Research Article

Cancer Epidemiology, Biomarkers & Prevention

Circulating Tumor DNA Is Effective for the Detection of EGFR Mutation in Non–Small Cell Lung Cancer: A Meta-analysis

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Abstract

Background: Circulating tumor DNA (ctDNA) has offered a minimally invasive and feasible approach for detection of EGFR mutation for non–small cell lung cancer (NSCLC). This meta-analysis was designed to investigate the diagnostic value of ctDNA, compared with current "gold standard," tumor tissues.

Methods: We searched PubMed, EMBASE, Cochrane Library, and Web of Science to identify eligible studies that reported the sensitivity and specificity of ctDNA for detection of EGFR mutation status in NSCLC. Eligible studies were pooled to calculate the pooled sensitivity, specificity, and diagnostic odds ratio (DOR). The summary ROC curve (SROC) and area under SROC (AUSROC) were used to evaluate the overall diagnostic performance.

Results: Twenty-seven eligible studies involving 3,110 participants were included and analyzed in our meta-analysis, and most studies were conducted among Asian population. The pooled sensitivity, specificity, and DOR were 0.620 [95% confidence intervals (CI), 0.513–0.716), 0.959 (95% CI, 0.929–0.977), and 38.270 (95% CI, 21.090–69.444), respectively. The AUSROC was 0.91 (95% CI, 0.89–0.94), indicating the high diagnostic performance of ctDNA.

Conclusion: ctDNA is a highly specific and effective biomarker for the detection of EGFR mutation status.

Impact: ctDNA analysis will be a key part of personalized cancer therapy of NSCLC. *Cancer Epidemiol Biomarkers Prev; 24(1); 206–12. ©2014 AACR.*

Introduction

One of the most exciting breakthroughs in cancer treatment is the application of personalized chemotherapy tailored according to the individual's genetic background. For non–small cell lung cancer (NSCLC), EGFR-TKIs, such as gefitinib and erlotinib, have been used for years (1, 2). It has been documented that EGFR mutation status is a sensitive and reliable biomarker for EGFR-TKIs therapy (3, 4). Patients carrying the point mutation in exon 21 (L858R) or deletion in exon 19 show good response to EGFR-TKIs (4); on the other hand, the point mutation (T790M) in exon20 indicates resistance to EGFR-TKIs and poor prognosis (5). It has also been reported that EGFR mutation status might change after chemotherapy. Bai and colleagues observed better response in patients whose EGFR mutation status switched from positive to negative after chemotherapy (6). Therefore, the examination of EGFR mutations is essential to determine an appropriate treatment strategy, especially for the administration of EGFR-TKIs and it is also necessary to monitor the dynamic change of EGFR mutation to identify acquired resistance at early time.

Currently, tumor tissue is the gold standard for detection of EGFR mutation, which is usually obtained by biopsy or surgery (7). Biopsy and surgery are invasive procedures, which cannot be performed repeatedly and cannot reflect the heterogeneity of tumor. Furthermore, biopsy is not without complications (7, 8). What is more important is that biopsy is only a snapshot, which is subjected to selection bias resulting from tumor heterogeneity (9).

In patients with cancer, dead tumor cells shed DNA into bloodstream and these DNA fragments carry tumor-specific sequence alterations (circulating tumor DNA, ctDNA; refs. 10, 11). Compared with tumor tissues, ctDNA is a potential source of tumor DNA for the identification of tumor-associated genetic and molecular alterations (12). Compared with biopsy, ctDNA is much more feasible, suitable for a general screening test for patients with cancer to characterize the genetic profile, which will greatly promote personalized cancer therapy. In addition, due to its nature of minimal invasiveness, ctDNA is suitable for real-time tumor monitoring (10, 13). Many studies have shown the feasibility and predictive value of using ctDNA to monitor tumor dynamics in various solid tumors (14–17), in which ctDNA even showed better test performance than circulating tumor cells and conventional serum biomarkers (18). As for NSCLC, many clinical centers have investigated the diagnostic accuracy of ctDNA for detection of EGFR mutation (19–21). The concordance rate of EGFR mutation between ctDNA and tumor tissues is largely dependent on detection techniques, and varies from 66% to

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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doi: 10.1158/1055-9965.EPI-14-0895

100% (22). In addition, these studies also differ in many aspects except for detection techniques, such as storage of tumor tissue, collection time of blood sample, and tumor–node–metastasis (TNM) stage; while no conclusion could be drawn for these factors.

