Medicine®

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

Shunkai Zhou, MD[a], Rongzhi Huang, MD[b], Yunpeng Cao, MM[c],* (iD)

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. I^2 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, $I^2 = 81.76$) and the pooled specificity was 0.98 (95% CI: 0.96–0.99, $I^2 = 88.33$). 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

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.[1] 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.[2] Non-small cell lung cancer (NSCLC) accounts for >80% of all types of lung cancer, which is the primary classification of lung malignancies.[3] 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),[4] which leads to the poor prognosis with a 5-year survival rate at ~18%.[5]

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.[6] 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.[7,8] 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.[9,10] 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.[11] 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.[12] According to previous studies, chest biopsy complications have been reported in ~17% of cases.[12] 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.[13] Compared with tumor tissue, ctDNA is a molecular-level biomarker for detecting tumor gene mutations.[14] Also, liquid biopsy has the advantages of less trauma, reproducible, real-time monitoring and easy acceptance by patients.[15] 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.[16] Current research shows that the consistency of ctDNAs biopsy and tissue biopsy ranges between 66 and 100%.[17] 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.[18]

2.1. Search strategy

The electronic databases, such as Pubmed (https://pubmed.ncbi.nlm.nih.gov/), Embase (https://www.embase.com/), Ovid Medline (https://www.ovid.com/) and the Cochrane library (https://www.cochranelibrary.com/), 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", "NSCLC", "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.[19] 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.[20]

Summary receiver operative curve (SROC) and area under the SROC (AUSROC) were measured.[21] The Fagan nomogram was created for visual presentation of diagnostic performance with pre- and post-test probabilities. The χ^2 test and I^2 statistics were used for detecting heterogeneity across studies ($I^2 \geq 50\%$ 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
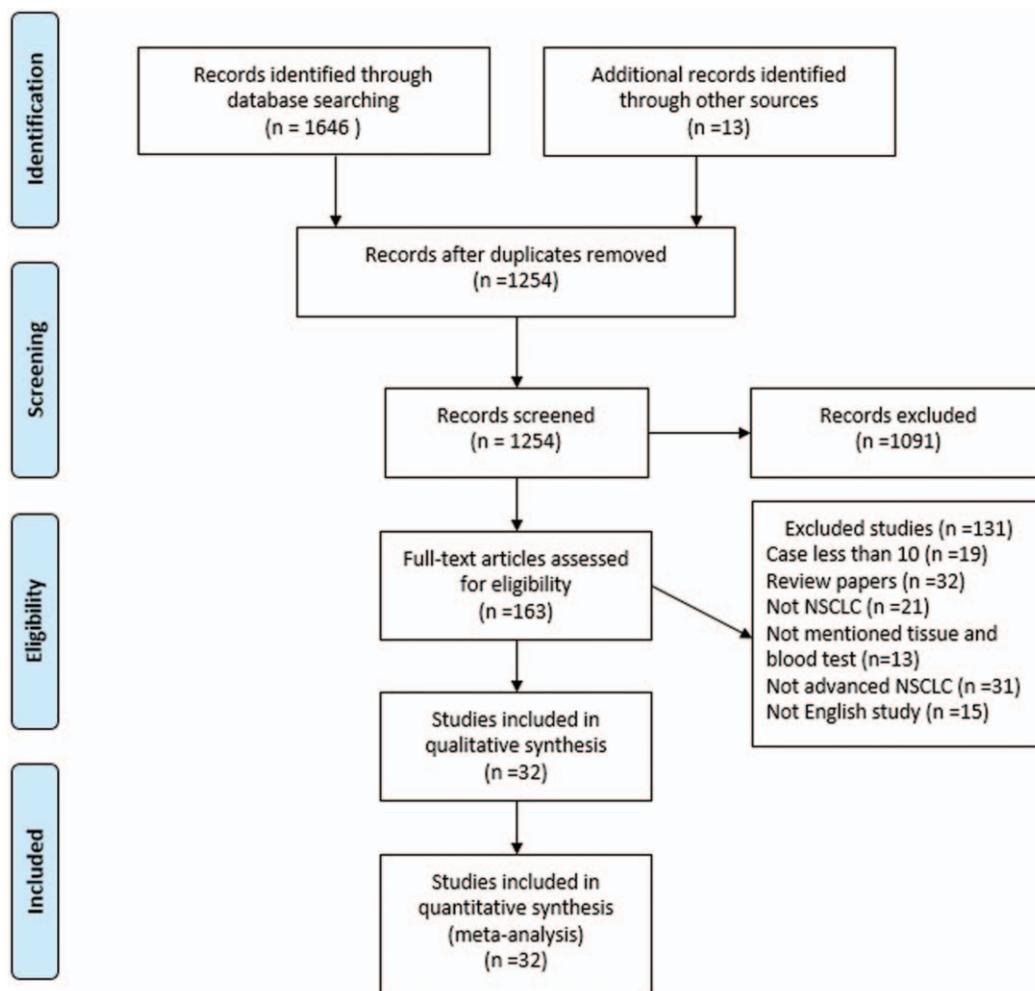

