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ORIGINAL RESEARCH

Diagnostic accuracy of droplet digital PCR for detection of EGFR T790M mutation in circulating tumor DNA

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Objectives: Although different methods have been established to detect epidermal growth factor receptor (EGFR) T790M mutation in circulating tumor DNA (ctDNA), a wide range of diagnostic accuracy values were reported in previous studies. The aim of this meta-analysis was to provide pooled diagnostic accuracy measures for droplet digital PCR (ddPCR) in the diagnosis of EGFR T790M mutation based on ctDNA.

Materials and methods: A systematic review and meta-analysis were carried out based on resources from Pubmed, Web of Science, Embase and Cochrane Library up to October 11, 2017. Data were extracted to assess the pooled sensitivity, specificity, positive likelihood ratio, negative likelihood ratio (NLR), diagnostic OR (DOR), and areas under the summary receiver-operating characteristic curve (SROC).

Results: Eleven of 311 studies identified have met the including criteria. The sensitivity and specificity of ddPCR for the detection of T790M mutation in ctDNA ranged from 0.0% to 100.0% and 63.2% to 100.0%, respectively. For the pooled analysis, ddPCR had a performance of 70.1% (95% CI, 62.7%–76.7%) sensitivity, 86.9 % (95% CI, 80.6%–91.7%) specificity, 3.67 (95% CI, 2.33–5.79) PLR, 0.41 (95% CI, 0.32–0.55) NLR, and 10.83 (95% CI, 5.86–20.03) DOR, with the area under the SROC curve being 0.82.

Conclusion: The ddPCR harbored a good performance for detection of EGFR T790M mutation in ctDNA.

Keywords: T790M, droplet digital PCR, circulating tumor DNA, lung cancer

Introduction

As reported, epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) could drastically increase the median survival time from 1 year to 3–5 years in EGFR-mutant nonsmall cell lung cancer (NSCLC) patients.[1,2] However, despite of initial response on first-generation TKIs, patients finally develop resistance within 1–2 years and about 50%–65% of resistance is gained because of EGFR T790M mutation.[3–6] Therefore, rebiopsy has been recommended to explore whether there is the T790M resistant mutation when disease has progressed. But, due to the invasiveness of biopsy procedures, sometimes the inaccessibility of tumor tissues, heterogeneity of the tumor or patients' unwillingness, it has always become difficult and problematic to carry out rebiopsy in routine clinical work.[7,8] Alternatively, circulating tumor DNA (ctDNA), which can be collected and extracted from peripheral blood, has been identified as clinically significant biomarker to help reveal the EGFR mutation status.[9–11]

Testing platforms of EGFR T790M mutation in ctDNA are numerous, including the real-time PCR [Cobas, Amplification Refractory Mutation System (ARMS)],

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digital platforms [droplet digital PCR (ddPCR), Beads, Emulsions, Amplification and Magnetic (BEAMing)], and next generation sequencing technologies, respectively.[10,12,13] Regarding to PCR-based techniques, data revealed that ddPCR had superior sensitivity compared with Cobas and ARMS.[14–18] Thress et al conducted a cross-platform comparison of these leading technologies and found the sensitivity of Cobas, ARMS, and ddPCR were 41%, 29%, and 71% in EGFR T790M mutation detection, respectively.[18] Besides, using Cobas tissue test as the reference, Zhang et al found the positive percent agreement (PPA) of Cobas plasmas, Super-ARMS, and ddPCR were 42%, 49%, and 56% respectively. The sensitivity for plasma T790M detection slightly increased with ddPCR compared with Super-ARMS and Cobas plasma test.[15]

The ddPCR technology harbors detection limit of 0.01%–0.04% for EGFR mutation.[19] Unlike EGFR sensitizing mutation, there is only a small fraction of mutant T790M alleles among plenty of wild-type alleles in clinical samples.[20] Therefore, the design of ddPCR assures the partitioning of competing backgrounds of wild-type alleles by thousands of even millions of droplets, leading to decrease in their PCR inhibitory effects and improvements in detection sensitivity.[21] However, the reported sensitivity and specificity of ddPCR for detection of EGFR T790M mutation in ctDNA varied. For example, Suzawa et al reported a sensitivity of 42.8%, whereas a high sensitivity of 100% was indicted by Yu et al.[22,23] Similar discordance in specificity was also observed.[24,25] Hence, the aim of the current study is to search related publications and then summarize data to provide pooled diagnostic accuracy values of ddPCR for detection of EGFR T790M mutation in ctDNA.

