Proceedings of Anticancer Research, 2023, Volume 7, Issue 4
http://ojs.bbwpublisher.com/index.php/PAR
ISSN Online: 2208-3553
ISSN Print: 2208-3545

The Diagnostic Value of Fecal *Fusobacterium nucleatum* Combined with FIT and CA199 in the Diagnosis of Colorectal Cancer

Tianhong Jia[1], Yang Yu[1], Yan Wang[1], Ming Li[2], Shuzhuo Liu[1], Wei Li[1]*

[1]Department of Laboratory Medicine, Affiliated Hospital of Hebei University, Baoding 071000, China

[2]School of Basic Medical Sciences, Hebei University, Baoding 071000, China

***Corresponding author:** Wei Li, 120073839@qq.com

Abstract: *Objective:* To analyze the diagnostic value of fecal *Fusobacterium nucleatum* detection, fecal immunochemical test (FIT), and carbohydrate antigen 19-9 (CA19-9) detection for colorectal cancer (CRC). *Method:* A total of 78 CRC patients and 60 healthy individuals were enrolled in this study. Stool and blood samples were collected for the 3 diagnoses, and ROC curves were analyzed for diagnostic value. *Result:* The 3 diagnoses' positive detection rates in CRC samples were significantly higher than those of healthy samples ($P < 0.05$). The combined CRC diagnoses showed significantly higher sensitivity as compared to individual fecal *F. nucleatum* detection ($\chi^2 = 6.495$, $P = 0.011$), FIT ($\chi^2 = 4.871$, $P = 0.027$), and serum CA19-9 detection ($\chi^2 = 7.371$, $P = 0.007$). The area under the ROC curve for fecal *F. nucleatum* detection was 0.63 [95% confidence interval (CI) = 1.124–6.238], with a sensitivity of 73.08% and specificity of 85.00%, whereas FIT was 0.65 (95% CI = 1.365–9.241), with a sensitivity of 51.28% and specificity of 96.67%, meanwhile, serum CA19-9 detection was 0.62 (95% CI = 1.517–12.342), with a sensitivity of 69.23% and specificity of 98.33%. The combined CRC diagnoses showed an area under the ROC curve of 0.76 (95% CI = 1.213–6.254), with a sensitivity of 87.18% and specificity of 70.00%. *Conclusion:* The combined diagnoses of fecal *F. nucleatum* detection, FIT, and serum CA19-9 detection can significantly improve the sensitivity and accuracy of CRC diagnosis, which has high clinical application value to provide guidance for clinical CRC screening and early intervention treatment.

Keywords: Fecal *Fusobacterium nucleatum*; Fecal immunochemical test; Carbohydrate antigen 19-9; Colorectal cancer

Online publication: July 27, 2023

1. Introduction

Colorectal cancer (CRC) is a common malignant tumor of the gastrointestinal tract. With the increase in its mortality rate, early diagnosis is of great significance in the prevention and treatment of CRC. At present, the clinical diagnosis of CRC is mainly based on stool tests, colonoscopy, and tumor marker determination. However, due to certain limitations of these methods, the diagnostic accuracy is not high. In order to reduce the mortality rate and early diagnosis of CRC, biomarkers and effective diagnostic methods have been investigated to improve the diagnosis of CRC [1,2]. The fecal immunochemical test (FIT) is a non-invasive, convenient, fast, but low-sensitivity examination [3]. Carbohydrate antigen 19-9 (CA19-9) is a tumor marker widely used in clinical practice and has important diagnostic value in tumor diseases [4]. Fecal *Fusobacterium nucleatum* (hereby *F. nucleatum*) can be enriched in the colon and rectum, which can promote the proliferation, metastasis, and invasion of intestinal cancer cells, and participate in the immune

regulation of tumor cells [5]. In this study, the joint diagnoses of fecal *F. nucleatum* detection, FIT, and serum CA19-9 were used to explore their significance in the diagnosis of CRC, aiming to provide an efficient and accurate early diagnosis method for clinical diagnosis, improve the early diagnosis rate of CRC, and further reduce the fatality rate.

2. Materials and methods

2.1. Background

A total of 78 CRC patients admitted to the Affiliated Hospital of Hebei University from January 2020 to December 2020 were continuously selected for retrospective analysis, including 49 males and 29 females, aged 28 to 79 years old, with an average age of 53.36 ± 3.42 years old. A total of 60 healthy individuals were also recruited for data comparison.

