OncoTargets and Therapy

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ORIGINAL RESEARCH

The diagnostic value of circulating tumor cells and ctDNA for gene mutations in lung cancer

This article was published in the following Dove Medical Press journal:
OncoTargets and Therapy

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Purpose: Detecting gene mutations by two competing biomarkers, circulating tumor cells (CTCs) and ctDNA has gradually paved a new diagnostic avenue for personalized medicine. We performed a comprehensive analysis to compare the diagnostic value of CTCs and ctDNA for gene mutations in lung cancer.

Methods: Publications were electronically searched in PubMed, Embase, and Web of Science as of July 2018. Pooled sensitivity, specificity, and AUC, each with a 95% CI, were yielded. Subgroup analyses and sensitivity analyses were conducted. Quality assessment of included studies was also performed.

Results: From 4,283 candidate articles, we identified 47 articles with a total of 7,244 patients for qualitative review and meta-analysis. When detecting *EGFR*, the CTC and ctDNA groups had pooled sensitivity of 75.4% (95% CI 0.683–0.817) and 67.1% (95% CI 0.647–0.695), respectively. When testing *KRAS*, pooled sensitivity was 38.7% (95% CI 0.266–0.519) in the CTC group and 65.1% (95% CI 0.558–0.736) in the ctDNA group. The diagnostic performance of ctDNA in testing *ALK* and *BRAF* was also evaluated. Heterogeneity among the 47 articles was acceptable.

Conclusion: ctDNA might be a more promising biomarker with equivalent performance to CTCs when detecting *EGFR* and its detailed subtypes, and superior diagnostic capacity when testing *KRAS* and *ALK*. In addition, the diagnostic performance of ctDNA and CTCs depends on the detection methods greatly, and this warrants further studies to explore more sensitive methods.

Keywords: lung cancer, circulating tumor cell, circulating tumor DNA, gene mutations

Introduction

Lung cancer has the highest incidence and mortality among cancer cases worldwide, with 2.1 million new lung cancer cases and 1.8 million lung cancer deaths in 2018.[1] Accumulating evidence confirms that driven gene mutations play a critical role in the oncogenesis, personalized treatment, and prognosis assessment of lung cancer.[2] Clearly, how to detect gene mutations more precisely is the cornerstone. Tissue biopsy is traditionally regarded as the gold standard for detecting gene mutations; however, invasiveness and high requirements for operation restrict its wide application.[3]

Currently, liquid biopsy focusing on the detection of ctDNA, circulating tumor DNA (ctDNA) and circulating tumor cell (CTCs) in the blood of cancer patients has shed new light on real-time monitoring of therapy, identifying drug resistance and surveillance of disease progression.[4] ctDNA refers to the single- or double-stranded DNA released from TCs into the bloodstream,[5] while CTCs are the cells released by primary tumors into peripheral blood.[6] ctDNA and CTCs have paved new diagnostic avenues: collecting blood samples from cancer patients and isolating CTCs or extracting ctDNA, thereby obtaining a wealth of information on gene mutations,

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http://dx.doi.org/10.2147/OTT.S195342

cancer phenotype, tumor-mutation burden, and drug resistance.[7] Noninvasiveness, predictability, and the same gene profile as primary tumors of ctDNA and CTCs have attracted enormous attention. However, which of the two competing biomarkers is better for detecting gene mutation in clinical practice is still a matter of debate. We undertook this meta-analysis to determine the diagnostic value of both ctDNA and CTCs in detecting different gene mutations in the blood of patients with lung cancer, including *EGFR*, *KRAS*, *ALK*, and *BRAF*, referred for tissue biopsy.

Methods

Search strategy

An electronic literature search of PubMed, Embase, and Web of Science as of July 2018 was performed by two independent reviewers. Search items were: lung, pulmonary AND cancer, carcinoma, tumor, neoplasm AND mutation AND serum, plasma, circulating. Some potential studies were manually searched from relevant reference lists. Any disagreements were discussed, and if necessary a third author would arbitrate.

Inclusion and exclusion criteria

Studies meeting all the following criteria were included: randomized controlled trials, cross-sectional studies, or cohort studies; focused on lung cancer patients; analyzed diagnostic value of CTCs or ctDNA for gene mutations; used tissue biopsy as the reference standard. Studies were excluded if they met one of the following criteria: reviews, letters, replies, case reports, conference abstracts, or animal experiments; articles not written in English; articles lacking essential information. Any disagreements were discussed.

Quality assessment

Two independent reviewers used RevMan version 5.3 to evaluate the quality of studies included based on the Quality Assessment of Diagnostic Accuracy Studies 2 tool.[8] Questions, including patient selection, index test, reference standard, and flow and timing, would be judged as "yes", "unclear", or "no" for each of the included studies.

Data extraction and management

Two independent authors extracted data: basic data (first author, publication year, countries/regions, number of patients, age, sex, blood volume, isolation methods, extraction methods, detection methods, and others) and diagnostic data (true positive, false positive, true negative and false negative). Disagreements were resolved by consensus.

Statistical analysis

Meta-Disc version 1.4 was used to calculate pooled sensitivity, pooled specificity, AUC, positive-likelihood ratio and negative-likelihood ratio, each with a 95% CI. Forest plots and a summary receiver-operating characteristic (sROC) curves were plotted to present the results visually. Both threshold effect and nonthreshold effect were assessed to find the potential source of heterogeneity. If the *P*-value of the Spearman correlation coefficient was <0.05, a threshold effect would exist. When the *P*-value of Cochran's *Q* test was <0.10, a nonthreshold effect would be identified. Subgroup analyses were performed one subtypes of *EGFR* mutations, detection methods of liquid biopsy, and consistency of detection methods between liquid biopsy and tissue biopsy. Sensitivity analyses were also carried out to test the robustness of the main results by removing low-quality studies one by one. Quantitative evaluation of heterogeneity was evaluated by calculating I^2, in accordance with the Cochrane Collaboration.[9]

Results

Study characteristics

A total of 47 of 4,283 studies were included in our analysis: nine[10–18] in the CTC group and 42[11,13,16,17,19–56] in the ctDNA group (four[11,13,16,17] studies were in both groups; Figure 1). Detected gene mutations in lung cancer were mainly in *EGFR*, *KRAS*, *ALK*, and *BRAF*. The volume of blood samples varied from 5.9 mL to 20.0 mL in the CTC group, and 1.5 mL to 20 mL in the ctDNA group. Detection methods for gene mutations were mainly sequencing and PCR in either liquid biopsy or tissue biopsy. The main characteristics of the CTC group and ctDNA group are shown in Tables 1 and 2, respectively.

