Diagnostic Performance of Intestinal *Fusobacterium nucleatum* in Colorectal Cancer: A Meta-Analysis

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Abstract

Background: Increasing evidence has supported the link of intestinal *Fusobacterium nucleatum* infection to colorectal cancer (CRC). However, the value of *F. nucleatum* as a biomarker in CRC detection has not been fully defined. In order to reduce the random error and bias of individual research, this meta-analysis aimed to evaluate the diagnostic performance of intestinal *F. nucleatum* in CRC patients and provide evidence-based data to clinical practice.

Methods: An article search was performed from PubMed, Embase, Cochrane Library, and Web of Science databases up to December 2017, using the following key words: "*Fusobacterium nucleatum*", "*Fusobacterium* spp.", "*Fn*", "colorectal cancer(s)", "colorectal carcinoma(s)", "colorectal neoplasm(s)", and "colorectal tumor(s)". Articles on relationships between *F. nucleatum* and CRC were selected according to the preestablished inclusion and exclusion criteria. This meta-analysis was performed using STATA 12.0 software, which included mapping of forest plots, heterogeneity tests, meta-regression, subgroup analysis, sensitivity analysis, and publication bias. The sensitivity, specificity, positive likelihood ratio (LR), negative LR, diagnostic odds ratio (DOR), and their corresponding 95% confidence interval (*CI*) of each eligible study were summarized.

Results: Finally, data for 1198 participants (629 CRC and 569 healthy controls) in 10 controlled studies from seven articles were included. The summary receiver operator characteristic curve was mapped. The diagnostic performance of intestinal *F. nucleatum* infection on CRC was as follows: the area under the curve: 0.86 (95% *CI*: 0.83–0.89), the pooled sensitivity: 0.81 (95% *CI*: 0.64–0.91), specificity: 0.77 (95% *CI*: 0.59–0.89), and DOR: 14.00 (95% *CI*: 9.00–22.00).

Conclusion: Intestinal *F. nucleatum* is a valuable marker for CRC diagnosis.

Key words: Colorectal Neoplasms; Diagnosis; *Fusobacterium nucleatum*; Meta-Analysis

INTRODUCTION

Colorectal cancer (CRC) ranks as the third-most common malignancy and the fourth leading cause of cancer-related deaths worldwide. CRC is more prevalent in Western than in developing countries. In recent years, the prevalence of CRC in Asian-Pacific areas increases rapidly.[1,2] The pathogenesis of CRC involves a multistep process including histological, morphological, and genetic changes. The transformation takes time from normal bowel epithelium to a precancerous state, i.e., adenomatous lesions (polyps), and finally to adenocarcinoma. Reduction of the incidence of CRC and its associated mortality primarily depend on the detection of premalignant and early-stage malignant colorectal neoplasia (polyps) and resection of the lesions before they become cancerous. As most CRCs grow slowly over time, development of diagnostic methods with good sensitivity and specificity for the early CRC is crucially important in clinical practice. Population-wide screening and prevention programs have been recommended by many consensus and guidelines, and various diagnostic strategies have been carried out in different countries according to their economic resources.[2]

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Colonoscopy, which can provide convincing diagnosis with histology-based protocols, has been well established as the gold standard in CRC diagnosis. However, it is not an ideal tool in practice because not all patients are willing to accept its invasive nature. Advancing age, male gender, family history of CRC, smoking, and obesity are the risk factors for CRC. Several risk-stratified scoring systems, such as High Risk Factor Questionnaire and Quantitative Risk-Assessment Method, have been recommended to evaluate the degrees of CRC risk.[3,4] Colonoscopy is obligatory for patients at high risk and patients with positive signs, suggesting neoplastic lesions in order to discover and manage the possible lesions.[3,5-7]

Noninvasive methods have been applied to appeal to patients at average risk. Fecal occult blood tests (FOBTs) including guaiac-based FOBT (gFOBT) and fecal immunochemical test (FIT) have been commonly used clinically for a long time. FOBT is an inexpensive, simple, and widely available test, but its accuracy needs improvement.[8] As the development of CRC requires the accumulation of gene alterations, identification of aberrant gene alterations is theoretically an effective method for CRC detection. Fecal DNA tests or stool DNA (sDNA) testing has been developed, which has the advantages of being highly sensitive and convenient. The sDNA tests measure molecular biomarkers including a wide variety of genetic alterations such as mutant adenomatous polyposis coli, *KRA*, actin, *FIT*, tumor protein P53, retinoic acid receptor beta 2, p16, vimentin (*VIM*), tissue factor pathway inhibitor 2 (*TFPI2*) and aberrantly methylated VIM, *TFPI2*, N-Myc downstream-regulated gene 4 protein, bone morphogenetic protein 3, and secreted frizzled-related protein 2. Some of them are based on detecting DNA alteration (mutations and methylations) in a single gene, while others in a number of genes. The pooled sensitivities in multigene testing were higher than that in single gene, but the specificity remained similar to each other.[5,9-11] In 2014, a stool multitarget sDNA (Mt-sDNA) test consisting of multitarget mutant DNA, aberrantly methylated DNA across CRC-related genes, and FIT was commercially available. Mt-sDNA had high sensitivity, similar specificity, and high costs compared with those of FIT alone.[12] In 2016, the first blood-based test, i.e., mSEPT9 assay was evolved, as hypermethylated DNA of septin 9 gene might be shed into the bloodstream from CRC tissues and be detected. The mSEPT9 serves to increase the participants in CRC detection, although its sensitivity and specificity are similar to FIT.[8,13] Other noninvasive screening tests, including microRNAs and stool proteins, have been explored, but still are in their infancy and need further evaluation.[14]

