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*Review*

# The NGS and PCR-based Detection of EGFR Mutations in Liquid Biopsy: A Systematic Review and Meta-analysis Compared With Tissue Biopsy in Treatment-naïve Patients With Non-small Cell Lung Cancer

MARÍA DEL SOCORRO GALEANO MOLINA[1,2], GABRIELA GUERRON-GÓMEZ[3], MERIDEIDY PLAZAS VARGAS[4] and RAFAEL PARRA-MEDINA[3,5,6]

[1]Department of Epidemiology, Universidad del Rosario, Bogotá, Colombia;
[2]Department of Epidemiology, Universidad CES, Medellín, Colombia;
[3]Research Institute, Fundación Universitaria de Ciencias de la Salud – FUCS, Bogotá, Colombia;
[4]Department of Epidemiology, Fundacion Universitaria de Ciencias de la Salud – FUCS, Bogotá, Colombia;
[5]Department of Pathology, Fundacion Universitaria de Ciencias de la Salud – FUCS, Bogotá, Colombia;
[6]Department of Pathology, Instituto Nacional de Cancerología, Bogotá, Colombia

## Abstract

*Background/Aim:* Liquid biopsy (LB) has demonstrated value in managing non-small cell lung cancer (NSCLC) and is widely used for monitoring disease progression in multiple cancers. While the role of baseline circulating tumor DNA (ctDNA) is not yet fully understood, emerging evidence suggests that LB should be integrated into NSCLC management, as pre-treatment ctDNA levels show a strong prognostic association with clinical outcomes. This systematic review and meta-analysis evaluated the diagnostic accuracy of molecular methods including PCR-based assays and next-generation sequencing (NGS) in detecting epidermal growth factor receptor (*EGFR)* mutations by comparing LB and tissue biopsy in treatment-naive patients with NSCLC.

*Materials and Methods:* A systematic search of MEDLINE and LILACS identified studies comparing matched LB and tissue biopsy in treatment-naive patients with NSCLC using polymerase chain reaction (PCR) or NGS. Sensitivity, specificity, positive likelihood ratio (PLR), and negative likelihood ratio (NLR) were calculated using a random-effects model.

*Results:* Twelve studies with 1,314 patients met the inclusion criteria. Most participants were male (59.5%), had adenocarcinoma (82.2%), and stage IV disease (77%). Pooled sensitivity and specificity for NGS in LB were 69% [95%

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Rafael Parra-Medina, Research Institute, Fundación Universitaria de Ciencias de la Salud – FUCS, Bogotá, Colombia. E-mail: Rafa.parram@gmail.com

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confidence interval (CI)=0.62-0.75] and 90% (95%CI=0.84-0.94), respectively. Regarding real time quantitative polymerase chain reaction (RT-qPCR) sensitivity was 56% (95%CI=0.46-0.65) and specificity 89% (95%CI=0.66-0.97). NGS yielded a PLR of 6.9 and NLR of 0.34; RT-qPCR had a PLR of 5.1 and NLR of 0.49.
*Conclusion:* NGS outperforms RT-qPCR in sensitivity and PLR for *EGFR* mutation detection *via* LB in patients with NSCLC prior to treatment, reinforcing its potential as a diagnostic tool.

**Keywords:** Non-small cell lung carcinoma, *EGFR*, liquid biopsy, cell-free nucleic acids, next generation sequencing, digital droplet PCR, real time q-PCR, review.

## Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide in women and men (1). Non-small cell lung cancer (NSCLC) accounts for 80-85% of all lung cancer cases (2). Numerous oncodriver alterations have been identified in NSCLC, defining the tumor mutation profile and contributing to cancer progression (3). Recent advances in understanding the molecular basis of lung cancer have led to the identification of various genomic signatures, facilitating the reclassification of NSCLC and accelerating the development of targeted therapies (4).

The epidermal growth factor receptor (*EGFR*) is a member of a subclass of receptor tyrosine kinases. Its signaling pathway is one of the most important mechanisms regulating growth, proliferation, and differentiation in mammalian cells (5). *EGFR* is frequently over-expressed during the development and progression of NSCLC; mutations in *EGFR* can lead to its constitutive activation, increased protein expression, and tumor progression. This is why alterations in *EGFR* have significant therapeutic implications for lung cancer (6). *EGFR* mutations are common among women, never smokers, and those with adenocarcinomas (7), the frequency of these mutations shows significant differences among various racial groups being higher in Asians (49.1%), followed by Latinos (23%) and Caucasians (12.8%) (8).