Risk of bias

In the CTC group, four studies were identified as low risk and one had unclear risk for the patient selection. Altogether, six publications were assessed as high risk and two had low risk on the index test. Low risk for reference standard was identified in all articles in this group. Four articles reported detailed information about flow and timing, assessed as low risk in this term. A total of four of nine, two of nine, and nine

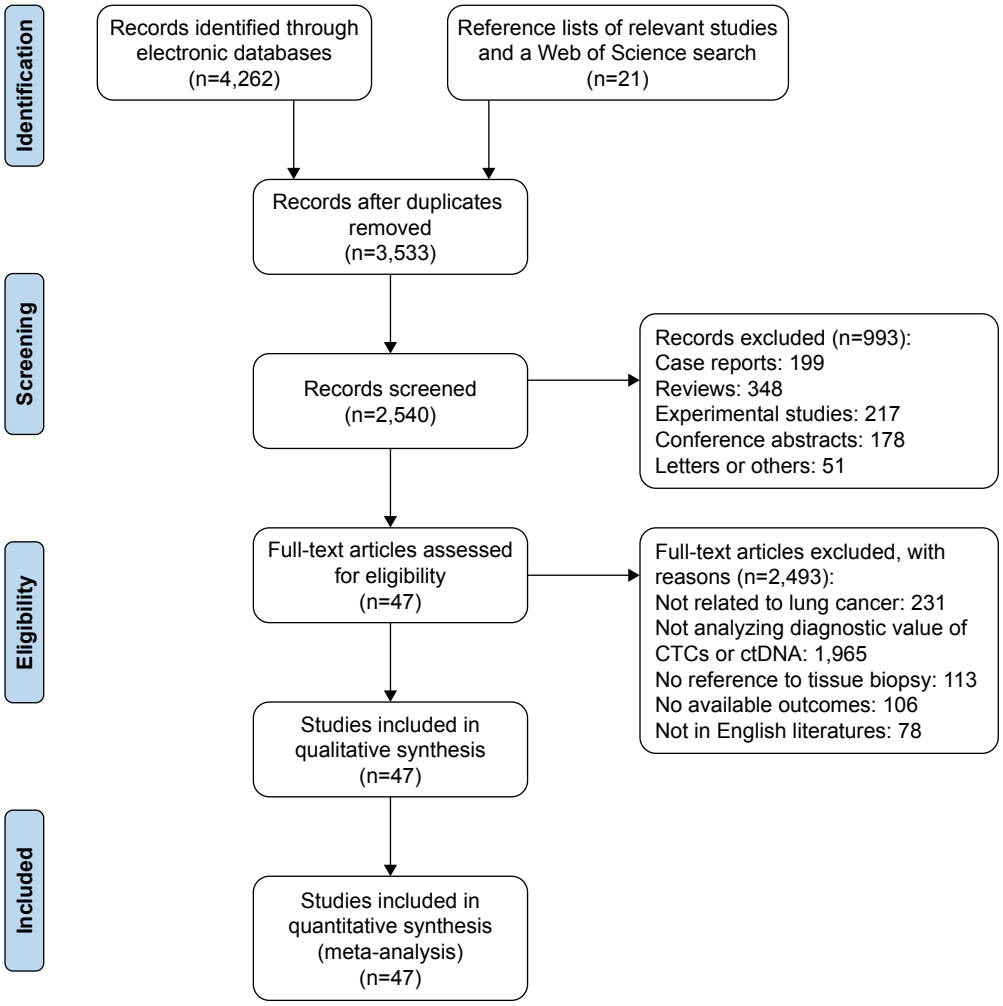


Figure 1 Flow diagram of article selection for this meta-analysis.
Abbreviations: CTCs, circulating tumor cells; ctDNA, circulating tumor DNA.

of nine articles had low concern regarding patient selection, index test, and reference standard, respectively. In the ctDNA group, 23 studies were assessed as low risk on patient selection, while two had unclear risk. There were 18 of 42 and 35 of 42 studies with low risk on the index test and reference standard, respectively. For flow and timing, 17 trials had low risk and the rest had high risk. A total of 23 of 42, 18 of 42, and 37 of 42 trials were identified as low concern for patient selection, index test, and reference standard, respectively. The risk of bias of the included studies is shown in Figure 2.

Heterogeneity

Using Spearman's correlation coefficient, we found that a threshold effect existed in the ctDNA group when detecting *ALK* (r=1.000, P<0.001). Cochran's Q indicated that a nonthreshold effect existed in the ctDNA group when testing *EGFR* (χ^2=90.39, P<0.001), *KRAS* (χ^2=22.73, P=0.007), and *BRAF* (χ^2=37.89, P<0.001). However, no nonthreshold effects were found in the CTC group regarding the detection of *EGFR* or *KRAS*. sROC curves for the CTC and ctDNA groups are shown in Figure 3.

Diagnostic accuracy

For *EGFR*, pooled sensitivity, specificity, and AUC were 75.4% (95% CI 0.683–0.817), 85.2% (95% CI 0.729–0.934), and 88.5% (95% CI 0.778–0.993) in the CTC group and 67.1% (95% CI 0.647–0.695), 96.1% (95% CI 0.954–0.968), and 83.91% (95% CI 0.759–0.919) in ctDNA group, respectively. For *KRAS*, they were 38.7% (95% CI 0.266–0.519), 92.1% (95% CI 0.850–0.965), and 74.1% (95% CI 0.472–1.000) in the CTC group and 65.1% (95% CI 0.558–0.736), 95.5% (95% CI 0.932–0.972), and 91.0% (95% CI 0.804–1.000) in the ctDNA group, respectively. For *BRAF*, they were 31.3% (95% CI 0.141–0.532), 99.5% (95% CI 0.978–1.000), and

