International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 9s, 2025
https://www.theaspd.com/ijes.php

Evaluating The Sensitivity, Specificity, And Predictive Accuracy Of Liquid Biopsy Across NSCLC Stages

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Abstract
The majority of lung cancer cases belong to the NSCLC category, yet patients often present with advanced disease, which reduces their treatment effectiveness. The current standard of tissue biopsy molecular profiling requires alternative solutions because of its invasive characteristics, along with restrictive sampling capabilities. Diagnostic precision of liquid biopsy through circulating tumor DNA (ctDNA) analysis remains insufficiently defined for all clinical stages. The research investigated liquid biopsy-based next-generation sequencing (NGS) against tissue-based NGS in 120 subjects who had never received treatment for NSCLC at a single medical facility. We tested matched tissue and plasma samples while using digital PCR to investigate cases showing differences between results. The analysis showed a 70.8% concordance rate and 85.9% sensitivity, together with 75.0% specificity. The recognition rate of Stage III–IV patients (94.3%) surpassed the exposure rate of patients in Stage I–II (68.0%). The positive predictive value (PPV) remained stable throughout all stages, but the negative predictive value (NPV) decreased in affected persons with early-stage NSCLC. The research findings demonstrate that liquid biopsy serves as an additional diagnostic method for NSCLC patients who have restricted tissue availability in advanced stages. This research enhances the case for using liquid biopsy in precision medicine by delivering performance results per disease stage and demonstrating the necessity to validate findings across larger patient groups in multiple centers.

Keywords: *Diagnostic accuracy, Circulating tumor DNA, Non-small cell lung cancer, Liquid biopsy, Precision oncology*

Introduction

NSCLC stands as the predominant lung cancer type responsible for 85% of cases, while continuing to be a foremost cause of global cancer-related deaths. NSCLC functions as a major public health concern because it causes more than 1.7 million annual lung cancer deaths. NSCLC management faces a critical challenge because patients often receive their diagnosis at advanced stages, thus reducing treatment effectiveness and affecting survival statistics. The field of precision oncology now revolutionizes treatment approaches through targeted therapy selection, which applies to mutations of EGFR and ALK rearrangements and ROS1 fusions [1]. The diagnosis and treatment planning process for NSCLC now depends on precise molecular profiling assessments, which must happen promptly. The gold standard for molecular testing depends on tissue biopsy as its traditional method. The invasive nature of tissue-based methods leads to procedural risks and produces insufficient material, especially when tumors become inaccessible in advanced disease stages (2). The heterogeneity of tumors and their evolutionary changes produce sampling bias through tissue biopsy because it provides only one point in time and space representation of the disease.

The clinical practice now utilizes liquid biopsy as a negligibly aggressive diagnostic technique to address existing challenges. Real-time assessment of tumor fruition and healing outcomes becomes possible through analyzing circulating tumor DNA (ctDNA) that occurs in blood tests with liquid biopsy (3,4). The operational sensitivity and specificity of the technique have improved through recent breakthroughs in next-generation sequencing technology (NGS) and digital PCR, which have extended its capacity within clinical practice (5). The serial sampling capabilities of liquid biopsy prove essential for medical situations that require repeated tissue biopsies to be unsafe or impractical. Scientists apply liquid biopsy measurements to detect cancers early and monitor diseases while identifying residual disease conditions (6,7). The International Association for the Study of Lung

Cancer, together with other international groups, has acknowledged through consensus statements that liquid biopsy provides clinical value in advanced NSCLC and should be used in diagnostic procedures (8). The achievements of recent research have not eliminated crucial missing knowledge areas. The existing research literature presents diagnostic statistics as aggregates without dividing results according to disease stages (9). The biological variation in ctDNA shedding between early-stage and late-stage tumors becomes a barrier to understanding test performance when no stratification methods are used (10). Systematic reviews together with meta-analyses create a broad population amalgamation of data from various patient groups and which reduces the clinical applicability of their findings (11).

