR, Next-generation sequencing, EGFR mutations, CtDNA, Personalized therapy

*Correspondence:
Parisa Mashayekhi
parisamashayekhi7@gmail.com
Mir Davood Omrani
davood_omrani@yahoo.co.uk
[1]Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Molecular Medicine Department, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran
[3]Department of Emergency Medicine, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[4]Department of Health in Disaster and Emergencies, Faculty of Management and Medical Information, Isfahan University of Medical Sciences, Isfahan, Iran
[5]Urogenital Stem Cell Research Center, Research Institute for Urology and Nephrology, Shahid Labbafinejad Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide, accounting for approximately 1,796,144 deaths in 2020. Non-small cell lung cancer (NSCLC) is the most common histological subtype, comprising about 90% of all lung cancer cases [1].

Unfortunately, NSCLC is often diagnosed at an advanced stage, resulting in a poor prognosis. Standard treatment typically involves multiple rounds of chemotherapy [2]. Early detection and precise molecular profiling are crucial for effective treatment and improved patient outcomes [3]. While traditional tissue biopsy remains the gold standard for diagnosing lung cancer, it is an invasive procedure that may not always be feasible or provide sufficient tissue for comprehensive molecular profiling. This limitation can hinder the identification of actionable genetic alterations essential for guiding personalized treatment decisions [4, 5].

Liquid biopsy, a minimally invasive technique that analyzes circulating tumor DNA (ctDNA) in blood plasma, has emerged as a promising alternative to traditional tissue biopsy [6, 7]. By capturing genetic alterations shed by tumor cells into the bloodstream, liquid biopsy offers a potential solution to the limitations of tissue biopsies [8]. This approach facilitates early detection, enables disease monitoring, and provides a real-time assessment of treatment response in a non-invasive manner [9, 10].

A growing body of evidence suggests that liquid biopsy can accurately detect a wide range of genetic alterations in lung cancer, including key driver mutations commonly associated with targeted therapies [11, 12]. Identifying these mutations allows clinicians to select appropriate targeted therapies, potentially improving patient outcomes and survival rates. Additionally, liquid biopsy can be used to monitor disease progression and assess treatment response, enabling timely modifications to therapeutic strategies [13, 14].

Despite its advantages, several challenges remain, including the sensitivity and specificity of ctDNA detection, the interpretation of complex mutational profiles, and the standardization of analytical methods [15]. Ongoing research aims to enhance the sensitivity and specificity of ctDNA detection assays, refine bioinformatics pipelines, and establish clinical guidelines for incorporating liquid biopsy into lung cancer management [16, 17].

This systematic review aims to comprehensively evaluate the efficacy of liquid biopsy in detecting genetic mutations in NSCLC. By systematically analyzing the available literature, we assess its sensitivity, specificity, and clinical utility across various clinical settings. Additionally, we identify knowledge gaps and potential directions for future research to further advance the clinical application of liquid biopsy in lung cancer diagnosis and treatment.

Method

Inclusion criteria

We conducted a systematic review, registered with PROSPERO (ID: CRD42024580227), in accordance with the PRISMA 2020 guidelines [18]. Studies were included if they met the following criteria:

- (A) Comparative studies analyzing genetic mutations in liquid biopsy samples from NSCLC patients.
- (B) Studies investigating the role of liquid biopsy in NSCLC diagnosis, prognosis, disease progression, and treatment response.

To ensure the quality and relevance of included studies, specific exclusion criteria were applied. We excluded studies that:

- Had incomplete or inadequate documentation.
- Were published in languages other than English.
- Were presented as letters to the editor, conference abstracts, case reports, or review articles.
- Included patients with small cell lung cancer or other lung cancer subtypes.

Information sources

A systematic search was conducted across Web of Science (WOS), Scopus, PubMed, Embase, and Cochrane Library to identify relevant articles published between 1990 and September 2024. To ensure comprehensive coverage, we extended our search to grey literature sources, including allconferences.com, conferencealerts.com, OpenGrey, and OATD.org, to capture conference presentations and open-access studies. Additionally, we performed a manual reference list analysis of included studies to identify any relevant articles that were not retrieved through the electronic search. Google Scholar was also searched for additional relevant studies.

Search strategy

A comprehensive search strategy was developed using the following keywords and Boolean operators:

Lung Cancer Terms: “Lung Neoplasms“[Mesh] OR “Lung Cancer” OR “Lung Neoplasm” OR “Pulmonary Neoplasm” OR “Pulmonary Cancer” OR “Pulmonary Tumor” OR “Bronchogenic Carcinoma” OR “Lung Adenocarcinoma” OR “NSCLC” OR “Non-Small Cell Lung Cancer”.

Biopsy Terms: “Biopsy, Liquid“[Mesh] OR “Liquid Biopsy” OR “Circulating Tumor DNA” OR “ctDNA” OR “Circulating Tumor Cells” OR “CTCs” OR “Plasma DNA” OR “Cell-Free DNA” OR “cfDNA” OR “Non-invasive Biopsy”.

Genetic Mutation Terms: “Genetic Markers“[Mesh] OR “Genetic Mutation” OR “Mutation” OR “Genetic

Alteration" OR "Genomic Mutation" OR "EGFR" OR "KRAS" OR "ALK" OR "BRAF" OR "TP53" OR "Genetic Profile".

Diagnostic Performance Terms: "Sensitivity and Specificity"[Mesh] OR "Diagnostic Accuracy" OR "Sensitivity" OR "Specificity" OR "Predictive Value" OR "Accuracy".

The search strategy was adapted for each database according to its indexing style and search filters.

Study selection process

Two independent reviewers (**MMJ and PM**) conducted the study selection in a two-stage process:

1. **Title and Abstract Screening**: Titles and abstracts of all identified records were screened for eligibility. Studies that clearly did not meet the inclusion criteria were excluded. Any discrepancies between reviewers were resolved through discussion.
2. **Full-Text Review**: Full-text articles of potentially eligible studies were retrieved and assessed against predefined eligibility criteria. Studies in languages other than English were excluded unless an English abstract or translation was available. If duplicate publications were identified, the study with the most comprehensive data was retained.

