Diagnostic value of fecal Fusobacterium nucleatum in colorectal cancer

Chen Xing, Li Zhihao, Di Ji

Department of Oncology, Affiliated Hospital of Qinghai University, Xining, China

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Corresponding author:
Ji Di
Department of Oncology
Affiliated Hospital
of Qinghai University
Xining, China
E-mail: luosangdj@126.com

Colorectal cancer (CRC) is the third most common malignant tumor and the second deadliest cancer worldwide [1]. Current research and practice have demonstrated that the progression of CRC precancerous lesions to cancer generally takes 5–7 years, which provides an opportunity where dysplastic lesions can be detected and excised at the precancerous phase, or at an early, localized cancerous stage, that is highly treatable. The discovery of biological markers specific to precancerous colorectal adenomas and early, localized CRC would aid their early detection [2]. Therefore, it is crucial to identify detection methods that are highly specific, easily tolerated, and more compliant to achieve early diagnosis of CRC. The gut microbiota has been shown to promote the occurrence and development of CRC, and screening of gut microbiota for CRC has the potential to be a simple, convenient, non-invasive, highly sensitive, and well-tolerated method for detecting early signs of CRC. Fusobacterium nucleatum (Fn) is a Gram-negative, specialized anaerobic bacterium that mainly colonizes the intestines and oral cavity [3]. Studies have increasingly demonstrated that fecal Fusobacterium nucleatum is an emerging biomarker with promising potential for the diagnosis of CRC. However, the accuracy of this biomarker in diagnosing CRC remains to be determined due to biases of individual research. This study aims to evaluate the diagnostic performance of fecal Fusobacterium nucleatum in CRC.

We retrieved systematic reviews in PubMed, Embase, Cochrane Library and CNKI up to and including July 1, 2022, by Boolean operator: ("Fusobacterium nucleatum" OR "Fusobacterium spp" OR "F. nucleatum" OR "Fn") AND ("Colorectal Neoplasms" OR "colorectal cancer" OR "colorectal carcinoma" OR "colorectal neoplasm" OR "colorectal tumor"). Inclusion criteria: 1) The study design is clearly divided into two groups: a case group and a control group; 2) Sensitivity and specificity values of Fn for detecting CRC can be obtained from relevant literature and used to construct a 2 × 2 diagnostic grid table; 3) The diagnosis of CRC must rely on histological examination, which is the microscopic examination of tissue specimens; 4) Fn detection should be based on quantitative polymerase chain reaction analysis, fluorescence in situ hybridization or 16SrRNA sequencing; 5) Samples (feces or tissues) should be stored at –20°C to –80°C after collection. Exclusion criteria: 1) Incomplete data prevent the creation of a 2 × 2 Diagnostic table; 2) The control group specimens were taken from adjacent non-tumor tissues of CRC patients rather than the healthy control group; 3) No pathology as the diagnostic basis for CRC; 4) Conference paper abstracts, reviews, lectures, case reports, expert opinions and comments, etc.

After screening the titles, abstracts and body of the retrieved papers, 16 articles were finally included in this study. Table I shows the General clinical characteristics of the articles [4–19]. A bivariate meta-analysis model was employed to merge sensitivity, specificity, diagnostic odds ratio (DOR), positive likelihood ratio (LR+), and negative likelihood ratio (LR-) to establish the diagnostic accuracy of Fn in identifying CRC and draw a summary receiver operating characteristic (SROC) curve. The forest plot (Figure 1) displays the performance of Fn in detecting CRC with a pooled sensitivity of 0.70 (95% CI: 0.65-0.75), specificity of 0.79 (95% CI: 0.76–0.82), LR+ of 3.3 (95% CI: 2.9–3.8), LR- of 0.38 (95% CI: 0.32–0.44), and DOR of 9 (95% CI: 7–11). The sensitivity heterogeneity (I^2 = 84.9%, 95% CI: 79.52–90.20, p < 0.00) and specificity heterogeneity (I^2 = 57.5%, 95% CI: 37.7–77.2, p < 0.00) indicated significant heterogeneity between studies. After conducting a meta-regression analysis (Table II), we found that the heterogeneity of specificity in the participants was influenced by their race. Additionally, we observed that both the heterogeneity of specificity and sensitivity were impacted by the sample size and the QUADAS score. The SROC showed an advisable distribution with an area under the curve (AUC) of 0.82 (95% CI: 0.78–0.85) (Figure 2).

