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

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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,3 while CTCs are the cells released by primary tumors into peripheral blood.4 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,
cancer phenotype, tumor-mutation burden, and drug resistance. 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 to 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, and if necessary a third author would arbitrate.

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. 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 on 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.

Results

Study characteristics

A total of 47 of 4,283 studies were included in our analysis: nine in the CTC group and 42 in the ctDNA group (four 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...
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 ($\chi^2=90.39$, $P<0.001$), KRAS ($\chi^2=22.73$, $P=0.007$), and BRAF ($\chi^2=37.89$, $P<0.001$). 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 | TNM (I–IV) | Mutation | Tissue | Treatment | Detection assay | Detection assay |
|-------|---------|-----|-----|---------|------------|----------|--------|-----------|----------------|----------------|
| Breitenbuecher et al | Germany | 82  | NA  | NA       | 11/21      | KRAS     | Plasma | Peripheral blood | Sanger sequencing | Cold PCR-HRM     |
| Freidin et al* | UK      | 82  | 77  | NA       | NA         | NA       | Plasma | Plasma    | Sanger sequencing | Direct sequencing |
| Guibert et al | France  | 32  | 32  | NA       | 11/21      | KRAS     | Plasma | Plasma    | dd-Pcr         | Direct sequencing |
| He et al | China   | 13  | 12  | 96       | 40/24/96   | EGFR     | Plasma | Plasma    | dd-Pcr         | Direct sequencing |
| Marchetti et al | Italy   | 32  | 27  | 25       | 15/12      | EGFR     | Plasma | Plasma    | dd-Pcr         | Direct sequencing |
| Pumposse et al | USA     | 32  | 32  | NA       | NA         | EGFR, KRAS| Plasma | Plasma    | dd-Pcr         | Direct sequencing |
| Yeo et al | Singapore | 7  | 7   | NA       | 6/1        | NA       | Plasma | Plasma    | dd-Pcr         | Direct sequencing |
| Sundaram et al* | USA     | 40  | 40  | NA       | 26/14      | EGFR, T790M| Plasma | Plasma    | dd-Pcr         | Direct sequencing |
|全是   | NA       | 8   | 8   | NA       | 6/1        | NA       | Plasma | Plasma    | dd-Pcr         | Direct sequencing |
| Note: *In both the CTC and ctDNA group, 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. |
| Study                  | Country     | n   | ADC | Smoker | F/M   | TNM (I–IV) | Mutation | ctDNA Sample | Detection assay                  | Tissue           | Detection assay                        |
|------------------------|-------------|-----|-----|--------|-------|------------|----------|--------------|-----------------------------------|-----------------|----------------------------------------|
| Arriola et al<sup>13</sup> | Spain       | 154 | 112 | 127    | 39/15 | 0/0/18/36  | EGFR     | Plasma       | PNA clamp, fragment-length analysis | NA              | Therascreen                            |
| Chai et al<sup>20</sup>   | China       | 61  | 58  | 34/27  | 0/0/21/40 | EGFR       | Plasma   | cSMART      | FFPE                | ARMS            |                                        |
