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ORIGINAL ARTICLE

Diagnostic Assessment of *septin9* DNA Methylation for Colorectal Cancer Using Blood Detection: A Meta-Analysis

Gongping Sun[1] · Jin Meng[2] · He Duan[1] · Dewei Zhang[1] · Yuanxin Tang[1]

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Abstract

This meta-analysis aimed to assess the diagnostic efficiency of blood-based septin 9 (*SEPT9*) methylation assay for the detection of colorectal cancer (CRC). Studies were searched in the Springer, Wiley, Cochrane Library, PubMed, Ovid, Embase, Web of Science, China BioMedicine, Wanfang and China National Knowledge Infrastructure databases until July 2017. Methodological quality assessment was performed based on the guidelines of the Quality Assessment of Diagnostic Accuracy Studies. According to 1/3 and 2/3 algorithms, the meta-analyses for the diagnostic effect of *SEPT9* in CRC were compared with healthy subjects and subjects with polyps, adenoma, and non-CRC, respectively. The random effects model was applied and publication bias was evaluated. The included 29 studies comprised 10,486 subjects (3202 patients with CRC and 7284 controls). In comparison with healthy subjects, the pooled sensitivity with 95% confidence intervals (CIs) of *SEPT9* methylation for the diagnosis of CRC was 0.74 (95% CI: 0.61–0.84) in the 1/3 algorithm group, whereas the specificity was 0.96 (95% CI: 0.95–0.97) in the 2/3 algorithm group. Additionally, positive likelihood ratio was less than 10 and negative likelihood ratio more than 0.1 in the 2/3 algorithm group for patients with CRC vs. polyps and adenoma. The *P* value of Deeks' funnel plot was 0.36, suggesting that there was no publication bias. *SEPT9* methylation can be used to diagnose CRC in healthy individuals under the 2/3 algorithm. The determination of *SEPT9* methylation does not distinguish well between CRC and polyps or adenoma.

Keywords SEPT9 methylation · Colorectal cancer · Positive likelihood ratio · Negative likelihood ratio · Summary receiver operating characteristic

Introduction

Colorectal cancer (CRC), also termed bowel and colon cancer, originates in the cells of the rectum or colon, regions of the large intestine, and takes several years to become cancerous [1]. CRC is the third most common type of cancer occurring worldwide [2]. More than 1.2 million people are diagnosed with CRC annually, and nearly 50% of patients die from the disease [3]. In addition, the incidence and mortality rates of CRC in China are 18.8% and 9.6%, respectively [4]. Although various techniques, including chemotherapy and radiation therapy, have been used for the CRC treatment, the survival rate remains unsatisfactory [5]. In patients with stage I disease, the 5-year survival rate is up to 90% but is slightly greater than 10% in patients with stage IV disease [5]. Therefore, diagnosis in the early stage is important to improve the survival rate of patients with CRC.

It is widely known and accepted that CRC can be grouped molecularly into chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylator phenotype (CIMP) [6]. It is estimated that 15%–20% of CRCs have CIMP, an epigenetic change resulting in the transcriptional silencing of many tumor suppressor genes by hypermethylation of cytosine residues at CpG-rich sequences (CpG islands) in the promoter regions [7]. CIMP is a phenomenon of concurrent methylation of a group of genes in a subset of tumors [8] including CRC. It is common knowledge that cancer-specific methylation occurs early in tumorigenesis and can be detected by an amplifiable signal [9]. Methylated genes in the blood and tumor tissues are key candidate markers for cancer detection in the early stage because methylation occurs in distinct genomic areas [10]. Aberrant DNA methylation

✉ Dewei Zhang
zhdeweizh@sina.com

[1] Department of the Third general surgery, The Fourth Affiliated Hospital of the China Medical University, No. 4 Chongshan Road, Shenyang 110032, China

[2] Shenyang Anorectal Hospital, No. 9 North of Nanjing Street, Heping District, Shenyang 110000, China

occurs in the blood, making it a feasible diagnostic CRC biomarker for the early detection of CRC [11]. Blood-based biomarkers for the early detection of CRC could complement current approaches to CRC screening [12].