The inclusion criteria included patients who meet the diagnostic criteria for CRC in the "Standards for Diagnosis and Treatment of CRC", diagnosed with CRC by postoperative histopathological examination, and aged > 18 years, yet to receive relevant anti-tumor treatment before admission, consist of complete clinical data, and able to fulfill the obligation of disclosure, know the content of the research, and voluntarily sign a consent form.

The exclusion criteria included CRC patients diagnosed with severe heart, liver, kidney, and other vital organ diseases, respiratory system diseases, immune and inflammatory diseases, or consisted of other malignant tumors, or consisted of chronic hepatitis, ulcerative colon cancer, and other digestive system diseases, or had mental disorders or communication difficulties. The research was approved by the Hospital Ethics Committee.

2.2. Methods

2.2.1. Fecal *Fusobacterium nucleatum*

Stool samples were taken from the patients in the morning, and the DNA of fecal *F. nucleatum* was extracted using Beijing Tiangen Biochemical Technology Co., Ltd. DP328 Fecal DNA Extraction Kit. The concentration and quality of the extracted DNA were analyzed using the Nanodrop One UV-Vis spectrophotometer (Thermo Scientific, USA). The ratio of $A_{260\ nm}$ and $A_{280\ nm}$ was 1.7~1.9. The relative expression of *F. nucleatum* DNA was detected using the 7900HT real-time PCR system (Applied Biosystems, USA), and the TB Green qPCR Master Mix reagent from Dalian Bao Biological Co., Ltd. (product number 639676) was used to prepare the reaction buffer and DNA template. The PCR reaction conditions were 45 cycles of pre-denaturation at 95°C for 30 s, denaturation at 95°C for 10 s, and annealing/extension at 60°C for 35 s. The sequence of the primers for *F. nucleatum* is as follows: the upstream primer is 5'-TTCAATAAAAGTGGCAGGTCAAG-3', and the downstream primer is 5'-TAACAACACATGCAGGTCAATGG-3'. The 16S rDNA primer sequence of the total bacterial internal reference gene is as follows: the upstream primer is 5'-CCATGAAGTCGGAATCGCTAG-3', and the downstream primer is 5'-GCTTGACGGGCGGTGT-3'. The relative expression level of target bacterial DNA is represented by 2-ΔCt, ΔCt=Ct target bacterial sequence-Ct16S rDNA.

2.2.2. Fecal immunochemical test (FIT)

Two stool samples were taken from the patient in the morning, and the fecal occult blood colloidal gold detection test paper was used for detection, and the relevant operations are strictly carried out according to the test paper instructions. The criteria for a positive fecal occult blood test are (1) there are differences in the positive or negative results of two fecal occult blood tests in one specimen, and (2) there are differences between positive and negative results in one test specimen.

2.2.3. Carbohydrate antigen 19-9 (CA19-9)

About 3 mL of fasting venous blood was taken from the patient, then placed in a test tube containing separating gel, centrifuged at 3000 r/min for 10 min, and the expression of CA19-9 was detected using a fully automatic electrochemiluminescence instrument and matching kits from Roche, Germany.

2.3. Observation indicators

The observation indicators of this study are as follows:

(1) Comparison of the positive detection rates of fecal *F. nucleatum*, FIT, and serum CA19-9: the positive rates of the three diagnostic methods were compared in CRC patients and healthy individuals.

(2) The diagnostic efficacy of fecal *F. nucleatum* detection combined with FIT and serum CA19-9 detection for CRC: the pathological results were used as the gold standard to evaluate the sensitivity and specificity of individual diagnostic methods and the combined CRC diagnoses for the CRC diagnosis degree and accuracy.

(3) ROC curve of fecal F. nucleatum detection combined with FIT and serum CA19-9 detection for CRC: the ROC curve was drawn according to each diagnostic method and the combined diagnosis of CRC. The larger the area under the ROC curve, the higher the diagnostic accuracy.

2.4. Statistical method

The research statistical data was analyzed using SPSS 21.0 software, the count data was described by number (percentage) [*n* (%)], the χ^2 test was used, and the difference was considered statistically significant when $P < 0.05$.

3. Results

3.1. Comparison of positive detection rates of fecal *F. nucleatum*, FIT, and serum CA19-9

The positive detection rates of fecal *F. nucleatum*, FIT, and serum CA19-9 in CRC patients were significantly higher than those of healthy individuals ($P = 0.000$), see **Table 1**.