All disagreements were resolved through discussion between the two reviewers, with a third reviewer (**MDO**) consulted if consensus could not be reached. To ensure transparency and reproducibility, the study selection process was documented using **EndNote (version 9)**.

Data extraction

Three researchers (**MMJ**, **PM**, **and AAM**) independently extracted data from the included articles using a standardized data extraction checklist. Any discrepancies were resolved through discussion or arbitration by a fourth researcher (**MDO**). Extracted data included:

- **Study details**: Author, publication year, country of origin.
- **Patient characteristics**: Study population, NSCLC stage.
- **Methodology**: Liquid biopsy techniques, methods for detecting mutations in tissue and plasma samples.
- **Mutation findings**: Types of detected mutations.
- **Key results**: Sensitivity, specificity, and overall conclusions of each study.

Risk of bias in individual studies

The quality of included studies was assessed using the **QUADAS framework** [19]. This tool evaluates the risk of bias across nine domains, rated as **"yes" (low risk)**, **"no" (high risk)**, **or "unclear" (insufficient information)**. Studies were classified as follows:

- **High-quality**: Low risk of bias in most domains, with minimal applicability concerns.
- **Moderate-quality**: Moderate level of "yes" ratings with some concerns about applicability.
- **Low-quality**: High proportion of "unclear" or "no" ratings, with significant concerns about applicability.

A study was considered **high quality** if it demonstrated:

(A) Low risk of bias in most domains, with no major flaws.
(B) High relevance to the target population and clinical setting.

Results

Characteristics of included studies

As depicted in Fig. 1, we utilized the PRISMA flow diagram to systematically identify relevant studies. Our initial database and grey literature searches yielded 8,376 records. After removing 3,618 duplicates, we screened the titles and abstracts of the remaining 4,758 articles. A total of 4,626 articles were excluded based on our inclusion and exclusion criteria. Following a full-text review of the remaining 132 studies, 102 were further excluded, resulting in 30 studies being included in this systematic review [11, 20–48].

Among these studies, six were conducted in China, five in the United States, three in Japan, and five in Italy. Additionally, two studies were conducted in Sweden, while the remaining studies originated from Portugal, Thailand, Australia, Mexico, Spain, Canada, the United Kingdom, Taiwan, India, and Denmark. A summary of the primary characteristics of the included studies is presented in Table 1.

The quality assessment results of the included studies are shown in Table 2. Of the 30 studies, 17 were classified as high quality, nine as medium quality, and four as low quality.

Biofluids and biomarkers for liquid biopsy in NSCLC

Liquid biopsy can be performed using multiple biofluids, each containing distinct biomaterials that provide valuable information for NSCLC diagnosis, prognosis, and treatment selection. Below is an overview of different biofluids, the biomaterials they contain, and their clinical relevance in NSCLC.

1. Blood (Plasma & Serum) – Most Widely Used Biofluid.

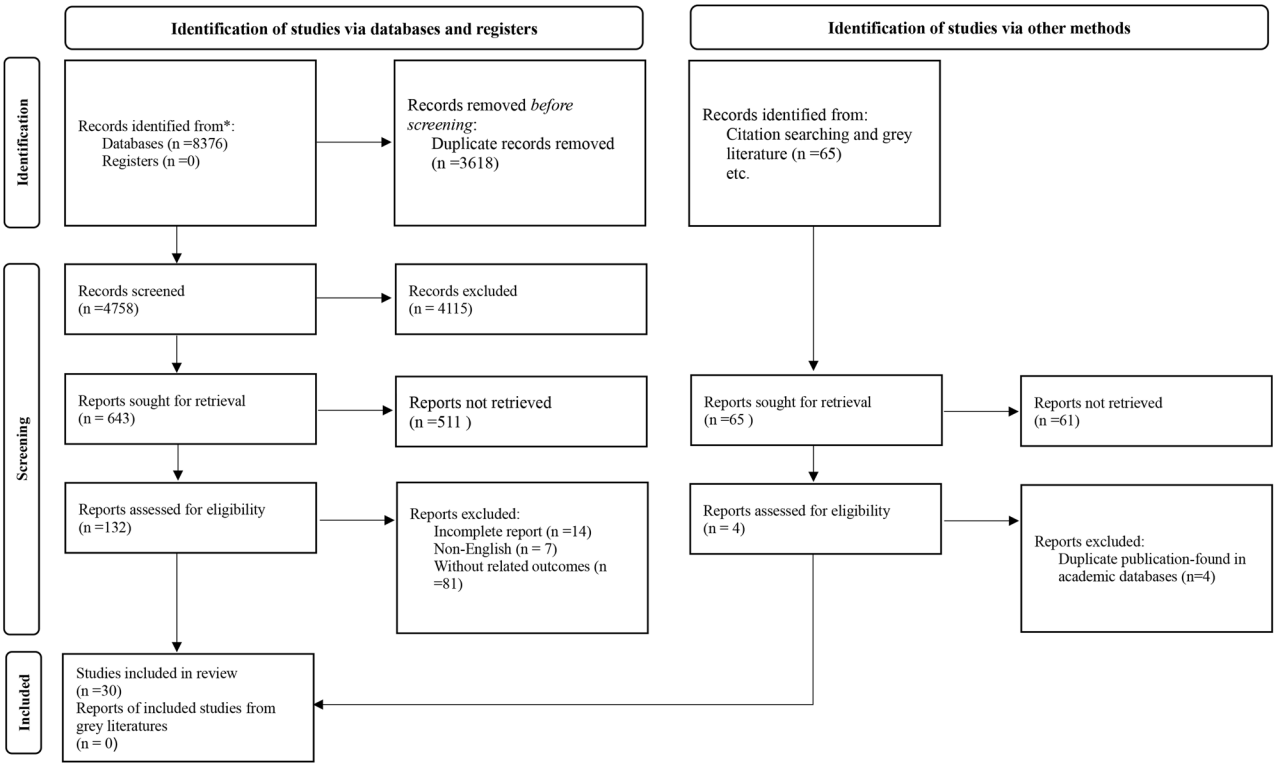

Fig. 1 Preferred Reporting Items for Systematic Reviews flow diagram (2020) of search process for studies examining the application of liquid biopsy in lung cancer diagnosis: A systematic review

- **Circulating Tumor DNA (ctDNA)**: Detects tumor-specific mutations (e.g., EGFR, KRAS, ALK, MET, BRAF, ROS1). Useful for treatment selection, resistance detection, and disease monitoring [26].
- **Circulating Tumor Cells (CTCs)**: Provides information on tumor heterogeneity and metastatic potential. CTC enumeration correlates with prognosis.
- **Extracellular Vesicles (EVs)/Exosomes**: Contains tumor-derived RNA, DNA, and proteins. Potential for early detection and monitoring response to therapy.
- **Tumor-Educated Platelets (TEPs)**: RNA profiling of TEPs can reveal tumor-specific alterations and serve as a potential biomarker for NSCLC detection [42].