Table 1 Characteristics of studies included in the CTC group

Study	Country	n	ADC	Smokers	F/M	TNM (I–IV)	Mutation	CTC		Tissue	
								Sample	Detection assay	Treatment	Detection assay
Breitenbuecher et al	Germany	8	NA	NA	5/3	NA	*EGFR*	Peripheral blood	Sanger sequencing	NA	Sanger sequencing
Freidin et al*	UK	82	27	NA	NA	NA	*KRAS*	Plasma	Cold PCR-HRM	FFPE	Therascreen, Cobas tissue test, cold PCR-HRM
Guibert et al	France	32	32	NA	11/21	NA	*KRAS*	Plasma	dd-PCR	FFPE	RT-PCR, HRM
He et al*	China	120	120	96	42/78	0/0/24/96	*EGFR*	Plasma	dd-PCR	NA	dd-PCR
Maheswaran et al	USA	27	25	NA	15/12	NA	*EGFR*	Plasma	ARMS	FFPE	Sanger sequencing, ARMS
Marchetti et al	Italy	37	NA	NA	NA	NA	*EGFR*	NA	Ultradeep NGS	NA	Ultradeep NGS
Punnoose et al*	USA, Australia	41	NA	NA	NA	NA	*EGFR*, *KRAS*	Plasma	TaqMan	NA	TaqMan
Sundaresan et al*	USA	40	NA	NA	26/14	0/0/6/34	$EGFR^{T790M}$	Plasma	Direct sequencing	NA	NA
Yeo et al	Singapore	7	NA	NA	6/1	NA	$EGFR^{L858R}$, $EGFR^{T790M}$	Plasma	Direct sequencing	NA	NA

Note: *In both the CTC and ctDNA groups, with CTC and ctDNA data analyzed in two independent articles.

Abbreviations: CTC, circulating tumor cell; ADC, adenocarcinoma; NA, not available; HRM, high-resolution melting; FFPE, formalin-fixed, paraffin-embedded; dd, droplet digital; RT, reverse transcription; ARMS, amplification-refractory mutation system; NGS, next-generation sequencing; ctDNA, circulating tumor DNA.

87.7% (95% CI 0–1.000) in the ctDNA group respectively. For *ALK*, only an sROC curve was plotted in ctDNA group, due to the threshold effect, and the ctDNA group had an AUC of 99.4% (95% CI 0.953–1.000). Summary plots of the CTC and ctDNA groups are shown in Figures 4 and 5, respectively.

Subgroup analyses

Although we did not find a nonthreshold effect in the CTC group, we still performed subgroup analyses to identify potential influencing factors of CTCs when detecting different gene mutations.

Subtypes of *EGFR* mutations

Seven subtypes of *EGFR* mutations – Del19, L858R, T790M, L861Q, E20ins, G719X, and S768I – were taken into consideration. For Del19, three and 18 studies were included in the CTC group and ctDNA groups, respectively. The CTC group and ctDNA group had summary sensitivity of 75.9% (95% CI 0.654–0.845) and 79.0% (95% CI 0.767–0.812), respectively. For L858R, the CTC group included four articles, while the ctDNA group had 20 studies. Pooled sensitivity was 62.2% (95% CI 0.501–0.732) in the CTC group and 76.7% (95% CI 0.731–0.800) in the ctDNA group. For T790M, the CTC group had slightly higher sensitivity than the ctDNA group (63.3% versus 61.2%). No significant findings were observed to explain the nonthreshold effect in the ctDNA group when detecting Del19, L858R, and T790M. However, a nonthreshold effect was not observed in ctDNA group when testing L861Q (χ^2=0.18, P=0.670), E20ins (χ^2=1.53, P=0.467), G719X (χ^2=0.09, P=0.765), or S768I (χ^2=0.27, P=0.606).

Detection methods of CTCs or ctDNA

The CTC group had higher sensitivity than the ctDNA group whether applying sequencing (85.1% versus 75.6%) or PCR (72.1% versus 67.2%) to detect *EGFR*. When sequencing was used to test *KRAS*, ctDNA showed excellent performance, with sensitivity of 66.9% (95% CI 0.535–0.786). When *KRAS* was detected by PCR, sensitivity was 30.8% (95% CI 0.170–0.476) and 66.9% (95% CI 0.535–0.786) in the CTC and ctDNA groups, respectively. When sequencing was employed to detect *BRAF*, sensitivity was 87.5% (95% CI 0.473–0.997) in the ctDNA group. Heterogeneity brought by nonthreshold effects was not found in the ctDNA group (χ^2=0.086, P=0.872) when detecting *KRAS* (χ^2=0.086, P=0.872) or *BRAF* (χ^2=0.62, P=0.892) by sequencing.

Table 2 Characteristics of articles included in the ctDNA group

Study	Country	n	ADC	Smoker	F/M	TNM (I–IV)	Mutation	ctDNA		Tissue	
								Sample	Detection assay	Treatment	Detection assay
Arriola et al[19]	Spain	154	112	127	39/115	0/0/18/136	*EGFR*	Plasma	PNA clamp, fragment-length analysis	NA	Therascreen
Chai et al[20]	China	61	58	NA	34/27	0/0/21/40	*EGFR*	Plasma	cSMART	FFPE	ARMS
Del et al[21]	Italy	33	NA	11	20/13	0/0/1/32	$EGFR^{T790M}$, *KRAS*	Plasma	dd-PCR	NA	dd-PCR, standard sequencing
Douillard et al[22]	13 countries	1,060	NA	NA	NA	NA	*EGFR*	Plasma	ARMS	NA	ARMS
Freidin et al[11,*]	UK	82	27	NA	NA	NA	*KRAS*	Plasma	Cold PCR/HRM	FFPE	Therascreen, Cobas tissue test, cold PCR/HRM assay
Gautschi et al[23]	USA	180	79	125	55/125	15/11/64/91	*KRAS*	Plasma	RFLP-PCR	FFPE	RFLP-PCR
Gu et al[24]	China	47	47	NA	26/21	0/0/11/36	*EGFR*	Plasma	d-PCR	FFPE	ARMS
Guo et al[25]	China	20	20	8	7/13	0/0/5/15	*EGFR*	Plasma	Tag sequencing	FFPE	ARMS
Han et al[26]	South Korea	208	164	131	72/136	0/0/15/193	*EGFR*, *KRAS*	Plasma	PNA clamp-assisted melting curve	FFPE	PNA clamp-assisted melting curve
He et al[27]	China	134	101	63	49/85	NA	*EGFR*	Plasma	Mutant-enriched PCR	NA	Direct sequencing
He et al[28]	China	200	200	188	54/146	0/0/44/156	*EGFR*	Plasma	dd-PCR	NA	dd-PCR
He et al[13,*]	China	120	120	96	42/78	0/0/24/96	*EGFR*	Plasma	dd-PCR	NA	dd-PCR
Jenkins et al[29]	UK	551	NA	NA	NA	NA	$EGFR^{del19}$, $EGFR^{L858R}$, $EGFR^{T790M}$	Plasma	Cobas plasma test	NA	Cobas tissue test
Kim et al[30]	South Korea	102	NA	31	62/40	0/0/0/102	$EGFR^{del19}$, $EGFR^{L858R}$	Plasma	PNA clamp-assisted melting curve	FFPE	PNA clamp-assisted melting curve
Kobayashi et al[31]	Japan	15	NA	7	10/5	NA	$EGFR^{T790M}$	Plasma, serum	Cobas plasma test	NA	PNA-LNA clamp, Cobas tissue test
Lee et al[32]	South Korea	57	57	16	39/18	0/0/0/57	$EGFR^{del19}$, $EGFR^{L858R}$	Plasma	PNA clamp-assisted melting curve	NA	Sanger sequencing, PNA clamp
Ma et al[33]	China	157	157	70	59/98	0/0/32/125	*EGFR*	Plasma	ARMS	FFPE	ARMS
Mao et al[34]	China	40	25	21	13/27	0/0/13/27	*EGFR*, *KRAS*, *ALK*, *BRAF*	Plasma	Targeted sequencing	FFPE	ARMS, FISH
Newman et al[35]	USA	66	NA	NA	NA	NA	*EGFR*	Plasma	iDES-enhanced CAPP sequencing	FFPE	iDES-enhanced CAPP sequencing
Pasquale et al[36]	Italy	96	84	64	36/60	NA	*EGFR*	Plasma	Therascreen, PNA clamp	NA	Therascreen
Pecuchet et al[37]	France	109	NA	73	60/49	0/0/12/97	*EGFR*, *KRAS*, *ALK*, *BRAF*	Plasma	Ultradeep-targeted NGS	FFPE	Ultradeep-targeted NGS
Punnoose et al[16,*]	USA, Australia	41	NA	NA	NA	NA	*EGFR*, *KRAS*, *BRAF*	Plasma	TaqMan	NA	TaqMan
Rachiglio et al[38]	Italy	44	NA	NA	21/23	0/0/1/43	*EGFR*	Plasma	Targeted sequencing	NA	Targeted sequencing