Figure 1. Flowchart of literature screening.

studies, 1254 studies were screened with titles and abstracts. After excluding 1091 inapposite publications, the full text of 163 studies was screened. A total of 32 studies were finally included in the meta-analysis after reading through the full text.[17,22–52] The flowchart is presented in Figure 1.

3.2. Characteristics of included studies

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 ($I^2 = 81.76$ for sensitivity, and $I^2 = 88.33$ 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
Leighl, N. B. et al	2019	2016–2018	America	69	129 (46)	221 (78)	IIIB-IV	7 (2)	275 (98)	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)	III-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	III-IV	NG	NG	55
Ito, K. et al	2018	2015–2016	Japan	76	54 (68)	59 (75)	III-IV	11 (14)	68 (86)	79
Arriola, E. et al	2018	NG	Spain	64	111 (72)	127 (82)	III-IV	9 (6)	136 (88)	154
Zhang, Y. et al	2017	2009–2014	China	NG	127 (59)	95 (44)	IIIB-IV	36 (17)	179 (83)	215
Zhang, X. et al	2017	2015–2016	China	59	65 (56)	51 (44)	III-IV	36 (31)	79 (68)	116
Wang, Y. et al	2017	NG	China	NG	133 (46)	107 (37)	IIIBm-IV	57 (20)	230 (80)	287
Vázquez, S. et al	2016	2011–2012	Spain	66	151 (76)	155 (78)	IIIB-IV	NG	NG	198
Sacher, A. G. et al	2016	NG	America	62	68 (38)	NG	IIIB-IV	3 (2)	172 (96)	180
Que, 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)	III-IV	2 (3)	70 (95)	74
Duan, H. et al	2015	2013–2014	China	58	61 (65)	48 (51)	III-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 alm.	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)	IIIB-IV	6 (12)	45 (88)	51
Huang, Z. et al	2012	2005–2009	China	58.4	438 (53)	340 (41)	IIIB-IV	NG	NG	822
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)	III-IV	1 (3)	30 (97)	31
Yung, K. F. et al	2009	NG	China	NG	NG	NG	III-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	28 (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.

Table 2

Diagnostic data of advanced NSCLC patients in paired tissue and blood sample.

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
Leighl, 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	Prospective	PNA-LNA PCR	Plasma	17	4	5	121	12
Zhang, Y. et al	2017	Retrospective	ddPCR	Plasma	57	4	36	118	9
Zhang, X. et al	2017	Retrospective	ARMS	Plasma	34	2	10	70	9
			ddPCR	Plasma	18	0	7	52	9
Wang, Y. et al	2017	Retrospective	ARMS	Plasma	32	8	30	47	9
Vázquez, S. et al	2016	Prospective	ARMS	Serum	13	1	12	148	11
Sacher, A. G. et al	2016	Prospective	ddPCR	Plasma	41	0	9	124	11
Que, D. et al	2016	Retrospective	ME-PCR	Plasma	33	6	7	58	9
Ma, M. et al	2016	Retrospective	ARMS	Plasma	54	4	36	125	11
Zhu, G. S. et al	2015	Retrospective	ddPCR	Plasma	18	1	4	63	9
Lam, D. C. et al	2015	Retrospective	PNA-LNA PCR	Plasma	34	9	1	30	9
Duan, H. et al	2015	Retrospective	RGQ-PCR	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	ddPCR	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.