2. Pleural Effusion – A Source of Tumor-Derived Biomolecules.

- **ctDNA**: Detects actionable mutations when blood ctDNA is insufficient.
- **CTCs**: Higher detection rates in pleural fluid compared to blood, offering additional diagnostic value.
- **Protein Biomarkers**: Elevated levels of VEGF, IL-6, and CEA indicate aggressive disease [45].

3. Bronchoalveolar Lavage Fluid (BALF) – Lung-Specific Sampling.

- **ctDNA & EVs**: Offers higher sensitivity for detecting lung cancer-specific mutations compared to plasma.
- **Proteins & Metabolites**: Useful for differentiating benign from malignant lung lesions [49].

4. Urine & Saliva – Emerging Non-Invasive Biofluids.

- **Urinary ctDNA**: Detects EGFR mutations in NSCLC patients, particularly those with brain metastases.
- **Salivary EVs & ctDNA**: Potential for early detection, though further validation is required [47].

Application of liquid biopsy in NSCLC

Zhao et al. conducted a cross-sectional study among 112 patients with stage III or IV NSCLC to evaluate the efficacy of liquid biopsy. They assessed the predictive value of specific tumor markers for concordance between plasma and tissue biopsies in detecting driver mutations in advanced NSCLC. Their findings indicated that when carcinoembryonic antigen (CEA) levels exceeded

Table 1 Characteristics of included studies

First author, year	Region	lung cancer type	Method for testing liquid specimens	Samples	Method for detecting tissue samples	Genes involved
Zungsontiporn et al.2024	Thailand	Advanced NSCLC	cobas or ddPCR method	23	ARMS	EGFR T790M
Zhao et al.2024	China	stage III or IV lung adenocarcinoma	NGS technology	1012	Not reported	CEA, CYFRA21-1
Martínez-Herrera. 2024	Mexico	adenocarcinoma subtype	NGS	127	Two allele-specific PCR assays	EGFR, KRAS
Uemura et al.2023	Japan	IIIB–IV or recurrent disease	ctDNA NGS	65	Not mentioned	ALK, ROS1, BRAF, KRAS, MET, RET, ERBB2, and NTRK
Ruiz et al. 2023	Spain	Stage I to IV	DHPLC	79	Immunohistochemistry techniques	ALK, EGFR, ROS1, and BRAF genes
Porta et al. 2023	Italy	Stage I to IV	DHPLC	229	fundationOne®Liquid CDx (F1LCDx®) assay	TP53, DNMT3A, EGFR, KRAS
Zhang et al.2022	Canada	advanced stages	NGS	24	Not mentioned	EGFR variants
Shah et al. 2022	UK	Stage I to IV	NGS	5036	Scorpion-ARMS	EGFR
Iaccarino et al. 2022	Italy	Stage I to IV	NGS	196	Not mentioned	BRAF
Ho et al. 2022	Taiwan	advanced stages	ctDNA NGS	231	ARMS	EGFR
Behel et al.2022	India	Stage IVA, IVB, IIIB	ME-PCR	158	Direct sequencing	EGFR
Zulato et al. 2021	Italy	Stage I to IV	NGS	86	ME-PCR	EGFR, KRAS, NRAS, PIK3CA
Zhu et al.2021	USA	Stage I to IV	Not mentioned	733	Not mentioned	KEAP1/NFE2L2
Ito et al.2021	Japan	Stage I to IV	ddPCR method	82	ddPCR	EGFR
Qvick et al.2021	Sweden	Stage II, IV	AVENIO ctDNA Surveillance kit (NGS)	60	Not mentioned	EGFR
Silveira et al. 2020	Portugal	stage IV	Cobas EGFR blood test	111	Cobas EGFR tissue test	EGFR
Schwartzberg et al.2020	USA	stage IV	NGS	140	ARMS	KRAS, MET, ALK, ERBB2, RET, BRAF and ROS1
Nacchio et al.2020	Italy	Stage I to IV	NGS	36	Not mentioned	BRAF, KIT, EGFR, PDGFRA
Minari et al. 2020	Italy	Stage I to IV	Cobas EGFR Mutation Test	120	Cobas EGFR tissue test	EGFR, MET, HER2
Mezquita et al.2020	France	Stage I to IV	DHPLC	128	DHPLC	ALK and ROS1
Ding et al.2019	Australia	advanced NSCLC	ddPCR	28	ARMS	EGFR
Bai et al.2019	China	Stage I to IV	NGS	79	Not mentioned	EGFR, ALK, ROS1
Akamatsu et al.2019	Japan	stage IV	ddPCR	57	ARMS	EGFR
Sorber et al. 2019	Belgium	Stage I to IV	ddPCR	234	ARMS	EGFR
Yang et al.2017	China	Stage I to IV	CastPCR	107	Not mentioned	BRAF, EGFR
Rachiglio et al.2016	Italy	Stage III and IV	NGS	44	Not mentioned	EGFR
Duan et al. 2015	China	Stage IIA-IV	Scorpion-ARMS	94	Scorpion-ARMS	EGFR mutations
Li et al.2014	China	Stage IIIb and IV	ARMS	164	ARMS	EGFR
Weber et al.2014	Denmark	Stage IIA-IV	Cobas EGFR blood test	199	Cobas EGFR tissue test	EGFR mutations in exons 18–21
Zhao et al.2013	China	Stage I to IV	ME-PCR	111	ME-PCR	EGFR mutations in exons 19 and 21

