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Review Article

Diagnostic value of circulating methylated SEPT9 DNA in colorectal cancer: a meta-analysis

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Abstract: The diagnostic value of circulating SEPT9 DNA methylation in patients with colorectal cancer remains controversial. Thus, we performed a systematic review and meta-analysis to assess diagnostic accuracy of SEPT9 DNA methylation for colorectal cancer (CRC). We conducted a comprehensive literature search in PubMed, the Cochrane library, Web of Science databases and Chinese National Knowledge Infrastructure (CNKI) up until January 20, 2016. The diagnostic sensitivity (SEN), specificity (SPE), positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and summary receiver operating characteristic (SROC) curve were pooled using STATA 12.0 software. A total of 6,079 subjects included 1,890 colorectal cancer patients in 20 studies were included in this meta-analysis. The summary estimates for circulating methylated SEPT9 DNA in the diagnosis of CRC in these studies were pooled SEN =0.72 (95% confidence interval [CI]: 0.65-0.78), SPE =0.88 (95% CI: 0.85-0.91), PLR 6.13 (95% CI: 4.90-7.67), NLR 0.32 (95% CI: 0.25-0.40]), and DOR 19.3 (95% CI: 13.14-28.31), and the area under the curve (AUC) was 0.90 (95% CI: 0.87-0.92). The current analysis indicated that circulating methylated SEPT9 DNA may be a valuable marker in the diagnosis of CRC, but this conclusion has to be interpreted cautiously owing to high heterogeneity and some limitations. Further studies with more well-designed should be required to confirm the results in the future.

Keywords: Colorectal cancer, SEPT9, circulating, meta-analysis, diagnostic test

Introduction

Colorectal cancer (CRC) has become the third most commonly diagnosed cancer in men and the second in women worldwide, with an estimated 1.4 million new cases are diagnosed and 693,900 deaths each year [1, 2]. The prognosis is poor due to its diagnosis at an advanced stage, and 5-year survival rate less than 10% for CRC cases who have distant metastases [3]. Its lethality may be due to the lack of typical symptoms and effective noninvasive screening and early diagnostic methods to detect the disease [1, 3, 4]. Evidence is mounting that screening can detect precursor lesions that can be removed before they become cancerous, as well as detects cancer at an early stage reduces mortality of the disease [4, 5]. Currently, several accepted screening options such as the guaiac-based fecal occult blood test (FOBT), the immunochemical FOBT, flexible sigmoidoscopy, stool DNA test, computed tomography colonography, double contrast barium enema and colonoscopy [1, 3, 4], have been implemented for years. But their cost, low sensitivity, requires a skilled examiner and invasive nature limit their applications [3, 4]. Thus, minimally invasive and highly accurate diagnostic methods for detection of early-stage CRC are urgently needed, so as to better improve the prognosis [3, 5].

Aberrant DNA methylation as a regulator of gene expression has been demonstrated in tumor biology in general [6], and its changes can be applicable as candidate biomarkers for detecting many types of malignant disease in easily accessible tissues, including CRC [6, 7]. Owing to methylated DNA derived from primary cancer cells into the bloodstream, it can be detected not only in tumor tissue, but also in peripheral blood [8]. Accumulated data indicate that the aberrant methylation of specific genes in the plasma or serum of patients with CRC

may be potential option with promising future for early detection and screening of CRC [6, 9-11]. In the past decade, DNA methylation-based candidates such as P16, ALX4, SEPT9, IGFBP3, GAS7 and TMEFF2 have been used as biomarkers for early detection of CRC, and more and more DNA methylation-based marker candidates are being identified [6]. Out of these candidates, the SEPT9 DNA-methylation test is among the most extensively studied [12].

SEPT9 gene is a member of the septin family involved in cytokinesis and cytoskeletal organization, and alterations in SEPT9 increases the risk of developing a multiple cancers [13]. The SEPT9 gene has been found to be hypermethylated in over 90% of tissues specimens and detected in peripheral blood of CRC patients [11, 14]. Based on previous research, SEPT9 gene methylation has been implicated as a diagnostic biomarker for CRC for more than 10 years and has been used clinically for more than 6 years [14]. Current, considerable attention has been focused on circulating methylated SEPT9 DNA has been proposed as a useful and powerful screening tool for CRC [6, 14]. Extensive confirmation and validation of circulating methylated SEPT9 DNA in large cohorts of CRC patients, the sensitivity and specificity for detecting CRC varied from 36.6% to 96.6% and from 72.9% to 100.0%, respectively [15-34]. The results of these studies are variable although inspiring. In the present study, we conducted the meta-analysis using data from multiple studies to systematically evaluate the potential of using circulating methylated SEPT9 DNA as non-invasive biomarkers in the diagnosis of CRC.

Materials and methods

Search strategy

The meta-analysis was conducted following the criteria of Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) [35]. We conducted a comprehensive literature search in PubMed, the Cochrane library, Web of Science databases and Chinese National Knowledge Infrastructure (CNKI) up until January 20, 2016. The keywords employed for literature retrieval included: (colorectal or colon or colonic or rectal or rectum) and (cancer or tumor or tumour or carcinoma or neoplasm) and (methylation or hypermethylation or hypomethylation or demethylation or epigenetic) and (SEPT9 or Septin 9 or mSEPT9). The languages were limited to English or Chinese. In addition, we also manually searched the references from included articles and relevant published reports.