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 were 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
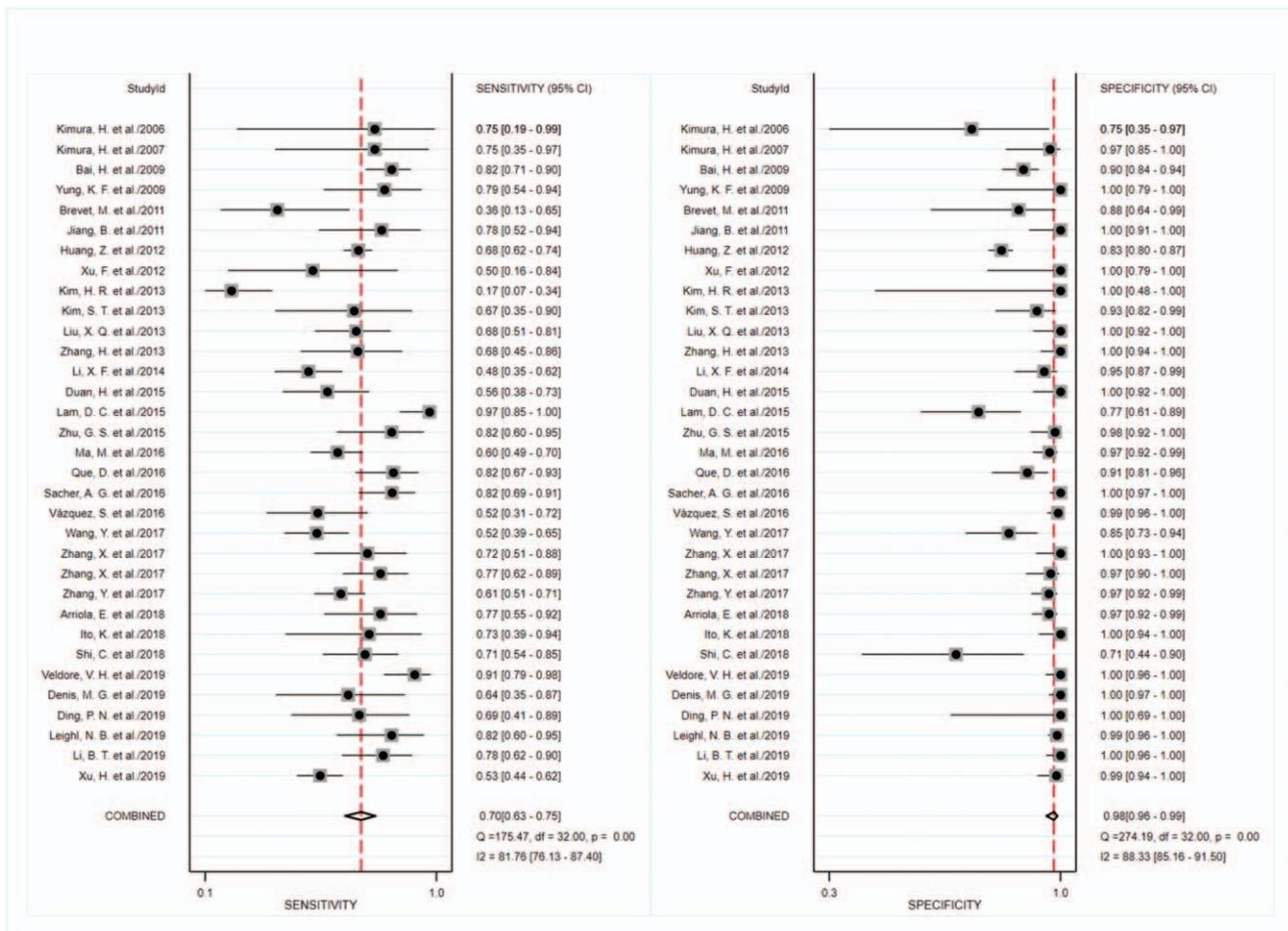
Figure 2. The forest plot of the pooled sensitivity and specificity of peripheral blood ctDNA in detecting EGFR mutation in advanced NSCLC patients.