Material and methods

Literature search strategy

We comprehensively searched various online sources including PubMed, Web of Science, Embase and Cochrane Library up to October 11, 2017, using key words "digital PCR" and "T790M". The language is limited to English and Chinese. After duplicates were removed, all searched results underwent title and abstract review and potentially eligible studies were reviewed through full texts. This analysis was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[26]

Inclusion and exclusion criteria

Searched studies were assessed by two reviewers independently and disagreements were solved by discussion with the third person until consensus was reached. Those that satisfied the following inclusion criteria were selected for final analysis: 1) enrolled the NSCLC patients treated with EGFR-TKI therapy; 2) studied diagnostic accuracy of ddPCR for detection of EGFR T790M mutation based on ctDNA; 3) taking biopsy samples as reference method; and 4) reported necessary data for calculating pooled index. Studies were excluded if they were 1) not published in full-text, such as meeting abstracts; 2) not original article, like case report or review; 3) unrelated to research topics; and 4) duplicate publications.

Data extraction and quality assessment

Eligible studies were checked again by the two reviewers and a consensus was reached prior to further process. All necessary data for calculating pooled index were extracted and two-by-two tables were reconstructed in each study for the true-positive (TP), false-negative (FN), true-negative (TN), and false-positive (FP) values. Other relevant information, including the name of the first author, year of publication, country of origin, sample size, basic characteristics of studied population, tumor histology, clinical stage, source of biopsy samples and related detection methods for EGFR T790M mutation, and the manufacturer of ddPCR platform used in the study, were extracted from the included studies.

The quality of all included studies were assessed by the revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 tool from four aspects, namely the methods of patients selection, the conduction and interpretation of index tests, reference standard, and flow and timing regarding to the index tests and reference standard. After being assessed, each study quality was categorized as being low, high, or unclear regarding to risk of bias, and applicability concerns, respectively. The same two reviewers carried out the assessment and disagreement was solved by consensus.[27]

Statistical analysis

Several pooled values were calculated in this meta-analysis, including sensitivity, specificity, the positive likelihood ratio (PLR), the negative likelihood ratio (NLR), and diagnostic OR (DOR). PLR equals to sensitivity/(1−specitity), and larger values mean high possibility of true positivity when the index test result is positive. Similarly, NLR is calculated by (1−sensitivity)/specificity, and the smaller the value, the higher the true negativity possibility of index test results. The DOR of a test is the ratio of the odds of positivity in disease relative to the odds of positivity in the nondiseased. Its value ranges from 0 to infinity, with higher values indicating better discriminatory test performance.[28]

Besides, PPA and negative percent agreement (NPA) were pooled, and summary receiver-operating characteristic curve (SROC) was drawn.

The Cochrane's Q and the I^2 statistic were used to analyze heterogeneity. For the Q statistic, P-value <0.10 was considered statistically significant for heterogeneity.

As for the I^2 statistic, which is the percentage of the observed variability between studies due to heterogeneity rather than chance, its value >50% represented that heterogeneity existed in pooled values. In the current meta-analysis heterogeneity existed, and all statistics were calculated and combined using the random effects model with 95% CI. To shed light on heterogeneity among studies, subgroup analyses were conducted.

In addition, the publication bias was assessed by Deek's funnel plot asymmetry test.[29] The professional statistical software Meta-Disc 1.4 (Ramón y Cajal Hospital in Madrid, Spain) program was used for pooled analysis. Publication bias was performed using STATA 12.0 (STATA Corporation, College Station, TX, USA).

Results

Study selection

According to PRISMA guidelines, the process of study selection was shown in Figure 1. Total 311 literatures were initially identified through online searching, and there were no additional records being found through other source searching. After 126 duplicates were removed, 185 studies were screened by title and abstract for potential eligibility of the meta-analysis. Then, 124 of them were excluded because of the publication format being meeting abstracts (n=112), reviews (n=11), and case (n=1). Finally, 61 studies were identified and reviewed through the whole text, with 11 being satisfied to our inclusion criteria. The other 50 studies were excluded because of insufficient data for meta-analysis (n=6) or unrelated to research topics (n=8).

Characteristics of included studies

The characteristics of the included 11 studies in this meta-analysis were summarized in Table 1. As a whole, there were 872 advanced NSCLC patients involved in these studies,

