Comparison between diagnostic performance of intestinal Fusobacterium nucleatum, Bacteroides fragilis and Escherichia coli in 5-fluorouracil resistance to colorectal cancer: A Meta-Analysis

Yuhang Zhang[1], wenyu wang[2], Hang Zhou[2], Zhi Wang[2], and Yi Min Cui[1]

[1]Peking University First Hospital
[2]Capital Medical University

April 05, 2024

Abstract

Background: Intestinal Fusobacterium nucleatum (F. nucleatum) infection has been implicated into the progression of colorectal cancer (CRC). However, F. nucleatum as a biomarker in 5-fluorouracil (5-FU) resistance of CRC has not been fully analyzed by comparing with other types of gut microbiota. This meta-analysis aimed to compare the diagnostic performance of intestinal Fusobacterium nucleatum, Bacteroides fragilis and Escherichia coli in 5-FU resistance to colorectal cancer and provide evidence-based data to clinical practice. Methods: Comprehensive searches of PubMed, Embase, Cochrane Library and Web of Science databases were conducted by the following key words: "Fusobacterium nucleatum", "5-Fluorouracil resistance", "Bacteroides fragilis", "Escherichia coli" and "colorectal cancer(s)". A total of 11 studies were selected according to the preestablished inclusion and exclusion criteria and analyzed by Review Manager 5.4 software. The sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR) and their corresponding 95% confidence interval (CI) of each eligible study were summarized. Results: Overall sensitivity and specificity of F. nucleatum detection in 5-FU resistance of CRC were 0.65 (95% CI:0.60-0.69) and 0.70 (95% CI:0.59-0.87), respectively. Its PLR and NLR in detecting colorectal cancer were 2.57 (95% CI:1.47-3.21) and 0.52 (95% CI:0.43-0.63). DOR value was 4.92 (95% CI:2.23-7.33), which significantly exceeds the performance of B. fragilis (DOR: 0.53, 95% CI:0.31-0.82) and E. coli (DOR: 0.63, 95% CI: 0.57-0.76) for indicating 5-FU resistance of CRC. Conclusion: Compared with B. fragilis and E. coli, intestinal F. nucleatum is a valuable biomarker for 5-FU resistance to colorectal cancer.

Introduction

5-Fluorouracil (5-FU) remains the cornerstone of palliative and adjuvant chemotherapy of colorectal cancer (CRC) [1, 2]. The majority of patients with advanced CRC are initially responsive to the implementation of 5-FU-based combination regimens and 5-FU pro-drugs [3, 4]. However, the 5-year survival rate eventually demonstrates lower than 10% in these CRC patients [5]. Unfortunately, colorectal tumors are generally not responsive to novel immune checkpoint therapy [6]. Thus, it is of paramount significance to elucidate the underlying risk factors of 5-FU resistance, which aims to reform the guidelines of 5-FU diagnostics and treatments for CRC patients.

CRC chemoresistance results from the complex crosstalk between gene regulation and the environment. Accumulating evidence indicates that gut microbiota is linked to the initiation and development of CRC via affecting intestinal inflammation and DNA mutations [7-10]. However, few studies have focused on their host response to CRC treatment. This systematic review has summarized the clinical correlations between 5-FU resistance of CRC and three common intestinal bacteria, including*Fusobacterium nucleatum* , *Bacteroides fragilis* and*Escherichia coli* .