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]

ctDNAs can also determine how the tumor DNA enters the blood. Diehl et al revealed that the amount of ctDNAs 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
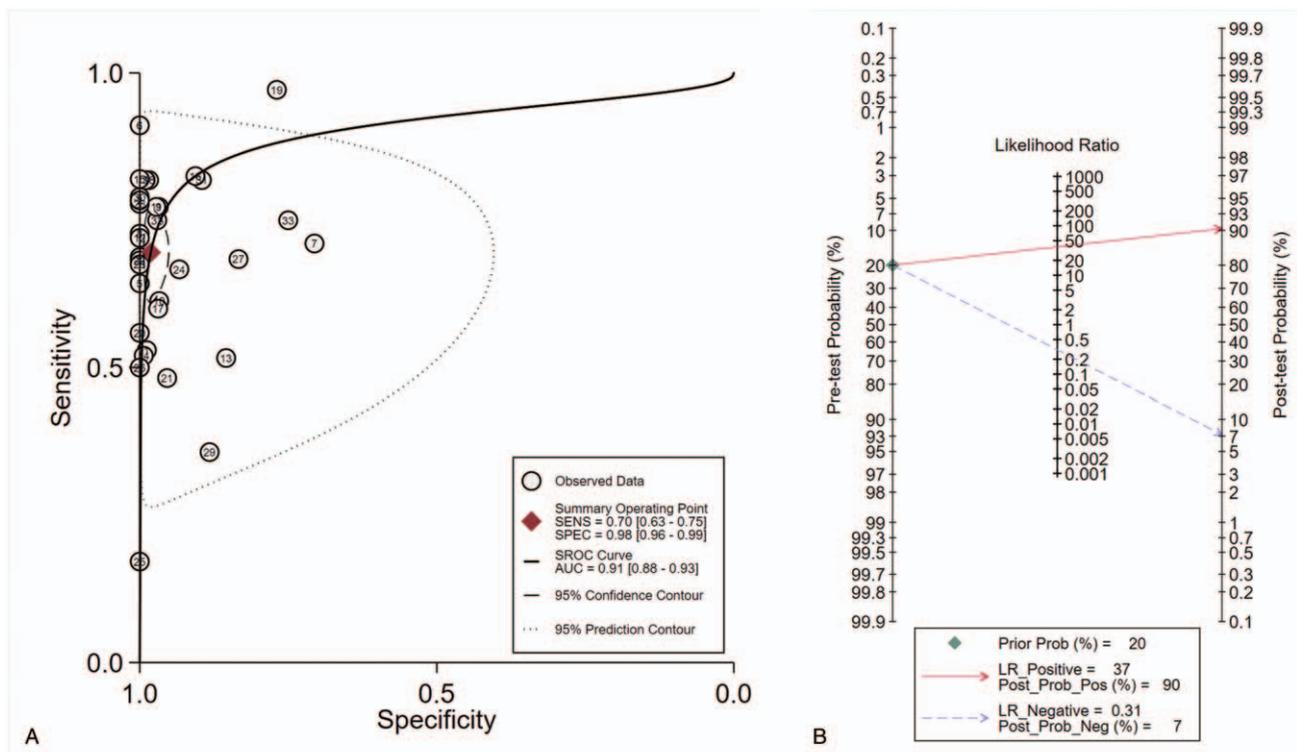
Figure 3. Summary receiver operating characteristics (SROC) plots showed a good diagnostic accuracy of peripheral blood ctDNA in detecting EGFR mutation in advanced NSCLC (A), Fagan plot (B).
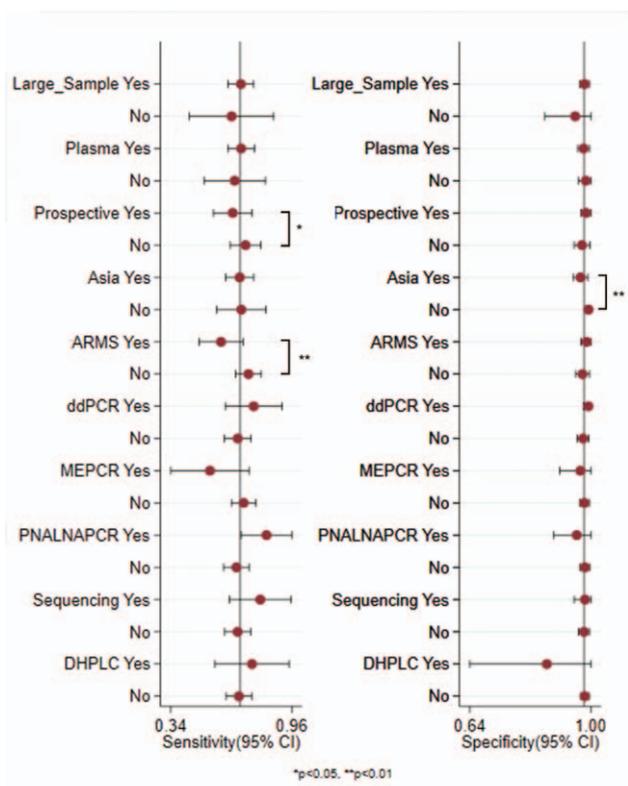
**Figure 4.** Forest plots of subgroup meta-regression for sensitivity and specificity.

