Systematic Review and Meta-Analysis

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Reliability of digital PCR in detecting KRAS mutation in colorectal cancer using plasma sample

A systematic review and meta-analysis

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Abstract

Background: Test on the KRAS somatic mutation status is necessary before cetuximab and panitumumab treatments are given to colorectal cancer patients. Metastatic colorectal cancer patients sometimes lack tumor tissue samples, and the testing of KRAS mutation in plasma samples requires highly sensitive methods.

Objectives: The aim of this study was to evaluate the accuracy of digital PCR in detecting KRAS mutation in plasma samples of colorectal cancer patients.

Data sources: Literature research was conducted in Pubmed, Embase, and Cochrane Library.

Study eligibility criteria, participants, and interventions: Database searching found 188 relevant studies. After removing duplicates, eligible studies were selected from 151 publications using the following exclusion criteria:

1. did not discuss colorectal cancer;
2. did not use digital PCR method;
3. lacked plasma sample or tissue sample;
4. did not measure KRAS status;
5. un-interpretable data.

Study appraisal and synthesis methods: Data were extracted from the eligible studies by 2 independent researchers. Pooled accuracy parameters were calculated from those extracted data using Meta-DiSc and STATA software.

Results: Twelve eligible studies were selected for the systematic review and meta-analysis. After calculation, the pooled sensitivity and specificity were 0.83 (95% CI: 0.79–0.86) and 0.91 (95%CI: 0.88–0.93), respectively. Pooled positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio were 7.30 (95%CI: 4.78–11.17), 0.22 (95%CI: 0.15–0.32), and 41.00 (95%CI: 21.07–79.78), respectively. Area under curve of the summarized ROC curve was 0.9322.

Limitations: Although no significant bias was identified, number of included studies was still quite small, especially in subgroup analysis.

Conclusions and implication of key findings: Digital PCR showed high accuracy and could be a reliable detection method for KRAS mutation in plasma samples. Large-cohort prospective study is required to further confirm the usefulness of digital PCR in KRAS mutation detection.

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Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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Abbreviations: ARMS = Scorpion amplified refractory mutation system, AUC = area under curve, CRC = colorectal cancer, ctDNA = circulating tumor DNA, DOR = diagnostic odds ratio, dPCR (QS3D) = QuantStuidio 3D Digital PCR System, ddPCR = droplet-based digital PCR, EGFR = epithelial growth factor receptor, mCRC = metastatic colorectal cancer, NGS = next generation sequencing, NLR = negative likelihood ratio, PLR = positive likelihood ratio, qPCR = quantitative PCR, SROC = summarized receiver operating curve.

Keywords: digital PCR, colorectal cancer, KRAS mutation

1. Introduction

Colorectal cancer (CRC) is a severe health threat around the world.[1] Surgery remains the mainstay of treatment for non-metastatic CRC, but for metastatic CRC (mCRC) when surgical resection is not possible, chemotherapy and targeted therapy are mostly used.[2] One example of the targeted therapies for CRC is anti-epithelial growth factor receptor (EGFR) therapy, e.g., cetuximab and panitumumab, which were approved for treatment of mCRC nearly 15 years ago.[3] However, similar to other targeted therapies, drug resistance is a severe problem for cetuximab and panitumumab.[3]

KRAS gene is a member of the RAS genes which are involved in signaling pathways regulating cell survival, proliferation, or metastasis.[4] Somatic mutations of *KRAS* gene are found in many types of cancer, especially in pancreatic cancer, lung cancer, and CRC.[5] As a downstream signaling factor of EGFR, mutations in *KRAS* gene can cause resistance to anti-EGFR therapy, leading to treatment failure.[6,7] It is therefore necessary to test *KRAS* mutation status before the anti-EGFR therapy is given.