Therefore, we conducted this meta-analysis to investigate the diagnostic accuracy of ctDNA for EGFR mutation detection compared with the "gold standard"-tumor tissues and address the effect of individual covariates.

Methods and Materials

Searching strategy

The present meta-analysis was performed and reported according to the guideline about diagnostic studies. MEDLINE (via PubMed), EMBASE (via OvidSP), the Cochrane library, and ISI Web of Knowledge were searched for potentially relevant studies. The searching strategy included the combination of following key words and medical subheadings: "lung neoplasms" or "lung cancer," "EGFR" or "erbB1," "serum" or "plasma" or "circulating," and "mutations." No limitation was performed. We searched the database between inception and September 28, 2014. Reference lists of included studies and relevant reviews were also manually screened.

Inclusion and exclusion criteria

Records retrieved from databases and reference lists were first screened by titles and abstracts and then full-text articles were further reviewed for eligibility. Eligible studies were selected according to the following inclusion criteria: (i) patients with NSCLC should be diagnosed histopathologically or cytologically; (ii) EGFR mutation status should be detected by circulating free DNA and tumor tissues; and (iii) providing sufficient information to construct the diagnostic 2×2 table, that is, false and true positives and negatives were provided.

The exclusion criteria were as follows: (i) tumor tissues and blood samples were not paired; (ii) EGFR mutation status was not compared with tumor tissues; and (iii) duplicate reports from the same center (refs. 23–25; the one with most patients with NSCLC were included; ref. 23) . All records were reviewed by the authors independently and they reached consensus on each eligible study.

Data extraction

Name of first author, year of publication, country where the study was performed, histologic type of NSCLC, TNM stage, techniques used for EGFR mutation detection in ctDNA, collection time of blood sample (before or after chemotherapy), serum or plasma, format of tumor tissues, true positive (TP), false positive (FP), false negative (FN), and true negative (TN) were collected from eligible studies. When EGFR mutation was detected by multiple methods, the one with best sensitivity or specificity was extracted. Two authors (M. Qiu and X. Ding) extracted these data independently and discrepancy between two authors was resolved by discussion with the third author (R. Yin).

Quality assessment

QUADAS-2 (quality assessment of diagnostic accuracy studies 2; QUADAS-2) is a tool (26) designed to evaluate the quality of primary diagnostic accuracy studies, which consists of four key domains (patient selection, index test, reference standard, and flow and timing). Methodologic quality of eligible studies was evaluated by QUADAS-2 by two investigators.

Statistical analysis

EGFR mutation status detected in tumor tissues was treated as the "gold standard." We tabulated true positives, false positives, false negatives, and true negatives stratified by study. These diagnostic numbers were used to calculate the pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and corresponding 95% confidence intervals (95% CI). The PLR is calculated as: sensitivity/(1−specificity) and the NLR is calculated as (1 − sensitivity)/specificity. A clinically useful test was defined with a PLR>5.0 and a NLR<0.2 (27, 28). DOR is a measure that combined sensitivity and specificity, which is calculated as: PLR/NLR19. The summary ROC curve (SROC) was generated and the area under the SROC (AUSROC) was calculated.

The heterogeneity caused by non-threshold effect was measured by the Q test and the inconsistency index (I^2), and a P value$\leq$0.05 and a I^2 value $\geq$50% indicated significant heterogeneity caused by non-threshold effect. Subgroup analyses were performed for detection techniques, collection time of blood sample, format of tissues, and TNM stages. Publication bias was detected by the Deek's funnel plot 21 and a P vale < 0.05 indicated the presence of publication bias (29).

All statistical analyses were performed using the STATA software (version 11.2, STATA Corp.) with the MIDAS module (30).