(*Continued*)

Table 2 (*Continued*)

Study	Country	n	ADC	Smoker	F/M	TNM (I–IV)	Mutation	ctDNA		Tissue	
								Sample	Detection assay	Treatment	Detection assay
Reck et al[39]	European nations, Japan	1,288	952	1,035	421/867	NA	*EGFR*	Plasma	Others**	NA	Others**
Schwaederle et al[40]	USA	88	88	50	58/30	NA	*EGFR*	Plasma	Digital sequencing	NA	NGS
Sun et al[41]	China	55	NA	NA	NA	NA	*EGFR*	Plasma	MST-PCR	FFPE	Direct sequencing
Sundaresan et al[17,*]	USA	40	NA	NA	26/14	0/0/6/34	$EGFR^{T790M}$	Plasma	Cobas plasma test	NA	NA
Thompson et al[42]	USA	102	83	65	69/33	0/2/2/98	*EGFR*, *KRAS*, *BRAF*	Plasma	Paired-end sequencing	NA	NGS
Thress et al[43]	USA	38	NA	NA	NA	NA	$EGFR^{L858R}$, $EGFR^{T790M}$	Plasma	ARMS, dd-PCR, d-PCR, Cobas plasma test	FFPE	Cobas tissue test
Uchida et al[44]	Japan	288	274	NA	119/169	64/46/26/146	*EGFR*	Plasma	PNA-LNA clamp	NA	PNA-LNA clamp
Veldore et al[45]	India	132	113	77	40/92	NA	*EGFR*	Plasma	NGS	FFPE	RT-PCR
Wang et al[46]	China	108	102	37	53/55	0/0/3/5	*EGFR*	Plasma	dd-PCR	FFPE	ARMS
Wang et al[47]	China	224	216	NA	NA	47/49/60/68	*EGFR*	Plasma	qRT-PCR	FFPE	qPCR
Wang et al[48]	China	287	249	64	104/83	0/0/31/156	*EGFR*	Plasma	DHPLC	FFPE	DHPLC
Wang et al[49]	China	103	103	33	55/48	0/0/25/78	*EGFR*,*** *KRAS*, *ALK*, *BRAF*	Plasma	cSMART	FFPE	ARMS
Wu et al[50]	China	45	42	NA	22/23	0/0/2/43	$EGFR^{del19}$, $EGFR^{L858R}$, $EGFR^{T790M}$	Plasma	ARMS	NA	ARMS
Xu et al[51]	China	51	43	19	20/31	0/0/6/45	$EGFR^{del19}$, $EGFR^{L858R}$	Plasma	DHPLC, MEL, ARMS	NA	ARMS
Yang et al[52]	China	73	73	20	44/29	NA	*EGFR*	Plasma	ddPCR	NA	dd-PCR
Yao et al[53]	China	39	34	10	20/19	0/0/8/31	*EGFR*, *KRAS*	Plasma	Targeted sequencing	Fresh or FFPE	Targeted sequencing
Yoshida et al[54]	Japan	31	NA	NA	NA	NA	$EGFR^{del19}$, $EGFR^{L858R}$, $EGFR^{T790M}$	Plasma	PNA-LNA clamp	NA	PNA-LNA clamp
Zheng et al[55]	China	117	108	29	71/46	0/0/5/91	$EGFR^{T790M}$	Plasma	dd-PCR	NA	ARMS
Zhou et al[56]	China	447	387	220	201/246	50/22/70/303	*EGFR*	Plasma	ARMS	NA	ARMS

Notes: *In both the CTC and ctDNA groups, with CTC and ctDNA data analyzed in two independent articles; **more than ten detection methods, eg, DNA sequencing and fragment length analysis, used in this study; ***$EGFR^{del19}$, $EGFR^{L858R}$, $EGFR^{T790M}$, $EGFR^{L861Q}$, $EGFR^{E20ins}$, $EGFR^{G719X}$, and $EGFR^{S768I}$ analyzed in this study.

Abbreviations: ctDNA, circulating tumor DNA; ADC, adenocarcinoma; NA, not available; cSMART, circulating single-molecule amplification and resequencing technology; FFPE, formalin-fixed, paraffin-embedded; ARMS, amplification-refractory mutation system; dd, droplet digital; HRM, high-resolution melting; RFLP, restriction fragment-length polymorphism; d-PCR, digital PCR; FISH, fluorescence in situ hybridization; NGS, next-generation sequencing; MST, microbial source tracking; qRT, quantitative real-time; DHPLC, denaturing high-performance liquid chromatography; MEL, ME liquid.