| Del et al<sup>21</sup>    | Italy       | 33  | NA  | 11     | 20/13  | 0/0/1/32   | EGFR<sup>TP53</sup>, KRAS | Plasma | dd-PCR | NA | dd-PCR, standard sequencing |
| Douillard et al<sup>22</sup> | 13 countries | 1,060 | NA  | NA  | NA | NA | EGFR | Plasma | ARMS | NA | ARMS |
| Freidin et al<sup>1, 8</sup> | UK          | 82  | 27  | NA     | NA    | NA | KRAS | Plasma | Cold PCR/HRM | FFPE | Therascreen, Cobas tissue test, cold PCR/HRM assay |
| Gautschi et al<sup>23</sup> | USA         | 180 | 79  | 125    | 55/125 | 15/11/64/91 | KRAS     | Plasma | RFLP-PCR | FFPE | RFLP-PCR |
| Gu et al<sup>24</sup>     | China       | 47  | 47  | 26/21  | 0/0/11/36 | EGFR | Plasma | d-PCR | FFPE | ARMS |
| Guo et al<sup>25</sup>    | China       | 20  | 20  | 8      | 7/13   | 0/0/5/15   | EGFR     | Plasma | Tag sequencing | FFPE | ARMS |
| Han et al<sup>26</sup>    | South Korea | 208 | 164 | 131    | 72/136 | 0/0/15/93  | EGFR, KRAS | Plasma | PNA clamp-assisted melting curve | FFPE | PNA clamp-assisted melting curve |
| He et al<sup>27</sup>     | China       | 134 | 101 | 63     | 49/85  | NA | EGFR | Plasma | Mutant-enriched PCR | NA | Direct sequencing |
| He et al<sup>28</sup>     | China       | 200 | 200 | 188    | 54/16  | 0/0/44/156 | EGFR | Plasma | dd-PCR | NA | dd-PCR |
| He et al<sup>13, 28</sup> | China       | 120 | 120 | 96     | 42/78  | 0/0/24/96  | EGFR     | Plasma | dd-PCR | NA | dd-PCR |
| Jenkins et al<sup>29</sup> | UK          | 551 | NA  | NA     | NA    | NA | EGFR<sup>del19</sup>, EGFR<sup>EGFR<sup>l858r</sup>, <sup>EGFR<sup>T790M</sup></sup> | Plasma | Cobas plasma test | NA | Cobas tissue test |
| Kim et al<sup>30</sup>    | South Korea | 102 | NA  | 31     | 62/40  | 0/0/0/102 | EGFR<sup>del19</sup>, EGFR<sup>l858r</sup> | Plasma | PNA clamp-assisted melting curve | FFPE | PNA clamp-assisted melting curve |
| Kobayashi et al<sup>31</sup> | Japan       | 15  | NA  | 7      | 10/5   | NA | EGFR<sup>T790M</sup> | Plasma, serum | Cobas plasma test | NA | PNA-LNA clamp, Cobas tissue test |
| Lee et al<sup>32</sup>    | South Korea | 57  | 57  | 16     | 39/18  | 0/0/0/57   | EGFR<sup>del19</sup>, EGFR<sup>l858r</sup> | Plasma | PNA clamp-assisted melting curve | NA | Sanger sequencing, PNA clamp |
| Ma et al<sup>33</sup>     | China       | 157 | 157 | 70     | 59/98  | 0/0/21/25  | EGFR     | Plasma | ARMS | FFPE | ARMS |
| Mao et al<sup>34</sup>    | China       | 40  | 25  | 21     | 13/27  | 0/0/13/27 | EGFR, KRAS, ALK, BRAF | Plasma | Targeted sequencing | FFPE | ARMS, FISH |
| Newman et al<sup>35</sup> | USA         | 66  | NA  | NA     | NA    | NA | EGFR | Plasma | iDES-enhanced CAPP sequencing | FFPE | iDES-enhanced CAPP sequencing |
| Pasquale et al<sup>36</sup> | Italy       | 96  | 84  | 64     | 36/60  | NA | EGFR | Plasma | Therascreen, PNA clamp | NA | Therascreen |
| Pecuchet et al<sup>37</sup> | France      | 109 | NA  | 73     | 60/49  | 0/0/1/97   | EGFR, KRAS, ALK, BRAF | Plasma | Ultradeep-targeted NGS | FFPE | Ultradeep-targeted NGS |
| Punnoose et al<sup>38, 39</sup> | USA, Australia | 41  | NA  | NA     | NA | NA | EGFR, KRAS, BRAF | Plasma | TaqMan | NA | TaqMan |
| Rachiglio et al<sup>40</sup> | Italy       | 44  | NA  | NA     | 21/23  | 0/0/1/43   | EGFR     | Plasma | Targeted sequencing | NA | Targeted sequencing |