Fusobacterium nucleatum (*F. nucleatum* , *Fn*), a human's oral cavity colonizer, has been frequently reported to enrich in stools from CRC patients as compared with the normal controls [11-13]. The previous study has revealed that *F. nucleatum*overabundance could impair the therapeutic efficacy of 5-FU by inducing LC3-II expression, autophagic flux and autophagosome synthesis in colorectal cancer [14]. Besides, the amount of *F. nucleatum*is associated with the upregulation of BIRC3 in CRC cells cocultured with *Fn* , which revealed *Fn* inhabiting in intestinal lumen of CRC patients can directly impair the efficacy of 5-FU [15].*Bacteroides fragilis* (*B. fragilis*), is the most frequent anaerobe isolated from clinical cases of diarrhea, peritonitis, intra-abdominal abscesses and sepsis [16-19]. Several studies showed the significant correlation between the presence of *B. fragilis*in stool or colonic biopsy specimens and poor prognosis of CRC [20-22]. The detection of *B. fragilis* may be a potential marker for the diagnosis of colorectal cancer. Despite the fact that*Escherichia coli* (*E. coli*) is a commensal bacterium of the human microbiota and represents the most common cultivable, gram-negative, aero-anaerobic bacteria [23, 24], various studies have demonstrated a clear link between mucosa-adherent *E. coli*and colorectal cancer [20, 25].

Although mechanisms and causalities between the above three intestinal bacteria and 5-FU resistance of CRC have been still uncovered, some studies have explored the diagnostic performance of their presence in feces or tumor tissues in 5-FU-treated CRC patients. This meta-analysis has summarized the published data which aims to evaluate the value of*F. nucleatum* , *B. fragilis* or *E. coli* detection in 5-FU efficacy for CRC treatment.

Results

Literature screening and characteristics of eligible studies. A total of 233 articles were identified using the search strategies, including 113 articles from PubMed, 5 articles from Cochrane library, 115 articles from Web of Science. Totally, 184 articles were excluded after careful filtration. Among them, 148 articles were duplicates and 36 had inappropriate abstracts and titles. Then the left 49 full-text articles were assessed for their eligibility, which contains 8 articles without diagnostic method, 13 articles without exact data on recurrence rates, 11 articles without using *F. nucleatum* , *B. fragilis*or *E. coli* as the single biomarker and 6 articles with other chemotherapeutics included. Finally, 11 articles were included in this meta-analysis, which involved 443 participants (200 CRC patients with high *F. nucleatum* abundance and 243 CRC patients with low*F. nucleatum* abundance, 144 CRC patients with high *B. fragilis* abundance and 121 CRC patients with low *B. fragilis*abundance, 400 CRC patients with high *E. coli* abundance and 26 CRC patients with low *E. coli* abundance). The selection process is illustrated in Figure 1.

Basic characteristics and diagnostic performance of *F. nucleatum* ,*B. fragilis* or *E. coli* in these 11 studies are shown in Table 1. Fecal gut microbiota was evaluated by RNA *in situ*hybridization (RNA-ISH), quantitative real-time PCR (RT-PCR), droplet digital PCR or multiplex PCR. All studies included in the meta-analysis used the histopathological examination as the gold standard. Figure 2 shows an overview of the methodological quality results. In general, the overall quality of the eligible studies was high.

Heterogeneity. It is known that the threshold effect, one source of heterogeneity, can be determined by calculating the Spearman correlation coefficient between sensitivity and specificity for all included studies. However, in our meta-analysis, Spearman's rank correlation coefficient was -0.94, calculated by an equation using logarithm of sensitivity and 1-specificity. SROC distributed advisably (AUC: 0.79, 95% CI: 0.74-0.81; Figure 3), which indicated no statistical significance ($P = 0.83$). Then we performed the meta-regression based on the variables, including ethnicity, median age, detection methods, CRC patients' number, sample size of 5-FU resistance to explain this heterogeneity (Table 1). Among the five factors, sample size of 5-FU resistance was identified as statistically significant ($P = 0.012$), indicating that sample size of CRC patients with 5-FU resistance was responsible for the relatively high heterogeneity.