Our analysis included a total of 15 English and 1 Chinese studies, examining 2513 CRC patients and 2370 healthy controls. The forest map shows that the missed diagnosis rate and misdiagnosis rate of Fn in diagnosing CRC are 30% and 21%, respectively. The AUC of SROC was 0.82, supporting the notion that Fn can be a reliable screening indicator for CRC with low missed and misdiagnosis rates. Fn testing has shown excellent performance among all current non-invasive screening methods. A different study [9] has demonstrated that combining Fn biomarkers with FIT significantly improves sensitivity in detecting CRC (92.3% vs. 73.1%, p < 0.001) compared to using FIT alone. Therefore, Fn has potential as a biomarker for non-invasive screening of CRC.

The analysis of this study demonstrates that detection of fecal Fn has high utility in diagnosing CRC. This method shows promise in moving toward a non-invasive and cost-effective detection method for CRC. However, this analysis has certain limitations: 1) Due to the lack of unified detection methods and thresholds for evaluating fecal microbiota, most of the included studies defined thresholds specific to their study through ROC curves and obtained different cutoff values. Further research is required through controlled clinical trials with robust designs to determine an optimal threshold for diagnosing fecal microbiota CRC and evaluate its performance. Therefore, we

Table I. 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*	Sen (%)	Spe (%)	PPV (%)	NPV (%)
Zeller *et al.* [4]	2014	France	Feces	16SrRNA sequencing	10	Colonoscopy	114	29	24	5	56	55	92	85.3	68.3
Mira-Pascual *et al.* [5]	2015	Spain	Feces	Real-time qPCR	10	Colonoscopy	16	6	1	2	7	85.7	77.8	75	87.5
Yu *et al.* [6]	2016	China	Tissues	FISH	10	Colonoscopy or surgical operation	113	62	31	4	16	66.7	80	93.9	34
Suehiro *et al.* [7]	2017	Japan	Feces	Droplet digital PCR	9	Colonoscopy or surgical operation	218	85	73	6	54	53.8	90	93.4	42.5
Liang *et al.* [8]	2017	China	Feces	Probe-based duplex qPCR	11	Colonoscopy	69	27	6	17	19	81.8	52.8	61.4	76
Liang *et al.* [8]	2017	China	Feces	Probe-based duplex qPCR	11	Colonoscopy	370	132	38	41	159	77.7	79.5	76.3	80.7

Table I. Cont.