(Continued)
| Study        | Country        | n    | ADC | Smoker | F/M | TNM (I–IV) | Mutation | ctDNA | Detection assay | Tissue                  | Treatment | Detection assay |
|-------------|----------------|------|-----|--------|-----|------------|----------|-------|----------------|-------------------------|-----------|-----------------|
| Reck et al59 | European nations, Japan | 1,288 | 952 | 1,035 | 421/867 | NA | NA | EGFR | Plasma | Others69 | NA | Others69 |
| Schwaederle et al40 | USA | 88 | 88 | 50 | 58/30 | NA | NA | NA | Plasma | Digital sequencing | NA | NGS |
| Sun et al41 | China | 55 | NA | NA | NA | EGR | Plasma | MST-PCR | FFP | Direct sequencing | NA | NA |
| Sundaresan et al7,8 | USA | 40 | NA | NA | 26/14 | 0/0/6/34 | EGR | Plasma | Cobas plasma test | NA | NA |
| Thompson et al43 | USA | 102 | 83 | 65 | 69/33 | 0/2/2/98 | EGR, KRAS, BRAF | Plasma | Paired-end sequencing | NA | NGS |
| Thress et al43 | USA | 38 | NA | NA | NA | EGR | Plasma | ARMS, dd-PCR, d-PCR, Cobas plasma test | FFP | Cobas tissue test |
| Uchida et al44 | Japan | 288 | 274 | NA | 119/169 | 64/46/26/146 | EGR | Plasma | PNA-LNA clamp | NA | PNA-LNA clamp |
| Veldore et al85 | India | 132 | 113 | 77 | 40/92 | NA | NA | EGR | Plasma | NGS | FFP | RT-PCR |
| Wang et al46 | China | 108 | 102 | 37 | 53/55 | 0/0/3/5 | EGR | Plasma | dd-PCR | FFP | ARMS |
| Wang et al47 | China | 224 | 216 | NA | NA | 47/49/60/68 | EGR | Plasma | qRT-PCR | FFP | qPCR |
| Wang et al48 | China | 287 | 249 | 64 | 104/83 | 0/0/31/156 | EGR | Plasma | DHPLC | FFP | DHPLC |
| Wang et al49 | China | 103 | 103 | 33 | 55/48 | 0/0/25/78 | EGR, KRAS, ALK, BRAF | Plasma | cSMART | FFP | ARMS |
| Wu et al50 | China | 45 | 42 | NA | 22/23 | 0/0/2/43 | EGR | Plasma | ARMS | NA | ARMS |
| Xu et al51 | China | 51 | 43 | 19 | 20/31 | 0/0/6/45 | EGR | Plasma | DHPLC, MEL, ARMS | NA | ARMS |
| Yang et al52 | China | 73 | 73 | 20 | 44/29 | NA | NA | EGR | Plasma | ddPCR | NA | ddPCR |
| Yao et al53 | China | 39 | 34 | 10 | 20/19 | 0/0/8/31 | EGR, KRAS | Plasma | Targeted sequencing | Fresh or FFP | Targeted sequencing |
| Yoshida et al54 | Japan | 31 | NA | NA | NA | NA | NA | EGR | Plasma | PNA-LNA clamp | NA | PNA-LNA clamp |
| Zheng et al55 | China | 117 | 108 | 29 | 71/46 | 0/0/5/91 | EGR | Plasma | dd-PCR | NA | ARMS |
| Zhou et al56 | China | 447 | 387 | 220 | 201/246 | 50/22/70/303 | EGR | 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; ***EGFRdel19, EGFRl858r, EGFRdel19, EGFRl858r, and EGFRl858r analyzed in this study.