NGS, next-generation sequencing; ME-PCR, mutant-enriched PCR; HRM, high-resolution melt; DHPLC, denaturing high-performance liquid chromatography; Scorpion-ARMS, scorpion amplification refractory mutation system; ARMS, amplification refractory mutation system; MEL, mutant-enriched liquidchip technology

established thresholds of 15.01 ng/ml and 51.15 ng/ml, the likelihood of concordance between diagnostic tests increased significantly, reaching positive predictive values of 90% and 95%, respectively. While CYFRA21-1 demonstrated some predictive ability, its performance was inferior to that of CEA. The area under the curve (AUC) for CYFRA21-1 was 0.727, compared to 0.741 for CEA ($p = 0.633$) [21].

In another study, Zungsontiporn et al. compared the sensitivity and accuracy of different liquid biopsy techniques for detecting EGFR mutations in NSCLC patients. Their results showed that combining digital droplet PCR

Table 2 Quality assessment of the included studies

First Author	Included disease spectrum	Object of study	Interval time	Reference test	Refer to the standard test	Interpretation of the results of the test to be evaluated	Interpretation of the results of reference standard tests	Intermediate test results	Case information	Overall quality
Zungsontiporn et al.2024	Yes	Yes	Yes	Yes	Yes	Yes	NR	No	Yes	High
Zhao et al.2024	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	High
Martínez-Herrera. 2024	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Medium
Uemura et al.2023	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Medium
Ruiz et al. 2023	Yes	Yes	No	Yes	Yes	Yes	Yes	NR	Yes	High
Porta et al. 2023	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	High
Zhang et al.2022	Yes	Yes	No	No	Yes	Yes	Yes	NR	Yes	Medium
Shah et al. 2022	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	High
Iaccarino et al. 2022	Yes	Yes	No	No	No	Yes	Yes	No	Yes	Low
Ho et al. 2022	Yes	Yes	No	Yes	Yes	Yes	Yes	NR	Yes	High
Behel et al.2022	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	High
Zulato et al. 2021	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Medium
Zhu et al.2021	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	High
Ito et al.2021	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Medium
Qvick et al.2021	Yes	Yes	No	No	Yes	Yes	No	Yes	Yes	Low
Silveira et al. 2020	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Medium
Schwartzberg et al.2020	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	High
Nacchio et al.2020	Yes	Yes	No	Yes	Yes	Yes	Yes	NR	Yes	High
Minari et al. 2020	Yes	Yes	Yes	Yes	NR	Yes	Yes	Yes	Yes	High
Mezquita et al.2020	Yes	Yes	No	No	No	Yes	Yes	No	Yes	Low
Ding et al.2019	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Medium
Bai et al.2019	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Medium
Akamatsu et al.2019	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	High
Sorber et al. 2019	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	High
Yang et al.2017	Yes	Yes	Yes	Yes	No	Yes	NR	Yes	Yes	High
Rachiglio et al.2016	Yes	Yes	No	No	Yes	Yes	No	Yes	Yes	Low
Duan et al. 2015	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	High
Li et al.2014	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	High
Weber et al.2014	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Medium
Zhao et al.2013	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	High

NR, not reported

(ddPCR) with the standard cobas assay significantly improved diagnostic accuracy [20]. The cobas EGFR Mutation Test, currently the standard method, detects EGFR mutations with a sensitivity of 61% and specificity of 79% for T790M plasma mutations. However, its 1% detection limit remains a constraint. Newer technologies, such as ddPCR, have demonstrated superior sensitivity, making them promising alternatives [25].

By partitioning DNA into individual droplets and independently amplifying each, ddPCR outperforms conventional PCR in detecting mutations at significantly lower frequencies. Compared to the cobas test, ddPCR offers superior sensitivity in identifying T790M mutations within circulating tumor DNA (ctDNA), even at exceptionally low frequencies of 0.01% [26, 27].

The European Society for Medical Oncology (ESMO) guidelines recommend comprehensive genetic testing for patients with advanced non-squamous NSCLC. Testing for genetic alterations in NSCLC includes EGFR mutations, ALK rearrangements, ROS1 fusions, BRAF mutations, MET exon 14 skipping mutations, and RET rearrangements. Additionally, PD-L1 expression is assessed separately using immunohistochemistry (IHC) rather than genetic testing, as it measures protein expression levels rather than genomic alterations. While squamous NSCLC historically had limited testing recommendations, recent evidence suggests molecular testing may be beneficial in select cases, particularly in never-smokers or those with mixed histology [29]. A broader testing panel incorporating KRAS, HER2, MET amplification, and NTRK rearrangements could further refine personalized treatment strategies [34, 35].

Table 3 illustrates the progression of liquid biopsy applications in NSCLC mutation detection over recent

Table 3 Evolution of liquid biopsy in NSCLC

Years	Evolution of Liquid Biopsy in NSCLC
Pre-2000s: Tissue Biopsy Era	• Tissue biopsy as the gold standard for NSCLC diagnosis. • Histological subtyping of lung cancer (squamous vs. non-squamous NSCLC).
2000s: Discovery of Genomic Aberrations and Targeted Therapies	• Identification of **EGFR mutations** and development of **EGFR-TKIs** (e.g., gefitinib, erlotinib). • Detection of **ALK rearrangements** using fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC). • Introduction of tissue-based genomic testing for treatment selection.
2010s: Emergence of Liquid Biopsy and ctDNA Testing	• 2011: **Liquid biopsy introduced for detecting** circulating tumor DNA (ctDNA). • 2014: **FDA approval of the** cobas EGFR Mutation Test v2, **first liquid biopsy test for NSCLC.** • 2015: **Detection of** EGFR T790M resistance mutation **enables targeted therapy shifts (osimertinib approval).** • 2016: **Expansion of genomic profiling to non-EGFR mutations**: KRAS, ALK, MET, BRAF, ROS1, RET, HER2. • 2018: **Next-generation sequencing (NGS) enhances liquid biopsy accuracy.**
2020s: Advancements in Liquid Biopsy Technologies	• **2020**: FDA approval of **Guardant360 CDx** for comprehensive genomic profiling. • **2021**: Emergence of **extracellular vesicles (EVs) and circulating tumor cells (CTCs)** in lung cancer research. • **2022**: Liquid biopsy expands to **monitor minimal residual disease (MRD)** and guide immunotherapy (PD-L1 assessment). • **2023-Present**: AI-driven bioinformatics tools improve liquid biopsy sensitivity and predictive value.
Future Directions (Beyond 2025)	• **Multi-modal liquid biopsy platforms** combining **ctDNA**, **CTCs**, **and EVs** for a holistic approach. • **Machine learning and AI-powered predictive models** for personalized treatment planning. • **Early detection and real-time monitoring** using non-invasive blood-based assays. • **Standardization of liquid biopsy protocols** for broader clinical adoption.