Table 1. Comparison of positive detection rates of fecal *F. nucleatum*, FIT, and serum CA19-9 [*n* (%)]

	Number of cases	Fecal *F. nucleatum*	FIT	Serum CA19-9
CRC patients	78	57 (73.1%)	40 (51.3%)	54 (69.2%)
Healthy individuals	60	9 (15.0%)	2 (3.3%)	1 (1.7%)
χ^2 value		45.841	23.698	51.386
P value		0.000	0.000	0.000

3.2. The diagnostic efficacy of fecal *F. nucleatum* combined with FIT and serum CA19-9 detection for CRC

Table 2 showed that the sensitivity of the combined diagnoses of the three was significantly higher than that of the individual detection of F. nucleatum ($\chi^2 = 6.495$, $P = 0.011$), FIT ($\chi^2 = 4.871$, $P = 0.027$), and serum CA19-9 ($\chi^2 = 7.371$, $P = 0.007$).

Table 2. The diagnostic efficacy of fecal *F. nucleatum* combined with FIT and serum CA19-9 detection for CRC [*n* (%)]

Detection method	Number of cases	Sensitivity	Specificity	Accuracy
F. nucleatum	138	73.08% (57/78)	85.00% (51/60)	78.26% (108/138)
FIT	138	51.28% (40/78)	96.67% (58/60)	71.01% (98/138)
CA19-9	138	69.23% (54/78)	98.33% (59/60)	71.74% (99/138)
Joint diagnoses	138	87.18% (68/78)	70.00% (42/60)	79.71% (110/138)

3.3. ROC curve of fecal *F. nucleatum* combined with FIT and serum CA19-9 detection for CRC

The area under the ROC curve for the diagnosis of CRC by fecal *F. nucleatum* detection was 0.63 [95% confidence interval (CI) = 1.124–6.238), and the sensitivity and specificity were 73.08% and 85.00%, respectively. The area under the ROC curve for the diagnosis of CRC by FIT was 0.65 (95% CI = 1.365–9.241), with a sensitivity of 51.28% and specificity of 96.67%. The area under the ROC curve for the diagnosis of CRC by serum CA19-9 detection was 0.62 (95% CI = 1.517–12.342), and the sensitivity and specificity were 69.23% and 98.33%, respectively. The area under the ROC curve for the combined three diagnoses of CRC was 0.76 (95% CI = 1.213–6.254), with a sensitivity of 87.18% and specificity of 70.00%, as shown in **Figure 1**.

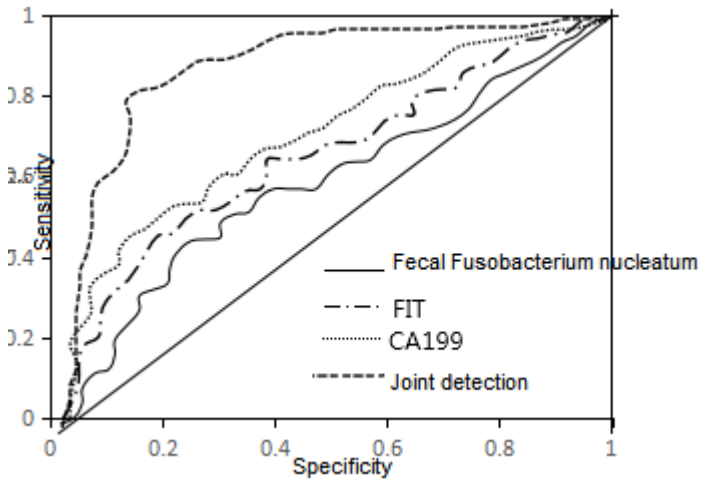

Figure 1: ROC curve of CRC diagnoses using fecal *F. nucleatum* detection, FIT, serum CA19-9, and a combination of all three diagnoses.

4. Discussion

In recent years, with the rapid economic development and changes in people's living habits, the incidence and mortality of CRC in China are gradually increasing [6], and roughly 20% of CRC patients are found to be associated with liver metastases at the early stage of diagnosis and progress to advanced stages. The 5-year survival rate of bowel cancer patients is about 60%. Moreover, due to the CRC patients are being not vigilant enough and performing limited examinations, the misdiagnosis rate of CRC is approximately 30% [7,8]. Therefore, improvement of the early diagnosis and screening of CRC is an essential clinical problem that needs to be solved urgently. In this study, the combined diagnoses of fecal *F. nucleatum* detection, FIT, and serum CA19-9 detection were found to have good sensitivity and accuracy, and it has high clinical application value for the early diagnosis of CRC.