Abbreviations: ctDNA, circulating tumor DNA; ADC, adenocarcinoma; NA, not available; SMART, circulating single-molecule amplification and resequencing technology; FFP, 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.
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 \textit{EGFR} when using the consistent method with tissue biopsy. Higher sensitivity was identified when using inconsistent methods to detect ctDNA for \textit{KRAS} (81.5%, 95% CI 0.673–0.914), as well as \textit{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 \textit{BRAF} analysis ($\chi^2=0.62$, $P=0.431$). 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 \textit{EGFR} and its detailed subtypes. However, ctDNA showed great strength for detecting \textit{KRAS} and \textit{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 \textit{EGFR}, which has been supported by some researchers.\textsuperscript{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.\textsuperscript{57} ctDNA quantity is prone to be only one genome per 5 mL plasma in the early stage of cancer.\textsuperscript{58} Therefore, the effective capture of ctDNA is still technically challenging, though Punnoose et al\textsuperscript{16} held the opposite opinion that ctDNA might outperform CTCs for \textit{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 \textit{KRAS}, ctDNA showed excellent diagnostic ability. Shen et al\textsuperscript{59} conducted a meta-analysis and came to a different conclusion than us. They included two studies that we excluded during literature screening.\textsuperscript{60,61} One did not describe clearly whether they analyzed the value of CTCs or ctDNA,\textsuperscript{60} while another extracted RNA from CTCs for detection.\textsuperscript{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 \textit{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 \textit{ALK}, in line with other investigators.\textsuperscript{62} For \textit{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.
ctDNA had low sensitivity, contrary to the results of the following two studies. Guibert et al analyzed only six samples, and did not regard tissue biopsy as the reference standard. Different sample size and reference standard were considered as the reasons for the discrepancy. Thierry et al 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
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, CTCs and ctDNA showed similar diagnostic performance. This was consistent with other researchers. 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. Although PCR is a cost-effective technology, it can analyze only limited genomic loci and has a high requirement for mutant-allele frequency. Notably, digital PCR, as distinct from traditional PCR, is considered a very sensitive detection method, 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. 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.

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### Table 1: Comparison of Diagnostic Performance Between CTCs and ctDNA for Various Gene Mutations

| Study          | Sensitivity (95% CI) | Specificity (95% CI) |
|----------------|----------------------|----------------------|
| **ctDNA KRAS group** |                      |                      |
| Del RM 2017    | 1.00 (0.29–1.00)     | 0.60 (0.15–0.95)     |
| Freidin MB 2015| 0.96 (0.78–1.00)     | 0.95 (0.86–0.99)     |
| Gautsch O 2007 | 1.00 (0.40–1.00)     | 0.60 (0.15–0.95)     |
| Han JY 2016    | 0.50 (0.21–0.79)     | 0.89 (0.83–0.94)     |
| Mao X 2017     | 1.00 (1.48–1.00)     | 0.97 (0.85–1.00)     |
| Pecuchet N 2016| 0.66 (0.46–0.82)     | 1.00 (0.95–1.00)     |
| Punnoose EA 2012| Not estimable        |                      |
| Thompson JC 2016| 0.67 (0.09–0.99)     | 1.00 (0.92–1.00)     |
| Wang Z 2017    | 0.59 (0.33–0.82)     | 1.00 (0.96–1.00)     |
| Yao Y 2017     | 0.75 (0.19–0.99)     | 1.00 (0.90–1.00)     |

| **ctDNA BRAF group** |                      |                      |
|----------------------|----------------------|----------------------|
| Mao X 2017           | 1.00 (0.16–1.00)     | 0.97 (0.86–1.00)     |
| Pecuchet N 2016      | 0.67 (0.09–0.99)     | 1.00 (0.97–1.00)     |
| Punnoose EA 2012     | 0.00 (0.00–0.22)     | Not estimable        |
| Thompson JC 2016     | 1.00 (0.03–1.00)     | 1.00 (0.93–1.00)     |
| Wang Z 2017          | 1.00 (0.16–1.00)     | 1.00 (0.96–1.00)     |

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**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.
Table 3 Results of subgroup analyses

|                         | n  | $\chi^2$ | P-value  | Sensitivity (95% CI) | $\phi$ | Specificity (95% CI) | $\phi$ |
|-------------------------|----|----------|----------|----------------------|-------|-----------------------|-------|
| **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)  | 0     | 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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