Inclusion criteria

All candidate publications identified by our search strategy were independently assessed by two reviewers. Any disagreement on controversial study was resolved by full discussion and arrived at consensus with a third investigator. Studies were included in this meta-analysis must meet to following criteria: (1) The diagnosis of CRC was based on colonoscopy or histological examination; (2) The diagnostic potential of methylated SEPT9 DNA for CRC being studies; (3) Study design being observational studies (cohort or case-control studies); (4) Sufficient data were presented to allow the construction of two-by-two tables; (5) Only studies that include at least 20 cases and matched controls were included since very small sample size may lead to selection bias. Studies with the following characteristics were excluded: (1) Duplicate publications or overlapping data; (2) Unsuitable publication types, including letters, editorials, meeting abstracts, case reports and reviews; (3) Insufficient data, data could not be retrieved or reconstructed for 2×2 tables.

Data extraction and quality assessment

The related data were retrieved from each included individual study independently by two reviewers. The data included the following characteristics: study details last name of the first author, year of publication, country of origin, sample size, assay methods, type of specimens), data for two-by-two table (sensitivity [SEN], specificity [SPE], true positive [TP], false-positive [FP], false negative [FN], and true negative [TN]) and study design. The reviewers were blinded to publication details, and disagreements between them were resolved by consensus.

To assess the quality of each study and potential risk of bias, we used the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool [36]. The 14 items in QUADAS were assessed in all the included articles. Each item was assessed as “yes”, “no” or “unclear”. The

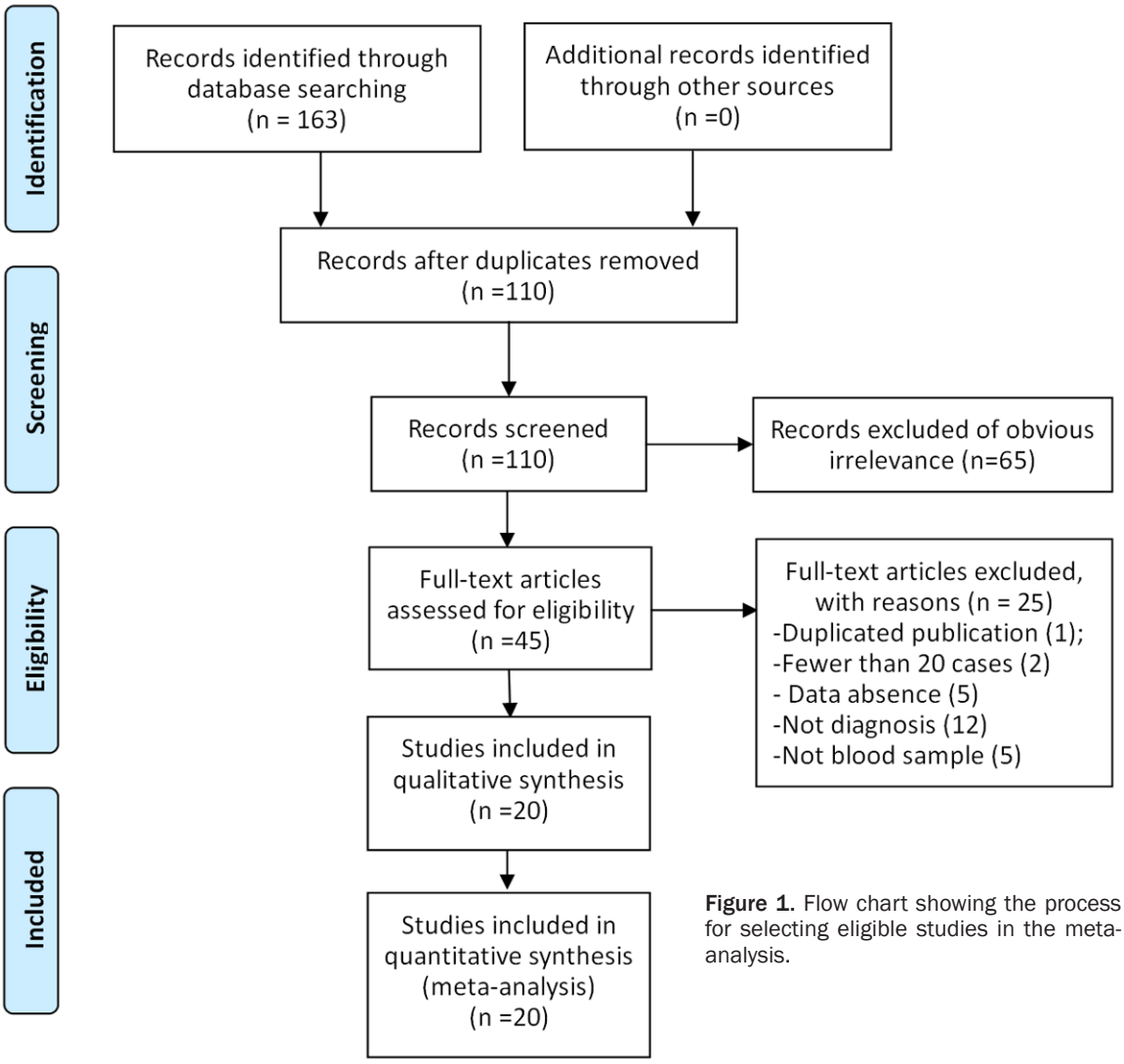

Figure 1. Flow chart showing the process for selecting eligible studies in the meta-analysis.