The tissue biopsy remains the gold standard for tumor diagnosis and molecular analysis due to its high level of standardization and accuracy of results (9); however, concerns regarding DNA integrity and the inadequate representation of the tumor's genetic landscape can limit the molecular diagnosis (10). LB is emerging as a valuable alternative due to its many advantages. It is a minimally invasive procedure that assesses a tumor's molecular profile when tissue samples are not available, it also allows for easy repeatability, provides a comprehensive tumor profile in real-time, and enables early detection of resistance mechanisms (11).

Despite advancements in the field and the evident advantages of LB, there are still significant limitations that need to be addressed. There is currently no standardized methodology for its use, leading to low reproducibility in preclinical studies (12). Additionally, LB may miss critical genetic alterations that occur in the early stages of disease (13). The risk of false negatives is a significant concern; this can occur due to a limited amount of ctDNA in the bloodstream (14).

Different molecular techniques are increasingly being used to detect *EGFR* mutations. However, the existing literature is inconsistent, making it difficult to determine the most effective method. Additionally, significant heterogeneity has been identified among the different testing methods used in plasma. LB utilizes technologies such as digital polymerase chain reaction (ddPCR), RT-qPCR, amplification refractory mutation system (ARMS) and NGS (15). In cases where there are limited variants of interest, targeted PCR methods can offer advantages over NGS, however, one limitation of all PCR methods compared to sequencing-based techniques is their potential to detect only known mutations, which may hinder the identification of new alterations (16). In contrast, technologies like NGS provide high-throughput sequencing, making it ideal for

large-scale analysis, allowing a more comprehensive understanding of the tumor's molecular profile due to their broader capabilities (17).

The clinical usefulness of LB to monitor disease progression is well known, having been studied across multiple cancers (18). Although the role of baseline ctDNA has not been thoroughly explored, monitoring levels of the initial activating *EGFR* mutation may facilitate more reliable detection of progression (19). Current studies recognize the significance of *EGFR* testing during initial diagnosis; however, few studies have shown that the yield of mutant allele burden in ctDNA at baseline was associated with aggressive disease (20). More research involving various molecular techniques and comparisons of data from multiple sources is necessary to define the effectiveness of LB in cancer as this remains an active development area (21).

Some systematic reviews have validated the accuracy of *EGFR* mutation detection using LB (22). Some of these explored treatment associations with response and survival outcomes (23) while others focused on establishing concordance among the two biopsy methods or testing the suitability of LB as an alternative to tissue biopsy (24). In contrast to these, our goal is to compare the diagnostic performance of *EGFR* mutation detection in LB to tissue biopsy in treatment-naive patients with NSCLC.

## Materials and Methods

*Registration and publication of study protocol.* The study protocol of this systematic review and meta-analysis has been registered on the International Prospective Register of Systematic Reviews (PROSPERO), and its registration number is CRD420251027919.

*Search strategy.* A systematic search in the Medline and LILACS databases identified studies that compare molecular techniques in tumor biopsy and LB to detect and quantify *EGFR* mutations in treatment -naive patients with NSCLC.

All searches were conducted during September 2024 and included articles published up to 2024. The PRISMA-DTA (25) guidelines were followed during data extraction, analysis, and reporting. No limits regarding language or publication period were considered. Appropriate truncations and word combinations were applied and adjusted for each database. The search was done with MeSH and DeCS. The terms considered included: Lung neoplasm, Lung carcinoma, Carcinoma, Non-small Cell Lung, Non-Small Cell Lung Carcinoma, Liquid Biopsy, Cell-free Nucleic Acids, "Exosome, Cells, Circulating Neoplastic, Protein Kinase Inhibitors, TKIs, Next generation sequencing, digital droplet PCR. Reference lists of previous systematic reviews were also reviewed to identify additional studies that might be eligible for inclusion in the analysis.

*Selection criteria.* The systematic review applied the following inclusion criteria: 1) descriptive studies, cohorts, and clinical trials; 2) comparative studies that evaluate the sensitivity and specificity between tissue biopsy and liquid biopsy in patients with *EGFR* mutations; and 3) untreated subjects.

The following exclusion criteria were applied: 1) the research methods and outcomes were not comprehensive, and the consistency between the two detection methods could not be established; 2) the study included patients with cancers other than the subtypes of NSCLC.