Diagnostic performance in meta-regression analysis. As forest plot revealed significant heterogeneity between studies, single-factor meta-regression analysis was applied to screen the potential variables impacting on the pooled data. The performance of *F. nucleatum* for 5-FU resistance of CRC is shown in forest plot (Figure 4): pooled sensitivity: 0.65 (95% CI:0.60-0.69), specificity: 0.70 (95% CI:0.59-0.87), PLR: 2.57 (95%

CI:1.47-3.21), NLR: 0.52 (95% CI:0.43-0.63) and DOR: 4.92 (95% CI:2.23-7.33). In contrast, the performance of *B. fragilis* for 5-FU resistance of CRC is lower than *F. nucleatum* (Figure 4): pooled sensitivity: 0.51 (95% CI:0.42-0.54), specificity: 0.36 (95% CI:0.21-0.53), PLR: 0.82 (95% CI:0.79-0.95), NLR: 1.55 (95% CI:1.01-1.62) and DOR: 0.53 (95% CI:0.31-0.82). The performance of *E. coli* for 5-FU resistance of CRC manifested remarkably higher sensitivity but poor specificity (Figure 4): pooled sensitivity: 0.93 (95% CI:0.90-0.95), specificity: 0.06 (95% CI:0.04-0.92), PLR: 0.99 (95% CI:0.94-1.05), NLR: 1.57 (95% CI:0.87-1.76) and DOR: 0.63 (95% CI:0.57-0.76).

Discussion

In this meta-analysis, *F. nucleatum* test showed better discrimination ability for detecting 5-FU resistance in CRC patients as compared with *B. fragilis* and *E. coli* . To the best of our knowledge, ours is the first meta-analysis to assess the diagnostic value of *F. nucleatum* , *B. fragilis* and *E. coli* test for discriminating CRC patients with 5-FU-based regimen. The pooled DOR was 4.92 (95% CI:2.23-7.33), indicating the test's relatively high discrimination ability. The pooled sensitivity and specificity were 64.9% and 69.6%, respectively, suggesting the test's superior ability for ruling out CRC patients which are not suitable for 5-FU-based regimen. However, the PLR and NLR of the test was 2.57 (95% CI:1.47-3.21) and 0.52 (95% CI:0.43-0.63), respectively, which shows that CRC patients with 5-FU resistance have approximately 2.57 times higher possibility of testing positive *F. nucleatum* compared with subjects without 5-FU resistance, as well as 52% chance of an CRC individual having 5-FU resistance if the test of *F. nucleatum* is negative. The performance of the fecal *F. nucleatum* test in the pooled PLR and NLR did not achieve the requirements of clinical practice, and remains to be modified for clinical confirmation and exclusion purposes. Nonetheless, the pooled results suggest that the fecal *F. nucleatum* test has better discrimination ability than*B. fragilis* and *E. coli* in clinical practice of suitable 5-FU-based therapy for CRC patients.

We identified the weaknesses of this study. First, the number of patients and studies included were relatively small, which may affect the overall quality of evidence. Second, these studies lacked a unified criterion for *F. nucleatum* , *B. fragilis* and *E. coli*positive expression. Thus, larger-scale, multicenter and higher-quality studies are required to confirm our findings in the future, allowing a better comparison of analytical results of *F. nucleatum* on clinical outcomes.

In conclusion, the fecal *F. nucleatum* test shows better discrimination ability for detecting whether 5-FU-based regimen is suitable for a colorectal cancer patient in our meta-analysis. We certainly recognize that our suggested method is incomplete, mainly from the limited sample size, but it is indicated that the fecal *F. nucleatum* test represents at least a starting point toward non-invasive and accurate procedures in the detection of 5-FU resistance for CRC treatment.

Methods

Search strategy. Four electronic databases were systematically searched from July 2005 to March 2021: PubMed, Embase, Cochrane Library and Web of Science. The following keywords were used in the literature search: "5-Fluorouracil (5-FU)", "5-Fluorouracil (5-FU) resistance", "Colorectal Cancer(s)", "colorectal carcinoma(s)", "colorectal neoplasm(s)", "colorectal tumor(s)", "Colorectal cancer(s) recurrence", "colorectal carcinoma(s) recurrence", "colorectal neoplasm(s) recurrence", "colorectal tumor(s) recurrence", "*Fusobacterium nucleatum* ", "*Fusobacterium* spp.", "*F. nucleatum* ", "*Fn* ", "*Bacteroides fragilis* ", "*Bacteroides* spp.", "*B. fragilis* ", "*Escherichia coli* ", "*Escherichia* spp." and "*E. coli* ". Furthermore, the listed referent studies and relevant review articles were also examined.