The current literature shows a significant deficiency in reporting essential diagnostic presentation metrics, including positive predictive value (PPV) and negative predictive value (NPV) for proper clinical decision assessment (12). The direct comparison of biopsy modalities becomes weaker because most studies lack matched plasma samples and tissue from the same affected person (13). Many cases of tissue and liquid biopsy mismatch fail to receive orthogonal confirmation through digital PCR testing because this method detects mutations that NGS platforms might miss (14). The implementation of algorithm-based subtyping alongside innovative biomarkers such as methylated DNA and tumor-educated platelets in liquid biopsy analysis exists mainly as exploratory research, which requires extensive testing in clinical environments [15]. Plenty of heterogeneity exists between diagnostic platforms as well as reporting methodologies, making their integration into standard operating procedures challenging (16). The findings lack generalization because research has insufficiently included diverse populations, particularly from regions with limited resources, where liquid biopsy presents unique implementation challenges (17). The unresolved issues demonstrate the necessity for stage-stratified studies that validate real-world methods for liquid biopsy performance assessment in well-defined patient populations.

Research Objectives

This research aims to conduct a comprehensive stage-stratified evaluation of liquid biopsy performance in NSCLC. Our research focuses on determining how well liquid biopsy NGS methods match tissue biopsy NGS methods for detecting relevant mutations for clinical purposes. Our research sample consists of 120 treatment-naive NSCLC patients who received histological confirmation, with 85 patients showing matching mutations between tissue and plasma tests and 15 patients with tissue-only mutations and 5 patients with plasma-only mutations, and 15 patients who were wild-type in both tests. The study analyzes liquid biopsy performance metrics, including detection and selectivity, and predictive value (PPV and NPV) for Stage I–II and Stage III–IV NSCLC-affected individuals through matched biopsy pair analysis. The accuracy of discordant results gets improved through additional validation using digital PCR methods. The research investigates both operational difficulties and diagnostic constraints of liquid biopsy implementation in medical practice when compared to conventional tissue-based diagnostic methods. The study follows STARD guidelines to provide data-based findings about NSCLC diagnostic changes.

Methodology

Study Design and Setting

The diagnostic accuracy study took place from January 2024 through January 2025 at two tertiary oncology facilities in the Pulmonology Department, Sri Lalithambigai Medical College and Hospital and Oncology Department, Kauvery hospital, Alwarpet, Chennai.,. The research examined how well liquid biopsy NGS testing performed against standard tissue biopsy NGS testing for NSCLC diagnosis. The researchers used consecutive sampling as their method to achieve representative results while reducing selection bias. The research design adhered to STARD 2015 (Standards for Reporting Diagnostic Accuracy Studies) guidelines during the development and reporting stages. The research was approved ethically by the Sri Lalithambigai Medical college and Hospital, Chennai.

Study Population

The research included 120 patients between 40 to 80 years of age who received an NSCLC diagnosis through histopathological examination. The study participants included sixty percent male patients and forty percent

female patients. The participants had an average age of 62.3 ± 9.8 years, while sixty-six percent of them were either active or previous tobacco users. The study enrolled treatment-naïve patients who had sufficient tissue and liquid biopsy material for genomic analysis. The study excluded patients who received chemotherapy or radiotherapy treatment, along with those who had concurrent malignancies or inadequate biopsy material quality or quantity. The American Joint Committee on Cancer (AJCC) 8th edition TNM cataloging served as the basis to stage diseases through radiological and histological evaluations. Each participant signed written consent documents before study admission.

Sample Collection Procedures

The research team obtained 10 mL EDTA tube blood samples from participants before they started any cancer treatment. The researchers processed blood samples within two hours to maintain the structural quality of circulating tumor DNA (ctDNA). The plasma separation process involved two centrifugation steps, starting with 1600 × g for 10 minutes, then continuing with 16,000 × g for another 10 minutes to eliminate cellular remains. The researchers stored plasma aliquots at −80°C before extracting DNA from them.

The researchers collected matched tumor tissue samples through CT-guided core needle biopsy or bronchoscopic biopsy procedures within seven days of blood collection, according to tumor location. The researchers conducted plasma sampling within a short timeframe to minimize clinical inconsistencies, which could be caused by tumor heterogeneity or clonal evolution. Standardized institutional protocols were used to fix and embed tissue samples in formalin before paraffin processing.