years and potential future developments. Initially, liquid biopsy was primarily used to detect EGFR mutations in advanced NSCLC patients [50]. Plasma-based EGFR testing at baseline has since become an established alternative when tissue biopsy is infeasible, guiding first-line treatment decisions for patients eligible for EGFR tyrosine kinase inhibitors (TKIs) [37]. Liquid biopsy also plays a crucial role in detecting the T790M resistance mutation, which is the primary cause of acquired resistance to first- and second-generation EGFR TKIs such as erlotinib, gefitinib, and afatinib [39, 40].

Detection and quantification of EGFR mutation in liquid biopsies

Table 4 summarizes the studies that employed liquid biopsy for EGFR mutation detection in NSCLC. Various methodologies, including ddPCR, next-generation sequencing (NGS), and the cobas EGFR Mutation Test, were used to identify actionable EGFR mutations in ctDNA from plasma samples.

Ruiz et al. conducted a comparative study evaluating the accuracy of liquid biopsy using the cobas technique to detect EGFR mutations. They found that in 71% of patients ($n = 27$), at least one liquid biopsy detected EGFR mutations [23]. Silveira et al. (2021) demonstrated that ddPCR could detect the T790M resistance mutation at allele frequencies as low as 0.01%, outperforming the cobas EGFR Mutation Test [33]. Similarly, Zungsontiporn et al. (2024) confirmed that ddPCR's compartmentalization of DNA into individual droplets enhances its detection capabilities, particularly for ctDNA mutations [20].

NGS offers comprehensive mutational profiling, as highlighted by studies conducted by Bai et al. (2019) and Martínez-Herrera et al. (2024). These studies demonstrated NGS's ability to uncover co-existing mutations and tumor heterogeneity, both of which are crucial for personalized therapeutic strategies. However, NGS requires high-quality plasma samples, limiting its applicability in cases with low ctDNA yield. Despite this limitation, its broad mutational detection capabilities make it an invaluable tool for assessing tumor dynamics and resistance mechanisms [22, 40].

While the cobas EGFR Mutation Test is widely used due to its accessibility and clinical utility, it has lower sensitivity compared to ddPCR, particularly for low-allele-frequency mutations. Combining cobas with ddPCR could improve diagnostic accuracy [11, 29, 35].

Additionally, innovative methods such as bronchial washing extracellular vesicle analysis, as explored by Park et al. (2020), offer a promising, minimally invasive alternative when ctDNA levels are insufficient. However, further validation and standardization are required before this approach can be widely implemented [50].

Comparative analysis of these techniques highlights the trade-offs between sensitivity, comprehensiveness, and practicality. ddPCR excels in detecting low-frequency mutations, making it ideal for monitoring resistance mutations like T790M. In contrast, NGS provides a broader mutational landscape but is limited by its sample quality requirements. The cobas test, while widely used, has lower sensitivity and benefits from being supplemented with more advanced detection methods [46–48].

Despite significant advancements, challenges remain. Variability in ctDNA levels across patients and tumor types can impact detection rates. Additionally, the lack

Table 4 Comprehensive summary of EGFR mutation detection studies using liquid biopsy in NSCLC