The CpG island 3 at the promoter region of the septin 9 (*SEPT9*) gene V2 transcript has been shown to be hypermethylated and DNA of the gene is released into the blood circulation of patients during CRC carcinogenesis [11, 13]. Importantly, *SEPT9* methylation has been shown to be a candidate diagnostic biomarker for CRC [14]. Certain blood-based diagnostic tests have verified the diagnostic value of methylated *SEPT9* for CRC with >70% and > 90% sensitivity and specificity, respectively [15, 16]. However, another study showed that the sensitivity was only 48.2% [9]. In addition, certain meta-analyses have been used to evaluate the diagnostic value of methylated *SEPT9* for CRC screening [17–19]. However, *SEPT9* assays have not shown similar sensitivity and specificity. It is clear that improving the detection rate and identifying novel assays [20] for the detection of SEPT9 methylation are important for developing SEPT9 as a blood-based methylation analysis biomarker for early CRC diagnosis. Algorithms, including the 1/1, 1/2, 1/3, and 2/3 algorithms, have also been used in screening studies to investigate the performance of the *SEPT9* gene methylation assay in CRC detection [9, 21]. However, no consensus has been found.

In this study, to obtain a better insight into the diagnostic value of *SEPT9* methylation for CRC detection, a novel and comprehensive meta-analysis was performed to investigate the diagnostic outcome of *SEPT9* gene methylation for CRC. Subsequently, the specificity, sensitivity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and area under the curve (AUC) of the summary receiver operating characteristic (SROC) of *SEPT9* methylation for the diagnosis of CRC were evaluated and pooled using the random effects model. Deeks' funnel plot was also used to discuss the possibility of publication bias.

Methods

Search Strategy

A comprehensive search of articles related to *SEPT9* gene methylation in CRC from the Springer, Wiley, Cochrane Library, PubMed, Ovid, Embase, Web of Science, China BioMedicine, Wanfang and China National Knowledge Infrastructure databases was conducted. The search strategy included the following terms: ("colorectal neoplasms" OR "rectal neoplasms" OR "colorectal neoplasm" OR "colorectal tumors" OR "colorectal carcinomas" OR "colorectal cancer" OR "rectal neoplasm" OR "rectum neoplasms" OR "rectal cancers" OR "rectum cancer" OR "CRC") AND ("SEPT9" OR "SEPT 9" OR "Septin 9" OR "Septin9"). Restrictions based on language and dataset were not applied in this study. The retrieval time for the present study was updated to July 26, 2017. In addition, manual searches were performed for the screening and selection of other eligible studies.

Study Selection

The inclusion criteria for the present meta-analysis were as follows: 1) the study was an observational study evaluating the diagnostic effects of *SEPT9* gene methylation in CRC using a blood assay; 2) the study used a standard diagnostic procedure for colonoscopy; 3) both case group (CRC patients) and control group (non-CRC) were included; 4) high integrity data, including sensitivity, specificity, PLR, and/or NLR calculation were available in the study. If more than one study was published by the same author, only the latest complete study was extracted. Studies unrelated to the research subjects, literature reviews, studies with incomplete data, and repeat publications were excluded.

Data Extraction and Quality Assessment

To reduce bias, the information from all selected studies was independently extracted by two investigators (Sun GP and Meng J). All investigators reached a consensus on all items via discussion and reexamination. The study information (name of the first author, year of publication, and country), and the patients' information (age and the number of cases, pathological type, detection methods, diagnostic power, and *SEPT9* gene source) were extracted from each eligible study.

Methodological quality assessment of the included studies was performed based on the guidelines of the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) criteria to ensure consistency in reviewing and reporting results. The QUADAS criteria include the following 11 items: QUADAS01 (representative spectrum), QUADAS02 (acceptable reference standard), QUADAS03 (acceptable delay between tests), QUADAS04 (partial verification avoided), QUADAS05 (differential verification avoided), QUADAS06 (incorporation avoided), QUADAS07 (reference standard results blinded), QUADAS08 (index test results blinded), QUADAS09 (relevant clinical information), QUADAS10 (uninterpretable results reported), and QUADAS11 (withdrawals explained).

Statistical Analysis

STATA statistical software (version 12.0, Stata Corp, College Station, TX, USA) was used for the statistical analyses. According to various algorithms of 1/1, 1/2, 1/3, 2/3, 3/3, and not reported, the meta-analyses for the diagnostic effect of *SEPT9* in CRC was compared with healthy subjects and subjects with polyps, adenoma, and non-CRC, respectively.

The 1/3 algorithm indicated that the final outcome was scored as positive if at least of one of three repeats were positive. Similarly, the 2/3 algorithm indicated that the final outcome was scored as positive if at least two of three repeats were positive. The diagnostic values of *SEPT9* methylation in CRC were then evaluated via the specificity, sensitivity, PLR, NLR, DOR, and AUC of the SROC. The diagnostic criteria were defined as follows: PLR > 10 and NLR < 0.1 represented exclusion and confirmation; PLR > 10 and NLR > 0.1 represented only confirmation; PLR < 10 and NLR < 0.1 represented only exclusion; PLR < 10 and NLR > 0.1 represented no exclusion or confirmation [22]. The random effects model was used to pool the results. Deeks' funnel plot was used to evaluate the possibility of publication bias. $P < 0.05$ indicated a high risk of bias.

