Detection of epidermal growth factor receptor mutations in peripheral blood circulating tumor DNA in patients with advanced non-small cell lung cancer
A PRISMA-compliant meta-analysis and systematic review
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Abstract

Background: The epidermal growth factor receptor (EGFR) mutation status related to the treatment approach for advanced non-small cell lung cancer (NSCLC) patients. This study aimed to evaluate the diagnostic accuracy of peripheral blood circulating tumor DNA (ctDNA) in EGFR mutated advanced NSCLC patients.

Method: The related database was systematically searched with keywords until January 19, 2020. Studies contained the histopathological and cytological advanced NSCLC samples were included, and the diagnostic data were recorded for calculating sensitivity and specificity. I² statistics were used for detecting heterogeneity across studies, and the meta-regression was performed to seek the source of heterogeneity.

Result: A total of 32 studies with 4527 advanced NSCLC patients were included in our meta-analysis. Among them, 87% of the patients were diagnosed as stage IV. The pooled sensitivity of peripheral blood ctDNA was 0.70 (95% CI: 0.63–0.75, I² = 81.76) and the pooled specificity was 0.98 (95% CI: 0.96–0.99, I² = 88.33). The meta-regression showed that the prospective study design and the ARMS detection method were the main source of heterogeneity for sensitivity (P < .05), and the publication country (Asia or non-Asia) was the main source of heterogeneity for specificity (P < .01).

Conclusion: ctDNA biopsy has high specificity and diagnostic accuracy in detection of EGFR mutation in advanced NSCLC patients. When the ctDNA gene test result is negative, we should fully consider the risk of missed diagnosis, and further tissue biopsy is still needed to undertake.

Abbreviations: ARMS = amplification blocking mutation system, ASCO = the American Society of Clinical Oncology, AUSROC = area under the SROC, ctDNA = circulating tumor DNA, ddPCR = droplet digital polymerase chain reaction, EGFR = epidermal growth factor receptor, EGFR-TKI = EGFR tyrosine kinase inhibitors, ESMO = the European Society for Medical Oncology, FN = false negatives, FP = false positives, MEPCR = meningitis/encephalitis (ME) panel PCR, NSCLC = non-small cell lung cancer, NLR = negative likelihood ratio, PLR = positive likelihood ratio, PNA-LNA PCR = the peptide nucleic acid-locked nucleic acid PCR, PRISMA = the preferred reporting items for systematic review and meta-analysis, QUADAS = the Quality Assessment for Studies of Diagnostic Accuracy Score, SROC = summary receiver operative curve, TP = true positives, TN = true negative, WCLC = the World Conference on Lung Cancer.

Keywords: circulating tumor DNA, EGFR, non-small cell lung cancer

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All the data were extracted from the published studies, and the ethical approval should be waived by our local ethical committee.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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1. Introduction

Lung cancer currently has highest incidence and mortality rates worldwide. With the intensification of air pollution and changes in lifestyles, the incidence of lung cancer is increasing. In the 2018 global cancer statistics, there were ~18.1 million new cases of cancer and 9.6 million patients that succumbed to cancer, of which lung cancer accounted for 11.6% of all new cases of cancer and 18.4% of all cancer deaths. Non-small cell lung cancer (NSCLC) accounts for >80% of all types of lung cancer, which is the primary classification of lung malignancies. Although the inspection equipment, technical methods and new drugs have developed rapidly in recent years, ~75% of patients with NSCLC are already at the advanced stage when they are clinically diagnosed (inoperable IIIA, IIIB and IV), which leads to the poor prognosis with a 5-year survival rate at ~18%.

Molecular targeted therapy has achieved great success in NSCLC and other types of cancer. The most representative and universal method is to target mutation-activated epidermal growth factor receptor (EGFR) in patients with NSCLC. EGFR mutations are found in >16% of patients with NSCLC in western countries, and up to 40% of EGFR mutations are found in East Asian patients with NSCLC. It has been reported that the deletion of the EGFR gene exon 19 and the point mutation (L858R) in exon 21 in NSCLC account for >80% of EGFR mutations, which induce constitutive activation of EGFR mutations in cancer cells. Therapies that target the activation of EGFR mutations have shown great success in patients with NSCLC with EGFR mutations. Therefore, this provides an opportunity for precision-targeted therapy for patients with NSCLC with EGFR mutations.