Currently, the gut microbiome is increasingly recognized to play an important role in the pathogenesis of diseases. The gut dysbiosis exposes the colon to different metabolic and inflammatory stimuli, increases DNA mutations, and eventually develops CRC. Several bacterial species including *Fusobacterium* spp*., Enterococcus faecalis, enterotoxigenic Bacteroides fragilis, enteropathogenic Escherichia coli,* *Parvimonas micra ATCC 33270, Streptococcus anginosus,* and *Proteobacteria* were significantly increased in stools from patients with CRC compared to controls.[15,16] Among them, *Fusobacterium nucleatum* (*F. nucleatum, Fn*), a human's oral cavity colonizer, has been frequently reported. Besides periodontal disease, *F. nucleatum* is involved in a wide spectrum of disorders including gastrointestinal diseases, respiratory tract infections, cardiovascular diseases, rheumatoid arthritis, Lemierre's syndrome, and Alzheimer's disease.[17] Accumulating evidence has revealed that *F. nucleatum* overabundance was present in tumor tissues and stool samples of CRC patients compared with normal controls.[18-20] Two previous studies also demonstrated that the prevalence of *F. nucleatum* was significantly higher in CRC tissues than those in controls and was associated with CRC progress and metastasis.[16,21] Although mechanisms and causalities between *F. nucleatum* and CRC have been still uncovered, some studies have explored the diagnostic performance of the presence of *F. nucleatum* in feces or tumor tissues in CRC patients.[22] This meta-analysis summarized the published data in order to evaluate the value of this novel technique in CRC diagnosis.

METHODS

Data sources

A comprehensive literature search was performed from the following electronic databases: PubMed, Embase, Cochrane Library, and Web of Science. The search period was up to December 2017. The following keywords were used in the database literature search: "*Fusobacterium nucleatum*", "*Fusobacterium* spp.", "*F. nucleatum*", "*Fn*", "colorectal cancer(s)", "colorectal carcinoma(s)", "colorectal neoplasm(s)", and "colorectal tumor(s)". The study was performed according to the Meta-analysis of Observational Studies in Epidemiology guidelines [Supplemental File 1].[23]

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) a clinical study of CRC patients and healthy controls with intact data of *F. nucleatum*-positive and *F. nucleatum*-negative cases; (2) studies that reported necessary data for calculating true-positive (TP), false-positive (FP), true-negative (TN), and false-negative (FN) *F. nucleatum* in CRC detection; (3) the diagnosis of CRC should be based on histology; (4) the detection of *F. nucleatum* should be based on quantitative polymerase chain reaction analysis, fluorescence *in situ* hybridization, or 16S rRNA sequencing; (5) the samples (feces or tissues) should be stored at −20°C to −80°C soon after collection; and (6) the articles should be original articles.

The exclusion criteria were as follows: (1) the samples in control group were collected from adjacent nontumor tissues of CRC patients, but not from healthy controls; (2) the results of studies did not include TP, FP, FN, and TN *F. nucleatum*; (3) only the abstract forms were presented; and (4) articles that were not original articles but reviews or other compositions.

Data extraction and assessment

The data from each study were independently extracted by two independent reviewers and then reciprocally verified using the predefined selection criteria. Disagreements of two reviewers were resolved in consultation with a third investigator. The following information from each article was extracted: the first author's name, the year of publication, patient ethnicity, sample size, sample type, diagnostic techniques of CRC and *F. nucleatum*, and clinicopathological characteristics of CRC patients and healthy controls. The parameters in results were summarized by bivariate mixed-effects models. The pooled TPs, FPs, TNs, and FNs, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and their 95% confidence interval (*CI*) were extracted for mapping forest plot.

The quality of each study was assessed according to the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) recommended by the Cochrane Collaboration. QUADAS tool includes 14 items covering patient spectrum, reference standard, disease progression bias, verification bias, review bias, clinical review bias, incorporation bias, test execution, study withdrawals, and indeterminate results. Each of the 14 items in the QUADAS checklist is scored as "yes", "no", or "unclear". QUADAS tool allows for more transparent rating of bias and applicability of primary diagnostic accuracy studies. If the QUADAS score is <10 points, the study is classified as having low methodological quality.