answer "yes" obtained a score of zero, whereas "no" or "unclear" gained a score of zero with a total score of 14. Quality assessment of the included studies was performed and cross-checked independently by two reviewers. In case of conflict, a third reviewer was consulted, and disagreement was settled through multilateral discussion.

Statistical analysis

We used Statistical analysis software (Stata 12.0) to perform the meta-analysis. The bivariate meta-analysis model was employed to summarize the SEN, SPE, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR), and to generate the bivariate summary receiver operator characteristic (SROC) curve [37]. The area under the curve (AUC) represents an analytical summary of the test performance and illustrates the trade-off between SEN and SPE. Spearman's rank correlation was performed as a test for threshold effect. Statistical heterogeneity was assessed using the chi-test based on Q-test, a value of P less than 0.1 or an I^2 higher than 50% indicated the existence of significant heterogeneity [38, 39]. The leave-one-out sensitivity analysis was conducted to determine whether our assumptions or decisions have a major effect on the results of the review by omitting each study. In addition, Meta-regression analyses were performed to explore the potential sources of between-study heterogeneity. Deeks' funnel plot asymmetry test was used to evaluate the potential publication bias. All statistical tests were two-sided, and a $P< 0.05$ was considered statistically significant.

Table 1. Summary of studies included in the meta-analysis

Study	Country	Year	Study design	No. of P/C	Assay method	Sample	TP	FP	FN	TN	SEN	SPE	QUADAS score
Ahlquist [15]	USA	2012	RE	30/48	qMSP	Plasma	18	13	12	35	60	72.9	11
Church [16]	USA	2013	PR	53/1457	Epi	Plasma	27	126	26	1331	50.9	91.4	12
Danese [17]	Italy	2015	RE	70/15	qMSP	Plasma	58	0	12	15	83	100	10
deVos [18]	USA	2009	RE	187/327	qMSP	Plasma	138	45	49	282	73.8	86.2	13
Grützmann [19]	Germany	2008	RE	285/378	qMSP	Plasma	193	25	185	260	51.1	91.2	12
He [20]	China	2010	RE	180/170	Methylight	Plasma	136	6	46	164	74.7	96.5	9
Jin [21]	China	2015	RE	135/341	Epi	Plasma	101	43	34	298	74.8	84.7	11
Johnson [22]	USA	2014	PR	97/193	Epi	Plasma	70	37	27	156	72.2	80.8	11
Kang [23]	China	2014	RE	80/52	Epi	Plasma	60	1	20	51	75	98.1	9
Lee [24]	South Korea	2013	RE	101/96	qMSP	Plasma	37	9	64	87	36.6	90.6	10
Liu [25]	Singapore	2013	RE	37/20	Epi	Serum	20	2	17	18	54.1	90	9
Lofton-Day [26]	USA	2008	RE	133/179	qMSP	Plasma	92	25	41	154	69.2	86	12
Ørntoft [27]	Denmark	2015	RE	150/150	Epi	Plasma	110	27	40	123	73.3	82	10
Potter [28]	Germany	2014	PR	44/444	Epi	Plasma	30	97	14	437	68.2	78.2	11
Ravegnini [29]	Italy	2015	RE	27/26	MSP	Serum	18	2	9	24	66.7	92.3	9
Tänzer [30]	Germany	2010	RE	33/34	Methylight	Plasma	27	4	6	30	81.8	88.2	12
Tóth [31]	Germany	2012	PR	92/92	Epi	Plasma	88	14	4	78	95.6	84.8	13
Wang [32]	China	2012	RE	36/20	MS-HRM	Plasma	25	2	11	18	69.4	90	8
Warren [33]	USA	2011	RE	50/94	qMSP	Plasma	45	11	5	83	90	88.3	11
Yu [34]	China	2015	RE	70/53	Epi	Plasma	57	7	13	46	81.5	86.7	8

Re = Retrospective; PR = Prospective; NR: not reported; No. of P/C = number of patients/control; Epi = EpiproColon; qMSP = quantitative methylation-specific PCR; MSP = Methylation-specific PCR; MS-HRM = methylation sensitive high-resolution melting; TP = true positive; FP = false positive; FN = false negative; TN = true negative; SEN = sensitivity; SPE = specificity.