Results

Included Studies

The study selection procedure was listed in Fig. 1. We initially identified 823 relative studies from electronic databases and one from a manual search. In total, 566 studies were included following the removal of 258 duplicates. Subsequently, 526 articles were excluded, which included reviews or meta-analyses ($n = 7$); studies without available data ($n = 12$); and studies unrelated to the research topics ($n = 507$). The remaining 40 studies were full text reviewed, and 11 of these studies were excluded because they were self-control studies ($n = 6$), included duplicate participants ($n = 3$), or used stool specimens ($n = 2$). Finally, a total of 29 publications in qualitative synthesis were used for the meta-analysis [9, 15, 21, 23–46]. Five studies were not suitable for quantitative analysis; therefore, the remaining studies were used for the meta-analysis.

Characteristics of the Included Studies

The main characteristics of the 29 studies are presented in Table 1. The 29 studies included a total of 10,486 subjects (3202 patients with CRC and 7284 controls) from studies published between 2008 and 2017. Among them, eight were published in Chinese, including three qualitative studies, and the others were in English. The detection methods and outcomes are showed in Table 2, including the numbers of true positives, false positives, false negatives, and true negatives. In addition, QUADAS quality evaluation results are showed in Fig. 2. The quality of each study was rated as "high,"

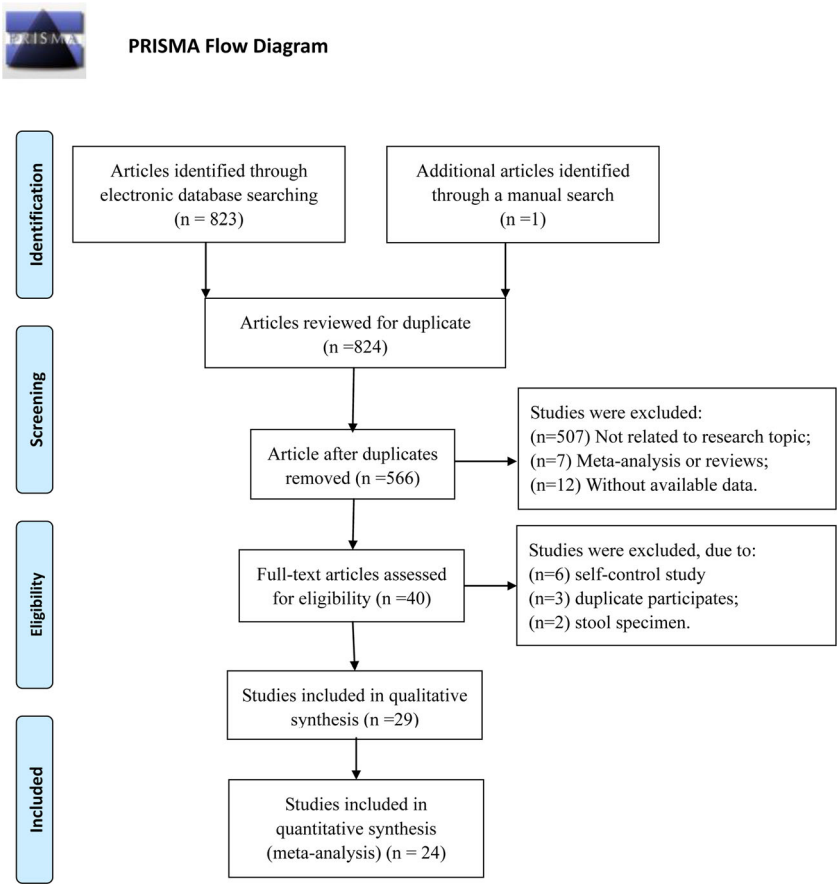

Fig. 1 Flow chart showing study selection procedure

Table 1 Main characteristics of all eligible studies

id	Author	Year	Area	Type of study	Case			Control			Cut-off	Participants of control group
					n	Age(y)	M/F	n	Age(y)	M/F		
1	Ahlquist DA	2012	USA	CC	30	69 (61–75)	NR	65	65 (52–75)	NR	Ct<45.0	43 healthy; 22 adenoma
2	Chen CH	2016	Taiwan	Cohort	51	NR	NR	9	NR	NR	NR	9 healthy
3	Church TR	2014	USA& Germany	Cohort	53	≥50	35/18	1457	≥50	654/803	Ct<50.0	934 healthy; 523 adenoma
4	deVos T	2009	Germany	CC	187	37–87	115/72	327	40–90	149/178	3.4 g/L	327 healthy
5	Ding QQ	2015	China	CC	82	60–82	47/35	100	60–99	NR	Ct<45.0	100 healthy; 80 polyps