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.[59] Furthermore, Yang et al found that ddPCR can detect 0.04% of mutations.[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 of sensitivity 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%.[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

Table 3

Subgroup analysis.

Subgroup	No. of studies	Summary sensitivity (95% CI)	P value	Summary specificity (95% CI)	P value
Sample size			.70		.21
Large than 40	28	0.70 (0.63–0.76)		0.98 (0.97–1.00)	
Less than 40	4	0.65 (0.44–0.87)		0.95 (0.85–1.00)	
Blood sample			.36		.39
Plasma	25	0.70 (0.63–0.77)		0.98 (0.96–1.00)	
Serum	7	0.67 (0.51–0.82)		0.99 (0.96–1.00)	
Design			.01		.42
Prospective	14	0.66 (0.56–0.76)		0.99 (0.97–1.00)	
Retrospective	18	0.72 (0.64–0.81)		0.97 (0.95–1.00)	
Reporting country			.11		<.01
Asia	24	0.69 (0.62–0.76)		0.97 (0.95–0.99)	
Non-Asia	8	0.70 (0.58–0.83)		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)	.88
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
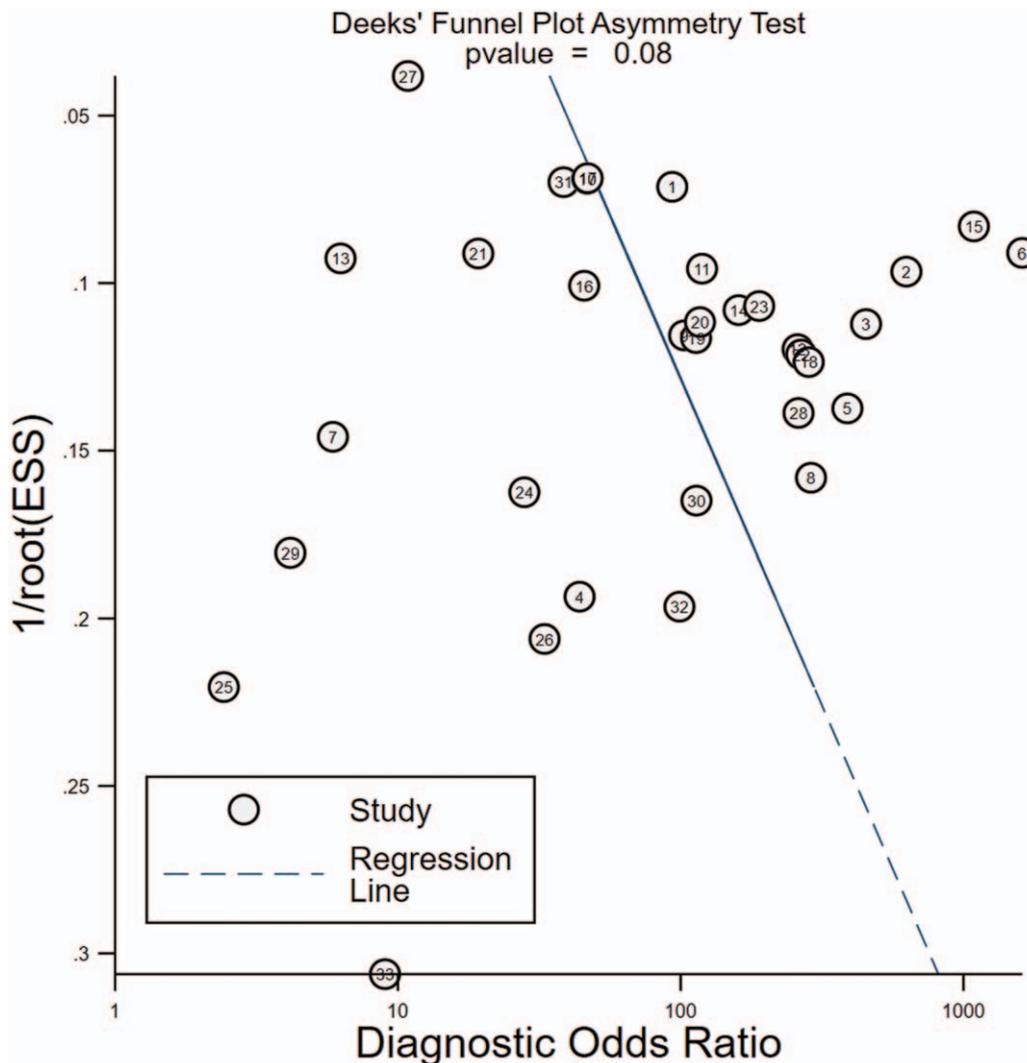
Figure 5. publication bias assessment in the Deek's funnel plot.