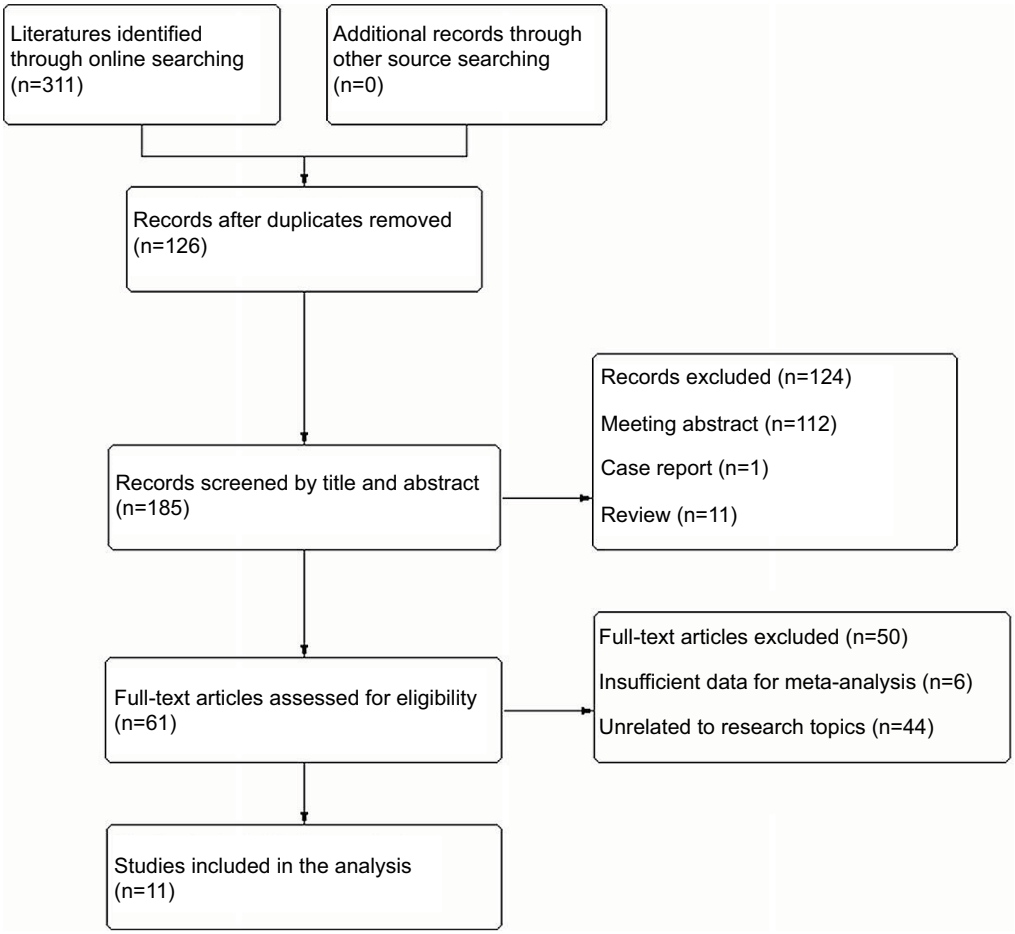

Figure 1 Flow chart of the included studies in this meta-analysis.

Table 1 Baseline characteristics of included studies

Study	Country	Ethnicity	Sample size	Age (years)	Male (%)	Smoker (%)	Histology	Clinical stage	Time for T790M detection
Ishii et al[36]	Japan	Japanese	18	50–81	11.1	5.6	Adenocarcinoma	Recurrent	Resistance developed
Thress et al[18]	UK	Hesperian	38	NA	NA	NA	NSCLC	M0/M1a/M1b	Resistance developed
Wei et al[42]	China	Chinese	50[a]	45–68	62.0	76.0	Adenocarcinoma	IV	No resistance
Wang et al[17]	China	Chinese	108	NA	50.9	34.3	NSCLC	IIIB /IV	Resistance developed
Takahama et al[43]	Japan	Japanese	260	36–90	30.0	27.3	NSCLC	IIIB/IV/inoperable/ recurrent	Resistance developed
Seki et al[44]	Japan	Japanese	35	47–74	40.0	31.4	Adenocarcinoma	IV/recurrent	Resistance developed
Zheng et al[24]	China	Chinese	117	NA	39.3	24.8	NSCLC	IIIB/IV/recurrent	Resistance developed
Sacher et al[25]	USA	Hesperian	180	NA	38.0	NA	NSCLC	IIIB/IV/recurrent	Resistance developed
Yu et al[22]	China	Chinese	22	35–74	45.5	NA	NSCLC	IIIB/IV	Resistance developed/ no resistance
Suzawa et al[23]	Japan	Japanese	24[a]	39–84	29.2	37.5	NSCLC	NA	NA
Xu et al[45]	China	Chinese	20	37–76	50	30	NSCLC	I–IV	NA

Note: [a]Healthy volunteers in original study were not included here.
Abbreviations: NSCLC, nonsmall cell lung cancer; NA, not available.

most of whom were Asians. Among all the patients, EGFR-TKIs were used as a targeted therapy for disease control. To evaluate ddPCR performance of EGFR T790M mutation detection in ctDNA, 298 patients (337 blood samples) who have underwent both T790M mutation test in tumor tissue or malignant fluid and plasma ctDNA, were identified. In 7 studies (63.6%), all studied patients have already developed EGFR-TKI resistance when ddPCR tests were carried out.