6	Grutzmann R	2008	Germany	CC	378	61	220/160	453	59	211/242	NR	285 healthy; 168 polyps
7	He N	2014	China	CC	76	29–84	41/38	205	35–76	132/87	Ct<45.0	136 healthy; 69 polyps
8	He Q	2010	China	CC	182	58(28–85)	121/61	170	60	116/54	PMR ≥ 4%	170 healthy
9	He Q	2015	China	CC	50	62	NR	50	58 (28–85)	31/19	PMR ≥ 4%	50 healthy
10	Herbst A	2011	Germany	CC	45	NR	NR	16	NR	NR	NR	16 healthy
11	Jin P	2015	China	CC	135	28–84	69/66	341	20–81	147/194	Ct<45.0	91 healthy; 81 polyps; 169 adenoma
12	Johnson DA	2014	USA	Cohort	101	50–84	69/33	197	50–84	75/122	NR	94 healthy; 77 polyps; 26 adenoma
13	Kang Q	2014	China	CC	80	61.2 ± 12.5	36/44	52	52.4 ± 11.4	18/34	NR	52 healthy
14	Lee, HS	2013	Korea	CC	101	63.6 ± 11.1	52/49	96	NR	NR	NR	96 healthy
15	Li SJ	2015	China	CS	91	60.1 ± 13.0	58/33	47	52.1 ± 11.3	NR	NR	47 healthy; 23 polyps
16	Liu YQ	2013	Singapore	CC	26	67.1(46–83)	NR	26	NR	NR	NR	26 healthy, test set
					37	NR	NR	20	NR	NR	NR	20 healthy, exploratory set
17	Lofton-Day C	2008	USA	CC	133	65	NR	179	56	NR	NR	179 healthy
18	Orntoft, MB	2015	Denmark	CC	128	NR	63/65	171	NR	85/86	Ct<45.0	150 healthy; 21 adenoma
19	Potter, NT	2014	USA& Germany	Cohort	44	≥50	30/14	1500	≥50	789/711	Ct<45.0	444 healthy; 435 polyps; 621 adenoma
20	Song LL	2016	China	CC	369	NR	192/177	490	NR	396/294	Ct<45.0	490 healthy; 87 polyps; 113 adenoma
21	Su XL	2014	China	CC	172	61.29 ± 11.40	106/66	62	53.00 ± 13.77	26/36	PMR ≥ 1%	healthy
22	Tanzer M	2012	Germany	CC	33	27–79	NR	128	20–75	NR	NR	34 healthy; 94 polyps
23	Toth K	2012	Hungary	CC	92	67.8 ± 9.8	45/48	92	62.6 ± 9.9	58/36	Ct<40.5	92 healthy
24	Toth K	2014	Hungary	CC	34	68.3 ± 9.3	15/19	50	56 ± 13.4	26/24	PMR ≥ 0.01%	24 healthy; 26 adenoma
25	Wang Z	2012	China	CC	36	NR	NR	20	NR	NR	PMR ≥ 1%	20 healthy
26	Warren JD	2011	USA, Russia	CC	50	62(42–85)	NR	94	58(40–86)	NR	Ct<45.0	94 healthy
27	Wu D	2016	China	CC	45	52.5 ± 18.3	NR	189	52.5 ± 18.3	NR	Ct<41.0	93 healthy; 28 polyps; 68 adenoma
28	Wu D	2016	China	CC	291	NR	183/108	625	NR	304/321	NR	295 healthy; 117 polyps; 213 adenoma
29	Yu D	2015	China	CC	70	66.1 ± 11.7	41/29	53	63.8 ± 13.5	31/22	Ct<45.0	53 non-CRC

n, number; *NR*, not reported; *USA*, United States of America; *CC*, case-control study; *M*, male; *F*, female; *PMR*, Percentage of Methylated Reference, *CRC*, colorectal cancer