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 I^2 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 ($P < .01$). 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
Write and revise: Shunkai Zhou, Rongzhi Huang and Yunpeng Cao

References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017;67:7–30.

[2] Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424.

[3] Ettinger DS, Wood DE, Aisner DL, et al. Non-small cell lung cancer, version 5.2017, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 2017;15:504–35.

[4] Tissot C, Gay P, Brun C, et al. Novel insights into the systemic treatment of lung cancer malignant pleural effusion. Clin Respir J 2019;13:131–8.

[5] Zappa C, Mousa SA. Non-small cell lung cancer: current treatment and future advances. Transl Lung Cancer Res 2016;5:288–300.

[6] Mok TS, Wu Y-L, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947–57.

[7] Rosell R, Moran T, Queralt C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 2009;361:958–67.

[8] Huang S-F, Liu H-P, Li L-H, et al. High frequency of epidermal growth factor receptor mutations with complex patterns in non-small cell lung cancers related to gefitinib responsiveness in Taiwan. Clin Cancer Res 2004;10:8195–203.

[9] Ladanyi M, Pao W. Lung adenocarcinoma: guiding EGFR-targeted therapy and beyond. Mod Pathol 2008;21(Suppl 2):S16–22.

[10] Karachaliou N, Mayo-de las Casas C, Queralt C, et al. Association of EGFR L858R mutation in circulating free DNA with survival in the EURTAC trial. JAMA Oncol 2015;1:149–57.

[11] Sequist LV, Heist RS, Shaw AT, et al. Implementing multiplexed genotyping of non-small-cell lung cancers into routine clinical practice. Ann Oncol 2011;22:2616–24.

[12] Overman MJ, Modak J, Kopetz S, et al. Use of research biopsies in clinical trials: are risks and benefits adequately discussed? J Clin Oncol 2013;31:17–22.

[13] Wan JCM, Massie C, Garcia-Corbacho J, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer 2017;17:223–38.

[14] Polivka J, Pesta M, Janku F. Testing for oncogenic molecular aberrations in cell-free DNA-based liquid biopsies in the clinic: are we there yet? Expert Rev Mol Diagn 2015;15:1631–44.

[15] Rolfo C, Castiglia M, Hong D, et al. Liquid biopsies in lung cancer: the new ambrosia of researchers. Biochim Biophys Acta 2014;2:539–46.

[16] Abbosh C, Birkbak NJ, Wilson GA, et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature 2017;545:446–51.

[17] Brevet M, Johnson ML, Azzoli CG, et al. Detection of EGFR mutations in plasma DNA from lung cancer patients by mass spectrometry genotyping is predictive of tumor EGFR status and response to EGFR inhibitors. Lung Cancer 2011;73:96–102.

[18] Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6:e1000097.

[19] Whiting P, Rutjes AW, Reitsma JB, et al. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. BMC Med Res Methodol 2003;3:25.

[20] Reitsma JB, Glas AS, Rutjes AW, et al. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. J Clin Epidemiol 2005;58:982–90.

[21] Devillé WL, Buntinx F, Bouter LM, et al. Conducting systematic reviews of diagnostic studies: didactic guidelines. BMC Med Res Methodol 2002;2:9.

[22] Xu H, Hakeem Baidoo AA, Su S, et al. A comparison of EGFR mutation status in tissue and plasma cell-free DNA detected by ADx-ARMS in advanced lung adenocarcinoma patients. Transl Lung Cancer Res 2019;8:135–43.

[23] Li BT, Janku F, Jung B, et al. Ultra-deep next-generation sequencing of plasma cell-free DNA in patients with advanced lung cancers: results from the Actionable Genome Consortium. Ann Oncol 2019;30:597–603.