Study	Methodology	Key Findings	Clinical Implications	Limitations
Zungsontiporn et al. (2024)	ddPCR vs. cobas PCR	ddPCR detected T790M mutations at lower frequencies (0.01%) than cobas PCR (1%).	Improved sensitivity for detecting resistance mutations; enables timely treatment adjustments.	Higher cost of ddPCR; requires specialized equipment.
Zhao et al. (2023)	NGS	Comprehensive mutation profiling achieved with high accuracy in advanced NSCLC.	Facilitates targeted therapy selection.	Potential false negatives in samples with low ctDNA levels.
Martínez-Herrera et al. (2024)	NGS	Detected co-existing mutations, showcasing tumor heterogeneity.	Aids in tailoring personalized therapeutic strategies.	Requires high-quality plasma samples.
Ruiz et al. (2023)	Targeted Liquid Biopsy	Positive EGFR mutations in liquid biopsies were significantly associated with improved overall survival.	Highlights the prognostic value of EGFR liquid biopsy results in patient management.	Limited exploration of other mutational markers.
Porta et al. (2023)	Comprehensive genomic profiling	Validated liquid biopsy use in diverse populations.	Supports real-world applicability of liquid biopsy in NSCLC management.	Variability in platform sensitivity.
Shah et al. (2022)	Liquid biopsy for EGFR testing	Effective for monitoring therapeutic responses.	Enables dynamic treatment adjustments based on mutational changes.	Lack of standardized protocols.
New Study (Efficacy of Liquid Biopsy)	Liquid biopsy for EGFR monitoring	Demonstrated efficacy in predicting tumor progression and monitoring treatment responses.	Provides an early warning system for disease progression and facilitates personalized treatment.	Limited by sample availability and variability in ctDNA levels.
Behel et al. (2022)	Liquid biopsy (cfDNA)	Demonstrated the utility of cfDNA for EGFR mutation detection post-treatment initiation in advanced NSCLC.	Provides dynamic disease monitoring and facilitates treatment adjustments.	Limited sample size and potential variability in cfDNA levels.
Silveira et al. (2021)	ddPCR	Accurate detection and quantification of T790M mutations in liquid biopsies.	Improves sensitivity and supports precision treatment decisions.	Requires highly specialized equipment and training.
Park et al. (2020)	Bronchial washing extracellular vesicles	Successful EGFR mutation detection in NSCLC using extracellular vesicles.	Offers an alternative to traditional plasma-based biopsies.	Limited generalizability; requires further validation.
Minari et al. (2020)	Liquid biopsy	Identified EGFR mutations in patients with disease progression during TKI therapy.	Supports real-time monitoring and subsequent treatment planning.	Retrospective nature and variability in patient treatment history.
Bai et al. (2019)	Target capture sequencing	Efficient detection of EGFR mutations in plasma using targeted sequencing.	Enables accurate identification of actionable mutations.	High cost and need for high-quality plasma samples.
Akamatsu et al. (2019)	Multiplexed digital PCR	Demonstrated the value of plasma EGFR mutation monitoring in afatinib-treated patients.	Supports longitudinal disease monitoring.	Small cohort size; findings may not generalize.
Sorber et al. (2018)	Optimized liquid biopsy workflow	Optimized workflow improves EGFR mutation detection accuracy in clinical settings.	Facilitates broader implementation of liquid biopsy in clinical practice.	Needs cross-platform validation.
Yang et al. (2017)	cfDNA	Detected EGFR and BRAF mutations in peripheral blood cfDNA of NSCLC patients.	Broadens mutation analysis scope, aiding personalized therapy.	Limited by cfDNA yield in certain patients.
Duan et al. (2015)	Scorpion ARMS method	Compared plasma and tumor EGFR mutation statuses, highlighting concordance.	Provides insights into plasma as a surrogate for tissue biopsy.	Lower sensitivity in plasma samples.
Weber et al. (2014)	Allele-specific PCR	Validated plasma as a reliable source for EGFR mutation detection.	Enables less invasive diagnostic approaches.	Potential for false negatives in low-burden cases.
Zhao et al. (2013)	Tissue vs. plasma comparison	Showed significant concordance of EGFR mutations between tissue and plasma samples.	Supports the use of plasma EGFR testing for early-stage NSCLC.	Lower mutation detection rates in early stages.

of standardized protocols and cross-platform validation contributes to inconsistencies in test performance and result interpretation [43]. Furthermore, the high costs associated with advanced technologies like ddPCR and NGS limit their accessibility in resource-constrained settings. Addressing these challenges requires continued innovation, multicenter validation studies, and efforts to standardize testing protocols [42].

Detection and quantification of non-EGFR mutation in liquid biopsies

Porta et al. in a cross-sectional study among the 229 lung cancer patients found that 90.4% of patients had at least one detectable alteration in plasma. The most frequently mutated genes were TP53 (47.6%), DNMT3A (33.2%), EGFR (20.1%), and KRAS (15.7%). Also, Elevated tumor fraction was observed in 18.3% of patients, signifying a high degree of test result reliability. Based on the ESCAT

classification, potentially actionable alterations (Tier I-II) were identified in 27.1% of samples. Furthermore, 5.2% of samples harbored alterations for which approved drugs are available in other cancer types (Tier III). They concluded that liquid biopsy NGS offers a promising avenue for tailoring treatment plans to individual patients [24]. Similarly, another study found a high sensitivity and accuracy of liquid biopsy in detecting KRAS mutations, HER2 mutations and MET amplification [30].

Moreover, researchers in a comparative study found that liquid biopsy could determine KEAP1/NFE2L2 mutations with high sensitivity among the patients with NSCLC [31]. Qvick et al. observed that liquid biopsy could detect more mutations in patients with stage IIIb-IV disease compared to patients with stage I-IIIa [34]. Similarly, Schwartzberg et al. found a considerable sensitivity and accuracy of liquid biopsy for ALK mutations detection [35].

In summary, liquid biopsy has demonstrated significant promise in NSCLC management. However, challenges such as ctDNA variability, methodological standardization, and cost barriers must be addressed for broader clinical integration. Combining multiple techniques may provide the most comprehensive approach for detecting and quantifying EGFR mutations, ultimately facilitating early intervention, personalized treatment, and improved patient outcomes.

Detection of extracellular vesicles and Circulating tumor cells in liquid biopsy

While ctDNA is the most widely used biomarker in liquid biopsy, other markers, including extracellular vehicles (EVs) and circulating tumor cells (CTCs), have been explored for their potential applications in NSCLC detection and monitoring. These alternative markers provide complementary information that may enhance the sensitivity and specificity of liquid biopsy-based approaches [37, 38].

Extracellular vesicles, including exosomes, contain tumor-derived nucleic acids, proteins, and lipids that can provide insights into tumor biology. Several studies have suggested that EVs may serve as promising biomarkers for detecting oncogenic mutations and monitoring treatment response. For example, Park et al. demonstrated that EGFR mutations could be detected in RNA extracted from bronchial washing-derived extracellular vesicles, indicating a potential role for EV-based liquid biopsy in lung cancer diagnostics. However, despite its promise, the use of EVs in routine clinical practice remains limited due to challenges in standardization, isolation, and validation of reliable biomarkers [50].

Circulating tumor cells represent another valuable component of liquid biopsy, offering direct insight into tumor heterogeneity and metastatic potential. CTC enumeration has been associated with prognosis in multiple cancers, including NSCLC, with higher CTC counts correlating with poorer survival outcomes [30]. Additionally, molecular profiling of CTCs allows for the identification of actionable mutations and resistance mechanisms. Despite these advantages, CTC detection remains technically challenging due to their rarity in circulation and the need for highly sensitive enrichment and isolation techniques [31].

Compared to EVs and CTCs, ctDNA remains the most widely used biomarker in liquid biopsy due to its higher detectability, ease of isolation, and well-established clinical utility. Advances in next-generation sequencing (NGS) and digital droplet PCR (ddPCR) have significantly improved the sensitivity and specificity of ctDNA analysis, making it the preferred option for genetic profiling and disease monitoring [27]. While emerging technologies continue to explore the potential of EVs and CTCs, ctDNA remains the primary liquid biopsy marker utilized in routine clinical practice due to its proven ability to guide targeted therapy decisions and track tumor evolution [26].