Table 2 The detection methods and outcomes of all eligible studies

Author	Year	Detection Methods	Kit used	Algorithm	Control	TP	FP	FN	TN
Ahlquist DA	2012	RT-PCR	Epi proColon 1.0	1/3	healthy	18	9	12	34
				1/3	adenoma	18	3	12	19
				1/3	Non-CRC	18	12	12	53
Chen CH	2016	RT-PCR	Epi proColon 1.0	1/3	healthy	24	1	27	8
Church TR	2014	RT-PCR	Epi proColon 1.0	1/2	healthy	27	80	26	854
				1/2	adenoma	27	46	26	477
				1/2	Non-CRC	27	126	26	1331
deVos T	2009	RT-PCR	Epi proColon 1.0	1/3	healthy	138	45	49	282
				2/3	healthy	105	11	82	316
Ding QQ	2015	RT-PCR	Epi proColon 2.0	2/3	healthy	60	4	22	96
				2/3	polyps	60	5	22	75
				2/3	Non-CRC	60	9	22	171
Grutzmann R	2008	RT PCR	NR	2/3	healthy	193	25	185	260
				2/3	polyps	193	25	185	143
				2/3	Non-CRC	193	50	185	403
He N	2014	RT-PCR	Epi proColon 2.0	2/3	healthy	54	5	22	131
				2/3	polyps	54	4	22	65
				2/3	Non-CRC	54	9	22	196
He Q	2010	MethyLight PCR	NR	NR	healthy	136	6	46	164
He Q	2015	MethyLight PCR	Research kit	NR	healthy	38	2	12	48
Herbst A	2011	MethyLight PCR	NR	NR	healthy	21	3	24	13
Jin P	2015	RT-PCR	Epi proColon 2.0	2/3	healthy	101	3	34	88
					polyps	101	5	34	76
					adenoma	101	35	34	134
					Non-CRC	101	43	34	298
Johnson DA	2014	RT-PCR	Epi proColon 1.0	NR	Non-CRC	74	37	27	163
Kang Q	2014	RT-PCR	Epi proColon 2.0	2/3	healthy	60	1	20	51
Lee, HS	2013	RT-PCR	Abbott Molecular	1/3	healthy	37	9	64	87
Li SJ	2015	RT-PCR	Epi proColon 2.0	2/3	healthy	66	4	25	43
					adenoma	66	1	25	22
					Non-CRC	66	5	25	65
Liu YQ	2013	RT PCR	NR	1/2	healthy	33	5	30	43
Lofton-Day C	2008	Heavy MethyLight PCR	NR	NR	healthy	92	25	41	154
Orntoft, MB	2015	RT-PCR	Epi proColon 2.0	1/3	healthy	93	27	35	123
					adenoma	93	3	35	18
					Non-CRC	93	30	35	141
				2/3	healthy	75	7	53	143
					adenoma	75	0	53	21
					Non-CRC	75	7	53	164
Potter, NT	2014	RT-PCR	Epi proColon 2.0	1/3	healthy	30	97	14	347
					polyps	30	87	14	348
					adenoma	30	134	14	487
					Non-CRC	30	318	14	1182
Song LL	2016	RT-PCR	Epi proColon 2.0	1/3	healthy	303	88	66	402
					polyps	303	23	66	64
					adenoma	303	42	66	71
					Non-CRC	303	153	66	537
				2/3	healthy	277	14	92	476
					polyps	277	8	92	79

Table 2 (continued)

Author	Year	Detection Methods	Kit used	Algorithm	Control	TP	FP	FN	TN
					adenoma	277	30	92	83
					Non-CRC	277	52	92	638
				3/3	healthy	214	6	155	484
					polyps	214	3	155	84
					adenoma	214	17	155	96
					Non-CRC	214	26	155	664
Su XL	2014	MS-PCR-DHPLC	NR	NR	healthy	150	4	22	58
Tanzer M	2010	Heavy MethyLight PCR	Epi proColon 1.0	1/3	healthy	27	4	6	30
					polyps	27	43	6	51
					Non-CRC	27	47	6	81
				2/3	healthy	24	3	9	31
					polyps	24	27	9	67
					Non-CRC	24	30	9	98
Toth K	2012	RT-PCR	Epi proColon 2.0	1/3	healthy	88	14	4	78
				2/3	healthy	73	1	19	91
Toth K	2014	RT-PCR	Epi proColon 2.0	NR	healthy	30	2	4	22
					adenoma	30	8	4	18
					Non-CRC	30	10	4	40
Wang Z	2012	MS-HRM PCR	research kit	1/1	healthy	25	2	11	18
Warren JD	2011	RT-PCR	Epi proColon 1.0	1/3	healthy	45	11	5	83
				2/3	healthy	38	1	12	93
				3/3	healthy	35	0	15	94
Wu D	2016	RT-PCR	Epi proColon 2.0	1/1	healthy	36	1	9	92
					polyps	36	0	9	28
					adenoma	36	7	9	61
					Non-CRC	36	8	9	181
Wu D	2016	RT-PCR	New SEPT9 Assay	NR	healthy	223	12	68	283
Yu D	2015	RT-PCR	Epi proColon 2.0	2/3	Non-CRC	57	7	13	46

RT, real time; *MS-PCR*, Methylation-specific polymerase chain reaction; *DHPLC*, Denaturing high-performance liquid chromatography; *MS-HRM*, Methylation-sensitive high resolution melting curve, *TP*, true-positive; *FP*, false-positive; *FN*, false-negative; *TN*, true-negative

"unclear," or "low." With the exception of the criteria for the QUADAS03 and QUADAS11 terms, all studies had a good consistency in reviewing and reporting results, which indicated a relatively high quality for the set of eligible studies.