Results

Our database search retrieved 976 records. After reviewing the title and abstracts, 927 records were excluded. By reviewing full-text articles, we excluded further 23 records, leaving 26 eligible articles (refs. 19–23, 31–51; Fig. 1). In the study reported by Li and colleagues (49), EGFR mutation was detected in both plasma and serum, and the data of plasma and serum were analyzed as two independently studies. Thus, 27 eligible studies were included in meta-analysis. A manual search of reference lists of eligible studies and related reviews did not identify more relevant articles.

Characteristics of 27 eligible studies are shown in Table 1. A total of 3,110 patients with NSCLC were included in analysis. Most studies were conducted in Asia and only recruited patients with advanced disease. Formalin-fixed paraffin-embedded (FFPE) tumor tissues were used for detecting EGFR mutation status in 14 studies. Only six studies reported the exact collection time point of both samples (tumor tissues and blood samples). Various detection methods were reported and the ARMS was the most frequently used method.

Methodologic quality of eligible studies was assessed by QUADAS-2. The overall quality of included studies was moderate (Supplementary Fig. S1). The Deek regression test was performed to detect potential publication bias and no significant publication bias was detected ($P = 0.896$, Supplementary Fig. S2).

The pooled specificity was 0.959 (95% CI, 0.929–0.977) and the pooled sensitivity was 0.620 (95% CI, 0.513–0.716). The AUSROC and the pooled DOR was 0.91 (95% CI, 0.89–0.94, Fig. 2) and 38.270 (95% CI, 21.090–69.444), respectively. Between-studies heterogeneity was detected among eligible studies (bivariate model 98.54, 95% CI, 97.88–99.21), while we did not find any evidence of threshold effect.

To investigate the effect of potential confounding factors, we conducted stratified analysis according to detection methods, TNM stages, collection time and format of blood sample, and

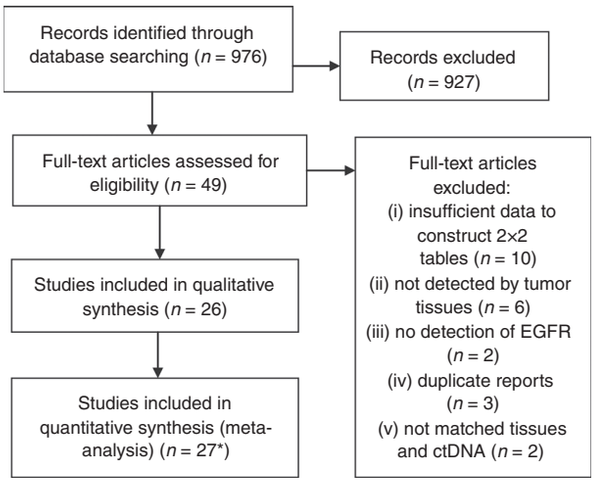

Figure 1.
Flow diagram of study selection. *, In the study reported by Li et al. (49), EGFR mutation was detected both in plasma and serum, and the data of plasma and serum were analyzed as two independent studies. Thus, 27 eligible studies were included in the meta-analysis.

treatment of tumor tissues. As measured by AUC, ME-PCR (0.97, 95% CI, 0.95–0.98) had higher diagnostic accuracy than other methods (significance not tested). For TNM stage, the diagnostic accuracy of ctDNA was higher in patients with advanced stage of NSCLC (0.96, 95% CI, 0.94–0.97; significance not tested). The diagnostic accuracy of ctDNA would be higher if the ctDNA was extracted from plasma and before chemotherapy (significance not tested). Unexpectedly, we found that the diagnostic performance of ctDNA would be better when compared with FFPE tissues than frozen tissues.

Discussion

Detection of EGFR mutation status in NSCLC has become a routine clinical test providing important information for patient prognosis and selection of EGFR-TKI therapy. In this meta-analysis, we found that compared with tumor tissues, detection of EGFR mutation status by ctDNA had high diagnostic accuracy. Detection EGFR mutation status by ctDNA will be widely applied in clinical practice and improve personalized cancer therapy and make real-time monitoring possible during chemotherapy (7, 11, 17).

The area under ROC serves as a global measure of diagnostic performance. According to the suggested guideline for interpretation of area under ROC (52), ctDNA had high diagnostic accuracy (0.9<AUC<1) for detection of EGFR mutation status in NSCLC. The value of DOR ranges from 0 to infinity, with higher values indicating better discriminatory test performance (53). Meta-analysis results showed that ctDNA had high diagnostic performance with a DOR of 38.270.