DNA Extraction and Library Preparation

The QIAamp Circulating Nucleic Acid Kit from Qiagen (Hilden, Germany) proved effective in extracting plasma ctDNA because it shows high sensitivity for recovering low-abundance fragmented DNA. The DNA extraction process for FFPE samples used the GeneRead DNA FFPE Kit (Qiagen) after performing deparaffinization and enzymatic digestion steps. The Qubit dsDNA High Sensitivity Assay from Thermo Fisher Scientific and the Agilent 2100 Bioanalyzer determined DNA concentration along with quality measurements.

The Ion AmpliSeq Library Kit 2.0 (Thermo Fisher Scientific) produced libraries from DNA inputs ranging from 10–50 ng by targeting 50 genes essential for NSCLC diagnosis (EGFR, ALK, KRAS, BRAF, MET, and ROS1). A cost-effective diagnostic tool emerged from this panel because it demonstrated clinical relevance according to both ESMO and NCCN criteria. The manufacturer's instructions guided the execution of library amplification and barcoding, and adapter ligation steps. The Ion S5 XL platform performed sequencing operations after the Ion Chef System executed the templating process.

Bioinformatics Analysis and Variant Calling

The Torrent Suite Software v5.12 analyzed sequencing data by aligning reads to hg19 before the Variant Caller plugin detected variants. The Ion Reporter Software v5.18 performed variant annotation through integration of dbSNP and ClinVar, and COSMIC databases for classification purposes. The analysis included variants that met two criteria: 500× read depth and VAF exceeding 0.5% for plasma-derived DNA and 2% for tissue-derived DNA. The established thresholds used the Ion S5 XL platform detection limits as their basis to achieve maximum analytical specificity while reducing noise. The analysis excluded variants that received benign or likely benign classifications.

The analysis of genomic alterations between matched patient samples used mutational concordance as the assessment method. The analysis considered mutations present in one sample type when they failed to appear in their corresponding matched sample. The presence of low-frequency variants was verified through orthogonal validation with digital PCR (dPCR) among 15% of discordant results to detect and decrease potential false-negative outcomes.

Diagnostic Performance Metrics

Tissue biopsy-based NGS testing is the benchmark for assessing the diagnostic efficacy of liquid biopsy. The capability of liquid biopsy to correctly recognize mutation-positive tissue samples defines sensitivity, while specificity measures its ability to detect mutation-negative tissue samples as negative in plasma. The number of

accurately detected instances was divided by the total number of cases to determine the prediction accuracy. This study performed subgroup analysis for clinical stage NSCLC patients by evaluating early-stage (Stage I–II) and late-stage (Stage III–IV) patients separately to determine stage-specific results. The discrimination power of the liquid biopsy technique was evaluated using the receiver operating characteristic (ROC) curves and area under the curve (AUC) computations. The study presented PPV and NPV values together with 95% CI for the analysis.

Statistical Analysis

The statistical study was carried out by the researchers using IBM SPSS Statistics for Windows version 26.0, which was created by IBM Corp. in Armonk, NY, USA. The study presented continuous variables as mean values with standard deviation (SD), while categorical data received frequency and percentage summaries. The Cohen's kappa coefficient measured the agreement level between tissue and liquid biopsy results. The appropriate statistical tests for categorical variable comparison included Chi-square tests alongside Fisher's exact tests. The research utilized independent-samples t-tests for analyzing continuous variables. The pROC package in R version 4.2.0 allowed the generation of ROC curves and AUC calculations. The research used a p-value threshold of 0.05 to govern statistical significance.

Ethical Considerations

The research protocol gained approval from , Sri Lalithambigai Medical College and Hospital, Chennai. Each participant provided written consent after researchers explained the complete study goals together with procedures and possible dangers. The study participants received guarantees for confidentiality and maintained the freedom to leave the study at their discretion. The diagnostic nature of this study exempted it from needing clinical trial registry registration according to existing national guidelines.

Results

Patient Characteristics

The research enrolled 120 patients who received an NSCLC diagnosis through histological examination. The average age of the patient group was 62.3 years, with a standard deviation of 9.8 years. The age range of the study participants was 40–80 years old. The study population included 72 male patients who made up 60.0% of the total, while 48 female patients formed 40.0% of the total population. The study revealed that 80 patients (66.7%) were smokers while 40 patients (33.3%) did not smoke. Stage I–II clinical classification included 50 patients (41.7%) while 70 patients (58.3%) received Stage III–IV clinical diagnosis. Among all cases of NSCLC adenocarcinoma proved to be the most prevalent histological type with 56.7%, while squamous cell carcinoma followed at 35.0%, and other subtypes accounted for 8.3%. The patient data in Table 1 shows advanced-stage disease and adenocarcinoma subtype as the most prevalent conditions among the cohort members.