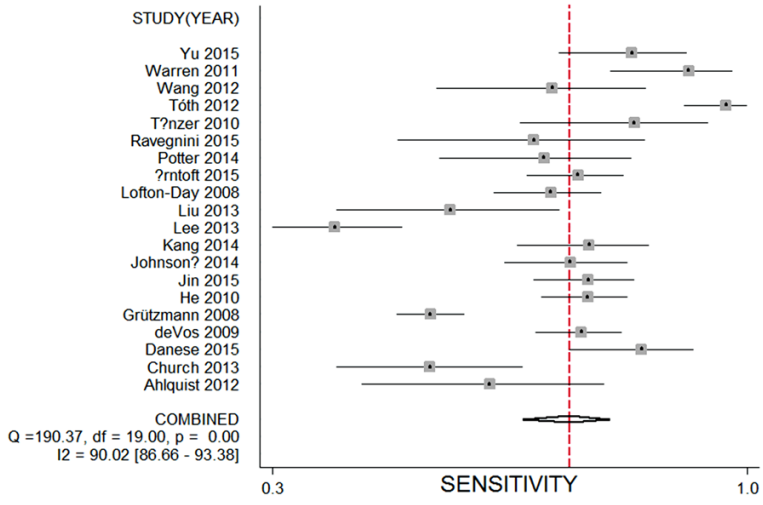

Figure 2. Forest plot of estimated sensitivity for circulating methylated SEPT9 DNA in the diagnosis of colorectal cancer.

Results

Search results

One hundred sixty-three publications were primitively identified according to the literature search strategy from databases and hand searching. After removal of duplicates, we obtained 110 literatures. Following the titles, abstracts, and key words were then carefully evaluated, and 65 studies were excluded (34 studies were excluded due to unsuitable publication types, 15 studies were excluded as non-diagnostic studies, and 16 were excluded for focusing on not CRC). Of these remained 45 literatures with full text were assessed, and 24 articles were excluded (1 study had significant overlap, 2 studies with fewer than 20 cases, 14 studies were not related to diagnosis, 3 data absence and 5 were not used blood sample. Thus, we obtained 20 publications [15-34] that met all of the eligibility criteria for this systematic review and meta-analysis. The flowchart for inclusion and exclusion of the studies is presented in **Figure 1**.

Characteristics of included studies

In this meta-analysis, the final set of 20 diagnostic studies [15-34] included a total of 2,062 subjects with colorectal cancer and 4,251 pa-

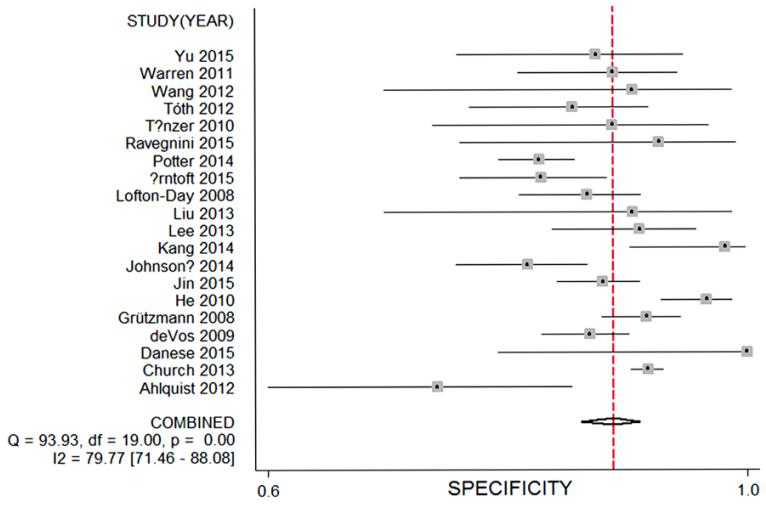

Figure 3. Forest plot of estimated specificity for circulating methylated SEPT9 DNA in the diagnosis of colorectal cancer.

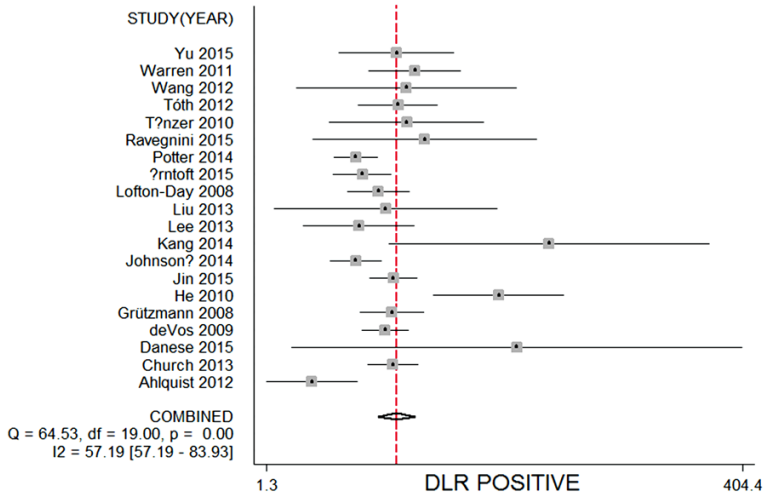

Figure 4. Forest plot of estimated PLR for circulating methylated SEPT9 DNA in the diagnosis of colorectal cancer.