*Data extraction.* Two reviewers independently assessed the studies eligible for inclusion and subsequently reached a consensus on which should be included in the analysis. In cases of disagreement, resolution was achieved through discussion or with the involvement of a third reviewer. The extracted data from each article included the author's name, year of publication, country of publication, study type, reference standard, objective, and methods. The diagnostic performance measures were true positives (TP), false positives (FP), true negatives (TN), false negatives (FN).

*Quality evaluation.* Data extraction and quality assessment were conducted independently. Quality was evaluated

using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool.

*Summary measures.* The primary outcomes evaluated were sensitivity and specificity. Positive predictive values (PPV) and negative predictive values (NPV) and likelihood ratios (LR) were also estimated.

*Data synthesis and analysis*. Contingency tables were generated for each study, including true positives and negatives, false positives and negatives cases. Meta-analysis was performed with a random effects model to calculate pooled sensitivity and specificity with their 95% confidence intervals (CIs) of the studies comparing the same technique in tissue biopsy and LB. These values are presented in forest plots. $I^2$ values were used to measure the heterogeneity between studies. $I^2$ values >50% suggested that there was significant heterogeneity. Statistical analysis was performed using Meta-disc software 2.0 (Clinical Biostatistics Unit of the Ramon y Cajal Research Institute, Madrid, Spain).

## Results

*Literature selection.* Figure 1 presents the flowchart for the literature selection process A total of 446 relevant articles were retrieved by searching the literature. After removing 192 duplicate studies, we screened the titles and abstracts of 254 articles. Of them, 209 were irrelevant, 45 were relevant and their full-text articles were assessed for eligibility. After assessment based on the inclusion and exclusion criteria, 33 articles were excluded because they included patients previously treated or under treatment or it was not possible to perform the correct data extraction, leaving a total of 12 eligible studies.

*Characteristics of included studies.* A total of 12 articles met the inclusion criteria for this analysis providing data from 1,314 patients with NSCLC (Table I) (26-37). These articles reported the diagnostic efficacy of detecting *EGFR* mutations in plasma, comparing the results to paired tumor tissue samples. The sample sizes of the studies ranged from 39 to 262 subjects. The most common characteristics of patients included: males: 59.5% (n=723); adenocarcinomas: 82.2% (n=929); and clinical stage IV: 77% (n=725).

Among the 12 studies, five performed NGS in plasma and seven applied PCR-based techniques. Six studies were prospective cohorts, and six were retrospective cohorts. Seven studies utilized the same detection method for both tissue and biopsy samples: four performed NGS, and three employed PCR-based methods.

*Diagnostic efficacy of NGS and PCR-based methods for detecting EGFR mutations in tissue and plasma.* As shown in Table I, all studies provided data for calculating the sensitivity and specificity related to LB's effectiveness in detecting *EGFR* mutations. NGS was used to detect *EGFR* mutations in 280 plasma samples across four eligible publications: Rachiglio *et al.* (26), Yao *et al.* (27), Park *et al*. (28), and Choudhury *et al*. (29). Choudhury *et al*. (29) analyzed two cohorts of patients: the first cohort compared NGS in plasma with targeted PCR in tissue, and the second cohort compared NGS in plasma and tumor tissue. Rachiglio *et al.* (26) reported the highest sensitivity for NGS in plasma at 77.2% while Park *et al.* (28) obtained the lowest at 67%. Regarding specificity, Yao *et al.* (27) achieved 100%, whereas Choudhury *et al*. (29) reported the lowest at 83% in the group that compared NGS in plasma and tumor tissue.

Three studies compared results from tissue biopsy with those from LB using RT-qPCR. A total of 334 plasma samples were analyzed. Weber *et al.* (30) reported the highest sensitivity and specificity for RT-qPCR in plasma at 61% and 96%, respectively, whereas Ulivi *et al*. (31) obtained the lowest sensitivity at 52%.

ddPCR was used to determine the *EGFR* mutational status in two eligible studies. Zhu *et al*. (32) used ddPCR in plasma and compared it with ARMS-PCR on tumor tissue. The results indicated a sensitivity of 76.19% and a specificity of 96.55% for ddPCR in liquid biopsy. Suryavanshi *et al.* (33) reported that ddPCR achieved a