Cancer-related mutations of *KRAS* gene are usually single-base mutations, which are predominantly at G12 and G13 in CRC.[5] This makes digital PCR a good choice for KRAS mutation detection. mCRC or recurrent CRC sometimes lacks tissue sample when surgical resection or biopsy is not possible. Circulating tumor DNA (ctDNA) in plasma could be used to detect mutation status, which requires highly sensitive detection method since the amount of ctDNA is usually very low.[8]

Digital PCR is known for its ability to detect very small amount of mutated DNA in the sample, e.g., ctDNA in plasma.[9] The reliability of digital PCR in detecting KRAS mutation status using ctDNA is, however, not fully determined yet. A few studies used digital PCR methods to detect KRAS mutation in plasma and compared the results with tissue samples.[10–21] The aim of this systematic review and meta-analysis was to investigate the reliability of digital PCR in KRAS mutation detection using plasma samples, using tissue samples as reference.

2. Materials and Methods

2.1. Literature searching and selection of publication

Literature research was performed independently by P.YE and PL.CAI in April 2019. Databases including Pubmed, Embase, and Cochrane Library were searched using "KRAS", "digital PCR", "BEAMing", and "colorectal". Alterations in spelling were also included in the search. Within the searching results, title and abstract of the publications were reviewed, and duplicated and irrelevant papers were excluded by the following criteria:

1. did not discuss CRC;
2. did not use digital PCR method;
3. lacked plasma sample or tissue sample;
4. did not measure KRAS status;
5. un-interpretable data.

Full text of the remaining publications were downloaded and carefully examined. KRAS testing results from paired plasma and tissue samples (mutated or wildtype) were extracted and numbers of sample showing true positive (TP), false positive (FP), false negative (FN), or true negative (TG) were summarized in a 2×2 table. If multiple digital PCR methods were used in the same publication, the following criteria were used to select a method for data extraction:

1. method used for a larger number of samples;
2. method which had similar detection region with the method used for tissue.

Any disagreement in the results between 2 investigators (P.YE and PL.CAI) was solved by careful reviewing of the searching results and discussion. Ethical approval was not necessary since the data obtained and analyzed in this study were extracted from existing literature and not on individual patients.

2.2. Statistical analysis

Pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR) were calculated using Meta-DiSc 1.4.[22] Summary receiver operating characteristic (SROC) curve and area under curve (AUC) were also generated using Meta-DiSc to evaluate diagnostic accuracy. Inter-study heterogeneity was evaluated using Q statistic test (Cochran-Q) and inconsistency index (I-square). In the case of significant heterogeneity ($I^2 \geq 50\%$ and $P \leq .05$), random effects model (DerSimonian-Laird) was used to calculate pooled results; otherwise, fixed effects model (Mantel–Haenszel method) was used. Threshold analysis and meta-regression were also performed using Meta-DiSc 1.4. Begg funnel plot and Egger test were performed by STATA 12.0 (STATA Corp.). Results were considered statistically significant if $P < .05$.

3. Results

3.1. Search results

As shown in Figure 1, after searching, 188 studies were obtained from Pubmed (63 studies), Embase (115 studies), and Cochrane Library (10 studies). Sixteen studies were left after duplicated and irrelevant publications were removed from the list. After reviewing the full texts, meta-analysis was performed, and 4 studies which did not include KRAS-wildtype tissue samples[23–26] were further excluded from meta-analysis by statistical software.

3.2. Review of eligible publications

The 12 eligible studies all included paired plasma and tissue CRC samples with either mutated or wildetype KRAS status (Table 1).