Tissue biopsy remains to be the “gold standard” for EGFR gene testing. Tissue specimens are mainly obtained through lung puncture, ultrasound bronchoscopy-guided transbronchial needle aspiration biopsy, or lung cancer resection, all of which are invasive procedures, which are difficult to repeat and where the risk of complications cannot be avoided. According to previous studies, chest biopsy complications have been reported in ~17% of cases. In addition, a large amount of literature has repeatedly reported tumor heterogeneity, and biopsies of one or more tumor partial regions may not account for all molecular changes in patients with tumors due to this heterogeneity. Therefore, patients with NSCLC urgently need a novel, non-invasive, and comprehensive method of disease detection.

Circulating tumor DNA (ctDNA) consists of small nucleic acid fragments, which are released from tumor cells with necrosis and apoptosis in primary and metastatic lesions, thereby reconstructing the solid tumor gene profile. Compared with tumor tissue, ctDNA is a molecular-level biomarker for detecting tumor gene mutations. Also, liquid biopsy has the advantages of less trauma, reproducible, real-time monitoring and easy acceptance by patients. ctDNA biopsy is more clinically feasible for screening EGFR gene mutations in patients with tumors, which can significantly promote personalized targeted therapy for patients with tumors. New clinical studies have demonstrated that detection of ctDNAs can predict treatment effects, such as resistance to postoperative chemotherapy, and in some cases can detect recurrence earlier than traditional clinical methods, which is expected to simplify the detection of cancer occurrence and evolution. Current research shows that the consistency of ctDNAs biopsy and tissue biopsy ranges between 66 and 100%. There is inconsistency among studies in ctDNAs gene detection technology, Tumor-Node-Metastasis staging, and study design. Thus, the present systematic review and meta-analysis was designed and performed to discuss the diagnostic value of peripheral blood ctDNAs in detection of EGFR mutations in patients with advanced NSCLC and attempted to discover the source of the heterogeneity.

2. Methods

The present study was designed and reported according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines.

2.1. Search strategy

The electronic databases, such as Pubmed, Embase, and the Cochrane library, were systematically searched to evaluate the specificity and sensitivity of peripheral blood tests in patients with EGFR-mutated advanced NSCLC. The key words were selected by an experienced librarian and searched on January 19th, 2020. Briefly, the key words contained “advanced”, “EGFR”, “liquid biopsy”, “circulating tumor DNA” and “circulating tumor cell”. Also, Google scholar and other similar websites were reviewed for relevant studies. The present study retrieved 3 conference databases, including the European Society for Medical Oncology (ESMO; https://www.esmo.org/), the World Conference on Lung Cancer (WCLC; http://wclc2017.iaslc.org/), and the American Society of Clinical Oncology (ASCO; https://www.asco.org/). All studies with titles and abstracts were imported into Endnote (Thomson Scientific, UK; version X7) for finding the duplications and for the further literature screening.

2.2. Selection criteria

Eligible studies were selected according to the following criteria: (i) all patients were diagnosed as stage III and IV NSCLC; (ii) the selected patients were diagnosed both histopathologically and cytologically; (iii) the data on true positives (TP), true negative (TN), false positives (FP) and false negatives (FN) were fully reported to construct the diagnostic 2 × 2 table; (iv) the EGFR mutation was detected. There was a limited number of prospective and retrospective studies. The reviews, other associated meta-analyses, comments, and conferences were screened for further inclusion in the studies. All studies were written in the English language.

The exclusion criteria were as follows: (i) Peripheral blood and tumor tissues were not paired; (ii) the case sample number was <10 in the case series studies; (iii) the study did not clarify the tumor stage and the data of advanced NSCLC could not be extracted.

2.3. Literature screening, data extraction and quality evaluation

There were 2 researchers (Zhou S.K. and Huang R.Z.) that independently screened the titles and abstracts based on the selection criteria. The full text was further evaluated if the abstracts could not be determined. Any discrepancy was resolved by discussion with the third author (Cao Y.P.).
The data were extracted by 2 researchers based on a standard form as follows: Author names, publication years, recruitment years, publication countries, study designs, detection methods of tissue and peripheral blood, median patient age, male percentage, smoking status, tumor stage and cases of TP, FP, FN, TN in comparison with the sensitivity and specificity between tumor tissue and peripheral blood.