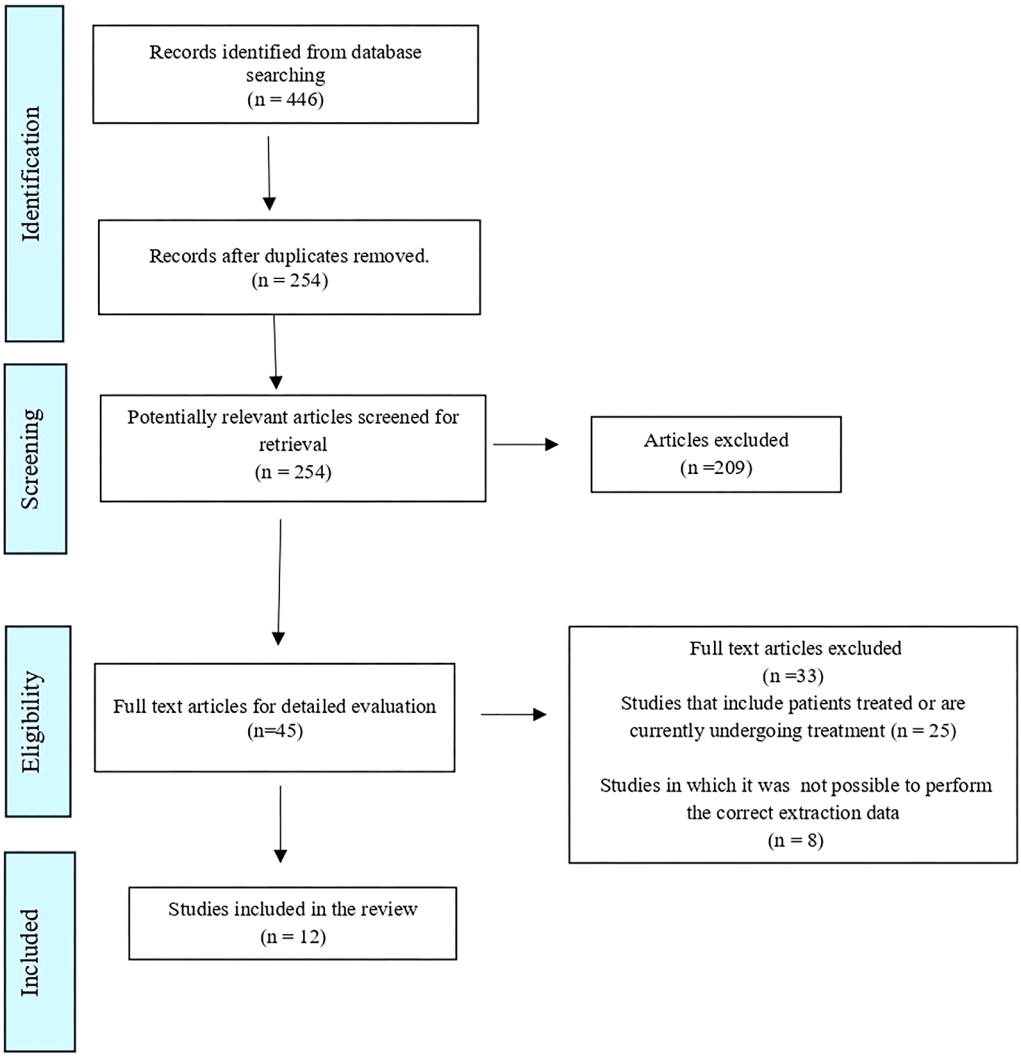


Figure 1. *PRISMA flow diagram.*

sensitivity of 87.5% in liquid biopsy samples when compared with RT-qPCR performed on tissue samples.

Only one eligible study evaluated the accuracy of ARMS-PCR for detecting *EGFR* mutations in plasma samples. Duan *et al.* (34) used ARMS-PCR to identify *EGFR* mutations in matched tumor tissue and plasma. The sensitivity of the test was found to be 50%, while the specificity was 100%.

*Meta-analysis comparing PCR-based methods and NGS*. In the meta-analysis, we compared the performance of NGS and RT-qPCR methods used in tissue biopsy and LB, focusing on the sensitivity and specificity (Table I).

NGS was performed in four articles (n=280) achieving an overall sensitivity of 69% (95%CI=62-75%) and specificity of 90% (95%CI=84-94%). The studies with the highest sensitivity were: Rachiglio *et al.* (26), 77% (95%CI=0.55-0.92), followed by Choudhury *et al.* (29) with 75% (95%CI=0.43-0.95). The highest specificities were achieved by Yao *et al.* (27) at 100% (95%CI=0.85-1.00) and Rachiglio *et al.* (26), at 91% (95%CI=0.71-0.99) (Figure 2).