Statistical analysis

All analyses were conducted by the STATA-12.0 software (Stata Corp., College Station, TX, USA). The numerical variables with normal distribution were expressed as mean ± standard deviation (SD), and qualitative variables were calculated as numbers and percentages. The 95% *CI*s were estimated for each predictive test. A two-tailed $P < 0.05$ was considered statistically significant. Bivariate mixed-effects models were used to process variable combination. The strength of association was represented as an overall odds ratio with corresponding 95% *CI*. The pooled sensitivity, specificity, and area under curve (AUC) of the summary receiver operator characteristic (SROC) with their 95% *CI*s were analyzed to determine the diagnostic accuracy of *F. nucleatum* for CRC detection. Between-study heterogeneity was assessed using Chi-square-based Q-statistic test and I^2 test. If the Q-test showed $P < 0.10$ and $I^2 > 50\%$, which indicated significant heterogeneity, the random effects model (DerSimonian-Laird method) was conducted; otherwise if the Q-test showed $P > 0.10$ and $I^2 < 50\%$, the fixed effect model (Mantel-Haenszel method) was used. The sources of heterogeneity between studies were evaluated by meta-regression and subgroup analyses. Funnel plots were used to evaluate a potential publication bias, which was quantitatively estimated with Egger's linear regression test. If a potential publication bias existed, the meta-trim method was conducted.

RESULTS

Study characteristics

A total of 142 articles were identified using the search strategy described above. Totally, 135 articles were excluded after careful filtration. Among them, 32 articles were duplicates, 93 had inappropriate abstracts and titles, and 10 articles did not provide necessary data mentioned in inclusion criteria, i.e., TP, FP, TN, or FN. Among these 10 articles, data were unavailable in 9 articles and insufficient in 1 article. Finally, 7 articles with 10 studies were included in this meta-analysis, which involved 1198 participants (629 CRC patients and 569 healthy controls). The selection process is illustrated in Figure 1. Basic characteristics and *F. nucleatum* performance in CRC detection of these 10 studies are shown in Table 1.

Heterogeneity assessment

The performance of *F. nucleatum* for CRC diagnosis is shown in forest plot [Figure 2]: pooled sensitivity: 0.81 (95% *CI*: 0.64–0.91), specificity: 0.77 (95% *CI*: 0.59–0.89), PLR: 3.60 (95% *CI*: 2.10–6.00), NLR: 0.25 (95% *CI*: 0.15–0.42), and DOR: 14.00 (95% *CI*: 9.00–22.00).

The sensitivity heterogeneity ($I^2 = 83.2\%$, 95% *CI*: 73.79–92.63, $P < 0.01$) and specificity heterogeneity ($I^2 = 86.8\%$, 95% *CI*: 79.85–93.73, $P < 0.01$) indicated significant heterogeneity between studies [Figure 2]. SROC distributed advisably [AUC: 0.86, 95% *CI*: 0.83–0.89; Figure 3], and Spearman's rank correlation coefficient was –0.98, calculated by an equation using logarithm of sensitivity and 1–specificity. Which indicated no statistical significance ($P = 0.97$). Both of them suggested that the pooled data in this meta-analysis were feasible since threshold effects did not exist.

Meta-regression analysis

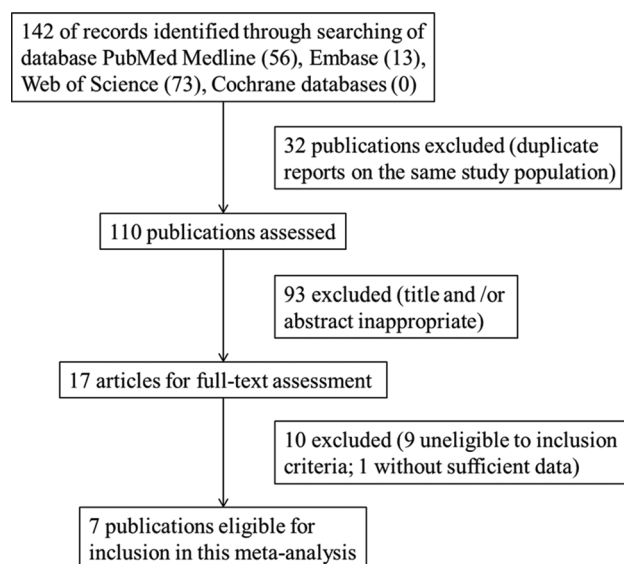As forest plot revealed significant heterogeneity between studies, single-factor meta-regression analysis was applied

Figure 1: Flow diagram of the step-wise selection for this meta-analysis.

Table 1: Basic characteristics and diagnostic performance of *F. nucleatum* in CRC

Authors	Year	Country	Sample types	Detection methods	QUADAS scores	Sample collection	Sample size, *n*	TP, *n*	FN, *n*	FP, *n*	TN, *n*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Wong *et al.*[24]	2017	China	Feces	Real-time qPCR	12	Colonoscopy	206	75	29	9	93	72.1	91.2	89.3	76.2
Wong *et al.*[24]	2017	China	Feces	Real-time qPCR	12	Colonoscopy	119	21	2	19	77	91.3	80.2	52.5	97.5
Liang *et al.*[25]	2017	China	Feces	Probe-based duplex qPCR	11	Colonoscopy	370	132	38	41	159	77.7	79.5	76.3	80.7
Liang *et al.*[25]	2017	China	Feces	Probe-based duplex qPCR	11	Colonoscopy	69	27	6	17	19	81.8	52.8	61.4	76.0
Yu *et al.*[26]	2016	China	CRC tissues	FISH	11	Colonoscopy	113	62	31	4	16	66.7	80.0	93.9	34.0
Suehiro *et al.*[27]	2017	Japan	Feces	Droplet digital PCR	11	Colonoscopy or surgical operation	218	85	73	6	54	53.8	90.0	93.4	42.5
Kostic *et al.*[20]	2013	USA	Feces	qPCR	10	Surgical operation	58	27	0	15	16	100.0	51.6	64.3	100.0
Mira-Pascual *et al.*[19]	2015	Spain	Feces	Real-time qPCR	10	Colonoscopy	16	6	1	2	7	85.7	77.8	75.0	87.5
Mira-Pascual *et al.*[19]	2015	Spain	CRC tissues	Real-time qPCR	10	Colonoscopy	12	2	5	0	5	28.6	100.0	100.0	50.0
Fukugaiti *et al.*[28]	2015	Brazil	Feces	Real-time qPCR	10	Colonoscopy	17	7	0	9	1	100.0	10.0	43.8	100.0