Similarly, 2 investigators evaluated the quality of the included studies using the Quality Assessment for Studies of Diagnostic Accuracy Score (QUADAS) tool. QUADAS is a useful tool that consists of 4 domains (patient selection, index test, reference standard, and flow and timing). A total of 14 questions focusing on the quality of the article was judged as “yes”, “no”, or “unclear”, with a maximum score of 14.

2.4. Statistical analysis

All statistical analyses were performed using Stata 15.0 software (Stata Corporation, College Station with the MIDAS module for diagnostic meta-analysis. The pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), positive predictive value (PPV), negative predictive value (NPV), diagnostic odds ratio (DOR), and corresponding 95% confidence intervals (95% CI) were calculated based on a bivariate regression model. The Fagan nomogram was created for visual presentation of diagnostic performance with pre- and post-test probabilities. The χ² test and I² statistics were used for detecting heterogeneity across studies (I² ≥ 50% indicated the presence of heterogeneity). When the heterogeneity was detected, the Spearman correlation coefficient was calculated to judge whether the threshold effect existed or not. The meta-regression was performed to detect heterogeneity using the covariates including large sample size (sample > 40 or not), blood sample (plasma or serum), study design (prospective or not), publication country (Asian or non-Asian), and method applied for detection, such as amplification blocking mutation system (ARMS), droplet Digital PCR (ddPCR), meningitis/encephalitis (ME) panel PCR, and the peptide nucleic acid (PNA)-locked nucleic acid (LNA) PCR. Publication bias was detected by the Deek’s funnel plot, and a P value <.05 indicated the presence of publication bias.

3. Results

3.1. Study selection

A total of 1,646 studies were identified based on the search strategy. After deleting duplications and adding the relevant
The baseline characteristics from the included studies are presented in Table 1. A total of 4527 patients with advanced NSCLC were included in the present study. The publication year ranged from 2006 to 2019 with a recruitment year between 2002 and 2018. A total of 31 studies were from single countries including Australia, America, Spain, India, Japan, Korea and China, and one study was performed in multiple countries. The median age of the patients was 61.8 years. A total of 58% of the patients were male and 72% of the patients had a history of smoking. In those patients with advanced NSCLC, 13% were classified with stage III, and 87% were classified with stage IV.

The data on detecting methods and the 2 × 2 data forms are presented in Table 2. A total of 10 studies used the ARMS, 15 studies mentioned PCR, and 4 studies used sequencing to detect EGFR mutations in samples. The quality of the included studies was assessed using the QUADAS guidelines, with moderate-to-high quality being observed throughout. Among them, 16 studies had QUADAS scores ≥10.

3.3. Accuracy of peripheral blood for detecting EGFR mutations

The pooled sensitivity of peripheral blood ctDNAs was 0.70 [95% confidence interval (CI), 0.63–0.75] and the pooled specificity was 0.98 (95% CI, 0.96–0.99) (Fig. 2). The pooled PPV of peripheral blood ctDNAs was 0.97 (95% CI, 0.95–0.99) and NPV was 0.75 (95% CI, 0.74–0.76). The positive and negative likelihood ratios were 37.5 (95% CI, 17.7–79.5) and 0.31 (95% CI, 0.25–0.38), respectively. The pooled DOR was 121 (95% CI, 54–271) and the AUSROC was 0.91 (95% CI, 0.88–0.93) (Fig. 3A), indicating that peripheral blood ctDNAs had high diagnostic accuracy. The Fagan plot was generated for the visual presentation of diagnostic performance (Fig. 3B).

3.4. Threshold effect and heterogeneity

Due to the higher heterogeneity existing among studies (I² = 81.76 for sensitivity, and I² = 88.33 for specificity), it was determined that the threshold effect is the major source of heterogeneity. The Spearman correlation coefficient and P value