Authors	Year	Country	Sample types	Detection methods	QUADAS scores	Sample collection	Sample size, *n*	TP, *n*	FN, *n*	FP, *n*	TN, *n*	Sen (%)	Spe (%)	PPV (%)	NPV (%)
Wong *et al.* [9]	2017	China	Feces	Real-time qPCR	11	Colonoscopy	119	21	2	19	77	91.3	80.2	52.5	97.5
Wong *et al.* [9]	2017	China	Feces	Real-time qPCR	11	Colonoscopy	206	75	29	9	93	72.1	91.2	89.3	76.2
Xie *et al.* [10]	2017	China	Feces	Real-time qPCR	10	Colonoscopy	569	177	150	51	191	54.1	79	77.6	55.8
Xie *et al.* [10]	2017	China	Feces	Real-time qPCR	10	Colonoscopy	180	69	49	19	43	58	69.3	78.4	46.7
Eklöf *et al.* [11]	2017	Sweden	Feces	qPCR	10	Colonoscopy	105	27	12	15	51	69.2	76.9	64.2	80.9
Amitay *et al.* [12]	2017	Germany	Feces	Multiplex PCR	10	Colonoscopy	277	25	21	58	173	54.3	74.8	30	89.1
Guo *et al.* [13]	2018	China	Feces	Real-time qPCR	10	Colonoscopy	254	131	21	28	74	85.9	72.6	82.7	77.1
Guo *et al.* [13]	2018	China	Feces	Real-time qPCR	10	Colonoscopy	371	162	53	31	125	75.3	80	83.9	70.2
Liang *et al.* [14]	2019	China	Feces	qPCR	9	Colonoscopy	491	158	45	62	226	77.8	78.5	71.8	83.3
Liang *et al.* [14]	2019	China	Feces	qPCR	9	Colonoscopy	168	42	29	16	81	59.2	83.5	72.4	73.6
Löwenmark *et al.* [15]	2020	Sweden	Feces	qPCR	11	Colonoscopy	101	23	15	12	51	61.1	81.4	65.7	77.2
Löwenmark *et al.* [15]	2020	Sweden	Feces	qPCR	11	Colonoscopy	332	143	95	20	74	60.0	79.1	87.7	43.7
Liu *et al.* [16]	2020	China	Feces	qPCR	6	Colonoscopy	173	93	22	18	40	80.8	68.9	83.7	64.5
Liang *et al.* [17]	2021	China	Feces	qPCR	12	Colonoscopy	227	140	37	15	35	79.1	70	92	48.6
Liang *et al.* [17]	2021	China	Feces	qPCR	12	Colonoscopy	248	24	9	41	174	72.7	80.9	37	95
Liu *et al.* [18]	2021	China	Feces	qPCR	10	Colonoscopy	102	42	18	11	31	69.2	73.9	79.2	63.2
Osman *et al.* [19]	2021	Malaysia	Tissues	qPCR	9	Colonoscopy or surgical operation	60	29	11	4	16	72.5	80	87.9	59.3

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.