TP: True positive; FN: False negative; FP: False positive; TN: True negative; PPV: Positive predictive value; NPV: Negative predictive value; CRC: Colorectal cancer; *F. nucleatum*: *Fusobacterium nucleatum*; qPCR: Quantitative polymerase chain reaction; FISH: Fluorescence *in situ* hybridization; QUADAS: Quality Assessment of Diagnostic Accuracy Study.
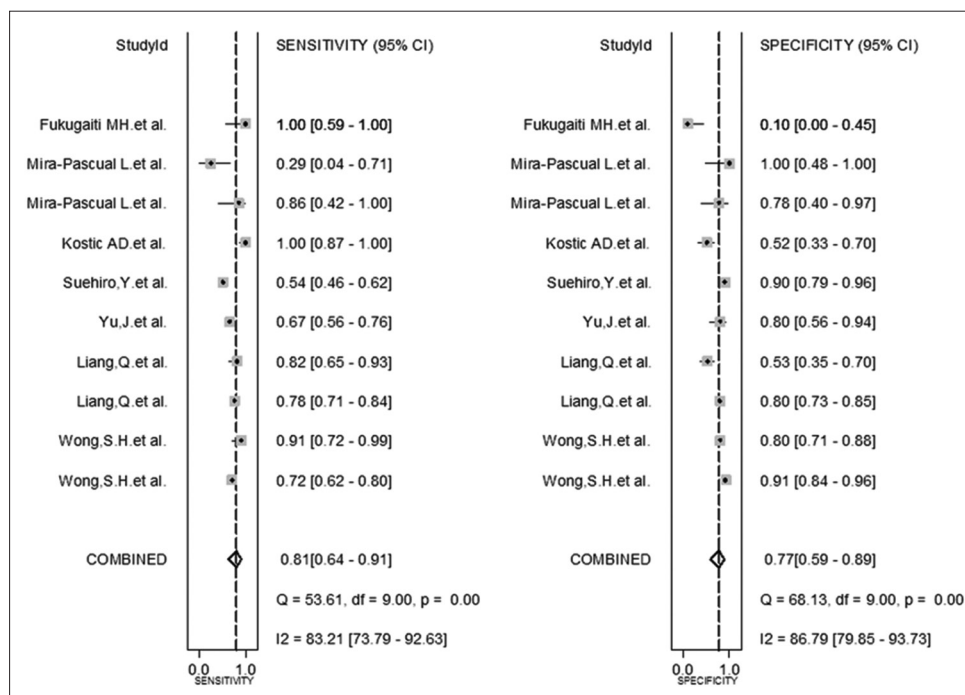

Figure 2: Forest plot of the pooled diagnostic accuracy of *Fusobacterium nucleatum* for colorectal cancer detection. *CI*: Confidence interval.

to screen the potential variables impacting on the pooled data. The results showed that race, sample type, sample size, and QUADAS scores were not the source of heterogeneity [all *P* > 0.05; Table 2].

Subgroup analysis

As meta-regression analysis failed to identify any source of heterogeneity, subgroup analyses were performed. Since the data from eligible literature were limited, only three subgroup analyses, i.e., race, sample type, and sample size were carried out. The results showed significant heterogeneity in Asian and non-Asian subgroups. In six Asian subgroups, the pooled sensitivity was 0.74 (95% *CI*: 0.63–0.82, $I^2 = 84.4\%$, $P < 0.01$) and specificity was 0.82 (95% *CI*: 0.72–0.90, $I^2 = 82.4\%$, $P < 0.01$), and in four non-Asian subgroups, the pooled sensitivity

was 0.97 (95% *CI*: 0.30–1.00, $I^2 = 88.5\%$, $P < 0.01$) and specificity was 0.65 [95% *CI*: 0.20–0.94, $I^2 = 78.9\%$, $P < 0.01$; Table 3]. Significant heterogeneity existed between the subgroups with different sample size. In five subgroups with sample size >100, the pooled sensitivity was 0.71 (95% *CI*: 0.61–0.80, $I^2 = 86.4\%$, $P < 0.01$) and specificity was 0.85 (95% *CI*: 0.79–0.90, $I^2 = 57\%$, $P = 0.05$), and in five subgroups, with sample size ≤100, the pooled sensitivity was 0.91 (95% *CI*: 0.47–0.99, $I^2 = 83.3\%$, $P < 0.01$) and specificity was 0.58 (95% *CI*: 0.27–0.84, $I^2 = 71.6\%$, $P = 0.01$). Significant heterogeneity was also found in sample type subgroups. In eight subgroups of stool studies, the pooled sensitivity was 0.86 (95% *CI*: 0.70–0.94, $I^2 = 85.5\%$, $P < 0.01$) and specificity was 0.72 (95% *CI*: 0.51–0.86, $I^2 = 89.5\%$, $P < 0.01$). Due to only two subgroups of CRC tissue studies, analysis data were unavailable [Table 3].