| Table 1 | Characteristics of included studies. |
|---------|-------------------------------------|
| Author  | Year          | Recruitment year | Country | Median age (yr) | Male, (%) | Smoker history (%) | Tumor stage | Stage III (%) | Stage IV (%) | Total number of samples |
| Xu, H. et al | 2019 | 2016–2017 | China | NG | 103 (51) | 64 (32) | IIIB-IV | 34 (17) | 169 (83) | 203 |
| Li, B. T. et al | 2019 | 2015 | America | 65 | 47 (37) | 7 (6) | IIIB-IV | 1 (1) | 126 (99) | 127 |
| Leight, N. B. et al | 2019 | 2016–2018 | America | 69 | 129 (46) | 221 (78) | IIIB-IV | 7 (2) | 275 (88) | 282 |
| Ding, P. N. et al | 2019 | 2015–2017 | Australia | 67 | 12 (43) | 7 (25) | IV | 0 (0) | 28 (100) | 28 |
| Denis, M. G. et al | 2019 | NG | Multi-center | 63.8 | 93 (64) | 120 (83) | IIIB-IV | 9 (6) | 124 (86) | 145 |
| Veldore, V. H. et al | 2019 | NG | India | NG | 92 (70) | 77 (58) | IV | 0 (0) | 132 (100) | 132 |
| Shi, C. et al | 2018 | NG | China | NG | NG | NG | NG | NG | 55 |
| Ito, K. et al | 2018 | 2015–2016 | Japan | 76 | 54 (68) | 59 (75) | IIIB-IV | 11 (14) | 68 (86) | 79 |
| Arriola, E. et al | 2018 | NG | Spain | 64 | 111 (72) | 127 (82) | IIIB-IV | 9 (6) | 136 (88) | 154 |
| Zhang, Y. et al | 2017 | 2009–2014 | China | NG | 127 (59) | 94 (44) | IIIB-IV | 36 (17) | 179 (83) | 215 |
| Zhang, Z. et al | 2017 | 2015–2016 | China | 59 | 65 (56) | 51 (44) | IIIB-IV | 30 (11) | 79 (88) | 116 |
| Wang, Y. et al | 2017 | NG | China | NG | 133 (46) | 107 (37) | IIIB-IV | 57 (20) | 239 (80) | 287 |
| Vázquez, S. et al | 2016 | 2011–2012 | Spain | NG | 151 (76) | 155 (78) | IIIB-IV | NG | NG | 198 |
| Sacher, A. G. et al | 2016 | NG | America | NG | 62 | 68 (38) | NG | IIIB-IV | 3 (2) | 172 (96) | 180 |
| Qu, D. et al | 2016 | 2011–2014 | China | NG | 80 (77) | 52 (50) | IIIB-IV | NG | NG | 104 |
| Ma, M. et al | 2016 | 2012–2014 | China | 58.7 | 145 (66) | 108 (49) | III-IV | 30 (14) | 171 (78) | 219 |
| Zhu, G. S. et al | 2015 | 2008–2012 | China | 55 | 56 (65) | 10 (12) | IIIB-IV | 4 (5) | 82 (95) | 86 |
| Lam, D. C. et al | 2015 | NG | China | 64 | 38 (51) | 25 (34) | IIIB-IV | 2 (3) | 70 (95) | 74 |
| Duan, H. et al | 2015 | 2013–2014 | China | 58 | 61 (65) | 48 (51) | IIIB-IV | 9 (10) | 80 (85) | 94 |
| Li, X. F. et al | 2014 | 2011–2012 | China | 58 | 96 (60) | 80 (50) | IIIB-IV | 14 (9) | 131 (81) | 161 |
| Zhang, H. et al | 2013 | 2011–2012 | China | 58 | 49 (57) | 44 (51) | IIIB-IV | 16 (19) | 70 (81) | 86 |
| Liu, X. Q. et al | 2013 | 2008–2012 | China | 55 | 56 (65) | 47 (55) | IIIB-IV | 4 (5) | 82 (95) | 86 |
| Kim, S. T. et al | 2013 | 2006–2009 | Korea | 64 | 35 (61) | 32 (56) | IIIB-IV | 7 (12) | 50 (88) | 57 |
| Kim, H. R. et al | 2013 | 2010–2011 | Korea | 62.5 | 21 (35) | 17 (28) | III-IV | 4 (7) | 53 (88) | 60 |
| Xu, F. et al | 2012 | 2007–2009 | China | NG | 31 (61) | 19 (37) | III-IV | 6 (12) | 45 (88) | 51 |
| Huang, L. et al | 2012 | 2005–2009 | China | 58.4 | 438 (53) | 340 (41) | IIIB-IV | NG | NG | 821 |
| Jiang, B. et al | 2011 | 2006–2008 | China | 56 | 40 (69) | 39 (67) | IIIB-IV | NG | NG | 58 |
| Brevet, M. et al | 2011 | NG | America | 62 | 15 (48) | 17 (55) | IIIB-IV | 1 (3) | 30 (97) | 31 |
| Yung, K. F. et al | 2009 | NG | China | NG | NG | NG | IIIB-IV | NG | NG | 35 |
| Bai, H. et al | 2009 | 2004–2007 | China | 60.7 | 123 (53) | 103 (45) | IIIB-IV | 80 (35) | 150 (65) | 230 |
| Kimura, H. et al | 2007 | 2002–2006 | Japan | 58 | 26 (67) | 28 (67) | IIIB-IV | NG | NG | 42 |
| Kimura, H. et al | 2006 | 2002–2003 | Japan | 64 | 18 (60) | 20 (67) | IIIB-IV | 4 (13) | 26 (87) | 30 |