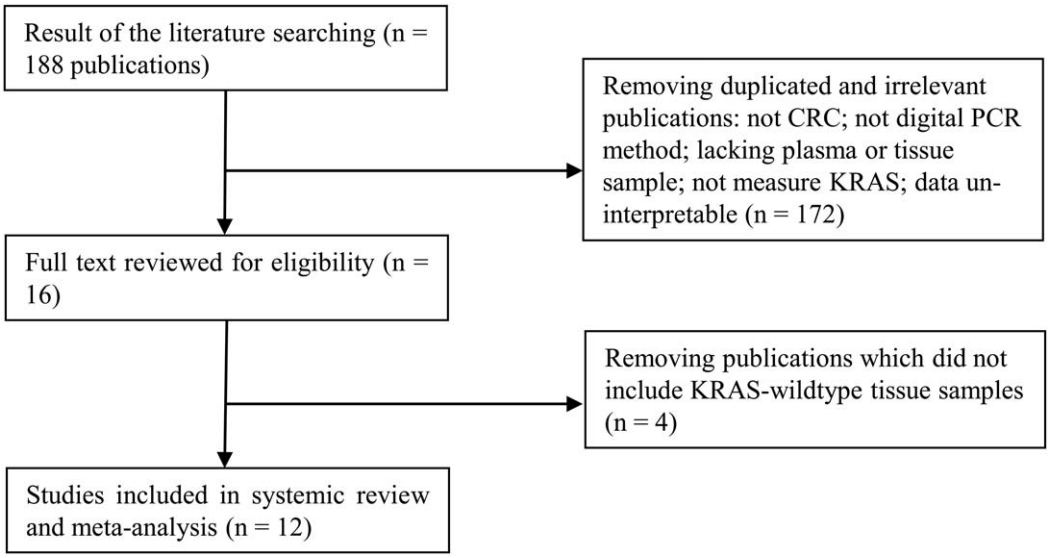

Figure 1. Flowchart of the literature searching and identification of eligible studies for systematic review and meta-analysis.

Takayama et al used droplet-based digital PCR (ddPCR) to monitor KRAS mutation status in a series of plasma samples from 85 mCRC patients at different time points during their chemotherapy treatment.[10] After comparing with baseline tissue sample, plasma samples at the first time point showed a concordance rate of 82% in KRAS status, and calculated sensitivity of 58.6% and specificity of 94.6%.[10] Galbiati et al compared COLD-PCR, microarray, and ddPCR in detecting KRAS mutations and results showed a modest concordance rate of 63.3% for ddPCR.[19] Garcia et al compared next-generation sequencing (NGS) and 2 digital PCR methods, droplet-based digital PCR (ddPCR) and BEAMing, in detecting KRAS mutation in plasma samples.[11] BEAMing showed 93% sensitivity and 69% specificity compared to tissue results, with overall concordance rate of 82%.[11] Results of ddPCR were not included in the data extraction since detection region of ddPCR was narrower than that of NGS, as used in tissue samples.[11] Another 5 studies also used BEAMing for plasma samples and showed concordance rates of 89.7%[13], 78.3%[15], 91.8%[16], 93%[20], and 89%[21] with corresponding tissue results. The calculated sensitivity and specificity from extracted data ranged from 69.7% to 96.4% (85.7%[13], 69.7%[15], 90.4%[16], 96.4%[20], and 86.3%[21]) and from 83.1% to 94% (94%[13], 83.1%[15], 93.5%[16], 90%[20], and 92.4%[21]).

Besides ddPCR and BEAMing, chip-based digital PCR (QuantStuidio 3D Digital PCR System, dPCR QS3D) and picodroplet digital PCR (RainDance Technologies) were also evaluated for KRAS detection in CRC. Three studies used dPCR (QS3D) and after data extraction and calculation, results showed concordance rates of 86.2%[12], 86.2%[17], and 85.3%[18], sensitivity of 85.1%[12], 79.5%[17], 68.8%[18], and specificity of 100%[12], 90.9%[17], 100%[18]. One study used picodroplet digital PCR and showed a concordance rate of 86%, and sensitivity and specificity of 73.7% and 93.5%.[14]

In conclusion, the 12 studies comprised 1008 CRC patients with paired plasma and tissue samples. Four types of digital PCR methods were used to detect KRAS mutation in plasma tissue: ddPCR, BEAMing, dPCR (QS3D), and picodroplet digital PCR. Majority (10 out of 12) of the publications showed highly concordant plasma and tissue KRAS mutation results (concor-

Table 1

Studies comparing KRAS mutation status in plasma and tissue samples of colorectal cancer.