Sensitivity analysis
In order to obtain the stability of the pooled data and evaluate the influence of single study on whole studies, a sensitivity analysis was conducted. The alteration of efficient variables was not statistically significant ($P > 0.05$), which suggested that the polled results were not interfered by the data from single studies [Table 4].

Fagan plot
When prior probability was set at 0.20, the LRs of positive postprobability and negative postprobability were 0.47 and 0.06, respectively. The results implied that a positive result of intestinal *F. nucleatum* infection increased 47.0% possibility of CRC presence, whereas a negative result decreased 6.0% in comparison with pretest possibility. When prior probability was set at 0.50, the LRs of positive postprobability and negative postprobability were 0.78 and 0.20, respectively. The results implied that a positive result increased 78.0% possibility of CRC presence, whereas a negative result decreased 20.0% in comparison with pretest possibility [Figure 4].

Bias of publication
Funnel plot and Egger's linear regression tests were conducted to evaluate potential publication bias. The spots were symmetrically distributed in Funnel plot and no significance was found in Egger's plot ($t = -0.19$, $P = 0.86$). Both suggested no publication bias in the analysis [Figures 5 and 6].

Figure 3: SROC assessment of diagnostic performance of *Fusobacterium nucleatum* for colorectal cancer. SROC: Summary receiver operator characteristic curve.

Discussion

The ideal strategies for CRC diagnosis should be efficient, safe, convenient, and cheap. So far, gFOBT/FIT followed by colonoscopy have been recommended as standard CRC program in most guidelines. Colonoscopy is obligatory for obtaining histological diagnosis.
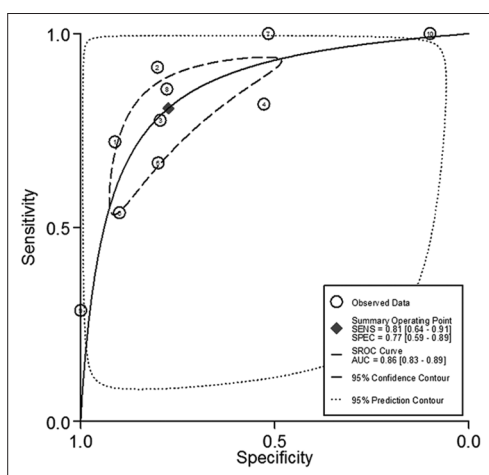
Table 2: Single-factor meta-regression analysis for sources of heterogeneity between studies

Variables	Coefficient	SE	*t*	*P*	95% *CI*
Race	0.02	0.85	0.02	0.98	−1.94–1.98
Sample type	−0.66	0.70	−0.95	0.37	−2.27–0.94
Sample size	0.00	0.00	0.54	0.60	−0.00–0.01
QUADAS scores	0.71	0.35	2.06	0.07	−0.09–1.52

SE: Standard error; QUADAS: Quality Assessment of Diagnostic Accuracy Study; *CI*: Confidence interval.

Table 3: Subgroup analysis for sources of heterogeneity between studies

Variables	AUC (95% *CI*)	Sensitivity (95% *CI*)	Specificity (95% *CI*)	PLR (95% *CI*)	NLR (95% *CI*)	DOR (95% *CI*)
Pooled data	0.86 (0.83–0.89)	0.81 (0.64–0.91)	0.77 (0.59–0.89)	3.60 (2.10–6.00)	0.25 (0.15–0.42)	14.00 (9.00–22.00)
Race						
Asian	0.84 (0.81–0.87)	0.74 (0.63–0.82)	0.82 (0.72–0.90)	4.20 (2.70–6.40)	0.32 (0.24–0.43)	13.00 (8.00–21.00)
Non-Asian	0.90 (0.87–0.92)	0.97 (0.30–1.00)	0.65 (0.20–0.94)	2.80 (0.80–9.60)	0.05 (0.00–1.82)	57.00 (3.00–1163.00)
Sample type						
Stool	0.87 (0.83–0.89)	0.86 (0.70–0.94)	0.72 (0.51–0.86)	3.00 (1.80–5.10)	0.20 (0.11–0.37)	15.00 (8.00–27.00)
CRC tissues	NA	NA	NA	NA	NA	NA
Sample size						
>100	0.87 (0.84–0.90)	0.71 (0.61–0.80)	0.85 (0.79–0.90)	4.90 (3.60–6.50)	0.34 (0.25–0.45)	14.00 (10.00–21.00)
≤100	0.81 (0.77–0.84)	0.91 (0.47–0.99)	0.58 (0.27–0.84)	2.20 (1.20–4.20)	0.16 (0.03–0.96)	14.00 (3.00–76.00)

AUC: Area under the curve; PLR: Positive likelihood ratio; NLR: Negative likelihood ratio; DOR: Diagnostic odds ratio; *CI*: Confidence interval; NA: Not applicable; CRC: Colorectal cancer.