NG = not given.
were calculated for evaluating the threshold effect. Although the P value was <.05, the correlation was −0.04, which suggested that there was no positive correlation among studies, and the threshold effect was not significant. Thus, the present study used the meta-regression analysis to detect the source of heterogeneity (Fig. 4). The meta-regression analysis revealed that the study design (prospective or retrospective) and the ARMS detection method were the main source of heterogeneity for sensitivity (P < .01), and the publication country (Asian or non-Asian) was the main source of heterogeneity for specificity (P < .01).

### 3.5. Subgroup analysis

The subgroup analysis is presented in Table 3. The subgroup analysis suggested that those prospective studies had poor pooled sensitivity (P = .66; 95% CI, 0.56–0.76) than retrospective studies (P = .72; 95% CI, 0.64–0.81) (P < .01). In addition, patients undertook ARMS detection had poor sensitivity (P = .60; 95% CI, 0.49–0.71) than those detections by other methods (P < .01). Although the P value was < .01, the difference of specificity between Asian countries and non-Asian countries was not significant (Asian country: P = .97; 95% CI, 0.95–0.99; non-Asian country: P = .99; 95% CI, 0.98–1.00). No significant difference was observed between the pooled sensitivities and specificities of the study samples and the blood samples (all P > .05).

### 3.6. Publication bias

As presented in Figure 5, Deek’s funnel plot was used to test the publication bias. The P value was 0.08 (P > .05), suggesting no evidence of publication bias among studies.

### 4. Discussion

The meta-analysis in the present study indicated that the peripheral blood ctDNAs pooled an acceptable sensitivity of 0.70 and a precise specificity of 0.98, which demonstrated its efficacy for the patients with advanced NSCLC. The large heterogeneity mainly came from the study design and detection method, which led to the difference in sensitivity. The present study suggested that gene mutations associated with tumor tissue can be detected in patients with advanced NSCLC, thus may provide important evidence for the treatment, postoperative monitoring and prognosis of lung cancer, particularly for those patients with NSCLC require EGFR tyrosine kinase inhibitors (EGFR-TKIs). However, different research methods, testing instruments, testing reagents and operator levels can affect