Future research should focus on integrating multiple liquid biopsy components, combining ctDNA, EVs, and CTCs to provide a more comprehensive assessment of tumor biology. As technological advancements improve the isolation and analysis of these markers, their role in NSCLC management is expected to expand.

Liquid biopsy and disease progression

Beyond the identification of actionable mutations, liquid biopsy serves as a valuable tool for monitoring disease progression and guiding treatment decisions in NSCLC. Due to its minimally invasive nature, serial liquid biopsy testing allows for real-time assessment of tumor evolution, treatment response, and the emergence of resistance mechanisms.

Several studies included in this review demonstrated that liquid biopsy effectively tracks changes in tumor burden over time by quantifying circulating tumor DNA (ctDNA) levels. For instance, Behel et al. reported that dynamic changes in ctDNA concentrations correlated with treatment response, with decreasing levels indicating therapeutic efficacy and rising levels signaling potential disease progression or resistance [29].

One of the most significant applications of liquid biopsy in treatment monitoring is the detection of resistance mutations, particularly in patients receiving targeted therapies. The emergence of the EGFR T790M resistance mutation in patients undergoing first- or second-generation tyrosine kinase inhibitors (TKIs) can be detected early through liquid biopsy, enabling a timely switch to third-generation TKIs such as osimertinib [32]. Similarly, MET amplification, a common resistance mechanism

to EGFR inhibitors, has been successfully identified via liquid biopsy, allowing for treatment adjustments with MET-targeted agents [26].

Furthermore, liquid biopsy facilitates early detection of minimal residual disease (MRD) following curative-intent treatments such as surgery or radiation therapy. Studies have shown that the presence of ctDNA post-surgery is associated with a higher risk of recurrence, highlighting the potential of liquid biopsy to guide adjuvant therapy decisions [37].

Collectively, these findings underscore the multifaceted role of liquid biopsy beyond mutation detection. Its utility in disease monitoring, treatment response assessment, and resistance detection makes it a powerful tool for personalized medicine in NSCLC. As technological advancements enhance the sensitivity and specificity of liquid biopsy assays, its integration into routine clinical practice is expected to improve patient management and outcomes.

Discussion

This systematic review highlights the growing potential of liquid biopsy as a minimally invasive tool for detecting genetic mutations in non-small cell lung cancer (NSCLC). The studies included in this review collectively emphasize the ability of liquid biopsy to identify actionable mutations, monitor disease progression, and guide personalized treatment decisions. Techniques such as ddPCR, NGS, and the cobas EGFR Mutation Test emerged as the most widely studied methodologies, each with distinct advantages and limitations. While ddPCR demonstrated exceptional sensitivity for low-frequency mutations such as T790M, NGS offered comprehensive mutational profiling, which is essential for understanding tumor heterogeneity. Although the cobas test is less sensitive, it remains a practical option due to its broad clinical adoption and accessibility.

Our findings align with previous reviews that emphasize the transformative role of liquid biopsy in precision oncology [51]. Repeated tissue biopsies, while essential for determining genetic mutations and tracking tumor evolution in NSCLC patients, are invasive procedures that rely on precise sampling and can be affected by tumor heterogeneity [52, 53]. Liquid biopsy, as a less invasive approach, may reduce the need for repeated tissue biopsies in patients with confirmed EGFR mutations, offering a more convenient and reliable method for monitoring disease progression and treatment response [54]. By using non-invasive methods, such as blood or urine samples, liquid biopsy is generally more tolerable for patients, facilitating convenient genetic testing and enabling personalized treatment strategies for individuals with NSCLC [55, 56].

Therefore, liquid biopsy can be effectively employed to track the molecular evolution of tumors over time and assess treatment response in advanced-stage cancers [57]. Notably, while liquid biopsy exhibits high sensitivity in advanced-stage tumors, it may not attain the same level of performance as tissue biopsy [58, 59]. To enhance the sensitivity of liquid biopsy, repeated blood sampling at intervals or the analysis of alternative biological fluids, such as saliva, urine, or sputum, could be considered, either independently or in conjunction with plasma [60]. However, liquid biopsy may not be optimal for early-stage tumors, as it frequently produces false-negative results. This limitation may be attributed to the scarcity or absence of ctDNA in the bloodstream or to the inherent limitations of current detection methodologies [61].

Another factor that can impact the diagnostic performance of liquid biopsy is the methodology employed for mutation detection. Currently, NGS and PCR-based techniques are utilized for ctDNA detection. However, the scarcity of ctDNA in the bloodstream remains a significant challenge, highlighting the need for the development of more sensitive techniques for its identification and quantification [51, 62].

A crucial aspect of the clinical applicability of liquid biopsy lies in its distinct role in early-stage versus advanced-stage NSCLC [63]. In advanced-stage NSCLC, where tumor burden is higher and ctDNA levels in the bloodstream are more abundant, liquid biopsy has shown significant promise in monitoring disease progression and detecting resistance mutations such as T790M [64]. The ability to track tumor evolution in real-time allows for personalized treatment adjustments, such as switching to second-line therapies or targeted treatments. Liquid biopsy in this setting provides a non-invasive, cost-effective alternative to traditional tissue biopsies, allowing for frequent and repeated monitoring without the risks associated with invasive procedures [65].

In contrast, the role of liquid biopsy in early-stage NSCLC remains less clear, with studies reporting variable sensitivity. Early-stage tumors typically shed lower amounts of ctDNA into the bloodstream, which can lead to false-negative results in liquid biopsy [66]. While there is potential for using liquid biopsy for early detection or monitoring minimal residual disease following surgery or adjuvant treatment, its current sensitivity may not be sufficient for widespread clinical application in early-stage NSCLC [67]. Nevertheless, emerging technologies, such as multi-biomarker liquid biopsies, could improve the sensitivity and specificity of these tests, potentially making them more useful for early-stage detection in the future [68]. Further studies are needed to optimize detection methods and validate the clinical utility of liquid biopsy in early-stage NSCLC.