Inclusion and exclusion criteria . The inclusion criteria were as follows: (1) Correlation of *F. nucleatum* to 5-FU resistance or CRC recurrence; (2) A clinical study of CRC patients with intact data of*F. nucleatum* positive and *F. nucleatum* negative cases,*B. fragilis* positive and *B. fragilis* negative cases as well as *E. coli* positive and *E. coli* negative cases; (3) Contains clear recurrence rates data; (4) All patients were treated with standard 5-FU-based regimen; (5) The diagnosis of CRC progression should be based on histology; (6) The detection of *F. nucleatum* ,*B. fragilis* and *E. coli* should be based on quantitative polymerase chain reaction analysis, fluorescence *in situ*hybridization or 16S rRNA sequencing; (7) The samples (feces or tissues) should

be stored at -20°C to -80°C soon after collection; (8) The articles should be original articles.

The exclusion criteria were as follows: (1) Exact data on recurrence rates are not given; (2) Studies that did not include the necessary data for calculating true-positive (TP), false-positive (FP), true-negative (TN) and false-negative (FN) of *F. nucleatum* , *B. fragilis*or *E. coli* in 5-FU resistance of CRC; (3) Use of other treatments or no chemotherapy; (4) Letters, reviews, conference abstracts and duplicate publications.

Data extraction and assessment. Based on the selection and inclusion criteria, two authors independently screened the title, abstract and full text of the retrieved studies. The third author excluded the irrelevant studies and cross-checked the data. The following information from each article was extracted: the first author's name, the year of publication, sample size, sample type,*F. nucleatum* , *B. fragilis* and *E. coli* positive and negative groups, and recurrence of CRC patients after 5-FU treatment. 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 data of the included studies were extracted into a spreadsheet and copied into Review Manager 5.4.

According to the Quality Assessment of Diagnostic Accuracy Studies (QUADAS), which is recommended by the Cochrane Collaboration. The quality of each study was assessed by QUADAS tool which 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 provides more transparent rating of bias and applicability of primary diagnostic accuracy studies. If the QUADAS score is less than 10 points, the study is identified as low methodological quality [35].

Statistical analysis. All analyses were conducted by the Review Manager 5.4 software. The available data were analyzed in the meta-analysis, and the outcomes are presented as forest plots and funnel plot. To calculate the combined OR and its 95% confidence interval (CI), heterogeneity was assessed using P -values in the pooled analyses which represents the percentage of total variation across the studies. If the P value was less than 0.01, then the summary estimate was analyzed in a random-effects model (DerSimonian-Laird method). Otherwise, a fixed-effects model (Mantel-Haenszel method) was applied. In addition, publication bias was detected by visual examination of the funnel plot symmetry, with asymmetry suggesting possible publication bias. It was also assessed by the Begg and Egger test in the meta-analysis. If the P value was less than 0.05, the study is classified as publication bias and the meta-trim method would be conducted.