### Table 2

| Author          | Year | Study type | Sample detection method | Blood type | TP  | FP  | FN  | TN  | QUADAS |
|-----------------|------|------------|-------------------------|------------|-----|-----|-----|-----|--------|
| Xu, H. et al    | 2019 | Prospective | ARMS                   | Plasma     | 63  | 1   | 56  | 83  | 12     |
| Li, B. T. et al | 2019 | Prospective | NGS                    | Plasma     | 29  | 0   | 8   | 90  | 12     |
| Leith, N. B. et al | 2019 | Prospective | Sequencing            | Plasma     | 18  | 2   | 4   | 201 | 10     |
| Ding, P. N. et al | 2019 | Prospective | ARMS, ME-PCR          | Plasma     | 11  | 0   | 5   | 10  | 12     |
| Denis, M. G. et al | 2019 | Prospective | ARMS                   | Plasma     | 9   | 0   | 5   | 112 | 10     |
| Veldore, V. H. et al | 2019 | Retrospective | PCR                   | Plasma     | 41  | 0   | 4   | 87  | 9      |
| Shi, C. et al   | 2018 | Retrospective | cSMART                | Plasma     | 27  | 5   | 11  | 12  | 11     |
| Ito, K. et al   | 2018 | Prospective | PNA-LNA PCR            | Plasma     | 8   | 0   | 3   | 59  | 9      |
| Arriola, E. et al | 2018 | Retrospective | PNA-LNA PCR          | Plasma     | 17  | 4   | 5   | 121 | 12     |
| Zhang, Y. et al | 2018 | Retrospective | PCR                   | Plasma     | 57  | 4   | 36  | 118 | 9      |
| Xu, H. et al    | 2017 | Retrospective | ARMS                   | Plasma     | 34  | 2   | 10  | 70  | 9      |
| Chen, C. et al  | 2019 | Prospective | ARMS                   | Plasma     | 32  | 8   | 30  | 47  | 9      |
| Vázquez, S. et al | 2016 | Prospective | ARMS                   | Plasma     | 13  | 1   | 12  | 148 | 11     |
| Sacher, A. G. et al | 2016 | Prospective | ARMS                   | Plasma     | 41  | 0   | 9   | 124 | 11     |
| Que, D. et al   | 2016 | Prospective | ME-PCR                 | Plasma     | 33  | 6   | 7   | 58  | 9      |
| Ma, M. et al    | 2016 | Prospective | ARMS                   | Plasma     | 54  | 4   | 36  | 125 | 11     |
| Zhu, G. S. et al | 2015 | Retrospective | ARMS                   | Plasma     | 18  | 1   | 4   | 63  | 9      |
| Lam, D. C. et al | 2015 | Retrospective | PNA-LNA PCR            | Plasma     | 12  | 1   | 3   | 30  | 9      |
| Duan, H. et al  | 2015 | Retrospective | ddPCR                 | Plasma     | 19  | 0   | 15  | 46  | 8      |
| Li, X. F. et al | 2014 | Retrospective | ARMS                   | Plasma     | 27  | 3   | 29  | 62  | 9      |
| Zhang, H. et al | 2013 | Retrospective | Liquid chip           | Plasma     | 15  | 0   | 7   | 64  | 11     |
| Liu, X. Q. et al | 2013 | Retrospective | ARMS                   | Serum      | 27  | 0   | 13  | 46  | 9      |
| Kim, S. T. et al | 2013 | Prospective | PNA-LNA PCR            | Serum      | 8   | 3   | 4   | 42  | 8      |
| Kim, H. R. et al | 2013 | Prospective | ME-PCR                 | Plasma     | 6   | 0   | 29  | 5   | 9      |
| Xu, F. et al    | 2012 | Retrospective | ARMS                   | Serum      | 4   | 0   | 4   | 16  | 11     |
| Huang, Z. et al | 2012 | Prospective | DHPLC                  | Plasma     | 184 | 79  | 85  | 396 | 8      |
| Jiang, B. et al | 2011 | Retrospective | ME-PCR                 | Serum      | 14  | 0   | 4   | 40  | 8      |
| Brevet, M. et al | 2011 | Prospective | ME-PCR                 | Plasma     | 5   | 2   | 9   | 15  | 11     |
| Yung, K. F. et al | 2009 | Retrospective | ARMS                   | Plasma     | 15  | 0   | 4   | 16  | 11     |
| Bai, H. et al   | 2009 | Prospective | DHPLC                  | Plasma     | 63  | 16  | 14  | 137 | 12     |
| Kimura, H. et al | 2007 | Retrospective | Sequencing            | Serum      | 6   | 1   | 2   | 33  | 11     |
| Kimura, H. et al | 2006 | Retrospective | Sequencing            | Serum      | 3   | 2   | 1   | 6   | 8      |

*FN* = false negative, *FP* = false positive, *TN* = true negative, *TP* = true positive.
genetic test results, leading to heterogeneity between different studies.

The key advantage of ctDNAs as a biomarker is its high specificity and extremely low misdiagnosis rate. When tissue biopsy is difficult to apply and the liquid biopsy result is positive, the patients could choose to try EGFR-TKI treatment. The diagnostic method of ctDNAs has numerous advantages compared with other biological materials. First, the sampling of ctDNAs is non-invasive or minimally invasive and can be collected by simple methods such as venous blood drawing. Secondly, ctDNAs include different information regarding all tumors rather than just tumor genomic DNA in one region. The analysis of ctDNAs can largely reveal almost all changes in the patient’s tumor genome and solve the problem of tumor heterogeneity. Thirdly, the detection of ctDNAs can monitor tumor progression at the molecular level in real-time and can guide clinical treatment dynamically. Fourthly, ctDNAs in a high-throughput manner can analyze tens of thousands of genomic sites in one test. In summary, plasma ctDNA detection provides a new method for clinicians to diagnose NSCLC, monitor tumor progression and treat clinical patients with its non-invasive, real-time and high-throughput advantages.[53,54]