tients healthy control individuals. All the colorectal cancer patients were diagnosed based on colonoscopy or histological examination. Regarding the origin of the studies, Seven studies [20, 21, 23-25, 32, 34] were conducted in Asia (5 in China, 1 in South Korea and 1 in Singapore), Six in America [15, 16, 18, 22, 26, 33], and Seven in Europe (2 in Italy, 4 in Germany and 1 in Denmark) [17, 19, 27-31]. All studies were published from 2008 to 2015, and the number of colorectal cancer patients in each study varied from 27 to 285. Several different methods were used in these studies to measure the circulating SEPT9 DNA methylation: Nine study conducted EpiproColon Assay (EpiproColon) [16, 21-23, 25, 27, 28, 31, 34], six studies used quantitative real-time polymerase chain reaction (qPCR) [15, 18, 19, 24, 26, 33], two studies used methylation-specific PCR (MSP) [17, 29], two studies performed fluorescence methylight [20, 30] and one study used methylation sensitive high-resolution melting (MS-HRM) [32]. Of the included 20 studies, 18 studies used plasma samples, and the remaining two studies [25, 29] used serum. The main characteristics of the 20 publications were demonstrated in **Table 1**.

Assessment of methodological quality

Quality assessment based on QUADAS guidelines was conducted on all 20 studies included for systematic review and meta-analysis. The QUADAS scores of studies were from 8 to 13, which satisfy the majority of the standards. As shown in **Table 1**.

Diagnostic accuracy analysis

In order to assess whether the heterogeneity of circulating SEPT9 DNA methylation are amongst the eligible studies, we first calculated the correlation coefficient between the logit of sensitivity and logit of 1-specificity by using Spearman test to exclude the threshold effect. As a result, the Spearman correction coefficient was 0.028 (P=0.907), indicating that the absence of heterogeneity from the threshold effect. However, I^2 test showed obvious inter-study heterogeneity (I^2=90.2% for SEN, I^2=79.8% for SPE, I^2=57.2% for PLR, I^2=90.8% for NLR and I^2=99.9% for DOR), suggesting high levels of heterogeneity in the 20 studies. The overall performance of circulating methylated SEPT9 DNA in diagnosis CRC. The pooled SEN 0.72 (95% confidence

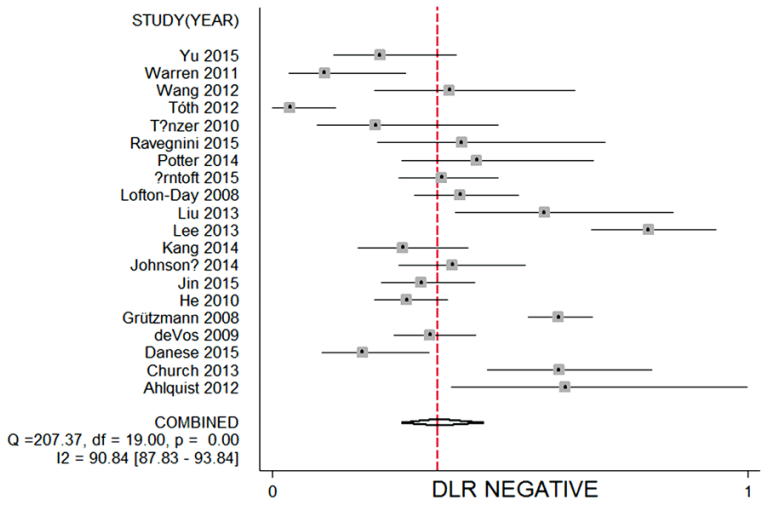

Figure 5. Forest plot of estimated NLR for circulating methylated SEPT9 DNA in the diagnosis of colorectal cancer.

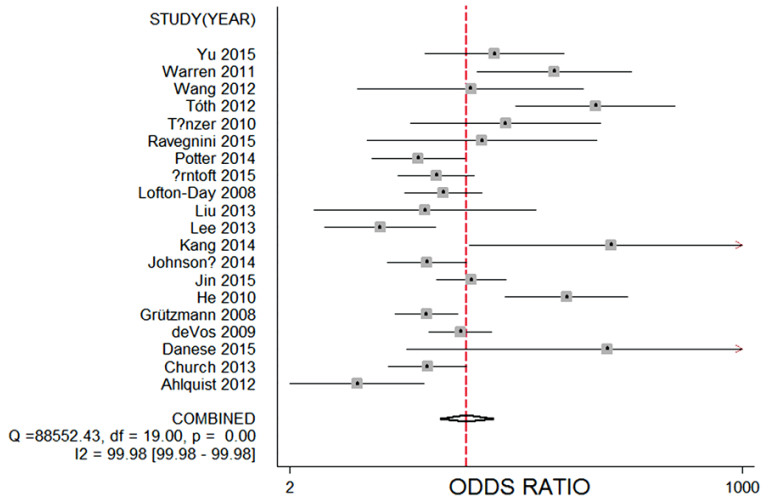

Figure 6. Forest plot of estimated DOR for circulating methylated SEPT9 DNA in the diagnosis of colorectal cancer.