Table 2 described the included sample size for 2×2 table, the samples were used for reference test and index text, as well as the time interval between tissue and plasma sampling. Most tissues were taken from primary sites. Almost all of the time interval were not specific, except for one study that told a median time of 12.5 months.

According to tissue or malignant fluid detection results, there were 165 T790M-mutation-positive patients (177 blood samples) and 133 T790M-mutation-negative patients (156 blood samples). The TP/ FP/ FN/ TN values, sensitivity, and specificity of ddPCR test in each study were summarized in Table 3.

Overall accuracy of the ddPCR test

For all studies, the sensitivity and specificity of ddPCR for the detection of T790M mutation in ctDNA ranged from 0.0% to 100.0% and 63.2% to 100.0%, respectively. The concordance between plasma and tissue test was 81.2%, with the PPA being 71.2% and NPA being 90.0%. When it came to the pooled analysis, ddPCR test had a performance of 70.1% (95% CI, 62.7%–76.7%) sensitivity, 86.9 % (95% CI, 80.6% –91.7%) specificity, 3.67 (95% CI, 2.33–5.79) PLR, 0.41 (95% CI, 0.32–0.55) NLR, and 10.83 (95% CI, 5.86–20.03) DOR in diagnosis of EGFR T790M mutation based on ctDNA in NSCLC patients. In addition, the area under the SROC curves (AUC) was 0.82 (Figure 2). Because significant heterogeneity exists regarding to pooled specificity (P=0.03, I^2=48.9%), the random-effect model was used for the whole analyses.

Subgroup analysis

As specificity heterogeneity existed, we performed subgroup analyses regarding to ethnicity, tumor histology, and index test (Table 4). The index test was the most possible cause of specificity heterogeneity, with I^2 changing from 48.9% to the maximum 8.1%. The picoliter-ddPCR and designed ddPCR assay presented higher specificity, 94.7% (95% CI, 74.0 %–99.9%) and 97.9% (95% CI, 88.7%–99.9%), respectively. In ethnicity and tumor histology subgroup analyses, specificity heterogeneity was still present.

Quality assessment

Based on QUADAS-2 tool, the details of quality assessment were listed in Table S1. As for risk of bias, the patient selection assessments were shown in column 2, index test assessments in column 3, reference standard assessments in column 4 and the flow and timing assessments in column 5. Except for one study, almost all patients were randomly enrolled without inappropriate exclusions. The results of ddPCR test were mostly interpreted independently, and reference methods were likely to correctly classify T790M mutation condition. However, regarding to the flow and timing, two studies showed the potential risk due to different detection strategies being used for T790M mutation in biopsy samples,

Table 2 The included sample for 2×2 table analysis

Study	Sample size	Included size for 2×2 table	Sample for reference test	Reference test	Sample for index test	Index test	Time interval[c]
Ishii et al[36]	18	18	Primary/ MF	ddPCR	Plasma ctDNA	ddPCR (Bio-Rad)	Not specific both following progression
Thress et al[18]	38	23	Primary	Cobas test	Plasma ctDNA	ddPCR (Bio-Rad)	Not specific both following progression
Wei et al[42]	50	50	Primary	NA	Plasma ctDNA	ddPCR (Bio-Rad)	Not specific both at baseline
Wang et al[17]	108	16	Primary	ARMS	Plasma ctDNA	ddPCR (AmoyDx)	Not specific both following progression
Takahama et al[43]	260	41	Primary/ MF	ARMS/Cobas test	Plasma ctDNA	ddPCR (Bio-Rad)	Not specific both following progression
Seki et al[44]	35	10	Primary	ARMS	Plasma ctDNA	Picoliter-ddPCR (RainDance)	Not specific both following progression
Zheng et al[24]	117	25	Primary/ MF	ARMS/ddPCR	Plasma ctDNA	PNA-TaqMan-ddPCR (Bio-Rad)	Not specific both following progression
Sacher et al[25]	180	54	Primary/ metastasis/MF	PCR/NGS	Plasma ctDNA	ddPCR (Bio-Rad)	Not specific
Yu et al[22]	22	20	Primary/ metastasis	ARMS	Plasma ctDNA	Picoliter-ddPCR (RainDance)	Not specific
Suzawa et al[23]	24	59[a]	Primary	PNA-LNA PCR	Plasma ctDNA	PNA-LNA-ddPCR (Bio-Rad)	Median 12.5 months both following progression
Xu et al[45]	20	21[b]	Primary/ metastasis/MF	ARMS/NGS	Plasma ctDNA	ddPCR (Bio-Rad)	Not specific

Notes: [a]59 plasma samples were withdrawn from 24 patients for analysis. [b]Total 20 patients, 17 were enrolled to compare EGFR mutation profiles between tissue and plasma, but 21 cases were analyzed. [c]Time interval between tissue and blood sampling used for T790M analysis.
Abbreviations: MF, malignant fluid; ddPCR, droplet digital polymerase chain reaction; ARMS, Amplification Refractory Mutation System; NGS, Next-Generation Sequencing; ctDNA, circulating tumor DNA; PNA, peptide nucleic acids; LNA, locked nucleic acids; NA, not available.