Table 1. Baseline Demographic and Clinical Characteristics of Patients with NSCLC

Characteristic	Category	n (%) / Value
Total patients	—	120
Age	Mean ± SD (years)	62.3 ± 9.8
	Range (years)	40–80
Sex	Male	72 (60.0%)
	Female	48 (40.0%)
Smoking status	Current/former smokers	80 (66.7%)
	Never smoked	40 (33.3%)
NSCLC Stage (AJCC 8th ed.)	Stage I–II	50 (41.7%)
	Stage III–IV	70 (58.3%)
Histological subtype	Adenocarcinoma	68 (56.7%)
	Squamous cell carcinoma	42 (35.0%)
	Other subtypes	10 (8.3%)

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 9s, 2025
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Mutation Concordance Between Liquid and Tissue Biopsy

The analysis of 120 matched plasma and tissue samples revealed identical mutations in 85 samples, which corresponded to 70.8% of the total cases. Mutations detected in tissue biopsy samples appeared without plasma presence in 15 cases (12.5%). The analysis of 5 samples (4.2%) revealed mutations that existed solely in plasma samples. The analysis of fifteen samples (12.5%) showed that no mutations were present in both biopsy types. Table 2 demonstrates the agreement between NGS analysis of plasma and tissue samples for detecting oncogenic mutations by showing that most cases produce matching results.

Table 2. Mutation Concordance Between Tissue and Liquid Biopsy (n = 120)

Mutation Status	No. of Cases (n)	Percentage (%)
Mutated in both tissue and plasma	85	70.8
Mutated in tissue only	15	12.5
Mutated in plasma only	5	4.2
No mutations in either sample	15	12.5

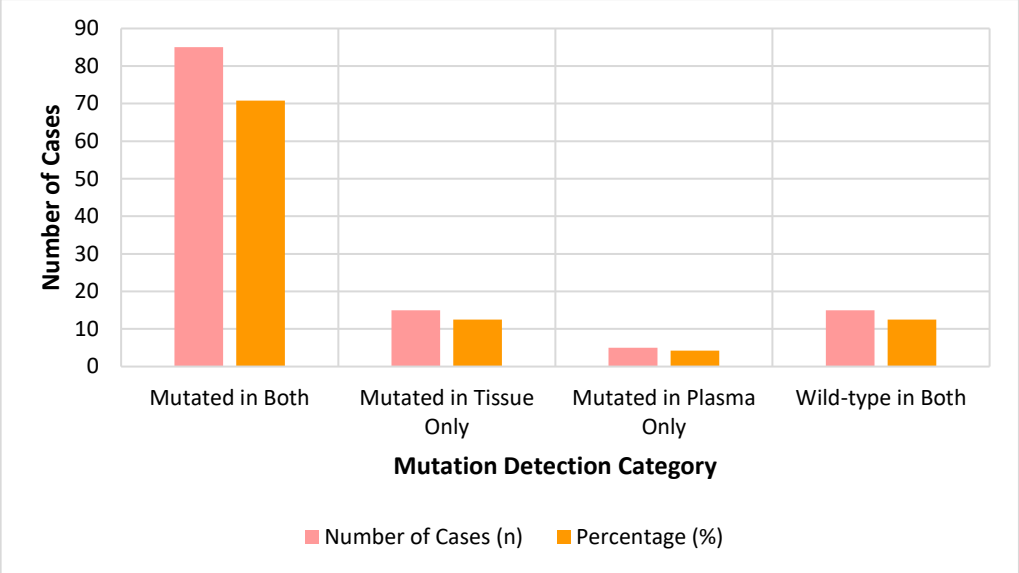

Figure 1. Mutation Concordance Between Liquid and Tissue Biopsy

Figure 1 demonstrates that most patients displayed identical mutation findings between tissue and liquid biopsy samples.

Digital PCR investigation was conducted on six discordant cases which represented 15% of the 40 non-concordant samples to verify erratic variant occurrence. Five out of these cases demonstrated mutations detected in plasma which tissue testing failed to detect or vice versa.