crDNAs can also determine how the tumor DNA enters the blood. Diehl et al revealed that the amount of crDNAs in colorectal cancer was associated with tumor aggressiveness. Together with the highly fragmented nature of ctDNAs, the authors proposed that ctDNAs came from necrotic tumor cells engulfed by macrophages.[55] It had also been proposed that ctDNAs were composed of apoptotic cell release.[56] Another potential source of ctDNAs was the breakdown of circulating tumor cells.[25] However, in a series of patients with malignant tumors, higher levels of ctDNAs were observed compared with circulating tumor cells, indicating that these cells were not the source of ctDNAs. Some studies have hypothesized that tumor cells may actively secrete DNA fragments, and patients with NSCLC secrete ctDNAs through microvesicles or exosomes.[57]

Due to the conjectures of ctDNA entry into peripheral blood, several studies had shown that detecting EGFR gene mutations by ctDNAs was more difficult than applying tumor tissues. To the best of our knowledge, there is currently no international standardized method for ctDNA extraction and detection. The type of blood specimen (plasma/serum), the selection of specimen storage reagent tubes, and blood specimen factors such as the time interval between collection and centrifugation, the temperature and time of specimen storage could also affect the test results. Thus, the sensitivity and specificity of ctDNAs detection of EGFR mutations in different laboratories were significantly different. Oxnard et al applied BEAMing to detect EGFR gene mutations in ctDNA. Compared with tumor tissues, the sensitivity was 86% and the specificity was 98%. [58] The lower
sensitivity of peripheral blood ctDNAs may be associated with the lower abundance of ctDNA mutations. EGFR mutations are abundant at 20% to 30% before they can be detected by direct sequencing and the lower detection limit of the ARMS is 1% mutations.\textsuperscript{[59]} Furthermore, Yang et al found that ddPCR can detect 0.04% of mutations.\textsuperscript{[60]} Therefore, the sensitivity of ctDNAs detection is limited by the abundance of EGFR mutations. However, as detection sensitivity increases, subclinical clonal signals that were not associated with treatment decisions may be detected. This raised the question as to whether the level of mutated DNA in the peripheral blood reflected the specific driver mutations of the primary tumor within a given time, so further study is required in order to discuss the clinical correlation between plasma DNA mutation levels and the probability of targeted drug response.

More recently, the liquid biopsy was believed to not only be useful for evaluating the advanced stage NSCLC, but can also be used for early-stage monitoring and screening. However, the tumor size or the malignancy status could affect the efficacy and specificity of liquid biopsy. Oellerich et al also suggested that the small tumor size (e.g. <1 cm) would result in the higher FN in detecting tumors, due to the insufficient mutant DNA fraction <0.01%.\textsuperscript{[61]} However, with the development of the technique in detecting ctDNAs in the blood, the sensitivity was increasing. For example, Newman et al have created cancer personalized profiling by deep sequencing (CAPP-seq) which constructed a highly sensitive ctDNA library. The sensitivity of this method is 50% in patients with stage I, and 100% in patients with stage II-IV. The specificity reaches 96% with a mutant gene ratio of 0.02%. More recently, the CAPP-seq technology platform was improved so that the proportion of mutant genes

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Figure 3. Summary receiver operating characteristics (SROC) plots showed a good diagnostic accuracy of peripheral blood ctDNA in detecting EGFR mutation in advanced NSCLC (A), Fagan plot (B).

Figure 4. Forest plots of subgroup meta-regression for sensitivity and specificity.
Table 3
Subgroup analysis.