[24] Leighl NB, Page RD, Raymond VM, et al. Clinical utility of comprehensive cell-free DNA analysis to identify genomic biomarkers in patients with newly diagnosed metastatic non-small cell lung cancer. Clin Cancer Res 2019;25:4691–700.

[25] Ding PN, Becker TM, Bray VJ, et al. The predictive and prognostic significance of liquid biopsy in advanced epidermal growth factor receptor-mutated non-small cell lung cancer: a prospective study. Lung Cancer 2019;134:187–93.

[26] Denis MG, Lafourcade MP, Le Garff G, et al. Circulating free tumor-derived DNA to detect EGFR mutations in patients with advanced NSCLC: French subset analysis of the ASSESS study. J Thorac Dis 2019;11:1370–8.

[27] Veldore VH, Choughule A, Routhu T, et al. Validation of liquid biopsy: plasma cell-free DNA testing in clinical management of advanced non-small cell lung cancer. Lung Cancer 2018;9:1–1.

[28] Shi C, Zheng Y, Li Y, et al. Association between clinical characteristics and the diagnostic accuracy of circulating single-molecule amplification and resequencing technology on detection epidermal growth factor receptor mutation status in plasma of lung adenocarcinoma. J Clin Lab Anal 2018;32:

[29] Ito K, Suzuki Y, Saiki H, et al. Utility of liquid biopsy by improved PNA-LNA PCR clamp method for detecting EGFR mutation at initial diagnosis of non-small-cell lung cancer: observational study of 190 consecutive cases in clinical practice. Clin Lung Cancer 2018;19:e219–26.

[30] Arriola E, Paredes-Lario A, Garcia-Gomez R, et al. Comparison of plasma ctDNA and tissue/cytology-based techniques for the detection of EGFR mutation status in advanced NSCLC: Spanish data subset from ASSESS. Clin Transl Oncol 2018;20:1261–7.

[31] Zhang Y, Xu Y, Zhong W, et al. Total DNA input is a crucial determinant of the sensitivity of plasma cell-free DNA EGFR mutation detection using droplet digital PCR. Oncotarget 2017;8:5861–73.

[32] Zhang X, Chang N, Yang G, et al. A comparison of ARMS-Plus and droplet digital PCR for detecting EGFR activating mutations in plasma. Oncotarget 2017;8:112014–23.

[33] Wang Y, Duan J, Chen H, et al. Analysis of EGFR mutation status in tissue and plasma for predicting response to EGFR-TKIs in advanced non-small-cell lung cancer. Oncol Lett 2017;13:2425–31.

[34] Vázquez S, Casal J, Afonso Afonso FJ, et al. EGFR testing and clinical management of advanced NSCLC: a Galician Lung Cancer Group study (GGCP 048-10). Cancer Manag Res 2016;8:11–20.

[35] Sacher AG, Paweletz C, Dahlberg SE, et al. Prospective validation of rapid plasma genotyping for the detection of EGFR and KRAS mutations in advanced lung cancer. JAMA Oncol 2016;2:1014–22.

[36] Que D, Xiao H, Zhao B, et al. EGFR mutation status in plasma and tumor tissues in non-small cell lung cancer serves as a predictor of response to EGFR-TKI treatment. Cancer Biol Ther 2016;17:320–7.

[37] Ma M, Shi C, Qian J, et al. Comparison of plasma and tissue samples in epidermal growth factor receptor mutation by ARMS in advanced non-small cell lung cancer. Gene 2016;591:58–64.

[38] Zhu G, Ye X, Dong Z, et al. Highly sensitive droplet digital PCR method for detection of EGFR-activating mutations in plasma cell-free DNA from patients with advanced non-small cell lung cancer. J Mol Diagn 2015;17:265–72.

[39] Lam DC, Tam TC, Lau KM, et al. Plasma EGFR mutation detection associated with survival outcomes in advanced-stage lung cancer. Clin Lung Cancer 2015;16:507–13.

[40] Duan H, Lu J, Lu T, et al. Comparison of EGFR mutation status between plasma and tumor tissue in non-small cell lung cancer using the Scorpion ARMS method and the possible prognostic significance of plasma EGFR mutation status. Int J Clin Exp Pathol 2015;8:13136–45.

[41] Li X, Ren R, Ren S, et al. Peripheral blood for epidermal growth factor receptor mutation detection in non-small cell lung cancer patients. Transl Oncol 2014;7:341–8.