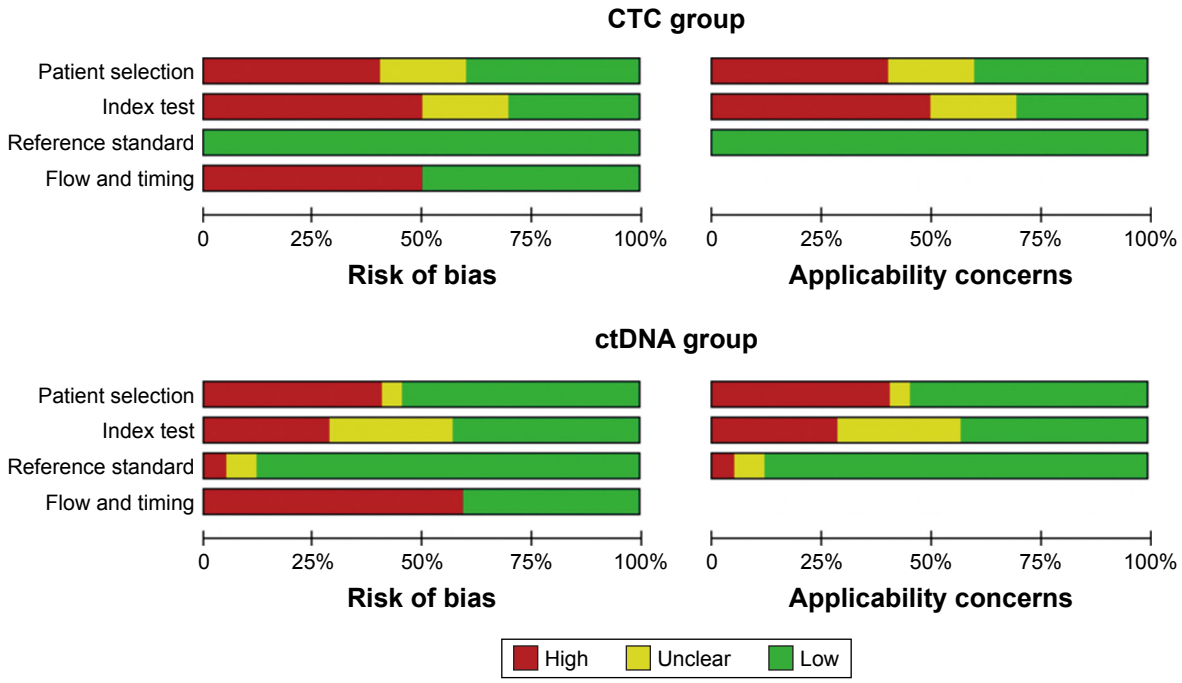


Figure 2 Risk of bias and applicability concerns in the CTC and ctDNA groups.
Abbreviations: CTC, circulating tumor cell; ctDNA, circulating tumor DNA.

Consistency of detection methods between liquid biopsy and tissue biopsy

If the same method were employed for liquid biopsy and tissue biopsy to test gene mutations, this would be grouped in the consistent subgroup and otherwise the inconsistent subgroup. CTCs and ctDNA showed similar capacity for testing *EGFR* when using the consistent method with tissue biopsy. Higher sensitivity was identified when using inconsistent methods to detect ctDNA for *KRAS* (81.5%, 95% CI 0.673–0.914), as well as *BRAF* (100%, 95% CI 0.398–1.000). Meanwhile, we did not find any nonthreshold effect in the ctDNA group when inconsistent methods were used for *BRAF* analysis (χ^2=0.62, *P*=0.431). Results of subgroup analyses are shown in Table 3.

Sensitivity analyses

No significant results were identified in sensitivity analyses.

Discussion

We found that ctDNA and CTCs had similar performance when detecting *EGFR* and its detailed subtypes. However, ctDNA showed great strength for detecting *KRAS* and *ALK*. Subgroup analyses indicated that detection method had a great impact on the diagnostic capacity of ctDNA and CTCs.

CTCs had slightly higher sensitivity than ctDNA when detecting *EGFR*, which has been supported by some researchers.[14] This may partly be attributed to the low abundance of ctDNA in peripheral blood. Although the level of ctDNA in cancer individuals was much higher than normal, it still accounted for <1% of cell-free DNA.[57] ctDNA quantity is prone to be only one genome per 5 mL plasma in the early stage of cancer.[58] Therefore, the effective capture of ctDNA is still technically challenging, though Punnoose et al[16] held the opposite opinion that ctDNA might outperform CTCs for *EGFR* detection. Treatment status may explain this inconsistency to some extent. The proportion of patients receiving treatment in their trial was higher than that in ours, while therapy can decrease CTC counts more effectively and increase the difficulty of detection.

For *KRAS*, ctDNA showed excellent diagnostic ability. Shen et al[59] conducted a meta-analysis and came to a different conclusion than us. They included two studies that we excluded during literature screening.[60,61] One did not describe clearly whether they analyzed the value of CTCs or ctDNA,[60] while another extracted RNA from CTCs for detection.[61] Great heterogeneity may exist between these two studies, which might have impacted the final results. Limited articles restricted us in analyzing the value of CTCs for *ALK* detection. In the ctDNA group, pooled sensitivity and specificity were not yielded, because of a threshold effect, while sROC curves and AUC indicated the high value of ctDNA in testing *ALK*, in line with other investigators.[62] For *BRAF*, the value of CTCs was not explored, due to limited studies.