| Subgroup            | No. of studies | Summary sensitivity (95% CI) | P value | Summary specificity (95% CI) | P value |
|---------------------|----------------|------------------------------|---------|------------------------------|---------|
| Sample size         |                |                              |         |                              |         |
| Large than 40       | 28             | 0.70 (0.63–0.76)             | .70     | 0.98 (0.97–1.00)             | .21     |
| Less than 40        | 4              | 0.65 (0.44–0.87)             | .86     | 0.95 (0.85–1.00)             | .39     |
| Blood sample        |                |                              |         |                              |         |
| Plasma              | 25             | 0.70 (0.63–0.77)             | .01     | 0.98 (0.96–1.00)             | .42     |
| Serum               | 7              | 0.67 (0.51–0.82)             | .01     | 0.99 (0.96–1.00)             |         |
| Design              |                |                              |         |                              |         |
| Prospective         | 14             | 0.66 (0.56–0.76)             | .11     | 0.99 (0.97–1.00)             | <.01    |
| Retrospective       | 18             | 0.72 (0.64–0.81)             | .01     | 0.97 (0.95–1.00)             |         |
| Reporting country   |                |                              |         |                              |         |
| Asia                | 24             | 0.69 (0.62–0.76)             | .01     | 0.97 (0.95–0.99)             | .65     |
| Non-Asia            | 8              | 0.70 (0.58–0.83)             | .01     | 0.99 (0.98–1.00)             |         |
| Detection methods   |                |                              |         |                              |         |
| ARMS                | 10             | 0.60 (0.49–0.71)             | <.01    | 0.99 (0.97–1.00)             | .65     |
| ddPCR               | 4              | 0.76 (0.62–0.91)             | .57     | 0.99 (0.98–1.00)             | .12     |
| ME-PCR              | 4              | 0.54 (0.34–0.74)             | .06     | 0.97 (0.91–1.00)             | .68     |
| PNA-LNA PCR         | 4              | 0.83 (0.70–0.96)             | .94     | 0.96 (0.89–1.00)             | .30     |
| Sequencing          | 4              | 0.80 (0.64–0.96)             | .93     | 0.98 (0.96–1.00)             | .73     |

Deeks’ Funnel Plot Asymmetry Test
p value = 0.08

Figure 5. publication bias assessment in the Deek’s funnel plot.
is as low as 0.004% and the sensitivity reaches 90%, which is the lowest ctDNA mutation concentration that can be detected on all technology platforms by far.\[62\]

The $P$ statistics test results suggested that heterogeneity existed among the included studies and thus, the present study performed a meta-regression analysis to investigate the source of heterogeneity. The results suggested that ARMS was a source of sensitivity heterogeneity ($P<0.01$). The pooled sensitivity of all the included studies using ARMS for peripheral blood ctDNAs was 60%, while the combined sensitivity of all included studies without ARMS was 74%. The reason was that ARMS required a higher abundance of EGFR mutations, which resulted in relatively low detection sensitivity. Also, the prospective study design was a source of sensitivity heterogeneity, so the present study suggested that it may be combined with different centers using different detection methods. The source of specificity heterogeneity was the publishing country. The pooled specificity of all studies with an Asian population is 97%, and the pooled specificity of all studies with non-Asian countries was 99%. The reason for the heterogeneity may be that the inequality in the number of subjects included in the 2 groups resulted in the sampling errors.

There were some limitations to the present study. Although a meta-regression analysis was performed to detect heterogeneity among the included studies, none of the characteristics of the analysis could explain the majority of the heterogeneity. Secondly, apart from the factors analyzed, the included studies differed in a number of aspects, such as lung adenocarcinoma percentage, treatments, blood collection time, methodological quality, and the association between blood collection and treatment. These unrecorded differences may be potential sources of heterogeneity. Furthermore, the number of factors included in the meta-regression analysis is relatively small, and the results are subject to bias. Although the detection threshold for mutant DNA in liquid biopsies will decrease as technology improves, it is not easy to determine a clinical correlation between low-level mutant DNA in plasma and the probability of responding to targeted drugs; further research is required in order to elucidate this situation.

5. Conclusion

In conclusion, ctDNA biopsy has high specificity and diagnostic accuracy in detection of EGFR mutations in patients with advanced NSCLC and can be used as a preliminary screening test for patients with NSCLC when it is difficult or unavailable to obtain tissue via biopsy. When the ctDNAs gene test result is negative, the risk of mis-diagnosis should be considered, and further tissue biopsy is required. With the advancement of gene detection technology and the standardization of gene detection methods, ctDNA gene detection will be an important part of precision treatment for patients with NSCLC.

Author contribution

Design of the meta-analysis: Shunkai Zhou, Rongzhi Huang and Yunpeng Cao

Literature screening: Shunkai Zhou and Rongzhi Huang

Quality assessment: Shunkai Zhou and Rongzhi Huang

Statistics analysis: Yunpeng Cao

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