Likelihood ratios and posttest probabilities are parameters used for evaluating clinical or patient-relevant utility of the diagnostic test (27). Likelihood ratios and posttest probabilities are also important for a biomarker. They provided information about the likelihood that a patient with positive or negative result has EGFR mutation or not. In our study, the PLR (PLR>10) and negative posttest probability (<0.1) were high (Fig. 3). Given the PLR and NLR, ctDNA is located in the right upper quadrant (Fig. 4), indicating that ctDNA could serve as a test to confirm EGFR mutation.

Table 1. Characteristics of eligible studies

Author	Year	Country	TNM	Treatment	Collection time	Sample	Detection methods	TP	FP	FN	TN
Sriram KB	2011	Australia	NA	Frozen	NA	Serum	ME-PCR	3	0	3	58
He C	2009	China	NA	FFPE	NA	Plasma	ME-PCR	8	0	1	9
Yung TK	2009	China	NA	FFPE	NA	Plasma	Digital PCR	11	1	0	17
Jiang B	2011	China	Advanced	NA	NA	Serum	Mutant-enriched sequencing	14	0	4	40
Hu C	2012	China	I-IV	Frozen	Yes	Serum	HRM	22	2	0	0
Huang Z	2012	China	NA	FFPE	NA	Plasma	DHPLC	188	81	108	445
Xu F	2012	China	Advanced	FFPE	NA	Serum	ARMS	4	0	4	26
Yam I	2012	China	I-IV	NA	NA	Plasma	AS-APEX	30	1	0	4
Jing CW	2013	China	I-IV	FFPE	NA	Plasma	HRM	29	2	16	73
Liu X	2013	China	Advanced	FFPE	NA	Plasma	ARMS	27	0	13	46
Lv C	2013	China	Advanced	FFPE	Yes	Plasma	DHPLC	0	0	3	3
Zhang H	2013	China	Advanced	FFPE	Yes	Plasma	MEL	15	0	7	64
Zhao X	2013	China	I-IV	FFPE	Yes	Plasma	ME-PCR	16	3	29	63
Wang S	2014	China	Advanced	FFPE	NA	Plasma	ARMS	15	2	53	64
Kimura H	2006	Japan	Advanced	FFPE	NA	Serum	ARMS	6	1	2	2
Kimura H	2007	Japan	Advanced	FFPE	NA	Serum	ARMS	6	1	2	33
Taniguchi K	2011	Japan	Advanced	NA	NA	Plasma	BEAMing	32	0	12	0
Goto K	2012	Japan	NA	NA	NA	Serum	AS-APEX	22	0	29	35
Nakamura T	2012	Japan	I-IV	NA	No	Plasma	Inhibiting PCR-quenching probe method	21	0	26	23
Kim HR	2013	Korea	Advanced	NA	No	Plasma	PNAClamp	6	0	29	5
Kim ST	2013	Korea	Advanced	FFPE	NA	Serum	PNA–LNA PCR clamp	8	3	4	42
Kuang Y	2009	USA	NA	NA	NA	Plasma	ARMS	21	2	9	11
Brevet M	2011	USA	Advanced	NA	NA	Plasma	Sequenom	8	2	10	11
Li X (plasma)	2014	China	I-IV	Frozen	NA	Plasma	ARMS	27	3	29	62
Li X (serum)	2014	China	I-IV	Frozen	NA	Serum	ARMS	19	2	29	42
Douillard JY	2014	Europe	NA	NA	YES	Plasma	ARMS	69	1	36	546
Weber B	2014	Denmark	I-IV	FFPE	YES	Plasma	Cobas EGFR blood test	17	6	11	162

Abbreviations: HRM, high-resolution melting; PNA-LNA, peptide nucleic acid-locked nucleic acid; AS-APEX, allele-specific arrayed primer extension; ME-PCR, mutant-enriched-PCR; DHPLC, denaturing high-performance liquid chromatography; BEAMing, beads, emulsion, amplification, and magnetics; ARMS, amplification refractory mutation system; MEL, mutant-enriched liquid chip.