Author, year	Sample size	Method (plasma)	Method (tissue)	Country	Location of tumor tissue
Takayama et al, 2018 [10]	85	ddPCR	ARMS	Japan	metastatic
Garcia et al, 2018 [11]	28	BEAMing	NGS	France	metastatic
Sefrioui et al, 2017 [12]	29	dPCR (QS3D)	SNaPshot multiplex assay	France	metastatic
Grasselli et al, 2017 [13]	56	BEAMing	MassARRAY	Spain	metastatic
Taly et al, 2013 [14]	50	dPCR (picodroplet digital PCR)	qPCR	France	either primary or metastatic
Normanno et al, 2018 [15]	92	BEAMing	NGS	Italy	metastatic
Schmiegel et al, 2017 [16]	98	BEAMing	NGS	Germany & Australia	metastatic
Yamada et al, 2016 [17]	94	dPCR (QS3D)	MEBGEN-Luminex method	Japan	metastatic
Sefrioui et al, 2015 [18]	34	dPCR (QS3D)	SNaPshot multiplex assay	France	metastatic
Galbiati et al, 2019 [19]	30	ddPCR	Sanger sequencing	Italy	metastatic
Vidal et al, 2017 [20]	115	BEAMing	qPCR	Spain	metastatic
Garcia-Foncillas et al, 2018 [21]	236	BEAMing	pyrosequencing	Spain	either primary or metastatic

ARMS = Scorpion amplified refractory mutation system, ddPCR = droplet-based digital PCR, dPCR (QS3D) = QuantStuidio 3D Digital PCR System, NGS = next generation sequencing, qPCR = quantitative PCR.

dance rate higher than 80%). Digital PCR showed high sensitivity (higher than 80%) in half of the studies, and high specificity (higher than 80%) in majority (10 out of 12) of the studies.

3.3. Meta-analysis of the accuracy of digital PCR in KRAS mutation detection using plasma samples

After pooling, the overall sensitivity and specificity of the 12 studies were 0.83 [95% confidence interval (CI): 0.79–0.86] and 0.91 (95%CI: 0.88–0.93) (Fig. 2A & B). The overall PLR, NLR, and DOR were 7.30 (95%CI: 4.78–11.17), 0.22 (95%CI: 0.15–0.32), and 41.00 (95%CI: 21.07–79.78), respectively (Fig. 2C-2E). SROC curve showed an AUC of 0.9322 (Fig. 2F).

Since forest plot results showed that inter-study heterogeneity was significant (Fig. 2), we further investigated the threshold effect and performed meta-regression analysis to find the potential source of heterogeneity. Spearman correlation coefficient was 0.245 ($P = .44$), indicating that no significant threshold effect was found. Two covariates (country and digital PCR methods) were included in the meta-regression, and results showed that inter-study heterogeneity was not associated to country of origin ($P = .88$) or digital PCR methods ($P = .46$).

Subgroup analysis was also carried out according to digital PCR methods, and detailed results were listed in Table 2. Since number of publications using ddPCR, dPCR(QS3D), or picodroplet digital PCR were limited (2, 3, and 1, respectively),