Table 4: Sensitivity analysis for stability of the pooled data and influence of single study on all studies

Studies	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)
Wong et al.[24]	NA	NA	NA	NA	NA	NA
Wong et al.[24]	0.85 (0.82–0.88)	0.80 (0.62–0.90)	0.77 (0.56–0.90)	3.50 (1.90–6.30)	0.26 (0.16–0.44)	13.00 (9.00–19.00)
Liang et al.[25]	0.86 (0.83–0.89)	0.82 (0.61–0.93)	0.77 (0.56–0.90)	3.50 (1.90–6.50)	0.23 (0.11–0.47)	15.00 (8.00–29.00)
Liang et al.[25]	0.88 (0.84–0.90)	0.83 (0.59–0.94)	0.79 (0.61–0.90)	4.00 (2.40–6.60)	0.22 (0.09–0.51)	18.00 (10.00–33.00)
Yu et al.[26]	0.87 (0.83–0.89)	0.83 (0.64–0.93)	0.76 (0.55–0.89)	3.50 (1.90–6.30)	0.23 (0.12–0.43)	15.00 (9.00–25.00)
Suehiro et al.[27]	0.86 (0.83–0.89)	0.82 (0.67–0.91)	0.76 (0.54–0.89)	3.40 (1.80–6.10)	0.24 (0.15–0.37)	14.00 (9.00–22.00)
Kostic et al.[20]	0.85 (0.81–0.88)	0.76 (0.61–0.87)	0.80 (0.61–0.91)	3.80 (2.10–7.20)	0.30 (0.20–0.44)	13.00 (8.00–22.00)
Mira-Pascual et al.[19]	0.86 (0.83–0.89)	0.81 (0.62–0.91)	0.77 (0.57–0.90)	3.60 (2.00–6.40)	0.25 (0.14–0.45)	14.00 (9.00–22.00)
Mira-Pascual et al.[19]	0.86 (0.83–0.89)	0.83 (0.69–0.92)	0.74 (0.56–0.86)	3.20 (2.00–5.10)	0.23 (0.13–0.38)	14.00 (8.00–23.00)
Fukugaiti et al.[28]	0.86 (0.83–0.89)	0.77 (0.63–0.86)	0.82 (0.70–0.89)	4.10 (2.80–6.20)	0.29 (0.19–0.44)	14.00 (10.00–22.00)
Pooled data	0.86 (0.83–0.89)	0.81 (0.64–0.91)	0.77 (0.59–0.89)	3.60 (2.10–6.00)	0.25 (0.15–0.42)	14.00 (9.00–22.00)

AUC: Area under the curve; PLR: Positive likelihood ratio; NLR: Negative likelihood ratio; DOR: Diagnostic odds ratio; CI: Confidence interval; NA: Not available.
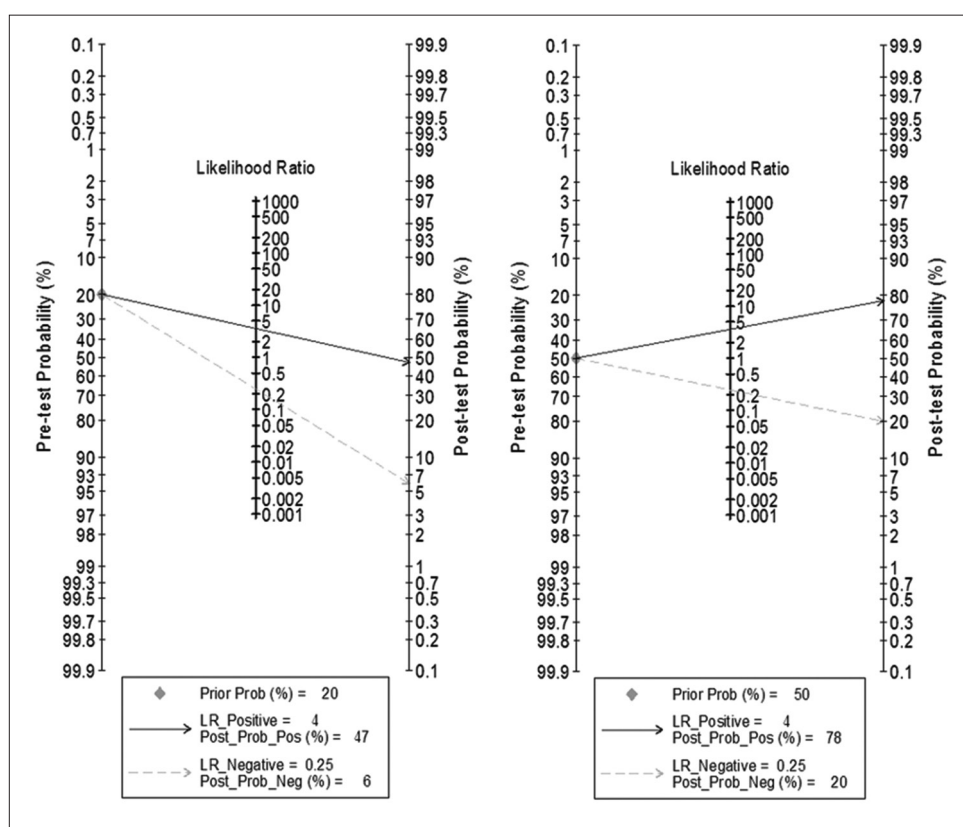

Figure 4: Fagan plot analysis to evaluate the intestinal *Fusobacterium nucleatum* infection for diagnosis of CRC. Prior Prob: Prior probability; Post Prob: Postprobability; LR: Likelihood ratio.