Diagnostic Performance of Liquid Biopsy

The diagnostic competence of liquid biopsy was evaluated via a reference standard method that used tissue biopsy-based NGS. The diagnostic test achieved 85.9% sensitivity and 75.0% specificity in its results. The probability of a true positive result reached 94.4% while the probability of a true negative result registered at 50.0%. The overall diagnostic accuracy was 83.3%. The ROC analysis generated an AUC value of 0.88. Table 3 demonstrates that liquid biopsy achieves high sensitivity and PPV but reveals lower NPV, especially when detecting cases without mutations.

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Table 3. Overall Diagnostic Performance Metrics

Metric	Value (%)
Sensitivity	85.9
Specificity	75.0
Positive Predictive Value (PPV)	94.4
Negative Predictive Value (NPV)	50.0
Diagnostic Accuracy	83.3
Area Under the Curve (AUC)	88.0

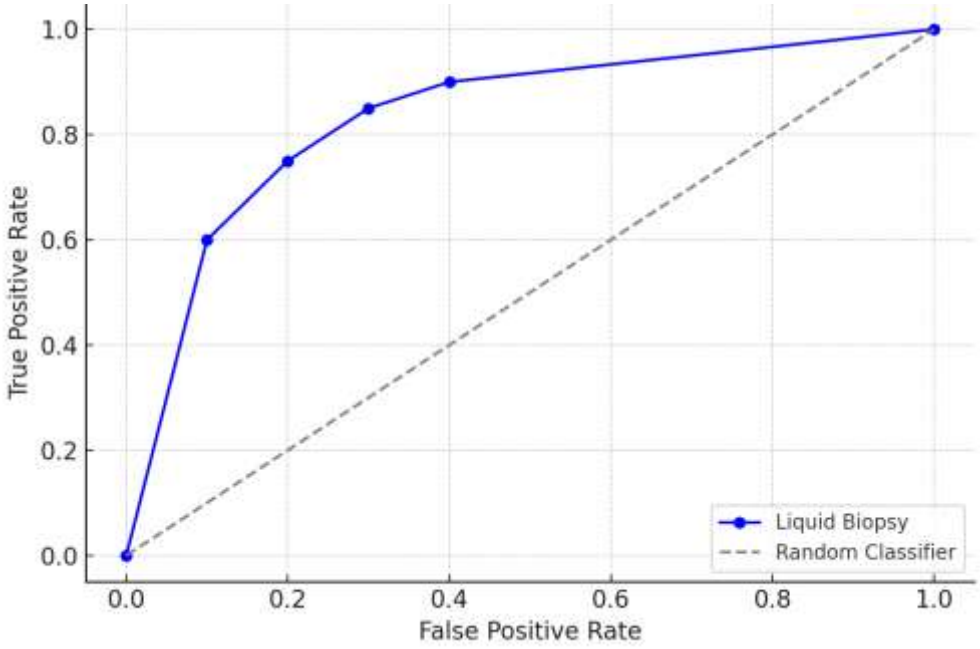

Figure 2. ROC Curve for Liquid Biopsy

Figure 2 displays the ROC curve for liquid biopsy, demonstrating its diagnostic presentation with an AUC of 0.88 relative to the tissue-based reference standard.