interval [CI]: 0.65-0.78), SPE 0.88 (95% CI: 0.85-0.91), PLR 6.13 (95% CI: 4.90-7.67), NLR 0.32 (95% CI: 0.25-0.40]), and DOR 19.3 (95% CI: 13.14-28.31), and the area under the curve (AUC) was 0.90 (95% CI: 0.87-0.92), indicating a relatively high accuracy of circulating methylated SEPT9 DNA for CRC diagnosis. Forest plots of the SEN, SPE, PLR, NLR, DOR, AUC for circulating SEPT9 DNA methylation in CRC diagnosis are shown in **Figures 2**-**7**.

Meta-regression analyses

The heterogeneity generated by non-threshold within the studies can be obviously observed in the forest plot of diagnosis index. To explore possible sources of the heterogeneity across these 20 studies, we performed a meta-regression analyses according to the "publication year", "Study location" (Region: Asia or not), "Study design" (Prospective or not), "Specimen types" (Sample: Plasma or Serum), "Sample size" (Sample size ≥200 or not), "Assay methods" (Methods), "Gene methylation assays" (Single or multiple) and "Quality of study" (QUADAS score ≥10 or not). Unfortunately, the meta-regression indicating that none of these factors showed any definite influence on heterogeneity (As shown in **Table 2**), indicating that these study characteristics did not substantially affect the diagnostic accuracy. Heterogeneity may have arisen due to other reasons, such as the number of included patients, age, tumor type, tumor size, metastasis, TNM staging and differences in the operating protocol, which could not be analyzed in the present study due to partial loss of the data or unrecognizable details.

Publication bias

Deeks' funnel plot asymmetry test was used to evaluate publication bias of the included studies. The slope coefficient was associated with *P* values of 0.373 (*t*=0.91), indicating no publication bias in the meta-analysis (As shown in **Figure 8**).

Sensitivity analysis

Sensitive analyses were conducted to determine to verify the effect of each study on the overall diagnostic value by repeating the meta-analysis, one by one single study involved in this meta-analysis was evaluated each time, there was almost no difference between the remaining 20 studies (As shown in **Table 3**).

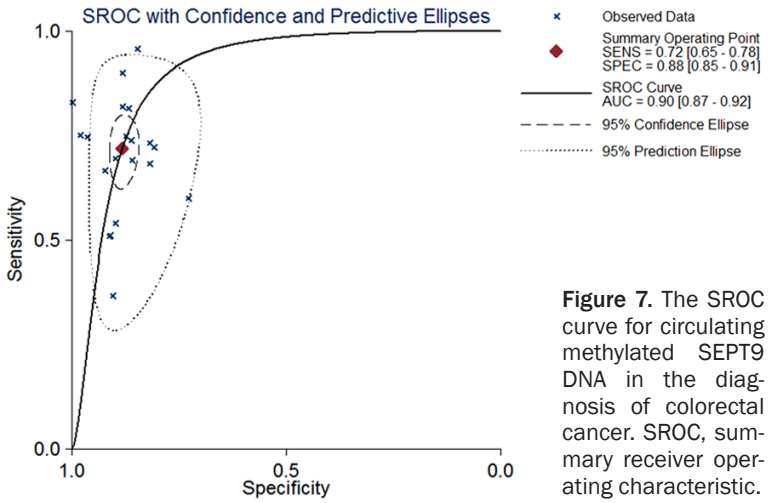

Figure 7. The SROC curve for circulating methylated SEPT9 DNA in the diagnosis of colorectal cancer. SROC, summary receiver operating characteristic.

Table 2. Results of the meta-regression performed to identify potential sources of heterogeneity

Covariates	RDOR	95% CI	Std.Err.	*p* value
Publication year	0.994	0.767-0.2881	0.116	0.963
Study location	1.489	0.640-3.464	0.571	0.321
Specimen types	0.174	0.012-0.521	0.211	0.178
Sample size	0.617	0.190-2.001	0.329	0.386
Assay method	0.995	0.503-0.964	0.307	0.987
Study design	0.924	0.211-4.045	0.620	0.909
Gene assays	1.471	0.390-0.549	0.887	0.535
QUADAS score	4.275	0.457-39.988	4.343	0.180

RDOR, Relative diagnostic odds ratio; CI, confidence interval; Std.Er, standard error.