Meta-Analysis for the Diagnostic Effect of SEPT9 Methylation in CRC

As shown in Table 3, the 1/3 and 2/3 algorithms were used in the present study. When compared with healthy subjects, the pooled sensitivity with corresponding 95% confidence intervals (CIs) of *SEPT9* methylation for the diagnosis of CRC in patients was 0.74 (95% CI: 0.61–0.84) in the 1/3 algorithm group, whereas the specificity was 0.96 (95% CI: 0.95–0.97) in the 2/3 algorithm group. In addition, AUC of *SEPT9* methylation for the diagnosis of CRC was high in the 2/3 algorithm group (0.95 (95% CI: 0.92–0.96) vs. 0.86 (0.83–0.89)) the 1/3 algorithm group. These indices indicated a high diagnostic value of *SEPT9* gene methylation CRC patients compared with the healthy ones. However, PLR was <10 and NLR was >0.1 in the 1/3 algorithm group.

When compared with patients with polyps, the pooled sensitivity and specificity of *SEPT9* methylation for the diagnosis of CRC in patients in the 2/3 algorithm group were 0.69 (95% CI: 0.61–0.76) and 0.90 (95% CI: 0.83–0.94), respectively. DOR and AUC of *SEPT9* methylation for the diagnosis of CRC in the 2/3 algorithm group were 19.43 (95% CI: 8.69–43.47) and 0.85 (95% CI: 0.82–0.88), respectively.

When compared with patients with adenoma, the sensitivity and AUC of *SEPT9* methylation for the diagnosis of CRC were similar in patients in the 1/3 and 2/3 algorithm groups. The specificity of *SEPT9* methylation for the diagnosis of CRC in the 2/3 algorithm group was 0.91 (95% CI: 0.65–0.98), whereas the specificity of *SEPT9* methylation for the diagnosis of CRC in 1/3 algorithm group was only 0.77 (95% CI: 0.67–0.84). In addition, PLR, NLR, and DOR of *SEPT9*

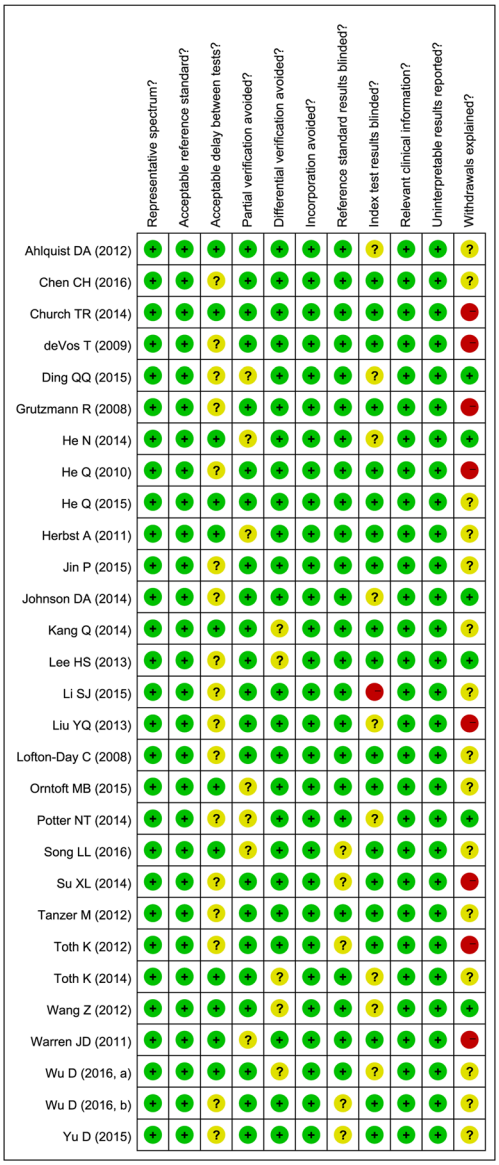

Fig. 2 Quality assessment of included studies by the guidelines of Quality Assessment of Diagnostic Accuracy Studies (QUADAS) criteria

methylation for the diagnosis of CRC in the 1/3 algorithm group were 3.13 (95% CI: 2.24–4.37), 0.35 (95% CI: 0.27–0.46), and 8.94 (95% CI: 5.76–13.88), respectively. PLR, NLR, and DOR in the 2/3 algorithm group were 7.49 (95% CI: 1.79–31.30), 0.33 (95% CI: 0.28–0.39), and 22.75 (95% CI: 5.28–98.07), respectively.