Table I. *Patient demographics and clinical characteristics.*

| Author (ref) | Study design | Tissue biopsy methods | Liquid biopsy methods | Male n (%) | PPV | NPV | TP | FP | TN | FN | Sensitivity % | Specificity % | PLR | NLR | Tumor type n (%) | Tumor stage n (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Weber (2014) (30) Denmark | Retrospective cohort n=199 | RT-qPCR n=196 | RT-qPCR n=196 | 101 (51) | 73 | 93 | 17 | 6 | 162 | 11 | 60.7 | 96 | 15.2 | 0.40 | Adenocarcinoma= 190 (95) Adenosquamous carcinoma=9 (5) | IIA + IIB= 2 (1) IIIA + IIIB=29 (15) IV=168 (84) |
| 2. Duan (2015) (34) China | Retrospective cohort n=94 | ARMS PCR | ARMS PCR | 61 (65) | 100 | 74 | 19 | 0 | 56 | 19 | 50 | 100 | N/A | 0.5 | Adenocarcinoma=72 Adenosquamous carcinoma=19 | IIA=1 (1) IIIA=4 (4) IIIIB=9 (10) IV=80 (85) |
| 3. Rachiglio (2016) (26) Italy | Prospective cohort n=44 | NGS n=22 | NGS n=22 | 23 (52) | 89 | 80 | 17 | 2 | 20 | 5 | 77.2 | 90.9 | 7.7 | 0.25 | N/A | III=1 (2) IV=43 (98) |
| 4. Yao (2016) (27) China | Prospective cohort n=39 | NGS n=39 | NGS n=39 | 19 (48.7) | 100 | 81 | 12 | 0 | 22 | 5 | 70.6 | 100 | N/A | 0.29 | Adenocarcinoma= 34(87.2) Squamous cell carcinoma=5(12.8) | IIIA=5 (13) IIIIB=3 (8) IV=31 (79) |
| 5. Zhu (2017) (32) China | Prospective cohort n=51 | ARMS-PCR n=51 | ddPCR n=51 | 31 (61) | 94 | 84. | 16 | 1 | 28 | 5 | 76.19 | 96.55 | 19 | 0.25 | Adenocarcinoma= 45 (88) squamous carcinoma=3 (6) | I=2 (4) IIII=4 (8) IV=44 (87) |
| 6. Suryavanshi (2018) (33) India | Retrospective cohort n=40 | RT-qPCR | ddPCR | 25 (62.5) | N/A | N/A | 35 | N/A | N/A | 5 | 87.5% | N/A | | N/A | N/A | N/A |
| 7. Veldore (2018) (36) India | Retrospective cohort n=132 | RT-qPCR | NGS | 92 (70) | 100 | 95 | 41 | 0 | 91 | 4 | 91.1 | 100 | N/A | 0.09 | Adenocarcinoma= 113 (86) squamous carcinoma=19 (14) | IV=132 (100) |
| 8. Denis (2019) (37) France | Prospective cohort n=145 | NGS/Sanger/ ARMS-PCR n=126 | ARMS-PCR n=126 | 93 (64.1) | 100 | 95 | 9 | 0 | 112 | 5 | 64.3 | 100 | N/A | 0.36 | Adenocarcinoma= 115 (79.3) squamous carcinoma= 11 (7.6) Non adenocarcinoma= 26 (17.9) Other=4 (2.8) | IIIA=12 IIIIB=9 IV=124 |
| 9. Park (2021) (28) Republic of Korea | Retrospective cohort n=262 | NGS n=195 | NGS n=195 | 176 (66.8) | 90 | 62. | 108 | 11 | 87 | 53 | 67 | 88.8 | 5.58 | 3.0 | Adenocarcinoma= 221 (84.3) Squamous cell carcinoma=17 (6.5) Other=24 (9.2) | N/A |
| 10. Ulivi (2021) (31) Italy | Prospective cohort n=137 | RT-qPCR n=38 | RT-qPCR n=38 | 50 (36.5) | 100 | 36.3 | 15 | 1 | 8 | 14 | 51.7 | 88.8 | 4.72 | 0.53 | Adenocarcinoma= 102 (86.3) Squamous carcinoma= 3 (2.6) Other=13 (11.1) | IIIA=5 (4.3) IIIIB=7 (6.1) IV=103 (89.6) |