Great efforts have been made to develop cost-effective noninvasive methods. gFOBT and FIT have advantages such as being convenient, inexpensive (US 1 dollar and US 10 dollar), and quick (30 min), but their results are instable (sensitivity: gFOBT 6.2–83.3%, FIT 5.4–98.0% and specificity: gFOBT 80.0–98.4%, FIT 77.0– 99.0%). FIT tests an antibody to human hemoglobin in stools instead of testing heme by gFOBT. Therefore, FIT is comparatively steady because of no cross-reaction to dietary meat. Stool DNA tests have comparatively high sensibility, i.e., stool DNA tests (48.0–77.8%) and multi-target stool DNA test (52.0–92.3%), but similar specificity (92.7–97.0% and 86.6–94.4%) to FIT. Both stool DNA test and multi-target stool DNA test are expensive ($300 and $600) and time-consuming (1 day). Serum methylated SEPT9 has advantages of being convenient, compliant, and available at an acceptable price (US 170 dollar), but its accuracy (sensitivity 64.0–80.0% and specificity 75.0–88.0%) needs further confirmation.[5,7-13,16,29-34] This meta-analysis demonstrated that the performance of *F. nucleatum* test (sensitivity 64.0–91.0%, specificity 59.0–89.0%, and cost US 70 dollar) ranked in the dominated level among the noninvasive methods so far available. It had a reasonable diagnostic value compared with that of
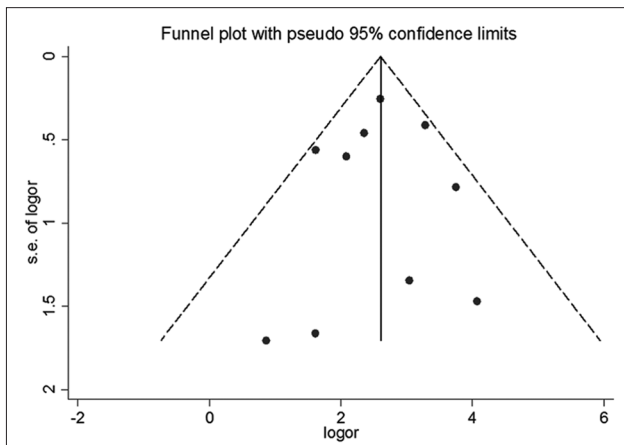
Figure 5: Funnel plot asymmetry test for publication bias. Each spot represents a separate study. logor: Log odds ratio; s.e. of logor: Standard error of log odds ratio.

Figure 6: Egger's linear regression test for publication bias. Each spot represents a separate study.

FIT and lower price compared with that of stool DNA and serum SEPT9 tests. A recent study showed that fecal *F. nucleatum* biomarker combined with FIT had significantly higher sensitivity than that of FIT alone in detecting CRC (92.3% vs 73.1%, *P* < 0.001) and advanced colorectal neoplasia (38.6% vs 15.5%, *P* < 0.001).[24] Intestinal *F. nucleatum* testing may be an additional option for detecting patients with CRC.

The association of *F. nucleatum infection* with clinical variables such as tumor node metastasis (TMN) stages and sites of CRC is an important issue for a new technique. In the eligible literatures, no study directly reported the data on early-stage CRC, but three articles documented that *F. nucleatum* was overabundance in malignant colorectal neoplasia (polyps, adenoma), compared with healthy controls.[19,20,27] Two articles revealed the sensitivity of *F. nucleatum* for CRC detection in different TMN stage,[24,25] one of which showed higher sensitivity in Stages 2, 3, and 4 than in Stage 1.[25] Two articles evaluated the link of *F. nucleatum* infection to the sites of CRC,[24,26] one of which concluded that *F. nucleatum* played more important roles in CRC development in proximal colon than in distal colon.[26] The above data were insufficient and not enough to perform further analysis.

In order to obtain an accurate result, this study selected healthy individuals as controls. The studies with CRC adjuvant tissues as controls were excluded because the abundance of *F. nucleatum* in CRC adjuvant mucosa was much higher than that in healthy controls.[16,29] Subgroup analyses were applied to identify the possible source of heterogeneity among studies. Significant heterogeneity between Asian and non-Asian groups in this study was consistent with the results in literatures. Gut microbial biomarkers had ethnical difference in patients with CRC.[35] Heterogeneity was also revealed between the subgroups of different sample sizes and sample types. In consistent with literatures, the amount of *F. nucleatum* in CRC tissues was significantly higher than that in stool.[36] Funnel plot and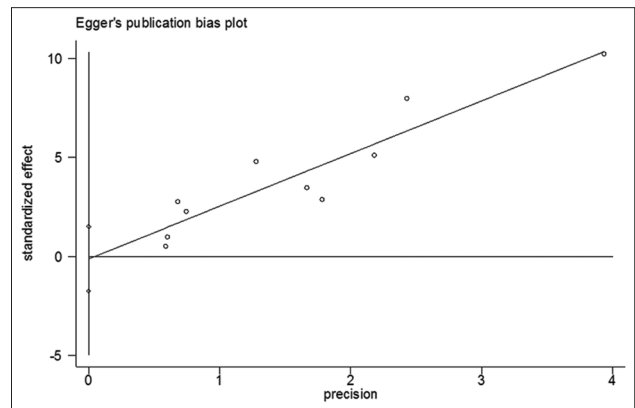 Egger's linear regression tests were applied to examine the heterogeneity, and no publication bias was found in this meta-analysis.