Table1. Characteristics and summary results of included studies

First Author	Year	Country	Median Age	Assay Method	Intestinal Bacteria	TP	FP	FN
G. Serna *et al.* [26]	2020	Spain	71	RNA-ISH	*F. nucleatum*	13	9	7
Sheng Zhang *et al.* [15]	2019	China	58	RT-PCR	*F. nucleatum*	34	20	17
Tachung Yu *et al.* [14]	2017	China	62	RT-PCR	*F. nucleatum*	58	21	29
Yanglong Chen *et al.* [27]	2019	China	59	droplet digital PCR	*F. nucleatum*	25	20	17
Jing Li *et al.* [28]	2020	China	58	RT-PCR	*B. fragilis*	22	13	17
Xingming Deng *et al.* [29]	2018	China	51	multiplex PCR	*B. fragilis*	6	13	11
Jun Li *et al.* [30]	2020	China	59	RNA-ISH	*B. fragilis*	54	36	48
John Nemunaitis *et al.* [31]	2013	America	52	RT-PCR	*E. coli*	3	2	1
Clemens Unger *et al.* [32]	2001	Germany	62	droplet digital PCR	*E. coli*	44	25	2
Maria Liljefors *et al.* [33]	2008	Sweden	63	RNA-ISH	*E. coli*	31	68	4
Jeremy Shapiro *et al.* [34]	1999	America	56	multiplex PCR	*E. coli*	42	147	3

RT-PCR: Real-Time polymerase chain reaction; RNA-ISH: RNA *in situ*hybridization; TP: True Positive; FN: False Negative; FP: False Positive; TN: True Negative; *F. nucleatum* : *Fusobacterium nucleatum* ; *B. fragilis* : *Bacteroides fragilis* ; *E. coli* : *Escherichia coli* ; PPV: Positive Predictive Value; NPV: Negative

Predictive Value; PLR: Positive Likelihood Ratio; NLR: Negative Likelihood Ratio.