Table I. *Continued*

Table I. *Continued*

| Author (ref) | Study design | Tissue biopsy methods | Liquid biopsy methods | Male n (%) | PPV | NPV | TP | FP | TN | FN | Sensitivity % | Specificity % | PLR | NLR | Tumor type n (%) | Tumor stage n (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 11. Choudhury (2022) (29) | Prospective cohort n=71 | 1.Group: Targeted PCR or Sanger n=37<br>2.Group: NGS n=24 | 1.Group: NGS<br>2.Group: NGS | 52 (73) | 100<br>81.8 | 89.2<br>76.9 | 9<br>9 | 0<br>2 | 25<br>10 | 3<br>3 | 75<br>75 | 100<br>83.3 | N/A<br>4.41 | 0.25<br>0.30 | NSCLC=54(76)<br>Adenocarcinoma= 37 (52)<br>Squamous carcinoma= 6 (8.5)<br>Other=8 (14.8) | N/A |
| 12. Paturu (2023) (35) | Prospective Cohort n=100 | RT-qPCR | RT-qPCR | N/A | 60.4 | 63.1 | 26 | 17 | 36 | 21 | 55.3 | 67.9 | 1.71 | 0.66 | N/A | N/A |

ARMS: Amplification refractory mutation system; PCR: polymerase chain reaction; ddPCR: digital droplet PCR; NGS: next-generation sequencing; RT-qPCR: real time quantitative PCR; PPV: positive predictive value; NPV: negative predictive value; TP: true positive; FP: false positive; TN: true negative; FN: false negative; PLR: positive likelihood ratio; NLR: negative likelihood ratio.

RT-qPCR was applied in three articles (n=334) achieving an overall sensitivity of 56% (95%CI=46-65%) and specificity of 89% (95%CI=66-97%). Weber *et al.* (30) achieved a sensitivity of 61% (95%CI=0.41-0.78). Following closely was Paturu *et al*. (35), who showed a sensitivity of 55% (95%CI=0.40-0.70). Additionally, Weber *et al*. (30) also achieved a specificity of 96% (95%CI=0.92-0.99), while Ulivi *et al*. (31) recorded a specificity of 89% (95%CI=0.52-1.00) (Figure 2).

*Positive likelihood ratio (PLR) and negative likelihood ratio (NLR).* As shown in Table I, PRL and NLR of NGS and RT-qPCR for detecting *EGFR* mutations were calculated. For NGS, PRL was 6.9 (95%CI=5.91570-7.88430) and NLR was 0.34 (95%CI=0.64430-1.32430). Regarding RT-qPCR the PRL was 5.1 (95%CI=4.11641-608359) and NLR was 0.49 (95%CI=0.49359-1.47359).

*Quality assessment of included articles.* Methodological quality assessment was performed using the QUADAS-2 tool a total of five domains were evaluated: patient selection, index test, reference standard, flow, and timing. Two review authors independently assessed the quality of the included studies, and the risk of bias (High, Low, or Unclear) was made for each domain. The results are shown in Table II.

Patient selection showed the highest risk of bias (25%), and flow, timing, and index test had the lowest risk of bias (0%). For the applicability of the studies, all the studies were classified as of low concern in the five aspects.

## Discussion

The present article, which included 12 studies involving 1.314 patients, is the first meta-analysis to assess the diagnostic performance of various molecular methods for detecting *EGFR* mutations in tissue biopsy compared to LB prior to treatment initiation. Our results showed that NGS had a sensitivity of 69% and specificity of 90%, whereas RT q-PCR had a sensitivity of 56% and specificity