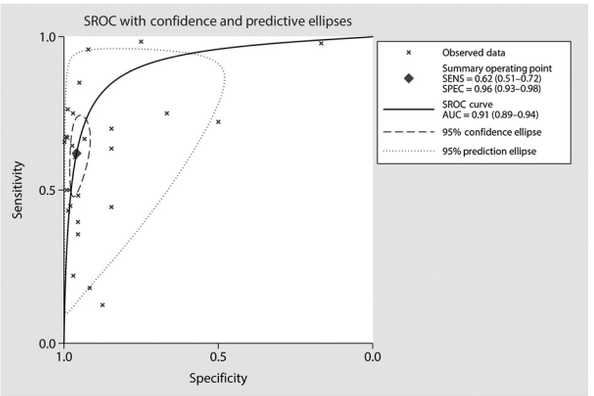

Figure 2.
SROC curve. The figure also shows 95% confidence contour and 95% prediction contour.

In overall analysis, ctDNA showed high diagnostic accuracy; however, no conclusion could be drawn for other important covariates, like detection methods, source of ctDNA, collection time of blood sample, TNM stage, and treatment of tumor tissues. Therefore, stratified analyses were performed to investigate whether these factors could influence diagnostic accuracy of ctDNA. ARMS was the most frequently used method and several commercial detection kits based on ARMS have been developed. Therefore, the diagnostic performance of ARMS was most useful for clinical practice. Our meta-analysis results showed that the specificity of ARMS was highest among all the methods assessed and the overall diagnostic performance was high (AUSROC and DOR, Table 2). In addition, the ME-PCR showed highest AUSROC, while the sample size of ME-PCR was relatively small and further studies are warranted. Although several highly sensitive methods such as digital PCR were also reported, subgroup analysis was not allowed because of too few studies.

Usually, ctDNAs were extracted from serum or plasma. Stratified analysis showed that ctDNA extracted from plasma had higher diagnostic accuracy than ctDNA extracted from serum. As measured by AUSROC, ctDNA had higher diagnostic accuracy when blood sample was collected before chemotherapy. Many investigators have reported that EGFR mutation status would change after chemotherapy (6) and this would lead to the inconsistence between tissues and ctDNA when blood sample was collected after chemotherapy. Compared with patients at early stage, those at advanced stage have high level of circulating-free DNA. It has been suggested that if the fraction of ctDNA in a sample is lower than 0.01%, it is considered negative for ctDNA (54, 55). These results suggested that the detection performance of ctDNA would be higher when ctDNA was in large amount. Currently, the exact mechanism that determines the release of ctDNA is unclear, but current hypotheses indicate that the amount of ctDNA is associated with tumor volume and status of metastasis. TNM stage is suitable marker that combines tumor volume and metastasis. By subgroup analysis, we found that in patients with advanced stage of NSCLC, ctDNA had higher AUSROC.

FFPE tissue is the most common method used for tissue storage, but this will lead to cross-link between nucleic acids and proteins, which then lead to false-positive or false-negative results. On the other hand, liquid nitrogen frozen tissues do not have the problem. However, stratified analysis found that concordance rate was higher in FFPE; this might be explained by the fact that too few studies were available for frozen tissues.