[42] Zhang H, Liu D, Li S, et al. Comparison of EGFR signaling pathway somatic DNA mutations derived from peripheral blood and corresponding tumor tissue of patients with advanced non-small-cell lung cancer using liquidchip technology. J Mol Diagn 2013;15:819–26.

[43] Liu X, Lu Y, Zhu G, et al. The diagnostic accuracy of pleural effusion and plasma samples versus tumour tissue for detection of EGFR mutation in

patients with advanced non-small cell lung cancer: comparison of methodologies. J Clin Pathol 2013;66:1065–9.

[44] Kim ST, Jung HY, Sung JS, et al. Can serum be used for analyzing the EGFR mutation status in patients with advanced non-small cell lung cancer? Am J Clin Oncol 2013;36:57–63.

[45] Kim HR, Lee SY, Hyun DS, et al. Detection of EGFR mutations in circulating free DNA by PNA-mediated PCR clamping. J Exp Clin Cancer Res 2013;32:50.

[46] Xu F, Wu J, Xue C, et al. Comparison of different methods for detecting epidermal growth factor receptor mutations in peripheral blood and tumor tissue of non-small cell lung cancer as a predictor of response to gefitinib. Onco Targets Ther 2012;5:439.

[47] Huang Z, Wang Z, Bai H, et al. The detection of EGFR mutation status in plasma is reproducible and can dynamically predict the efficacy of EGFR-TKI. Thorac Cancer 2012;3:334–40.

[48] Jiang B, Liu F, Yang L, et al. Serum detection of epidermal growth factor receptor gene mutations using mutant-enriched sequencing in Chinese patients with advanced non-small cell lung cancer. J Int Med Res 2011;39:1392–401.

[49] Yung TKF, Chan KCA, Mok TSK, et al. Single-molecule detection of epidermal growth factor receptor mutations in plasma by microfluidics digital PCR in non-small cell lung cancer patients. Clin Cancer Res 2009;15:2076–84.

[50] Bai H, Mao L, Wang HS, et al. Epidermal growth factor receptor mutations in plasma DNA samples predict tumor response in Chinese patients with stages IIIB to IV non-small-cell lung cancer. J Clin Oncol 2009;27:2653–9.

[51] Kimura H, Suminoe M, Kasahara K, et al. Evaluation of epidermal growth factor receptor mutation status in serum DNA as a predictor of response to gefitinib (IRESSA). Br J Cancer 2007;97:778–84.

[52] Kimura H, Kasahara K, Shibata K, et al. EGFR mutation of tumor and serum in gefitinib-treated patients with chemotherapy-naive non–small cell lung cancer. J Thorac Oncol 2006;1:260–7.

[53] Schwarzenbach H, Hoon DSB, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer 2011;11:426–37.

[54] Donaldson J, Park BH. Circulating tumor DNA: measurement and clinical utility. Annu Rev Med 2018;69:223–34.

[55] Diehl F, Li M, Dressman D, et al. Detection and quantification of mutations in the plasma of patients with colorectal tumors. Proc Natl Acad Sci USA 2005;102:16368–73.

[56] Newman AM, Bratman SV, To J, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med 2014;20:548–54.

[57] Anker P, Stroun M, Maurice PA. Spontaneous extracellular synthesis of DNA released by human blood lymphocytes. Cancer Res 1976;36:2832–9.

[58] Oxnard GR, Thress KS, Alden RS, et al. Association between plasma genotyping and outcomes of treatment with osimertinib (AZD9291) in advanced non-small-cell lung cancer. J Clin Oncol 2016;34:3375–82.

[59] Ellison G, Donald E, McWalter G, et al. A comparison of ARMS and DNA sequencing for mutation analysis in clinical biopsy samples. J Exp Clin Cancer Res 2010;29:132.

[60] Yang X, Zhuo M, Ye X, et al. Quantification of mutant alleles in circulating tumor DNA can predict survival in lung cancer. Oncotarget 2016;7:20810–24.

[61] Oellerich M, Christenson RH, Beck J, et al. Plasma EGFR mutation testing in non-small cell lung cancer: a value proposition. Clin Chim Acta 2019;495:481–6.

[62] Newman AM, Lovejoy AF, Klass DM, et al. Integrated digital error suppression for improved detection of circulating tumor DNA. Nat Biotechnol 2016;34:547–55.