Table 3 The ddPCR performance of each included study

Study	Included size	Reference method		ddPCR for ctDNA				Sensitivity (%)	Specificity (%)
		T790M +	T790M –	Ref +/ ddPCR +	Ref–/ ddPCR +	Ref +/ ddPCR –	Ref–/ ddPCR –		
Ishii et al[36]	18	11	7	9	1	2	6	81.8%	85.7%
Thress et al[18]	23	17	6	12	1	5	5	70.6%	83.3%
Wei et al[42]	50	25	25	19	3	6	22	76.0%	88.0%
Wang et al[17]	16	9	7	6	2	3	5	66.7%	71.4%
Takahama et al[43]	41	31	10	20	3	11	7	64.5%	70.0%
Seki et al[44]	10	7	3	5	0	2	3	71.4%	100.0%
Zheng et al[24]	25	16	9	13	0	3	9	81.3%	100.0%
Sacher et a [25]	54	35	19	27	7	8	12	77.1%	63.2%
Yu et al[22]	20	4	16	4	1	0	15	100.0%	93.8%
Suzawa et al[23]	59	21	38	9	1	12	37	42.8%	97.3%
Xu et al[45]	21	1	20	0	2	1	18	0.0%	90.0%

Abbreviations: ddPCR, droplet digital polymerase chain reaction; ctDNA, circulation tumor DNA; Ref, reference.

and in addition one study showed long-time interval between tissue and plasma sampling. The applicability was high for most studies.

Publication bias

Deek's funnel plot asymmetry test (P=0.84) did not show argument for publication bias (Figure 3).

Discussion

Since the best detection method for EGFR T790M-resistant mutation is still in debate, a meta-analysis was conducted to evaluate the diagnostic performance of ddPCR. When testing in plasma ctDNA with tissue or malignant fluid test as reference method, ddPCR harbored a high sensitivity of 70.1%, a specificity of 86.9 %, and a DOR of 10.83. In