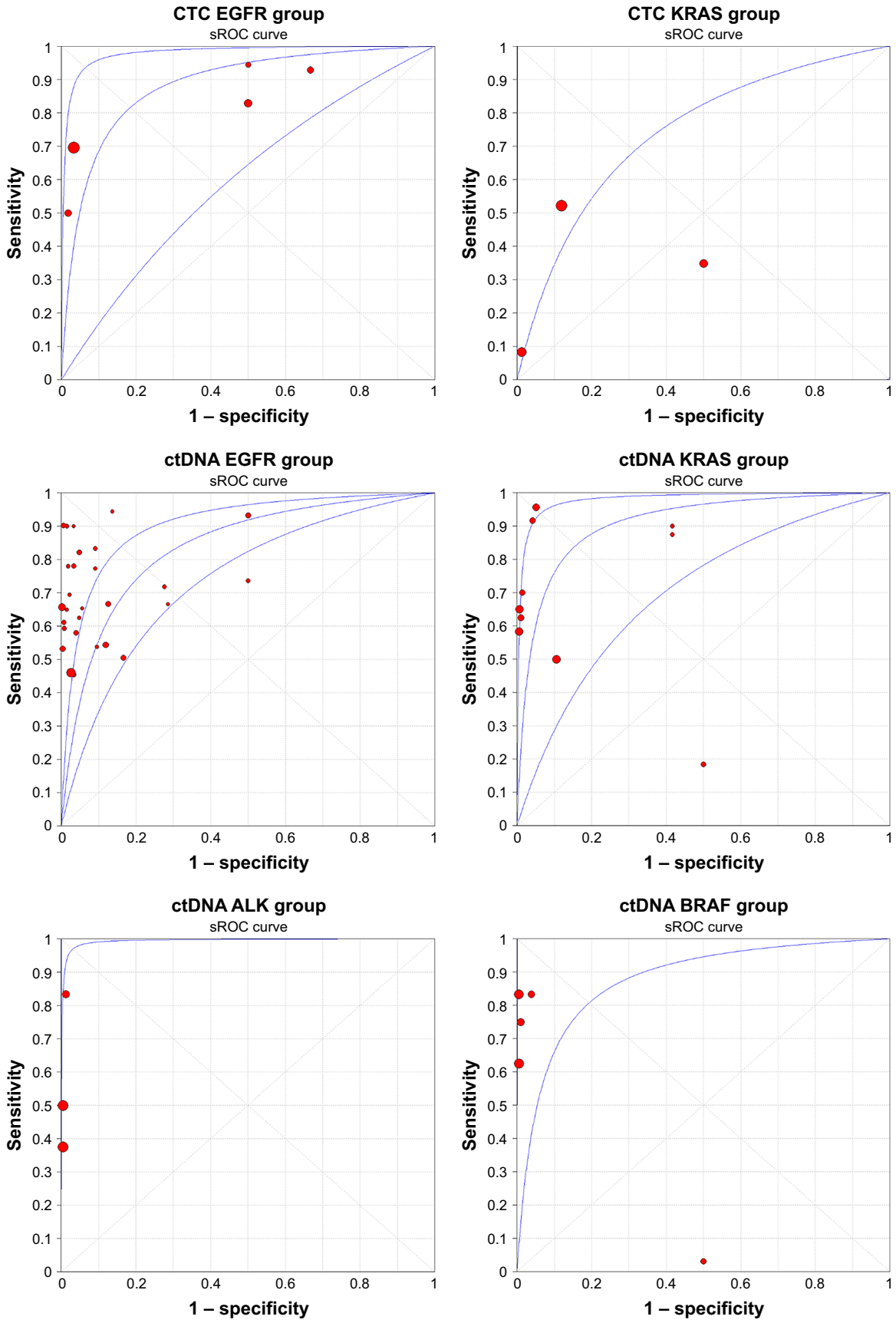


Figure 3 sROC curves for the CTC and ctDNA groups.
Abbreviations: CTC, circulating tumor cell; KRAS, kirsten rat sarcoma viral oncogene homolog; sROC, summary receiver operating characteristic curve; ctDNA, circulating tumor DNA; ALK, anaplastic lymphoma kinase; BRAF, B-Raf proto-oncogene, serine/threonine kinase.

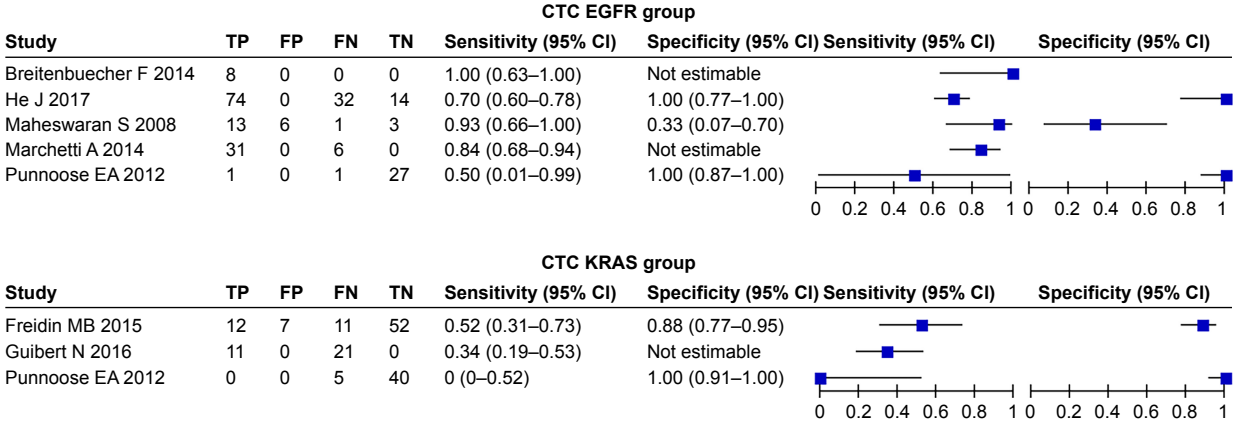


Figure 4 Summary plots of sensitivity and specificity of the CTC group.
Abbreviations: CTC, circulating tumor cell; EGFR, epidermal growth factor receptor; TP, true positive; FP, false positive; TN, true negative; FN, false negative; KRAS, kirsten rat sarcoma viral oncogene homolog.

ctDNA had low sensitivity, contrary to the results of the following two studies.[63,64] Guibert et al analyzed only six samples, and did not regard tissue biopsy as the reference standard.[63] Different sample size and reference standard were considered as the reasons for the discrepancy. Thierry et al[64] concentrated on the value of ctDNA in colorectal cancer. Different *BRAF* mutational load between lung cancer and colorectal cancer may have led to the difference in results. CTCs and ctDNA showed great variance in performance for different gene mutations and different detection kits, and methods may have contributed also.