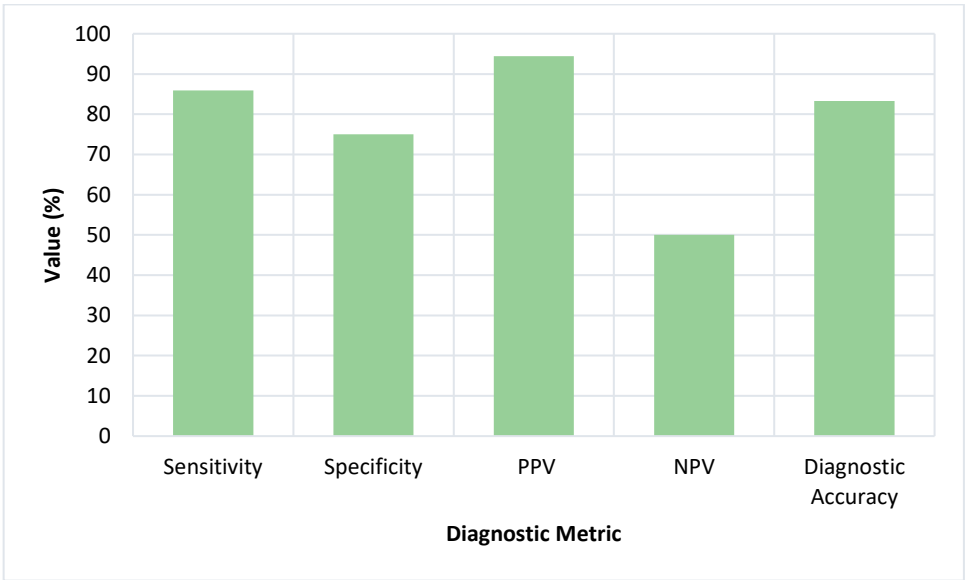

Figure 3. Summary of Diagnostic Performance Metrics

Figure 3 provides a visual summary of the diagnostic metrics for liquid biopsy, highlighting high sensitivity and PPV, with lower NPV values observed.

Stage-wise Diagnostic Performance

The analysis included 50 affected individuals with Stage I–II NSCLC and 70 persons with Stage III–IV NSCLC who collectively made up 100 patients or 100% of the total cohort. The Stage I–II subgroup analysis revealed a sensitivity of 68.0%, and specificity of 85.7%, and a PPV of 91.7%, together with an NPV of 50.0%. The sensitivity rate for Stage III–IV patients reached 94.3%, along with 70.0% specificity and 95.7% PPV, and 63.6% NPV. Table 4 shows that the performance of liquid biopsy tests differs according to disease stage because advanced NSCLC patients achieve better sensitivity results.

Table 4. Stage-wise Diagnostic Performance of Liquid Biopsy

Metric	Stage I–II	Stage III–IV
Sensitivity (%)	68.0	94.3
Specificity (%)	85.7	70.0
Positive Predictive Value (%)	91.7	95.7
Negative Predictive Value (%)	50.0	63.6

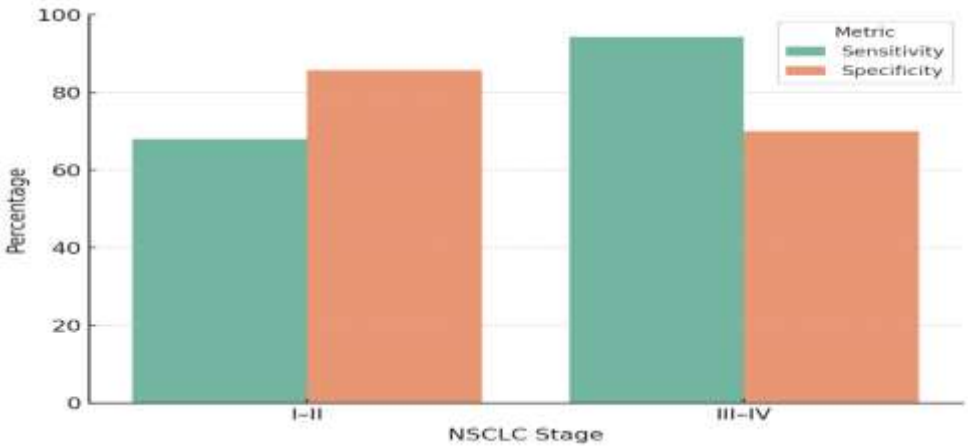

Figure 4. Sensitivity and Specificity by NSCLC Stage

Figure 4 compares the sensitivity and specificity of liquid biopsy across early-stage (I–II) and advanced-stage (III–IV) NSCLC, illustrating that sensitivity increases with disease stage while specificity slightly declines.