For patients in the non-CRC group, the sensitivity and AUC of *SEPT9* methylation for the diagnosis of CRC were similar in the 1/3 and 2/3 algorithm groups. The specificity of *SEPT9* methylation for the diagnosis of CRC in the 2/3 algorithm group was 0.91 (95% CI: 0.87–0.94), whereas the specificity of *SEPT9* methylation for the diagnosis of CRC in the 1/3 algorithm group was 0.78 (95% CI: 0.72–0.82). In addition, PLR, NLR, and DOR in the 1/3 algorithm group were 3.41 (95% CI: 2.78–4.19), 0.31 (95% CI: 0.22–0.46), and 24.71 (95% CI: 15.34–39.81), respectively. PLR, NLR, and DOR in the 2/3 algorithm group were 8.08 (95% CI: 5.54–11.79), 0.33 (95% CI: 0.27–0.40), and 11.15 (95% CI: 7.46–16.67), respectively.

Publication Bias

The publication bias was assessed based on the group with the most included studies (CRC vs. healthy, Algorithm = 2/3). The *P* value of Deeks' funnel plot was 0.36 (Fig. 3), suggesting that no significant publication bias existed in this meta-analysis.

Discussion

It is clear that patients with CRC benefit from early diagnosis. *SEPT9* methylation has been reported as a good biomarker for the early detection of CRC [47]. In this meta-analysis, we evaluated the diagnostic effect of *SEPT9* gene methylation on CRC. Two types of algorithm, 1/3 and 2/3, were used in the present study. Five studies were not suitable for use for meta-analysis because they did not use this algorithm [9, 15, 27, 40, 41]; for examples, Church et al used multiple polymerase chain reaction replicates according to the following algorithm: the final outcome was positive when determined from at least one positive outcome from two repeats [9]. He *at al* detected *ALX4* and *SEPT9* methylation in CRC using multiplex MethyLight assay according to the percentage of methylated reference [27]. Therefore, the remainder 24 studies were used for meta-analysis.

Sensitivity was high in the 1/3 algorithm group but specificity was low. Sensitivity, also known as probability of detection, measures the proportion of positives that are correctly identified as having CRC in the study [48]. A high specificity in our study will increase the positive predictive value of *SEPT9* in screening CRC and thereby reduce the number of false positives. In addition, AUC and DOR were applied to investigate the overall test performance and the compactness between cases and diagnostic efficiency. Consequently, AUC of SROC was >0.8 in all algorithm groups, and DOR in the 2/3 algorithm was high, suggesting a good diagnostic effect of SEPT9 methylation. Therefore, these results confirm that *SEPT9* methylation is a good biomarker for CRC in both 1/3 and 2/3 algorithm. However, PLR was <10 and NLR was >0.1 in the 1/3 algorithm group for patients with CRC vs. healthy controls. The diagnostic criteria were as follows: PLR > 10 and NLR < 0.1 represented exclusion and confirmation; PLR > 10 and NLR > 0.1 represented only confirmation; PLR < 10 and NLR < 0.1 represented only exclusion; PLR < 10 and NLR > 0.1 represented no exclusion or confirmation [22]. In addition, PLR was >10 and NLR was >0.1 in the 2/3 algorithm group for patients with CRC vs. healthy controls. Therefore, the 2/3 algorithm was recommended for *SEPT9* methylation detection between CRC and health controls.

Table 3 The results of meta-analysis for the diagnostic effect of SEPT9 methylation in CRC