This review provides a distinct perspective by not only synthesizing the current evidence but also addressing key gaps in the literature. Specifically, we emphasize the dynamic role of liquid biopsy in personalized treatment regimens, including its capacity to monitor disease progression, identify actionable mutations, and detect resistance mutations such as T790M. Moreover, our review critically examines the current methodologies, such as ddPCR, NGS, and the cobas EGFR Mutation Test, to highlight the strengths, weaknesses, and challenges specific to their clinical application in NSCLC.

In addition to summarizing existing methodologies, this review proposes novel directions for the future use of liquid biopsy. For example, while previous reviews have predominantly focused on ctDNA as a sole biomarker, we expand the scope to include emerging techniques such as exosome-based analyses, the use of alternative biological fluids, and multimodal liquid biopsy approaches [69]. Furthermore, we explore the clinical implications of integrating liquid biopsy into routine practice, with an emphasis on standardization of methodologies and overcoming technical challenges related to cost, variability in ctDNA levels, and methodological discrepancies.

Our findings also highlight the underexplored potential of liquid biopsy in early-stage NSCLC, providing new insights into how this non-invasive approach could be leveraged for early diagnosis and monitoring, especially for patients who may not yet present with detectable levels of tumor-specific DNA. The identification of non-EGFR mutations, including KRAS, ALK, and MET amplifications, is another area where liquid biopsy shows significant promise, broadening the scope of actionable targets beyond EGFR mutations [70]. These findings position liquid biopsy as not only a diagnostic tool but also an integral part of the evolving landscape of personalized cancer therapy, where it could provide a comprehensive, real-time understanding of tumor evolution.

In addition to EGFR mutations, liquid biopsy plays a critical role in detecting a wide range of non-EGFR mutations in NSCLC, significantly broadening its clinical utility. Studies have shown that liquid biopsy effectively identifies mutations in KRAS, ALK, MET, BRAF, ROS1, RET, and NTRK genes, many of which serve as actionable targets for personalized therapy [71].

KRAS mutations, particularly KRAS G12C, are detected with high sensitivity using next-generation sequencing (NGS)-based liquid biopsy, enabling targeted therapy with KRAS inhibitors such as sotorasib and adagrasib. In a study by Porta et al., 90.4% of NSCLC patients analyzed via liquid biopsy had at least one detectable alteration, with KRAS mutations identified in 15.7% of cases [72].

ALK and ROS1 rearrangements, which are traditionally detected using fluorescence in situ hybridization (FISH) or immunohistochemistry (IHC) in tissue biopsy, can also be identified through liquid biopsy [73]. Schwartzberg et al. demonstrated a high concordance rate between liquid biopsy and tissue biopsy for detecting ALK fusions, reinforcing its role in guiding targeted treatment with ALK inhibitors such as alectinib and lorlatinib [74].

Additionally, MET exon 14 skipping mutations and MET amplifications, both of which confer sensitivity to MET inhibitors like tepotinib and capmatinib, have been successfully detected via liquid biopsy. A study by Qvick et al. found that liquid biopsy had a higher detection rate for MET alterations in patients with advanced-stage NSCLC compared to early-stage cases, highlighting its potential utility in disease monitoring [75, 76].

Moreover, liquid biopsy has been used to detect rare but clinically significant alterations, such as RET and NTRK fusions. Although these mutations occur at a lower frequency in NSCLC, their detection is essential for guiding treatment with RET inhibitors (selpercatinib, pralsetinib) and NTRK inhibitors (larotrectinib, entrectinib) [77].

These findings underscore the expanding role of liquid biopsy in NSCLC beyond EGFR testing, providing a minimally invasive approach for comprehensive genomic profiling and precision medicine. As liquid biopsy technologies continue to improve, their application in detecting a broader range of oncogenic drivers will further enhance clinical decision-making and patient outcomes.

While previous reviews have addressed the potential of liquid biopsy in NSCLC [63], few have connected its application to both diagnosis and ongoing treatment modifications across diverse patient populations. The findings from this review extend existing literature by offering a clearer roadmap for the implementation of liquid biopsy in clinical practice, addressing both its advantages and limitations, and suggesting practical solutions to overcome barriers for broader adoption.

This review provides a comprehensive analysis of the current landscape of liquid biopsy in NSCLC, synthesizing findings from diverse methodologies and clinical contexts. However, certain limitations must be acknowledged. The included studies exhibited heterogeneity in patient populations, ctDNA yield, and detection platforms, which may affect the generalizability of the results. Additionally, the retrospective nature of several studies limits the ability to draw causal inferences about clinical outcomes. Future research should prioritize prospective studies with standardized protocols to enhance comparability and robustness.

Conclusion

Liquid biopsy represents a significant advancement in NSCLC diagnostics and treatment, offering a minimally invasive alternative to traditional tissue biopsy. While

ddPCR and NGS stand out for their sensitivity and comprehensiveness, broader clinical adoption will require addressing cost, standardization, and technical challenges. By bridging the gap between precision diagnostics and personalized therapy, liquid biopsy has the potential to revolutionize cancer care, improving outcomes for patients worldwide.

Emerging technologies such as machine learning-assisted mutation analysis and multimodal liquid biopsy (combining ctDNA, exosomes, and circulating tumor cells) represent exciting avenues for future research. Additionally, multicenter trials with diverse patient populations are needed to validate findings and refine clinical guidelines. Expanding the application of liquid biopsy beyond NSCLC to other cancer types also warrants exploration, given its potential for early detection and precision treatment.

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Author contributions
MR conceived the study, MF, MR, RR and FMS collected and analyzed the data. MR, and GB interpreted the statistical analyses and MR wrote the first draft of the manuscript. FMS contributed to the manuscript revising and editing. All of the authors critically revised the manuscript. The author(s) read and approved the final manuscript.

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Declarations

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Transparency declaration
The lead author affirms that this manuscript is an honest, accurate, and transparent account of the studies being reported. The lead author affirms that no important aspects of the studies have been omitted and that any discrepancies from the studies as planned have been explained.

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