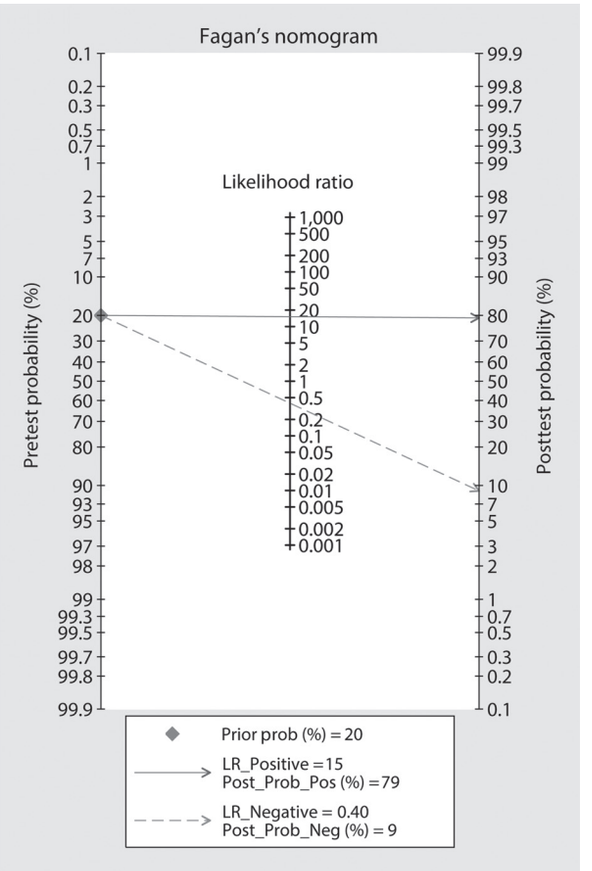

Figure 3.
Fagan nomogram of ctDNA for the detection of EGFR mutation.

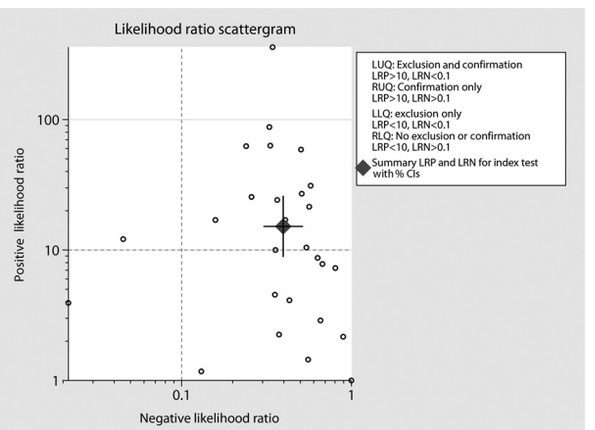

Figure 4.
The likelihood ratio matrix of ctDNA for the detection of EGFR mutation.

Table 2. Meta-analysis results

	Studies	AUSROC	Sensitivity	Specificity	PLR	NLR	DOR
Overall	27	0.91 (0.89–0.94)	0.620 (0.513–0.716)	0.959 (0.929–0.977)	15.176 (8.924–25.807)	0.397 (0.305–0.515)	38.270 (21.090–69.444)
Format of blood sample							
Plasma	18	0.92 (0.89–0.94)	0.599 (0.468–0.717)	0.960 (0.925–0.979)	14.952 (7.876–28.386)	0.418 (0.305–0.572)	35.798 (16.375–78.259)
Serum	9	0.90 (0.87–0.92)	0.658 (0.463–0.811)	0.954 (0.864–0.986)	14.428 (5.440–38.268)	0.359 (0.219–0.587)	40.223 (16.538–97.826)
TNM stage							
I-IV	6	0.94 (0.91–0.95)	0.786 (0.420–0.949)	0.921 (0.751–0.978)	9.935 (3.771–26.175)	0.233 (0.071–0.761)	42.703 (17.732–102.837)
Advanced	14	0.96 (0.94–0.97)	0.521 (0.399–0.641)	0.962 (0.940–0.977)	13.865 (7.861–24.454)	0.497 (0.382–0.647)	27.877 (13.047–59.565)
Storage method of tumor tissues							
FFPE	14	0.93 (0.90–0.95)	0.607 (0.484–0.718)	0.957 (0.925–0.975)	14.011 (7.942–24.720)	0.411 (0.304–0.555)	34.104 (16.564–70.217)
Frozen	4	0.84 (0.81–0.87)	0.627 (0.253–0.893)	0.908 (0.479–0.991)	6.785 (1.292–35.636)	0.411 (0.181–0.935)	16.507 (4.647–58.635)
Detection methods							
ARMS	9	0.88 (0.85–0.91)	0.549 (0.419–0.672)	0.975 (0.937–0.991)	22.283 (8.244–60.230)	0.463 (0.347–0.617)	48.168 (15.479–149.887)
AS-APEX	2	0.96 (0.94–0.98)	0.859 (0.189–0.994)	0.935 (0.527–0.995)	13.313 (1.635–108.404)	0.151 (0.010–2.212)	88.339 (8.851–881.676)
DHPLC	2	0.82 (0.78–0.85)	0.628 (0.572–0.681)	0.846 (0.813–0.874)	4.086 (3.286–5.081)	0.439 (0.377–0.511)	9.303 (6.672–12.973)
HRM	2	0.91 (0.88–0.93)	0.887 (0.402–0.989)	0.736 (0.042–0.994)	3.360 (0.197–57.314)	0.153 (0.035–0.673)	21.974 (1.522–317.223)
ME-PCR	3	0.97 (0.95–0.98)	0.556 (0.290–0.794)	0.975 (0.906–0.994)	22.469 (4.628–109.078)	0.455 (0.241–0.858)	49.369 (6.522–373.727)
Collection time of blood sample							
BC	6	0.89 (0.86–0.91)	0.647 (0.375–0.848)	0.967 (0.773–0.996)	19.572 (2.931–130.691)	0.365 (0.184–0.725)	53.568 (8.694–330.045)
AC	2	0.81 (0.78–0.85)	0.307 (0.149–0.528)	0.961 (0.732–0.995)	7.784 (0.711–85.209)	0.721 (0.522–0.997)	10.790 (0.767–151.869)