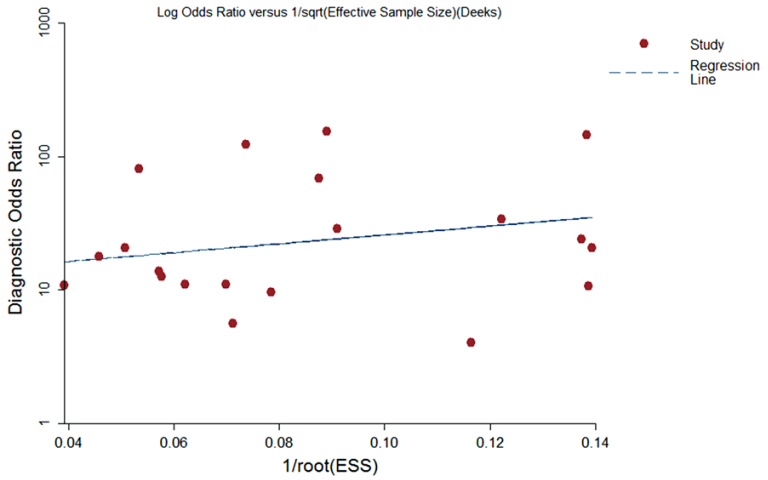

Figure 8. The Deeks' funnel plot for the assessment of potential publication bias of the included studies.

Therefore, the results of our meta-analysis were statistically robust.

Discussion

It is widely accepted that DNA methylation is an important manner of regulation at the epigenetic level for gene expression, cell growth, and cell differentiation [40]. It has found that DNA methylation of different genes was associated with the risk of CRC, which implicated a potential role of DNA methylation in the prediction and prognostication for CRC. One of the latest DNA methylation-based biomarkers for CRC is the SEPT9 promoter methylation analyses in serum, plasma, and stool samples, which has consistently demonstrated utility in detecting CRC in several clinical studies [41]. Moreover, increasing evidence suggests that the analysis of DNA methylation in blood or fecal specimens could represent a promising auxiliary diagnostic tool in early CRC detection to be used in screening programs, but with considerable controversial results [15-34]. To our knowledge, there is no evidence-based evaluation for circulating SEPT9 DNA methylation as a novel biomarker to diagnosis CRC since it was first reported on the quantitative assessment in patients with CRC. Therefore, in the present study, we performed a systematic review and meta-analysis to assess diagnostic value of circulating SEPT9 DNA methylation in peripheral blood of patients with CRC.

In this meta-analysis, we finally included 2,062 CRC patients and 4,251 controls with 20 diagnostic clinical studies. The pooled SPE of the circulating SEPT9 DNA methylation was high 0.88 (95% CI: 0.85-0.91); however, it has moderate

Table 3. Results of sensitivity analysis to verify the effect of each study on the overall diagnostic value

Study	SEN (95% CI)	SPE (95% CI)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)
Overall	0.72 (0.65-0.78)	0.88 (0.85-0.91)	6.13 (4.90-7.67)	0.32 (0.25-0.40)	19.3 (13.14-28.31)
Ahlquist [15]	0.72 (0.65-0.78)	0.88 (0.86-0.90)	6.36 (5.14-7.87)	0.31 (0.24-0.39)	20.46 (14.06-29.78)
Church [16]	0.72 (0.65-0.78)	0.88 (0.85-0.90)	6.09 (4.78-7.75)	0.30 (0.24-0.39)	19.77 (13.13-29.75)
Danese [17]	0.71 (0.64-0.77)	0.88 (0.85-0.90)	5.95 (4.79-7.38)	0.32 (0.25-0.41)	18.24 (12.50-26.63)
deVos [18]	0.71 (0.64-0.78)	0.88 (0.85-0.90)	6.23 (4.88-7.96)	0.31 (0.24-0.40)	19.62 (12.97-29.68)
Grützmann [19]	0.73 (0.66-0.79)	0.88 (0.85-0.90)	6.12 (4.82-7.78)	0.30 (0.24-0.38)	19.99 (13.33-29.97)
He [20]	0.71 (0.64-0.78)	0.87 (0.84-0.89)	5.61 (4.69-6.71)	0.32 (0.25-0.41)	17.35(12.19-24.70)
Jin [21]	0.71 (0.64-0.78)	0.88 (0.85-0.90)	6.19 (4.84-7.91)	0.31 (0.24-0.40)	19.41 (12.84-29.35)
Janson [22]	0.72 (0.64-0.78)	0.88 (0.85-0.90)	6.35 (5.04-8.00)	0.31 (0.24-0.40)	20.09 (13.44-30.04)
Kang [23]	0.71 (0.64-0.78)	0.87 (0.85-0.89)	5.83 (4.75-7.16)	0.32 (0.25-0.41)	18.15 (12.44-26.48)
Lee [24]	0.73 (0.67-0.78)	0.88 (0.85-0.90)	6.21(4.91-7.84)	0.30 (0.24-0.37)	20.65 (14.16-30.12)
Liu [25]	0.72 (0.65-0.78)	0.88 (0.85-0.90)	6.17 (4.90-7.75)	0.30 (0.24-0.39)	19.96 (13.44-29.62)
Lofton-Day [26]	0.72 (0.64-0.78)	0.88 (0.85-0.90)	6.25 (4.91-7.96)	0.31 (0.24-0.40)	19.86 (13.17-29.94)
Ørntoft [27]	0.71 (0.64-0.78)	0.88 (0.85-0.91)	6.32 (4.99-8.00)	0.31 (0.24-0.40)	19.94 (13.27-29.97)
Potter [28]	0.72 (0.64-0.78)	0.88 (0.85-0.90)	6.36 (5.06-8.00)	0.31 (0.24-0.40)	20.27 (13.63-30.13)
Ravegnini [29]	0.72 (0.64-0.78)	0.88 (0.85-0.90)	6.09 (4.85-7.64)	0.31 (0.24-0.40)	19.30 (12.97-28.73)
Tänzer [30]	0.71 (0.64-0.77)	0.88 (0.85-0.90)	6.10 (4.83-7.69)	0.32 (0.25-0.41)	18.85 (12.68-28.00)
Tóth [31]	0.69 (0.63-0.75)	0.88 (0.85-0.90)	6.07 (4.76-7.73)	0.34 (0.28-0.41)	17.79 (12.27-25.80)
Wang [32]	0.72 (0.64-0.78)	0.88 (0.85-0.90)	6.12 (4.87-7.70)	0.31 (0.24-0.40)	19.35 (12.98-28.85)
Warren [33]	0.70 (0.63-0.76)	0.88 (0.85-0.90)	6.04 (4.77-7.64)	0.33 (0.26-0.41)	18.23 (12.41-26.78)
Yu [34]	0.71 (0.64-0.77)	0.88 (0.85-0.90)	6.13 (4.84-7.77)	0.32 (0.25-0.41)	18.93 (12.66-28.30)