Discussion

Liquid biopsy proved to match tissue-related NGS for detecting clinically significant mutations in NSCLC patients with advanced disease stages. The diagnostic sensitivity rate was higher in persons with Stage III–IV NSCLC because of their increased tumor burden and elevated ctDNA levels. However, diagnostic specificity remained stable across all disease stages. The high PPV confirms that plasma-based mutation detection methods deliver reliable results in oncology practice when screening for EGFR and KRAS mutations. Stage I–II NSCLC patients showed lower NPV when liquid biopsy was used independently for exclusion testing. Digital PCR validation of tissue–plasma discrepancies showed that the differences resulted from variants that required higher sensitive platforms to detect subclonal or low-allele frequency variants. The research demonstrates that disease stage directly impacts diagnostic accuracy and demonstrates a practical model where liquid biopsy works together with tissue sampling for diagnostic purposes, especially when tissue sampling is not possible or when therapeutic decisions need repeated testing or disease monitoring. The liquid biopsy's competence to monitor dynamic changes and resistance patterns in tumors stands out as an important benefit because it obtains better heterogeneity data than static tissue measurements (18). The collected data support the execution of liquid biopsy as a precision oncology technique that should follow mutation-based protocols at specific disease stages.

These research findings receive direct affirmation from analysis carried out in current literature studies. Solid concordance between tissue and plasma mutation profiles in metastatic NSCLC has been reported, aligning with the high sensitivity observed in Stage III–IV cases [19,20]. The diagnostic accuracy achieved in high-burden tumors supports the implementation of liquid biopsy across multiple stages of cancer care, including baseline genotyping and longitudinal monitoring [21,22]. While the feasibility of plasma-based diagnostics has been demonstrated, previous studies did not provide stage-specific disaggregation or validate discordant results, both of which are addressed in the matched tissue-plasma approach used here [23,24]. Tissue biopsy accuracy is affected by spatial heterogeneity, whereas plasma-only positive results in the present analysis demonstrate that plasma-based testing can detect mutations inaccessible to traditional biopsy methods [25]. The limited diagnostic capacity of liquid biopsy in early-stage recognition has been previously noted, which is consistent with the reduced NPV and higher false-negative rates observed in low-burden cases in this study [26,27]. Similar limitations during early-stage assessment have also been documented, alongside proposals for machine-learning-based models aimed at enhancing early detection accuracy [28,29]. The diagnostic utility of liquid biopsy has additionally been supported in other tumor types such as gastric cancer, reinforcing its broader applicability in comprehensive oncology practice [30]. The present study benefits from three essential contributions, which include stage-specific diagnostic metrics from real-world matched data and digital PCR validation of conflicting results, and a defined role for liquid biopsy as a reliable secondary tool in disease areas with high prevalence. Several important restrictions exist despite the strong findings. The research featured DNA mutations as its sole criterion while operating from one institution, using a modest subject number that might hinder universal application. The research findings create groundwork toward studying clinical outcomes in longitudinal and treatment-linked investigations. The research data demonstrate that liquid biopsy should be incorporated into NSCLC diagnostic procedures when tissue samples are unavailable or inadequate. Further research needs to study larger multi-center patient groups while performing multi-omic testing to assess the cost-effectiveness and clinical benefits alongside patient results. Early-stage detection sensitivity will benefit from both AI-based decision-support systems integration with multi-analyte assays exploration. This analysis provides additional support for liquid biopsy as a practical diagnostic tool if physicians use it within its correct application scope and maintain awareness of its technical constraints.

Conclusion

The performance of liquid biopsy testing for NSCLC was compared to tissue-based NGS across different clinical stages. Stage III–IV NSCLC patients showed high sensitivity and PPV in diagnostic tests through liquid biopsy, which makes it a reliable diagnostic tool for inaccessible or insufficient tissue cases or repeated molecular profiling needs. The ctDNA-based detection method shows reduced sensitivity and NPV in Stage I–II tumors because early-stage cancers shed less material, which demonstrates the necessity of tissue analysis for confirmation in low-burden cases. The diagnostic validity and interpretation depth of the approach were reinforced by a double sample design and digital PCR analysis to validate conflicting results. The research supports liquid biopsy as an additional diagnostic tool that works best in high-risk disease conditions instead of replacing tissue biopsy at all disease stages. The study presents diagnostic thresholds for each stage, together with hands-on validation methods, which previous literature lacks. The study provides essential operational information that helps healthcare providers execute liquid biopsy testing in their daily practice. The methodology shows clinical relevance even when faced with the constraints of limited sample centers and sample size, and genomic mutation-based analysis. Studies of the future should expand their research by using multi-institutional groups of patients while including multiple biomarkers, including methylation markers and exosomal RNA, in addition to developing artificial intelligence-enhanced diagnostic systems to detect cancer early and increase precision oncology applications for liquid biopsy testing.

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