Abbreviations: BC, before chemotherapy; AC, after chemotherapy.

In our meta-analysis, we showed that ctDNA had high diagnostic accuracy, especially the high degree of specificity. As Diaz and Bardelli pointed, the key advantage of ctDNA is the high degree of specificity (7), since the mutations are defined by their presence in the tumor DNA and absence in matched normal DNA. As the likelihood ration scattergram showed, ctDNA is suitable for a screening test to identify those with sensitive EGFR mutation. Because of its noninvasive nature, ctDNA is a perfect marker for real-time monitoring during management of NSCLC and acquired resistance timely (11, 18).

We would like to acknowledge and discuss the potential limitations of present study to prevent misinterpretation of our findings. First, several point mutations and deletion of EGFR were reported, while we did not perform stratified analysis for individual mutations specifically. Second, substantial heterogeneity was detected but none of the analyzed characteristics could account for the majority of heterogeneity. Except for the factors analyzed, the included studies differ in many aspects, like ethnicity, percentage of lung adenocarcinoma, and methodologic quality. These unrecorded differences might be the potential sources of heterogeneity. Third, for several stratified analyses, the number of included studies was relatively small and the results were easily biased. And results of these stratified analyses should be interpreted with caution. In addition, some important information was not available in all studies, such as collection time of blood sample, the relationship between treatment and blood sample collection, and the detailed chemotherapy regimens. Further studies are warranted to investigate these issues.

In conclusion, ctDNA is an effective method to detect EGFR mutation status in NSCLC. Given the high diagnostic accuracy and specificity, ctDNA could be a primary screening test for NSCLC and development of standardized methodologies for ctDNA analyses and validation is necessary.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: M. Qiu, L. Xu, R. Yin

Development of methodology: M. Qiu, L. Xu

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Qiu, J. Wang, Y. Xu, X. Ding, M. Li, F. Jiang, L. Xu

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Qiu, J. Wang, Y. Xu, X. Ding, L. Xu

Writing, review, and/or revision of the manuscript: M. Qiu, J. Wang, Y. Xu, X. Ding, L. Xu, R. Yin

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Qiu, M. Li, F. Jiang, L. Xu

Study supervision: M. Qiu, L. Xu, R. Yin

Grant Support

This work was funded by the Natural Science Foundation of China (81372321 to L. Xu; 81201830 and 81472200 to R. Yin), Natural Science Foundation for High Education of Jiangsu Province (13KJB320010 to R. Yin), Research and Innovation Program for Graduates of Jiangsu Province (CXLX13_571 to M. Qiu), Human Resource Summit Grant of Jiangsu Province (10-D-078 to R. Yin and WS-116 to M. Li), and Jiangsu Provincial Special Program of Medical Science (BL2012030 to L. Xu).

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Received July 31, 2014; revised October 11, 2014; accepted October 13, 2014; published OnlineFirst October 22, 2014.

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