To our knowledge, this meta-analysis early evaluated the value of intestinal *F. nucleatum* test in CRC detection. This approach may take one step further toward a noninvasive, accurate, and affordable method for CRC detection. There were several limitations in this study. First, the number of eligible literatures and the sample size might not be large enough to support a powerful conclusion. In order to find more literatures, we searched Chinese articles and found 39 additional articles. However, the methodological quality of them was not good enough to meet the inclusion criteria. Second, there was heterogeneity between the subgroups, although no publication bias was found. Third, the techniques of intestinal *F. nucleatum* testing were not standard.

On the whole, this meta-analysis proved that intestinal *F. nucleatum* is a valuable marker for CRC diagnosis. However, it remains to be proven whether this method can improve diagnostic uptake compared with the established strategies.

Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.

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Conflicts of interest
There are no conflicts of interest.

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肠道具核梭杆菌检测对结直肠癌诊断价值– Meta分析

摘要

背景： 近年，肠道具核梭杆菌感染和结直肠癌发病的相关性的研究增多，但其作为生物标志物的临床诊断价值尚未完全明确。为减少单个研究随机误差及偏倚，本研究采用荟萃（Meta）分析评估肠道具核梭杆菌检测对结直肠癌诊断的价值, 为临床应用提供循证医学依据。

方法： 检索从建库起至2017年12月PubMed、Embase、 Cochrane Library、Web of Science，关键词为"具核梭杆菌", "梭菌属", "*Fn*", "结直肠癌", 和 "结直肠肿瘤"。按照预先制定的纳入和排除标准筛选文献。本文使用Stata12.0软件进行统计学分析，包括绘制森林图、异质性检验、Meta回归、亚组分析、敏感性分析、发表偏倚等。再汇总每项纳入研究的诊断的敏感性，特异性，阳性似然比，阴性似然比，诊断比值比以及其相应的95%可信区间等参数。

结果： 最终本分析共纳入1198参与者，（结直肠癌629例，健康人群569例），资料来源于7篇文献，共10项研究。具核梭杆菌诊断结直肠癌的综合参数如下：曲线下面积0.86（95% *CI*: 0.83–0.89）、敏感性0.81（95% *CI*: 0.64–0.89）、特异性0.77（95% *CI*: 0.59–0.89）、诊断比值比14.00（95% *CI*: 9.00比0.89积）。

结论： 具核梭杆菌可作为诊断结直肠癌有价值的标志物。

Supplemental File 1: MOOSE checklist

Item number	Recommendation	Reported on page number
Reporting of background should include		
1	Problem definition	2
2	Hypothesis statement	2
3	Description of study outcome(s)	2
4	Type of exposure or intervention used	None
5	Type of study designs used	2
6	Study population	2
Reporting of search strategy should include		
7	Qualifications of searchers (e.g., librarians and investigators)	3
8	Search strategy, including time period included in the synthesis and keywords	3
9	Effort to include all available studies, including contact with authors	3
10	Databases and registries searched	2
11	Search software used, name and version, including special features used (e.g., explosion)	2
12	Use of hand searching (e.g., reference lists of obtained articles)	None
13	List of citations located and those excluded, including justification	3,4
14	Method of addressing articles published in languages other than English	None
15	Method of handling abstracts and unpublished studies	2
16	Description of any contact with authors	None
Reporting of methods should include		
17	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	3
18	Rationale for the selection and coding of data (e.g., sound clinical principles or convenience)	None
19	Documentation of how data were classified and coded (e.g., multiple raters, blinding, and interrater reliability)	None
20	Assessment of confounding (e.g., comparability of cases and controls in studies where appropriate)	3
21	Assessment of study quality, including blinding of quality assessors, stratification or regression on possible predictors of study results	3
22	Assessment of heterogeneity	3
23	Description of statistical methods (e.g., complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	3
24	Provision of appropriate tables and graphs	3
Reporting of results should include		
25	Graphs summarizing individual study estimates and overall estimate	3
26	Table giving descriptive information for each study included	4
27	Results of sensitivity testing (e.g., subgroup analysis)	4,5
28	Indication of statistical uncertainty of findings	None
Reporting of discussion should include		
29	Quantitative assessment of bias (e.g., publication bias)	7
30	Justification for exclusion (e.g., exclusion of non-English language citations)	None
31	Assessment of quality of included studies	7
Reporting of conclusions should include		
32	Consideration of alternative explanations for observed results	7
33	Generalization of the conclusions (i.e., appropriate for the data presented and within the domain of the literature review)	7
34	Guidelines for future research	7
35	Disclosure of funding source	None

MOOSE: Meta-Analysis of Observational Studies in Epidemiology.