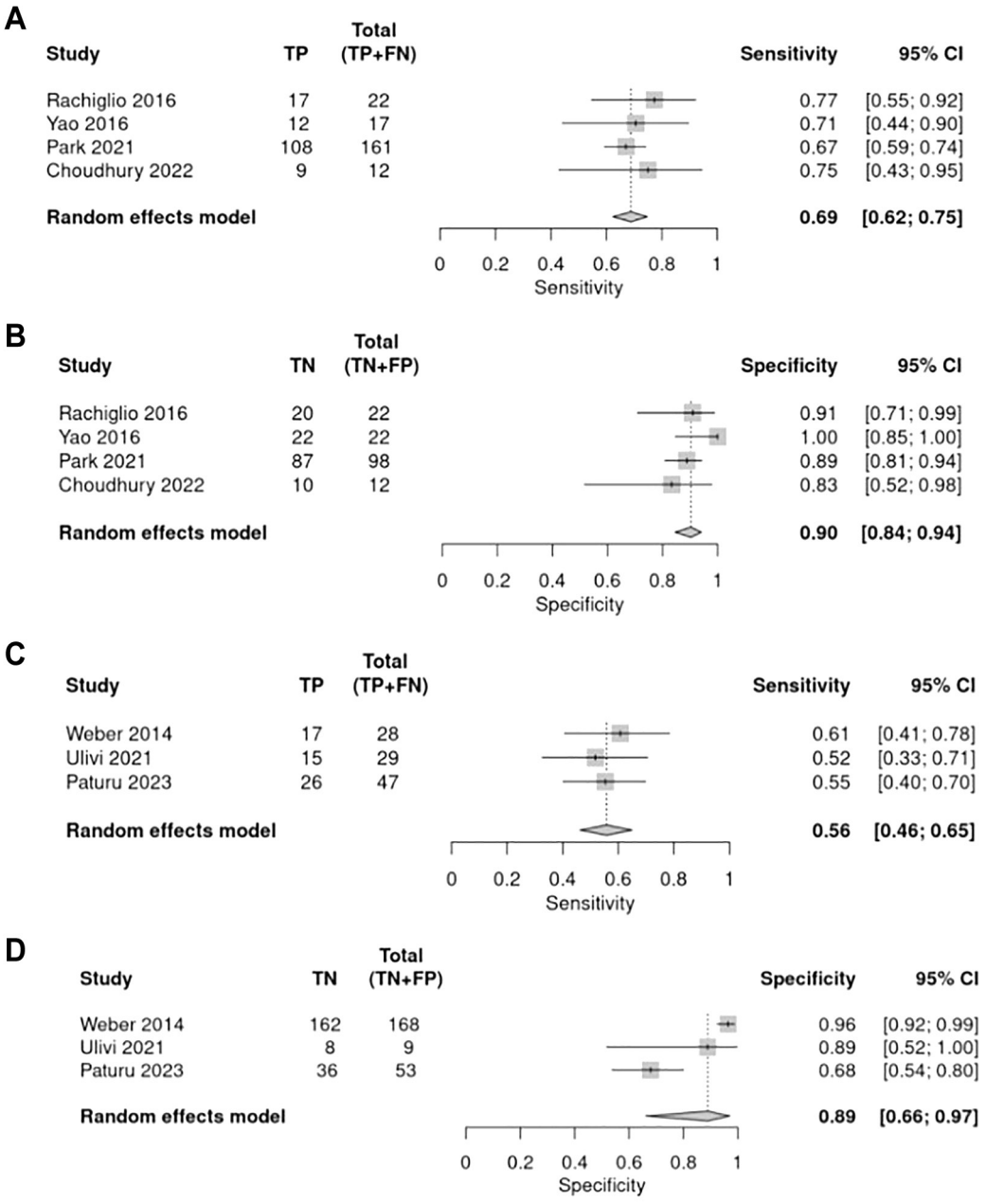


Figure 2. *The forest plot of the pooled sensitivity (A) and specificity (B) of next-generation sequencing (NGS) and pooled sensitivity (C) and specificity (D) of RT-q PCR in LB for detecting EGFR mutations in treatment naive patients with non-small cell lung cancer.*

of 89%. The NGS is generally considered less sensitive than PCR (38). However, our results showed that NGS has better sensitivity and higher LPR compared to RT-qPCR.

Some systematic reviews and meta-analyses have analyzed the diagnostic accuracy of LB, with Wang *et al.* (23) being the only study to report differential results based on the techniques analyzed. The overall LB sensitivity and specificity were 68% and 98%, respectively, slightly higher than ours. In contrast, Franzy *et al*. (22) analyzed LB using various techniques, such as

Table II. *QUADAS-2 entries for evaluation of literature quality.*

| Author | Patient selection | Index test | Reference standard | Flow and timing |
|---|---|---|---|---|
| 1.Weber *et al.* (30) | Low | Low | Low | Low |
| 2. Duan *et al.* (34) | High | Low | Low | Low |
| 3. Rachiglio *et al.* (26) | Low | Low | Low | Low |
| 4. Yao *et al.* (27) | Low | Low | Low | Low |
| 5. Zhu *et al.* (32) | Low | Low | Low | Low |
| 6. Suryavanshi *et al.* (33) | High | Low | Low | Low |
| 7.Veldore *et al.* (36) | High | Low | Low | Low |
| 8. Denis *et al.* (37) | Low | Low | High | Low |
| 9. Park *et al.* (28) | Low | Low | Low | Low |
| 10. Ulivi *et al.* (31) | Low | Low | Low | Low |
| 11. Choudhury *et al.* (29) | Low | Low | High | Low |
| 12. Paturu *et al.* (35) | Low | Low | Low | Low |