Subgroup analyses

In view of individual treatment, analyzing detailed *EGFR*-mutation subtypes is critical. Therefore, we focused on the value of CTCs and ctDNA in testing detailed *EGFR*-mutation

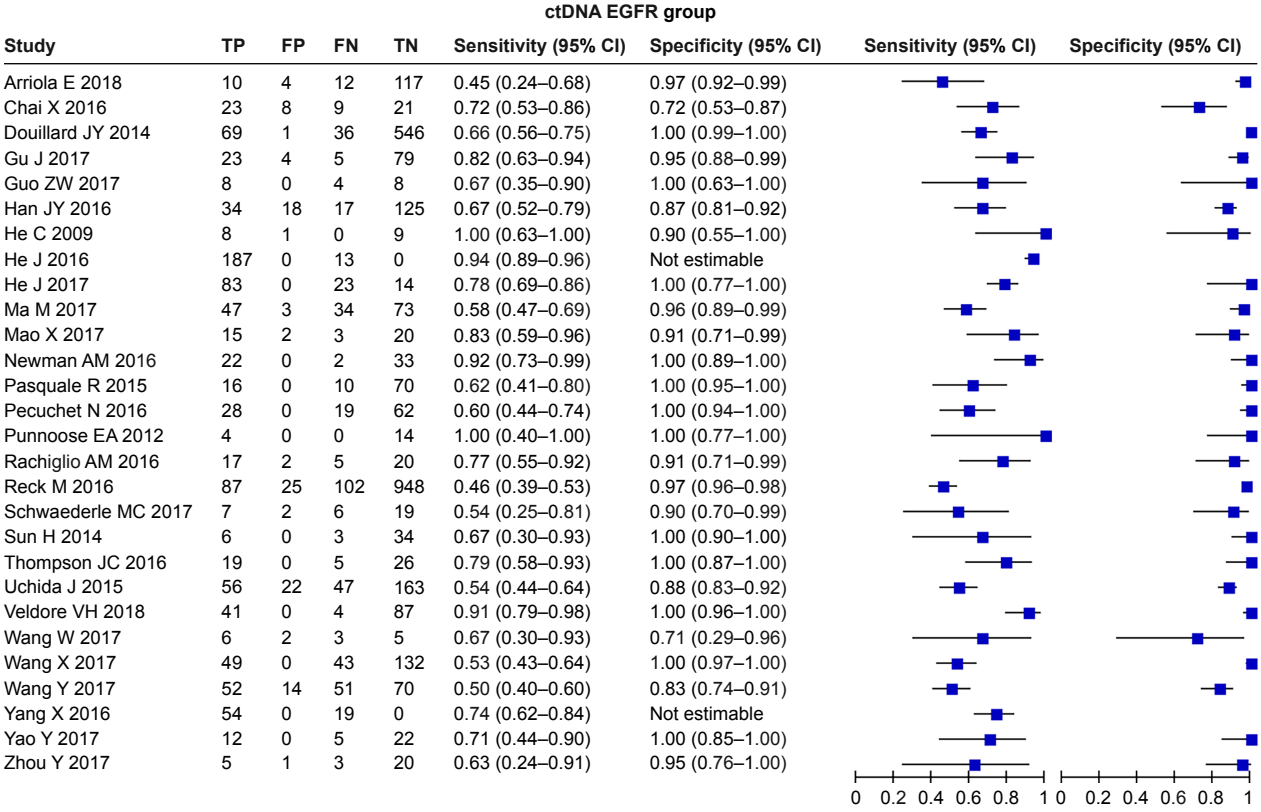
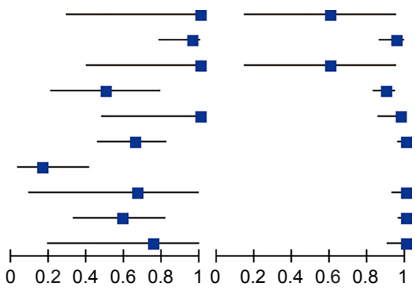


Figure 5 (*Continued*)

ctDNA KRAS group

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Del RM 2017	3	2	0	3	1.00 (0.29–1.00)	0.60 (0.15–0.95)
Freidin MB 2015	22	3	1	56	0.96 (0.78–1.00)	0.95 (0.86–0.99)
Gautschi O 2007	4	2	0	3	1.00 (0.40–1.00)	0.60 (0.15–0.95)
Han JY 2016	6	13	6	110	0.50 (0.21–0.79)	0.89 (0.83–0.94)
Mao X 2017	5	1	0	34	1.00 (0.48–1.00)	0.97 (0.85–1.00)
Pecuchet N 2016	19	0	10	80	0.66 (0.46–0.82)	1.00 (0.95–1.00)
Punnoose EA 2012	3	0	15	0	0.17 (0.04–0.41)	Not estimable
Thompson JC 2016	2	0	1	47	0.67 (0.09–0.99)	1.00 (0.92–1.00)
Wang Z 2017	10	0	7	86	0.59 (0.33–0.82)	1.00 (0.96–1.00)
Yao Y 2017	3	0	1	35	0.75 (0.19–0.99)	1.00 (0.90–1.00)



ctDNA BRAF group

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Mao X 2017	2	1	0	37	1.00 (0.16–1.00)	0.97 (0.86–1.00)
Pecuchet N 2016	2	0	1	106	0.67 (0.09–0.99)	1.00 (0.97–1.00)
Punnoose EA 2012	0	0	15	0	0.00 (0.00–0.22)	Not estimable
Thompson JC 2016	1	0	0	49	1.00 (0.03–1.00)	1.00 (0.93–1.00)
Wang Z 2017	2	0	0	101	1.00 (0.16–1.00)	1.00 (0.96–1.00)

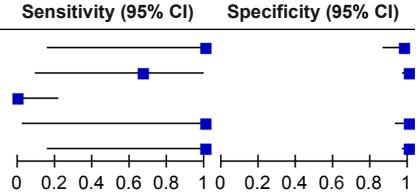


Figure 5 Summary plots of sensitivity and specificity of the ctDNA group.
Abbreviations: CtDNA, circulating tumor DNA; EGFR, epidermal growth factor receptor; TP, true positive; FP, false positive; TN, true negative; FN, false negative; KRAS, kirsten rat sarcoma viral oncogene homolog; BRAF, B-Raf proto-oncogene, serine/threonine kinase.

subtypes. We found that ctDNA had slightly higher accuracy for del19 and L858R. Different-accuracy detection methods may have an impact. More sensitive methods, including droplet digital PCR and circulating single-molecule amplification and resequencing technology, were used in the ctDNA group. For T790M, which is largely responsible for resistance to first-generation or irreversible tyrosine-kinase inhibitors,[65] CTCs and ctDNA showed similar diagnostic performance. This was consistent with other researchers.[14,66]

Various detection methods had great influence on the accuracy of CTCs and ctDNA; therefore, subgroup analyses based on different detection methods were necessary. In both the CTC and ctDNA groups, sequencing outperformed other detection methods, whether detecting *EGFR*, *KRAS*, or *BRAF*. To our knowledge, the low limit of detection and ability to determine lower mutant-allele frequency confers excellent capacity upon sequencing.[67,68] Although PCR is a cost-effective technology, it can analyze only limited genomic loci and has a high requirement for mutant-allele frequency.[58] Notably, digital PCR, as distinct from traditional PCR, is considered a very sensitive detection method,[69,70] and our study also confirmed this (data not shown).