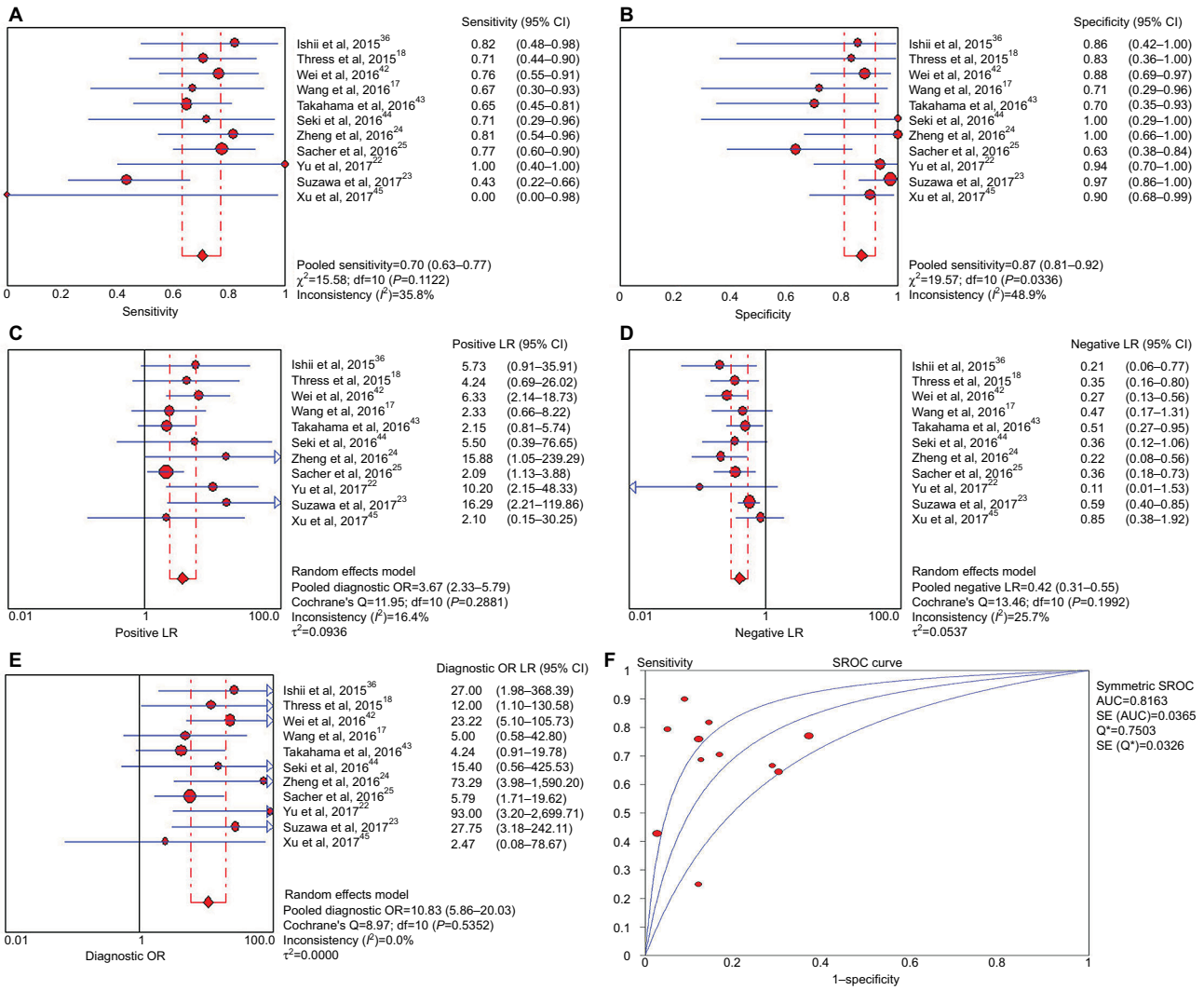

Figure 2 Meta-analysis of (**A**) sensitivity, (**B**) specificity, (**C**) positive likelihood ratio, (**D**) negative likelihood ratio, (**E**) diagnostic odds ratio, (**F**) and SROC curve for droplet digital PCR for diagnosing epidermal growth factor receptor T790M mutation in circulating tumor DNA.
Abbreviations: LR, likelihood ratio; SROC, summary receiver-operating characteristic; AUC, area under the ROC curve; PCR, polymerase chain reaction..

stratified analysis, the picoliter-ddPCR performed well with the sensitivity, specificity, and DOR being 81.8%, 94.7%, and 37.35% respectively.

Compared to Cobas and ARMS, the pooled results confirmed that ddPCR performed a high sensitivity but relatively low specificity. Two previous meta-analyses, which studied the diagnostic value of ctDNA for the detection of EGFR mutation status in NSCLC, found that the sensitivity and specificity of ARMS were 54.9% (0.41.9%–0.67.2%) and 52.5% (35.7%–0.68.8%), 97.5% (93.7%–99.1%), and 94.7% (86.0%–98.1%), respectively.[30,31] Therefore, ARMS has exhibited a low sensitivity but a definitely higher specificity. As for Cobas, in the ongoing ASTRIS study, the detection rate of T790M mutation in ctDNA was much lower than that in tissues, 27.5%–62.8%.[32] That could be related to its lower sensitivity. Besides, Jenkins et al evaluated the performance of Cobas plasma test from patients screened for the AURA extension and AURA2 Phase II studies, finding the concordance between the Cobas plasma and the tissue tests for detection of T790M mutation was only 65.4%.[33] However, the concordance of ddPCR was 81.2% in the current study.

For the T790M mutation detection in ctDNA, digital PCR platforms generally outperform the nondigital platforms.[16,18] The familiar digital platforms include ddPCR, BEAMing, and Quant Studio 3D dPCR (QS3D dPCR), with a detection limit of around 0.01%–0.04%, 0.01%, and 0.1%, respectively.[19] For the same samples, Thress et al reported the sensitivity and specificity for T790M mutation detection were 71% and 83%, respectively, with the ddPCR, and 71% and 67%, respectively, with BEAMing.[18] Besides, the study by Karlovich et al also revealed that the sensitivity and specificity of BEAMing was 73.3% and 50.0%.[34] Hence, the ddPCR