NGS and PCR-based methods. The results indicated that NGS exhibited slightly higher sensitivity than PCR-based techniques (0.62% *vs*. 0.56%). This aligns with our findings; although we investigated the same methods, we focused on a different population, specifically the group of treatment-naive patients with NSCLC.

A study by Lawrence *et al.* (39), reported a tissue and plasma NGS sensitivity of 94.8%, and 52.6%, respectively. The sensitivity of detection in tissue compared to plasma varies across different studies; tissue NGS has shown significantly higher sensitivity across various patient subgroups and disease stages (39); in comparison, on LB, NGS exhibits high specificity, almost 100%, especially for detecting *EGFR* mutations (40). While RT-qPCR is highly specific, it can only detect a limited number of genetic alterations. Pisapia *et al.* (41) found that 8.9-9.4% of EGFR mutation went undetected using RT-qPCR but were identified through NGS.

We found that the pooled sensitivity of RT-q-PCR was 56%, which fell within the range of 51.7% to 60.7% reported in other studies (30, 31, 35). For specificity, our study recorded a pooled specificity of 89% (0.65; 0.97), aligning with data from other authors (30, 31, 35). Kanaoka *et al* (42) analyzed causes of discordant results in *EGFR* detection between two platforms: cobas® *EGFR* Mutation Test v2, based on RT-qPCR, and the Oncomine Dx Target Test *(ODxTT*), based on targeted NGS. The main objective was to identify factors that contribute to differences in method sensitivity. The study determined that specific mutations are key factors in certain patient populations and tumor types. Cobas® *EGFR* Mutation Test v2 could generate false positives for exon 20 insertion mutations in smokers and patients with squamous cell carcinoma. Unfortunately, we could not meta-analyze ddPCR data. Studies included in this systematic review, using ddPCR, have reported sensitivities ranging from 80% to 90%, with specificities exceeding 90% (33).

Recently, LB has gained recognition not only for its usefulness in evaluating advanced NSCLC but also for its potential in early-stage monitoring and screening (43). There is limited data on the effectiveness of LB in early diagnosis, and the results regarding the accuracy of pre-treatment LB are still inconclusive (12). It can lead to false-negative outcomes, primarily due to the small quantity of ctDNA present in the bloodstream (44). The sensitivity of LB when compared to corresponding tissue samples ranges from 70% to 85% in advanced-stage disease, though it diminishes in earlier stages of the disease (45). As a result, it remains unclear which method is the most efficient for molecular profiling in LB during the early stages of diagnosis (46).

The present article has limitations such as the low number of studies that tested tissue biopsy and LB to detected *EGFR* mutations prior the treatment. The included studies varied across several factors, including the cut-off levels for ctDNA, sample sizes, tumor stages, methodological approaches, pre-analytical conditions, and detection methods. These differences could introduce potential sources of bias and heterogeneity. Despite these limitations, this study provides important insights for selecting the most effective technique for patients who have not undergone treatment in the early stages of diagnosis.

In conclusion, these results contribute to the understanding of the utility of LB in clinical practice for detecting *EGFR* mutations in treatment-naive patients with NSCLC. We identified that NGS has better diagnostic performance than RT-qPCR in LB compared to tissue biopsy

samples tested with the same methodology. In our data, the majority of patients were in advantage stages. Therefore, we recommend more studies that compare the diagnostic performance of the different techniques in treatment-naive patients with NSCLC in different stages mainly in early stages.

## Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study.

## Authors' Contributions

(I) Conception and design: M Galeano, R Parra-Medina; (II) Administrative support: M Galeano, R Parra-Medina (III) Provision of study materials or patients: All Authors; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All Authors; (VI) Manuscript writing: All Authors; (VII) Final approval of manuscript: All Authors.

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## Artificial Intelligence (AI) Disclosure

No artificial intelligence (AI) tools, including large language models or machine learning software, were used in the preparation, analysis, or presentation of this manuscript.

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