SEN for diagnosing CRC (0.72 (95% CI: 0.65-0.78)). Compared to conventional screening methods (FOBT and the immunochemical FOBT), with a sensitivity of 23.8% and 61% for CRC diagnosis, respectively [42]. The detection accuracy of circulating SEPT9 DNA methylation was higher. The PLR and NLR are employed to evaluate the clinical applicability of circulating SEPT9 DNA methylation as screening tools, which LRs of >10 or <0.1 generate large and often conclusive shifts from pretest to posttest probability [43]. In our study, the pooled PLR and NLR was 6.13 (95% CI: 4.90-7.67) and 0.32 (95% CI: 0.25-0.40]), respectively. this result indicated that CRC patients have six times greater chance of being circulating SEPT9 DNA methylation-positive compared with healthy controls, and an approximately 32% error rate would be present when the true negative was determined in the circulating SEPT9 DNA methylation-negative test. The value of DOR ranges from zero to infinity, with higher values indicating better discriminatory test performance [44]. Despite of the unsatisfactory pooled PLR and NLR value, the DOR value of 19.3 (95% CI: 13.14-28.31) indicates that the circulating SEPT9 DNA methylation could be a useful biomarker for CRC patients' diagnosis. Apart from that, AUC is calculated to evaluate accuracy of the selected indicator, and SROC is usually used to summarize overall test performance [45]. In our study, The AUC of SROC was 0.90 (95% CI: 0.87-0.92). It is still better than the other promising blood-based biomarkers. For example, the AUC of serum carcinoembryonic antigen (CEA) [46], serum Interleukin-6 [47], and circulating microRNA-21 [48] were 0.75, 0.79 and 0.87 for diagnosing CRC, respectively. Thus, circulating SEPT9 DNA methylation has a relatively high accuracy for CRC diagnosis. Unfortunately, rare studies directly compare the diagnostic value of circulating SEPT9 DNA methylation with other markers, and combine other diagnostics marker for CRC diagnosis, thus our study could not fully assess whether circulating SEPT9 DNA methylation improves the diagnostic accuracy of commonly used tumor marker for CRC screen.

Exploring the sources of heterogeneity is one major purpose of meta-analysis [49]. In the present meta-analysis, significant heterogeneity was detected among the included studies.

The Spearman correction coefficient of the current study was 0.028 (P=0.907) indicated that the heterogeneity was not caused by the threshold effect. In order to explore the potential source of heterogeneity, we investigated the characteristics of included studies such as publication year, study location, sample type, study design and assay methods using meta-regression, but no covariables were found to contribute to the heterogeneity, indicating that these study characteristics did not substantially affect the diagnostic accuracy and the influencing factors are unclear. In addition, the publication bias was not significant, indicating that the results of our meta-analysis are reliable.

It must be pointed that some limitations may affect the objectivity of our meta-analysis. Firstly, selection bias is inevitable due to only articles published in English or Chinese were included in this meta-analysis. Secondly, some large between-study heterogeneity existed in our meta-analyses, and it could not be analyzed in the present study due to partial loss of the data or unrecognizable details in the involved studies. Thirdly, most included studies that have a smaller number of cases and have a retrospective design. Therefore, the results of the trials in a pooled analysis were not robust. Further validations of circulating methylated SEPT9 DNA in large and prospective studies are needed. Fourthly, this meta-analysis was based on published studies; the exclusion of unpublished data is generally associated with an overestimation of the true effect, thus resulting in a publication bias.

In conclusion, this meta-analysis demonstrated that circulating methylated SEPT9 DNA may be a valuable marker in the diagnosis of CRC, but this conclusion has to be interpreted cautiously owing to high heterogeneity and some limitations. Further studies with more well-designed should be required to confirm the results in the future.

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Disclosure of conflict of interest

None.

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