Table 4 Subgroup analysis of ddPCR performance

Subgroup	Number of studies	Sensitivity % (95% CI)	Specificity % (95% CI)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)	AUC	I^2 [b]	P-value[c]
Ethnicity									
Hesperian	2	75.0 (61.1–86.0)	68.0 (46.5–85.1)	2.25 (1.26–4.03)	0.36 (0.21–0.61)	6.73 (2.27–19.96)	NA	0.0%	0.34
Japanese	4	61.4 (49.0–72.8)	91.4 (81.0–97.1)	4.27 (1.66–10.95)	0.52 (0.38–0.70)	10.48 (3.59–30.56)	0.78	56.3%	0.08*
Chinese	5	76.4 (63.0–86.8)	89.6 (80.6–95.4)	5.12 (2.60–10.09)	0.37 (0.19–0.70)	17.13 (5.51–53.24)	0.90	3.9%	0.39
Tumor histology									
Adenocarcinoma	3	76.7 (61.4–88.2)	88.6 (73.3–96.8)	6.10 (2.53–14.69)	0.28 (0.16–0.48)	22.70 (6.71–76.86)	0.92	0.0%	0.67
NSCLC	8	67.9 (59.3–75.7)	86.4 (79.1–91.9)	3.35 (1.90–5.91)	0.47 (0.34–0.63)	8.46 (4.13–17.34)	0.79	62.5%	0.01*
Index test									
Picoliter-ddPCR (RainDance)	2	81.8 (48.2–97.7)	94.7 (74.0–99.9)	8.70 (2.28–33.19)	0.29 (0.09–0.95)	37.35 (3.51–397.16)	NA	0.0%	0.55
ddPCR (Bio-Rad)	7	72.1 (63.5–79.6)	79.8 (70.2–87.4)	2.72 (1.80–4.11)	0.41 (0.30–0.57)	8.11 (4.09–16.03)	0.80	8.1 %	0.37
Others[a]	2	59.5 (42.1–75.2)	97.9 (88.7–99.9)	16.14 (3.23–80.57)	0.39 (0.15–1.05)	38.28 (6.51–225.05)	NA	0.0 %	0.51
Pool	11	70.1 (62.7–76.7)	86.9 (80.6–91.7)	3.67 (2.33–5.79)	0.41 (0.32–0.55)	10.83 (5.86–20.03)	0.82	48.9%	0.03*

Notes: [a]Others including technologies of PNA-TaqMan-ddPCR (Bio-Rad) and PNA-LNA-ddPCR (Bio-Rad). [b]The I^2 for specificity in each subgroup. [c]Heterogeneity test for specificity in each subgroup. *Statistically significant for specificity heterogeneity

Abbreviations: ddPCR, droplet digital polymerase chain reaction; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnosis odd ratio; AUC, area under the ROC curve; NA, not available.

and BEAMing performed similar sensitivity but BEAMing showed a lower specificity. As for QS3D dPCR, Gu et al studied 39 samples, finding its sensitivity, specificity, and concordance for T790M mutation detection in ctDNA was 100.0%, 97.3%, and 97.44%, respectively.[35]

The overall concordance rate of T790M testing between the paired tumor tissues and plasma was 81.2% in the pooled analysis, which was similar to the previous studies.[24,36] The PPA and NPA were 71.2% and 90.0%, respectively. Therefore, ddPCR can be a practical and alternative method for T790M mutation detection in clinics. Due to its noninvasiveness and short turnaround time, serial monitoring of EGFR mutation status using ddPCR has been carried out in recent clinics. Related studies found the detection of T790M mutation in plasma ctDNA was around 2–12 months earlier than the clinical manifestation of disease progression.[12,24,37] For example, Zheng et al found almost half of the T790M ctDNA-positive patients were identified at a median time of 2.2 months prior to clinically progressive disease.[24] Oxnard et al revealed that the detection of resistance mutations was up to 3.5 months before radiographic progression.[37] When resistance developed, the AURA3 phase 3 trial confirmed that osimertinib is superior to standard platinum–pemetrexed chemotherapy regarding to median progression-free survival (10.1 months vs. 4.4 months), objective response rate (71% vs. 31%) as well as the quality of life.[38] Nevertheless, more reliable data need to be demonstrated in future trials.

On the other hand, Soria et al recently identified that the median progression-free survival was significantly longer with osimertinib as first line than with standard EGFR-TKIs (18.9 months vs. 10.2 months).[39] Therefore, there is the prediction that the detection of T790M mutation will be less important.[40] However, US Food and Drug Administration (FDA) has not approved the indications of osimertinib using as first-line treatment yet, and the data from Soria's study have not shown overall survival benefits. Thus, ddPCR can be of great use clinically in a long time. Except for T790M, ddPCR is also being used to detect other EGFR aberrations in ctDNA from lung cancer patients. When compared with tissue samples, the sensitivity, and specificity are 62.5%–90.9% and 88.9%–100% for Exon 19 del, 74.0%–88.9% and 96.6%–100% for L858R, respectively.[18,22,25,41]

In spite of the thoroughly search of related articles as well as careful selection and evaluation, there were several limitations of our meta-analysis. First, included cases for analysis were limited and most publications were retrospective studies. Second, different biopsy materials and detection strategies were used regarding to the reference method. And, there could