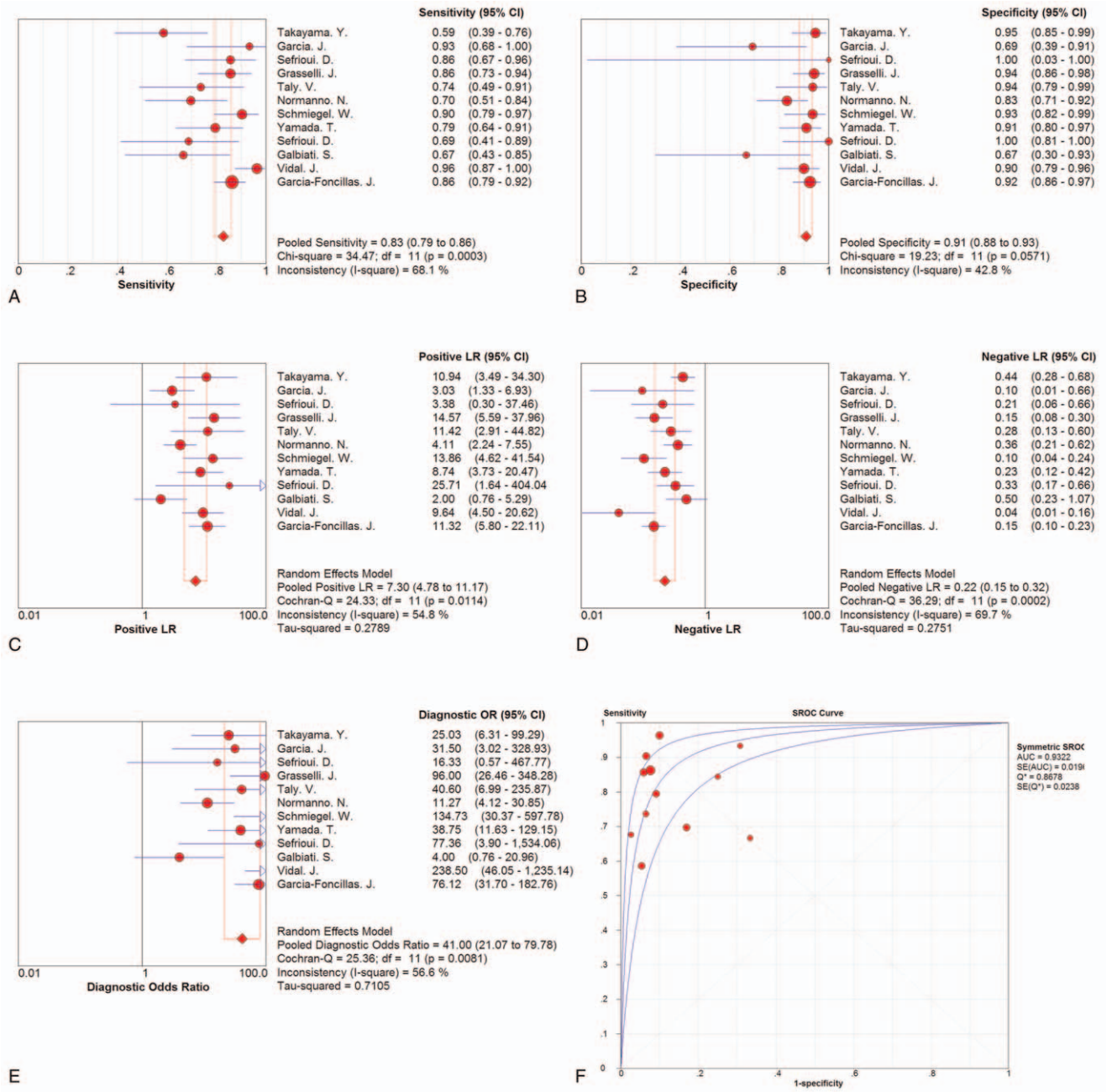

Figure 2. Detailed and pooled sensitivity, specificity, PLR, NLR, DOR, and SROC of the eligible studies.

Table 2

Meta-analysis results.