Recently, the research on the relationship between intestinal microecology and CRC has progressed, and the relationship between intestinal microecology and CRC has gradually been recognized. Gut bacteria

have certain carcinogenicity and can drive the occurrence of CRC and change with the development of CRC [9,10]. *F. nucleatum* can form a Fap2/Gal-Gal NAc complex through its bacterial protein Fap2 and become a passive bacterium in the process of CRC lesions, indicating that *F. nucleatum* is closely related to CRC lesions [11,12]. Therefore, fecal *F. nucleatum* has a certain clinical value in the diagnosis of CRC. Although the detection of fecal microbial markers is convenient and economical, the DNA of fecal microorganisms is stable, and the extraction method is mature and simple, but due to the diversity of gastrointestinal flora and its micro-ecological environment, it is easy to obtain errors in the diagnostic results, thereby leading to misdiagnosis, which is inconsistent with several reports in related studies of using fecal *F. nucleatum* as a biomarker for early screening of bowel cancer, which may be due to the differences in the gastrointestinal flora of patients [11,13]. The fecal occult blood test is a simple immunological detection method. However, when the blood concentration in the stool sample is too high, the result will appear as a false negative, resulting in misdiagnosis [3,14]. Tumor marker CA19-9 has good specificity and accuracy for the diagnosis of CRC [15], and CA242 also has a certain value in the diagnosis of CRC [16]. However, there are many tumor markers available and it is impossible to detect them one by one. Therefore, the detection of a single tumor marker has certain exclusivity. It can be seen that a single detection method cannot meet the needs of clinical early diagnosis. In recent years, the application of joint detection has become the preferred method for the diagnosis of CRC [17,18], but there is still a lack of unified standards for joint detection indicators. Several detection methods that have been popular in recent years have been integrated to find a more stable and reliable early diagnosis method for early intervention and treatment, thereby reducing the mortality rate of CRC.

The combined detection results of the three showed that the sensitivity and sensitivity of fecal *F. nucleatum* combined with FIT and CA19-9 detection were significantly higher than that of individual diagnoses ($P < 0.05$), indicating that the combination diagnoses can improve the sensitivity of early diagnosis of CRC, and the ROC curve proves that the accuracy of combined diagnosis of CRC is higher. In the current clinical diagnostic experiments of CRC, combined detection is often used to improve the sensitivity or specificity of diagnosis, thereby improving the efficiency of clinical diagnosis. However, many experiments show that the premise of improving the sensitivity of diagnosis is to reduce the specificity, and the improvement of specificity is at the expense of reducing the sensitivity. In this study, the combined detection of fecal *F. nucleatum*, FIT, and serum CA19-9 significantly improved the sensitivity of diagnosing CRC. The combined detection can make up for the limitation of single index diagnosis to a certain extent, and the ROC curve proves that its diagnostic accuracy is high, indicating that combined detection can improve the sensitivity and accuracy of CRC diagnosis, and can be used as a fast and effective detection method for screening and diagnosing CRC.

In conclusion, fecal *F. nucleatum* combined with FIT and serum CA19-9 detection can efficiently and accurately diagnose CRC with high sensitivity and high diagnostic value, which can provide a reference for early diagnosis of CRC, and is conducive to timely intervention and treatment to improve the quality of life of CRC patients.

Funding

The In-Hospital Fund of the Affiliated Hospital of Hebei University (2017Q004) and the Medical Research Project of Hebei Provincial Health Commission (20190924)

Disclosure statement

The authors declare no conflict of interest.

References

[1] González-González M, Garcia JG, Monstero JAA, et al, 2013, Genomics and Proteomics Approaches for Biomarker Discovery in Sporadic CRC with Metastasis. Cancer Genomics Proteomics, 10(1): 19–25.