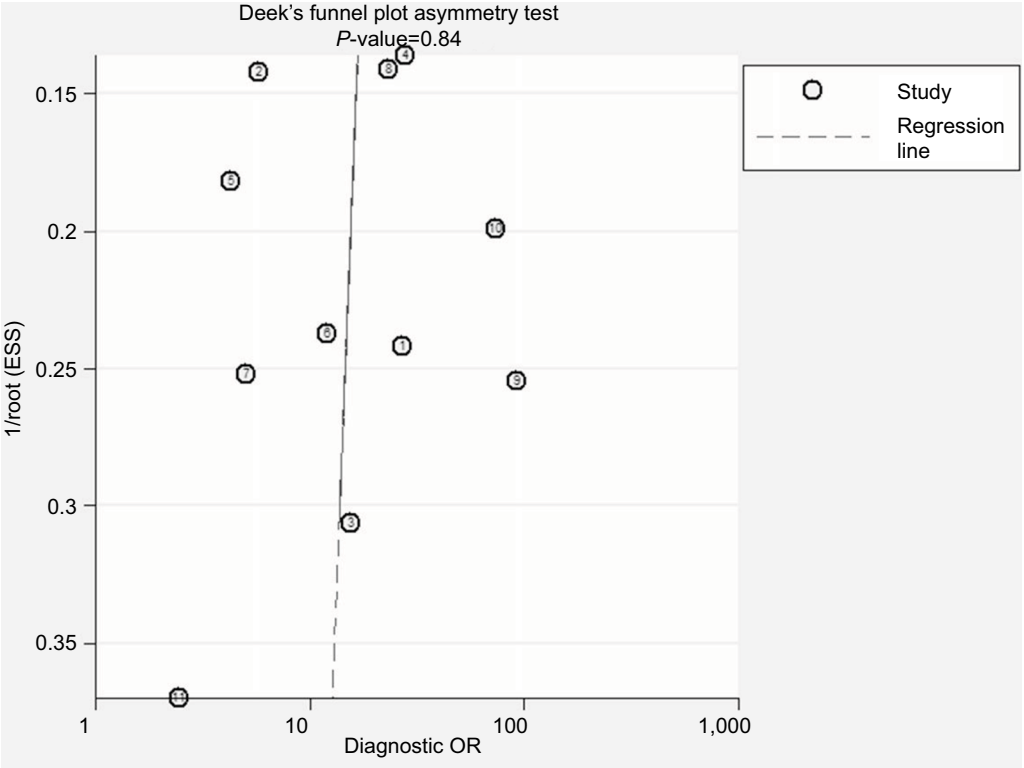

Figure 3 Assessment of publication bias by Deek's funnel plot asymmetry test.
Abbreviation: ESS, effective sample size.

be FN even for the mutation detection in biopsy samples due to tumor heterogeneity or incorrect operation.[42] Third, the time between index tests and reference tests were not provided in most studies. In one study, the median time interval between plasma collection and rebiopsy test was 12.5 months.[23] That meant the discrepancy between the two results could be caused by real genomic change of tumor instead of technological capability. Last but not the least, specificity heterogeneity exited due to index tests. To sum up, the limitations above should be considered when interpreting our results.

In conclusion, we found ddPCR harbored a performance of 70.1% sensitivity and 86.9 % specificity for T790M mutation detection in ctDNA. Compared to ARMS and Cobas plasma test, it showed a higher sensitivity. Besides, though the ddPCR and BEAMing performed similar sensitivity, ddPCR showed a higher specificity. In addition to the utilization of third-generation TKIs as first-line treatment for EGFR mutant lung cancers, ddPCR could still play an important role in detecting T790M resistant mutation.

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Author contributions

PT and WL were involved in the study design, whereas RZ, BC, and XT carried out data collection, data analysis, and drafting of the manuscript. YW, CW, and JJ participated in the interpretation of study results and critical revision of the manuscript. All authors contributed toward data analysis, drafting and critically revising the paper 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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Supplementary materials

Table S1 Quality assessment of included studies according to QUADAS-2 tool

Study	Risk of bias				Applicability concerns		
	Patient selection	Indexed test	Reference methods	Flow and timing	Patient selection	Indexed test	Reference methods
Ishii et al[1]	☺	☺	☺	☺	☺	☺	☺
Thress et al[2]	☺	☺	☺	☺	☺	☺	☺
Wei et al[3]	□	☺	?	☺	□	☺	?
Wang et al[4]	☺	☺	☺	☺	☺	☺	☺
Takahama et al[5]	☺	☺	☺	□	☺	☺	☺
Seki et al[6]	☺	☺	☺	☺	☺	☺	☺
Zheng et al[7]	☺	☺	☺	□	☺	☺	☺
Sacher et al[8]	☺	☺	?	☺	☺	☺	?
Yu et al[9]	☺	☺	☺	☺	☺	☺	☺
Suzawa et al[10]	☺	☺	☺	☺	☺	☺	☺
Xu et al[11]	☺	☺	☺	□	☺	☺	☺

Notes: ☺=low risk; □=high risk; ?=unclear risk.
Abbreviation: QUADAS-2, Quality Assessment of Diagnostic Accuracy Studies-2.

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