	No. of studies	Sensitivity	Specificity	PLR	NLR	DOR	AUC of SROC
Overall	12	0.83 (0.79–0.86)	0.91 (0.88–0.93)	7.30 (4.78–11.17)	0.22 (0.15–0.32)	41.00 (21.07–79.78)	0.9322
BEAMing	6	0.87 (0.83–0.91)	0.90 (0.86–0.93)	7.73 (4.41–13.54)	0.15 (0.08–0.25)	63.84 (24.67–165.22)	0.9503
Other digital PCR methods[#]	6	0.73 (0.65–0.80)	0.92 (0.87–0.96)	6.73 (3.22–14.04)	0.34 (0.26–0.45)	23.22 (10.55–51.09)	0.8405

[#] droplet-based digital PCR, dPCR (QuantStuidio 3D Digital PCR System), or picodroplet digital PCR.

AUC = area under curve, DOR = diagnostic odds ratio, NLR = negative likelihood ratio, PLR = positive likelihood ratio, SROC = summarized receiver operating curve.

they were included as 1 group (other digital PCR methods, see Table 2) in subgroup analysis. Compared to other digital PCR methods, BEAMing showed the higher pooled sensitivity [0.87 (95%CI: 0.83-0.91)] but lower pooled specificity [0.90 (95%CI: 0.86-0.93)]. The pooled DOR [63.84 (95%CI: 24.67-165.22)] for BEAMing was higher than other digital PCR methods [23.22 (95%CI: 10.55-51.09)]

Publication bias was evaluated by Begg funnel plot (see Fig. 3) and Egger test. Result showed a *P* value of .11, indicating no obvious publication bias.

4. Discussion

Anti-EGFR targeted therapy (cetuximab or panitumumab) was approved for clinical usage more than a decade ago[3] and is widely used for treatment of mCRC nowadays. It is usually required to test somatic KRAS mutations before the anti-EGFR therapy is given, because those mutations may lead to drug resistance and treatment failure.[6] Digital PCR is a good choice for testing of KRAS mutation in mCRC patients since those patients sometimes lack tissue sample and the alternative (plasma sample or ctDNA) requires highly sensitive detection methods.[8,9] Several studies used digital PCR methods to detect KRAS mutation in plasma samples of CRC patients and compared the results with tissue samples. However, those studies were all limited in sample size and the results varied greatly, e.g., sensitivity ranged from 67%[19] to 96%[20] in the 12 studies. Therefore, a meta-analysis is needed to achieve a more generalized understanding on the accuracy of the digital PCR methods on the KRAS mutation detection using plasma sample.

After database searching and selection of eligible studies, we included 12 studies in our meta-analysis. After pooling the 12 studies using random effects model, results showed pooled sensitivity of 83% and specificity of 91%, with AUC of ROC curve of 0.9322. The pooled PLR and NLR were 7.30 and 0.22, respectively, and DOR, an important indicator of diagnostic test performance, was 41.00 in our meta-analysis. All of the above results indicated that digital PCR methods had an overall high accuracy in KRAS mutation detection in plasma samples of CRC patients. A previous meta-analysis by Xie et al focused on the diagnostic accuracy of ctDNA for KRAS mutation detection and revealed a pooled sensitivity of 63.7%, specificity of 94.3% and DOR of 37.883.[27] This difference in sensitivity between our study and Xie's meta-analysis may be due to the difference in