[2] Kumar M, Cash BD, 2017, Screening and Surveillance of CRC Using CT Colonography. Curr Treat Options Gastroenterol, 15(1): 168–183. http://doi.org/10.1007/s11938-017-0121-7

[3] Lee JK, Liles EG, Bent S, et al, 2014, Accuracy of Fecal Immunochemical Tests for CRC: Systematic Review and Meta-Analysis. Ann Intern Med, 160(3): 171. http://doi.org/10.7326/M13-1484

[4] Zhu Y, Zhao W, Mao G, 2022, Perioperative Lymphocyte-to-Monocyte Ratio Changes Plus CA199 in Predicting the Prognosis of Patients with Gastric Cancer. J Gastrointest Oncol, 13(3):1007–1021. http://doi.org/10.21037/jgo-22-411

[5] Castellarin M, Warren RL, Freeman JD, et al, 2012, Fusobacterium nucleatum Infection is Prevalent in Human Colorectal Carcinoma. Genome Res, 22(2): 299–306. http://doi.org/10.1101/gr.126516.111

[6] Sung JJY, Lau JYW, Goh KL, et al, 2005, Increasing Incidence of CRC in Asia: Implications for Screening. Lancet Oncol, 6(11): 871–876. http://doi.org/10.1016/S1470-2045(05)70422-8

[7] Aghagolzadeh P, Radpour R. 2016, New Trends in Molecular and Cellular Biomarker Discovery for CRC. World J Gastroenterol, 22(25): 5678–5693. http://doi.org/10.3748/wjg.v22.i25.5678

[8] Sung JJY, Ng SC, Chan FKL, et al, 2015, an Updated Asia Pacific Consensus Recommendations on CRC Screening. Gut, 64(1): 121–132. http://doi.org/10.1136/gutjnl-2013-306503

[9] Yu J, Feng Q, Wong SH, et al, 2017, Metagenomic Analysis of Faecal Microbiome as a Tool Towards Targeted Non-Invasive Biomarkers for CRC. Gut, 66(1): 70–78. http://doi.org/10.1136/gutjnl-2015-309800

[10] Liang Q, Chiu J, Chen Y, et al, 2017, Fecal Bacteria Act as Novel Biomarkers for Noninvasive Diagnosis of CRC. Clin Cancer Res, 23(8): 2061–2070. http://doi.org/10.1158/1078-0432.CCR-16-1599

[11] Ahn J, Sinha R, Pei Z, et al, 2013, Human Gut Microbiome and Risk for CRC. J Natl Cancer Inst, 105(24): 1907–1911. http://doi.org/10.1093/jnci/djt300

[12] McCoy AN, Araújo-Pérez F, Azcárate-Peril A, et al, 2013, Fusobacterium is Associated with Colorectal Adenomas. PLoS One, 8(1): e53653. http://doi.org/10.1371/journal.pone.0053653

[13] Lopez-Siles M, Martinez-Medina M, Surís-Valls R, et al, 2016, Changes in the Abundance of Faecalibacterium prausnitzii Phylogroups I and II in the Intestinal Mucosa of Inflammatory Bowel Disease and Patients with CRC. Inflamm Bowel Dis, 22(1): 28–41. http://doi.org/10.1097/MIB.0000000000000590

[14] Haug U, Hundt S, Brenner H, 2010, Quantitative Immunochemical Fecal Occult Blood Testing for Colorectal Adenoma Detection: Evaluation in the Target Population of Screening and Comparison with Qualitative Tests. Am J Gastroenterol, 105(3): 682–690. http://doi.org/10.1038/ajg.2009.668

[15] Gao Y, Wang J, Zhou Y, et al, 2018, Evaluation of Serum CEA, CA19-9, CA72-4, CA125 and Ferritin as Diagnostic Markers and Factors of Clinical Parameters for CRC. Sci Rep, 8(1): 2732. http://doi.org/10.1038/s41598-018-21048-y

[16] Rao H, Wu H, Huang Q, et al, 2021, Clinical Value of Serum CEA, CA24-2 and CA19-9 in Patients with CRC. Clin Lab, 67(4). http://doi.org/10.7754/Clin.Lab.2020.200828

[17] Wong SH, Kwong TNY, Chow T-C, et al, 2017, Quantitation of Faecal Fusobacterium Improves Faecal Immunochemical Test in Detecting Advanced Colorectal Neoplasia. Gut, 66(8): 1441–1448.

http://doi.org/10.1136/gutjnl-2016-312766

[18] Steer T, Collins MD, Gibson GR, et al, 2001, Clostridium hathewayi sp. nov., from Human Faeces. Syst Appl Microbiol, 24(3): 353–357. http://doi.org/10.1078/0723-2020-00044