Strengths and limitations

Although several meta-analyses were carried out, they focused on the diagnostic value of ctDNA or CTCs in only one type of gene mutation.[59,71,72] This is the first comprehensive study to analyze the diagnostic value of both ctDNA and CTCs for various gene mutations in lung cancer. We found that ctDNA might have better diagnostic performance than CTCs; however, clinical application of ctDNA for gene-mutation detection in lung cancer still needs to consider cost, operation process, and other factors. Meanwhile, subgroup analyses based on detailed *EGFR*-mutation subtypes, the detection methods of CTCs or ctDNA, and consistency of detection methods between liquid biopsy and tissue biopsy, were also carried out to explore potential influencing factors. However, other gene mutations in lung cancer, such as *PIK3CA* and *TP53*, were not included in our study, due to limited literature, which is the subjects of further investigations.

Conclusion

For lung cancer, ctDNA showed equivalent diagnostic ability as CTCs when detecting *EGFR* and its subtypes, and excellent performance for *KRAS*- and *ALK*-mutation detection. In general, ctDNA might be more suitable for clinical application of gene-mutation detection in lung cancer. Furthermore, our study also implies the significance of effective extraction kits and detection methods for improving the diagnostic capacity of ctDNA and CTCs.

Table 3 Results of subgroup analyses

	n	χ^2	*P*-value	Sensitivity (95% CI)	I^2	Specificity (95% CI)	I^2
CTC							
EGFR-mutation types							
del19 subgroup	3	1.00	<0.001	75.9% (0.654–0.845)	85.2%	98.0% (0.917–0.999)	66.4%
L858R subgroup	4	6.01	0.111	62.2% (0.501–0.732)	0	98.7% (0.929–1.000)	45.1%
T790M subgroup	3	2.02	0.365	63.3% (0.353–0.860)	60.8%	75.0% (0.522–0.908)	57.5%
Detection methods							
EGFR sequencing	2	0.15	0.695	85.1% (0.717–0.938)	0	50.0% (0.013–0.987)	0
EGFR PCR	3	1.85	0.396	72.1% (0.633–0.799)	56.1%	88.0% (0.757–0.955)	92.1%
KRAS PCR	2	0.84	0.358	30.8% (0.170–0.476)	50.8%	97.6% (0.874–0.999)	62.5%
Consistent or inconsistent							
EGFR consistent	4	2.83	0.418	69.8% (0.611–0.775)	41.0%	97.7% (0.877–0.999)	55.4%
KRAS consistent	2	0	0.963	42.0% (0.227–0.632)	76.6%	90.9% (0.836–0.956)	84.6%
KRAS inconsistent	2	1.67	0.197	42.0% (0.289–0.559)	40.1%	87.5% (0.764–0.946)	0
ctDNA							
EGFR-mutation types							
del19 subgroup	19	143.29	<0.001	79.0% (0.767–0.812)	91.5%	95.8% (0.948–0.967)	93.1%
L858R subgroup	20	58.54	<0.001	76.7% (0.731–0.800)	70.2%	97.2% (0.964–0.979)	70.9%
T790M subgroup	17	31.41	0.012	61.2% (0.570–0.654)	41.3%	92.7% (0.909–0.943)	86.7%
L861Q subgroup	2	0.18	0.670	100% (0.292–1.000)	0	99.4% (0.966–1.000)	50.5%
E20ins subgroup	3	1.53	0.467	83.3% (0.359–0.996)	24.1%	98.3% (0.964–0.994)	0.6%
G719X subgroup	2	0.09	0.765	100% (0.398–1.000)	0	97.4% (0.935–0.993)	71.5%
S768I subgroup	2	0.27	0.606	75.0% (0.061–1.000)	0	99.5% (0.979–1.000)	21.0%
Detection methods							
EGFR sequencing	10	24.13	0.004	75.6% (0.698–0.807)	59.0%	95.8% (0.93–0.977)	78.5%
EGFR PCR	15	45.27	<0.001	67.2% (0.643–0.701)	91.0%	97.2% (0.965–0.979)	83.3%
EGFR others	3	6.15	0.046	54.5% (0.469–0.621)	55.7%	89.7% (0.86–0.926)	83.9%
KRAS sequencing	6	7.37	0.195	66.9% (0.535–0.786)	0	97.8% (0.954–0.991)	87.9%
KRAS PCR	4	8.05	0.045	63.3% (0.477–0.772)	91.0%	84.5% (0.742–0.918)	41.5%
KRAS others	2	8.92	0.003	80.0% (0.631–0.916)	90.2%	91.2% (0.861–0.949)	38.8%
BRAF sequencing	4	0.62	0.892	87.5% (0.473–0.997)	0	99.7% (0.981–1.000)	27.1%
Consistent or inconsistent							
EGFR consistent	16	62.81	<0.001	69.3% (0.664–0.720)	88.5%	95.7% (0.945–0.967)	88.2%
EGFR inconsistent	10	23.25	0.006	74.6% (0.682–0.804)	65.4%	95.5% (0.933–0.972)	78.6%
KRAS consistent	7	15.14	0.019	62.8% (0.519–0.727)	82.5%	92.1% (0.886–0.949)	79.9%
KRAS inconsistent	4	8.21	0.042	81.5% (0.673–0.914)	73.6%	95.0% (0.908–0.976)	90.2%
BRAF consistent	2	10.06	0.002	13.2% (0.023–0.364)	84.7%	99.5% (0.957–1.000)	79.9%
BRAF inconsistent subgroup	2	0.62	0.431	100% (0.398–1.000)	0	99.3% (0.961–1.000)	61.7%

Abbreviations: CTC, circulating tumor cell; CtDNA, circulating tumor DNA.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Acknowledgments

We are grateful to Hong Xie for her advice on English language editing of this manuscript. This work was supported by the National Natural Science Foundation of China (grants 81472026 and 81672095).

Author contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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