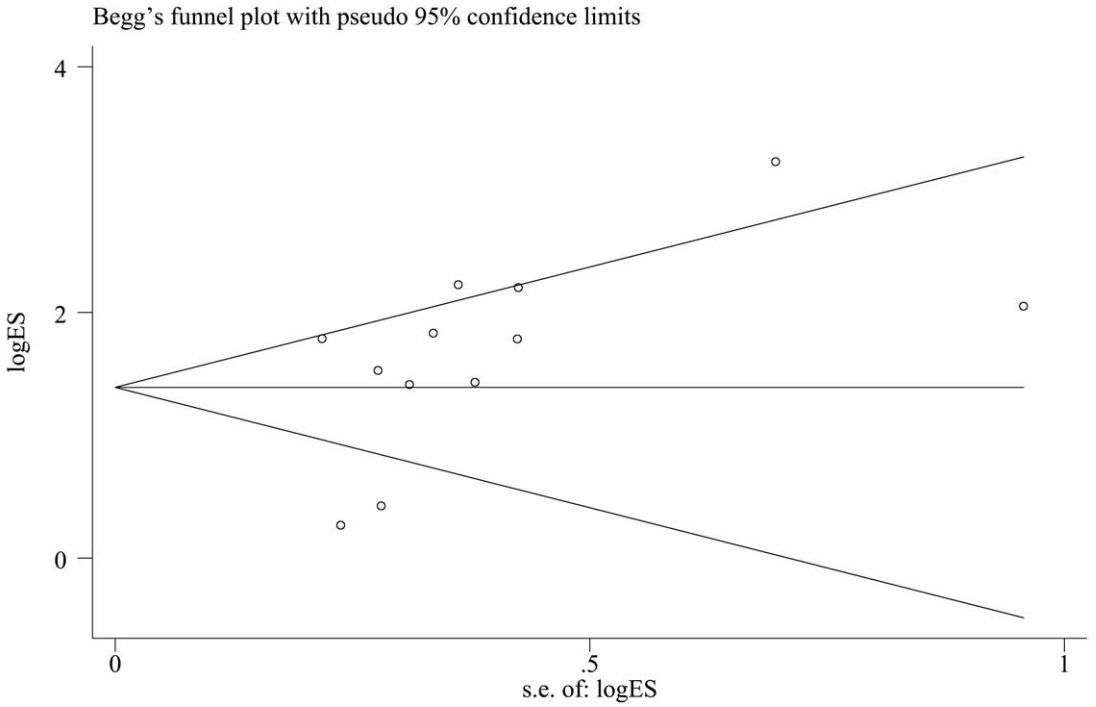

Figure 3. Funnel plot.

detection methods. Our study only included digital PCR methods and Xie's meta-analysis included studies using various detection methods (direct sequencing, quantitative PCR, digital PCR, next-generation sequencing, etc.).

The forest plot also revealed significant heterogeneity among the 12 studies, and therefore, we further investigated the possible explanation of this heterogeneity. We found no threshold effect, and meta-regression also showed that country of origin or digital PCR methods was not associated to heterogeneity among those studies. Subgroup analysis was also conducted and we found that among different digital PCR methods, BEAMing had the highest pooled sensitivity (87%) and DOR (63.84), while ddPCR showed pooled sensitivity of 62% and DOR of 11.93. Those subgroup analysis results might partially explain the inter-study heterogeneity observed. Other than threshold effect and covariates, we also investigated the publication bias among the 12 studies using Begg funnel plot and Egger test, and result showed no significant publication bias.

5. Conclusion

In all, our study showed that overall, digital PCR has high accuracy in detecting KRAS mutation in plasma samples, and could be used to guide anti-EGFR therapy in mCRC patients. Compared to other digital PCR methods, BEAMing showed higher accuracy and is therefore a recommended method for KRAS mutation detection in plasma sample. Limitation of this study may include that although no significant bias was identified, the number of included studies was still quite small, especially in subgroup analysis, which should be handled carefully. In addition, KRAS mutation status in tissue samples was detected using various methods. Although the accuracy of those methods did not differ much due to the highly abundant KRAS-mutated DNA in tissue samples, the difference in detection methods still may cause potential bias to the results. A large-cohort prospective study may be required to further confirm the usefulness of digital PCR in KRAS mutation detection in plasma samples.

Author contributions

Conceptualization: Peng Ye, Jie Zhang.
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Funding acquisition: Peng Ye.
Supervision: Jie Zhang.
Writing – original draft: Peng Ye.
Writing – review & editing: Peng Ye, Peiling Cai, Jing Xie, Jie Zhang.

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