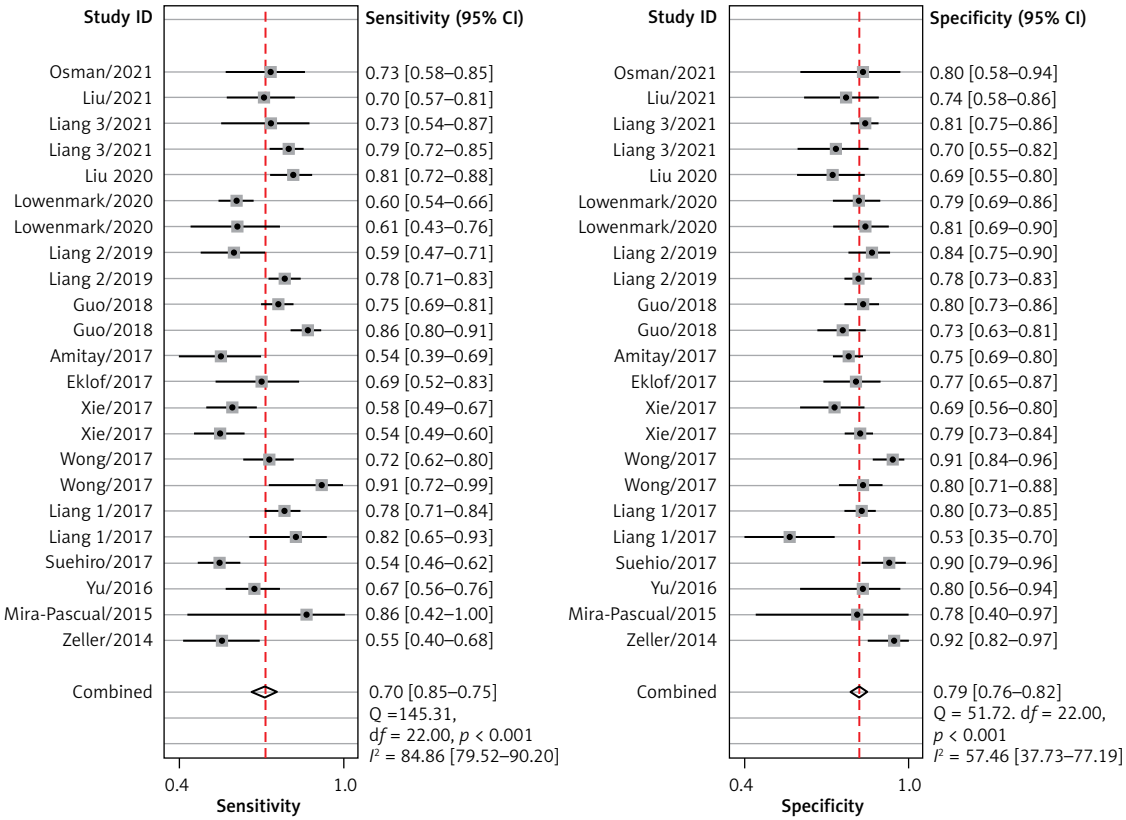

Figure 1. Forest plot of the pooled diagnostic accuracy of Fusobacterium nucleatum for colorectal cancer detection

CI – confidence interval.

Table II. Results of meta-regression for CRC

Parameter	Category	Cohorts, *n*	Sensitivity	P1	Specificity	P2	*P*-value
Race[a]	Yes	16	0.73 (0.68–0.77)	0.21	0.78 (0.75–0.82)	0.00	0.22
	No	7	0.63 (0.54–0.73)		0.80 (0.74–0.85)		
Sample types[b]	Yes	21	0.70 (0.66–0.75)	0.25	0.79 (0.76–0.82)	0.09	0.98
	No	2	0.70 (0.53–0.86)		0.80 (0.66–0.95)		
Sample size[c]	Yes	11	0.71 (0.64–0.77)	0.00	0.80 (0.76–0.83)	0.00	0.64
	No	12	0.70 (0.63–0.77)		0.77 (0.73–0.82)		
QUADAS scores[d]	Yes	18	0.70 (0.65–0.76)	0.02	0.78 (0.75–0.82)	0.00	0.77
	No	5	0.70 (0.60–0.80)		0.81 (0.75–0.87)		

[a]All participants who had CRC patients from China. [b]Sample type sourced from feces. [c]Number of samples > 200. [d]QUADAS scores ≥ 10. P1: The univariable regression results of sensitivity. P2: The univariable regression results of specificity. P-value: The joint model regression results of the overall heterogeneity.

cannot rule out that different cutoff values can significantly affect the diagnostic value. 2) The study's sample size is relatively small. It is essential to increase the sample size to enhance the accuracy of the consolidation effect.

At present, a large number of studies have confirmed the mechanism by which Fn promotes the occurrence of CRC. However, the relationship between Fn and CRC still lacks evidence-based basis. We have demonstrated a positive correlation between Fn and CRC through meta-analysis. Therefore, this study provides valuable guidance for the early diagnosis of CRC in clinical practice.

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Conflict of interest

The authors declare no conflict of interest.

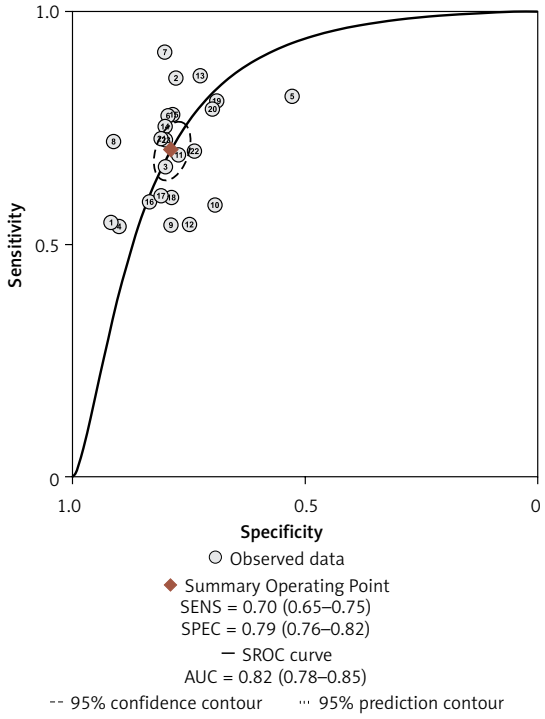

Figure 2. SROC assessment of diagnostic performance of Fusobacterium nucleatum for colorectal cancer

SROC – summary receiver operator characteristic curve

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