Algorithm	n	Sensitivity	Specificity	PLR	NLR	DOR	SROC
CRC vs. Healthy							
1/3	10	0.74 (0.61, 0.84)	0.84 (0.81, 0.87)	4.63 (3.67, 5.84)	0.31 (0.19, 0.48)	15.11 (7.95, 28.74)	0.86 (0.83, 0.89)
2/3	12	0.69 (0.64, 0.74)	0.96 (0.95, 0.97)	19.58 (13.36, 28.71)	0.32 (0.26, 0.38)	61.87 (36.48, 104.93)	0.95 (0.92, 0.96)
NR	6	0.75 (0.66, 0.83)	0.93 (0.89, 0.96)	11.52 (6.23, 21.30)	0.26 (0.18, 0.38)	43.63 (17.37, 109.55)	0.93 (0.90, 0.95)
CRC vs. polyps							
2/3	6	0.69 (0.61, 0.76)	0.90 (0.83, 0.94)	6.69 (3.71, 12.06)	0.34 (0.26, 0.45)	19.43 (8.69, 43.47)	0.85 (0.82, 0.88)
CRC vs. adenoma							
1/3	4	0.73 (0.64, 0.81)	0.77 (0.67, 0.84)	3.13 (2.24, 4.37)	0.35 (0.27, 0.46)	8.94 (5.76, 13.88)	0.81 (0.78, 0.84)
2/3	4	0.70 (0.63, 0.76)	0.91 (0.65, 0.98)	7.49 (1.79, 31.30)	0.33 (0.28, 0.39)	22.75 (5.28, 98.07)	0.79 (0.75, 0.82)
CRC vs. non-CRC							
1/3	5	0.76 (0.67, 0.83)	0.78 (0.72, 0.82)	3.41 (2.78, 4.19)	0.31 (0.22, 0.42)	24.71 (15.34, 39.81)	0.84 (0.80, 0.87)
2/3	9	0.70 (0.64, 0.76)	0.91 (0.87, 0.94)	8.08 (5.54, 11.79)	0.33 (0.27, 0.40)	11.15 (7.46, 16.67)	0.88 (0.85, 0.91)

PLR, positive likelihood ratio; *NLR*, negative likelihood ratio; *DOR*, diagnostic odds ratio; *SROC*, summary receiver operating characteristic

Although the 2/3 algorithm was accepted to be a good test for *SEPT9* methylation in the diagnosis of CRC, PLR was <10 and NLR was >0.1 in the 2/3 algorithm group for patients with CRC vs. polyps and adenoma. Therefore, *SEPT9* methylation for the diagnosis of CRC partly caused misdiagnosis based on the 2/3 algorithm between CRC vs. polyps or adenoma. Therefore, the 2/3 algorithm with a high specificity should be applied for early detection of CRC other than screening the difference among CRC and polyps and adenoma.

Some limitations in our study deserve consideration. First, the sample sizes of the included studies were relatively small. Second, the age and gender data in certain studies were missing, which may have affected the overall results. Third, the research methods were not unified, which may have led to minor differences in the results of this meta-analysis. Therefore, further prospective studies with larger sample sizes are required to confirm our findings and provide a more accurately representative statistical analysis.

Conclusion

In conclusion, under the 2/3 algorithm, *SEPT9* methylation can diagnose CRC from healthy individuals, but it also causes a certain degree of misdiagnosis. In addition, the determination of *SEPT9* methylation does not distinguish well between CRC and types of precancerous lesions (such as polyps and adenoma).

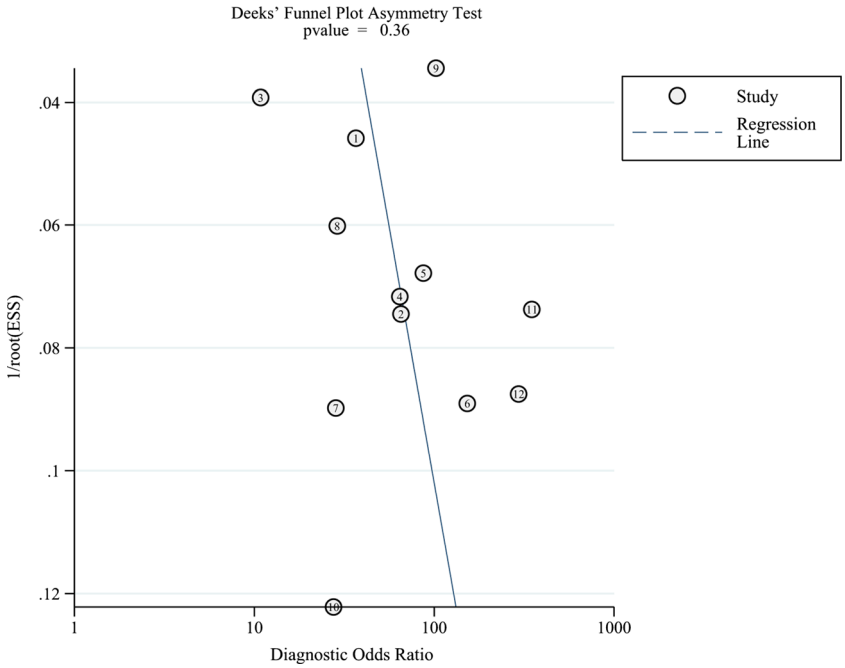

Fig. 3 Results of publication bias. *P* value of Deeks' funnel plot was 0.36

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest.

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