Figures

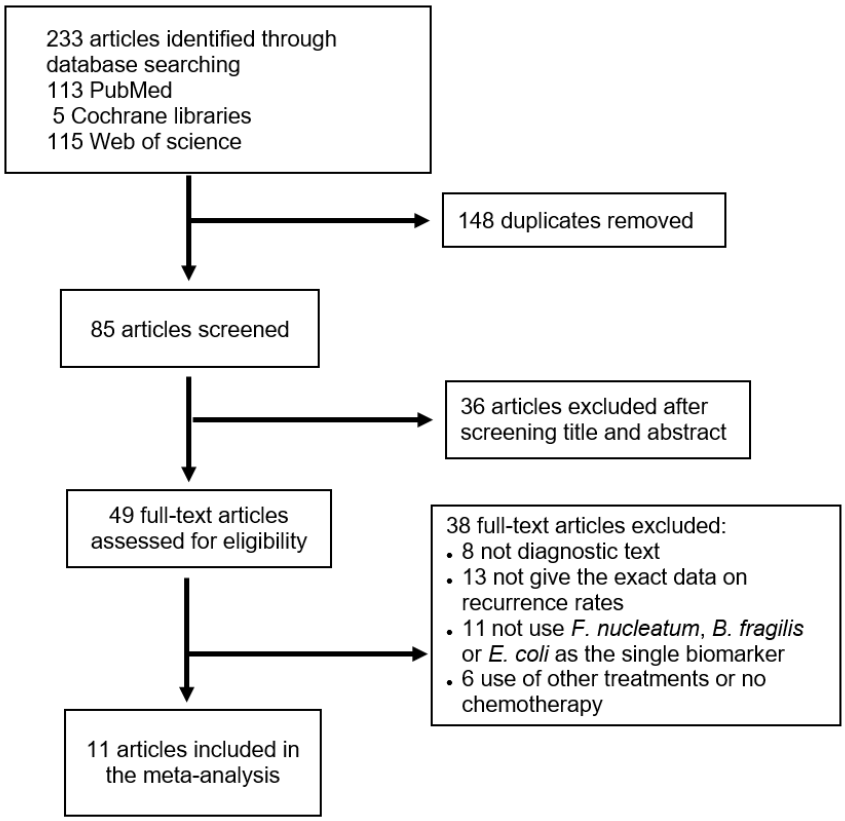
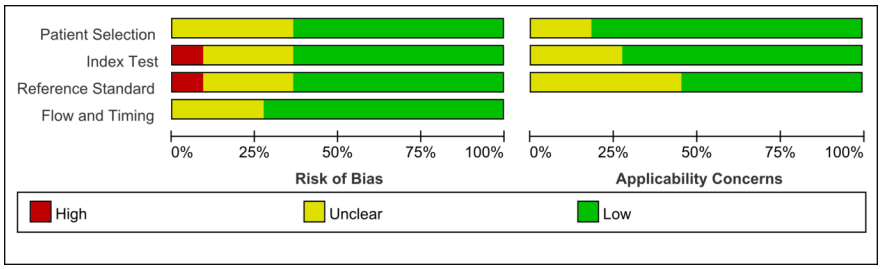
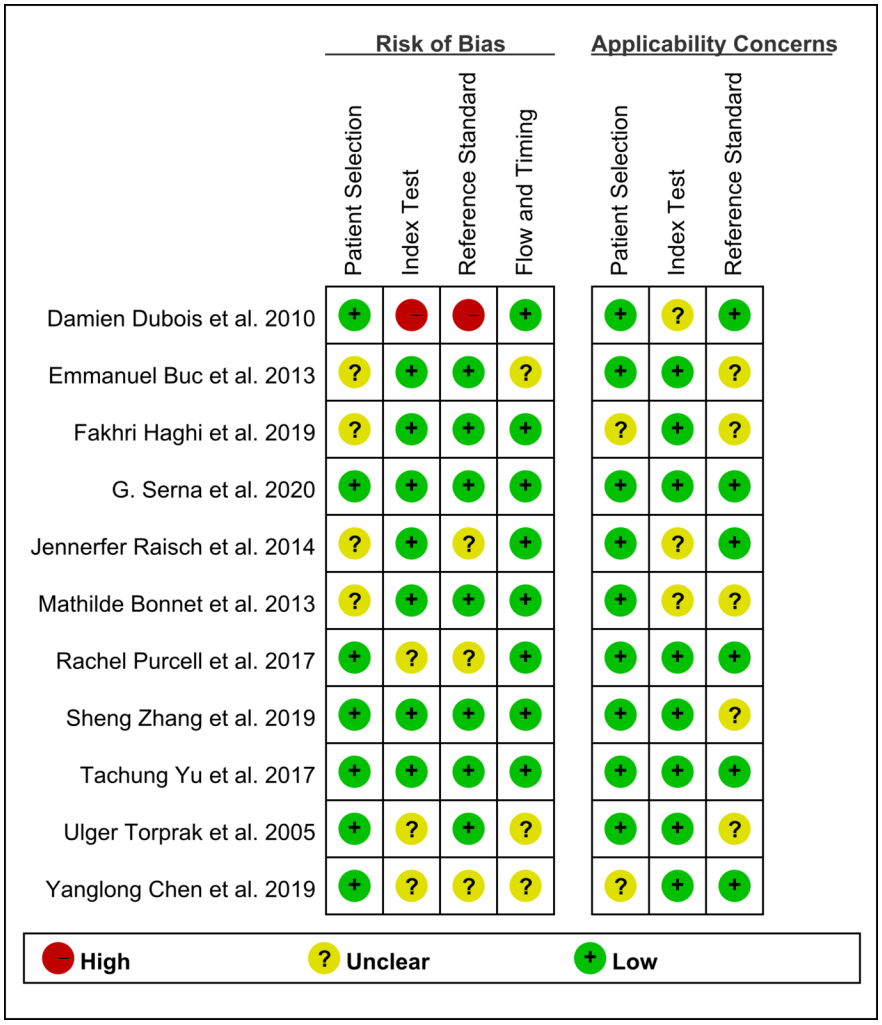


Figure 1. Flow chart of the systematic review and meta-analysis process.



(a)



(b)

Figure 2. An overview of the methodological quality results. (a) Risk of bias and applicability concerns summary. (b) Risk of bias and applicability concerns graph.

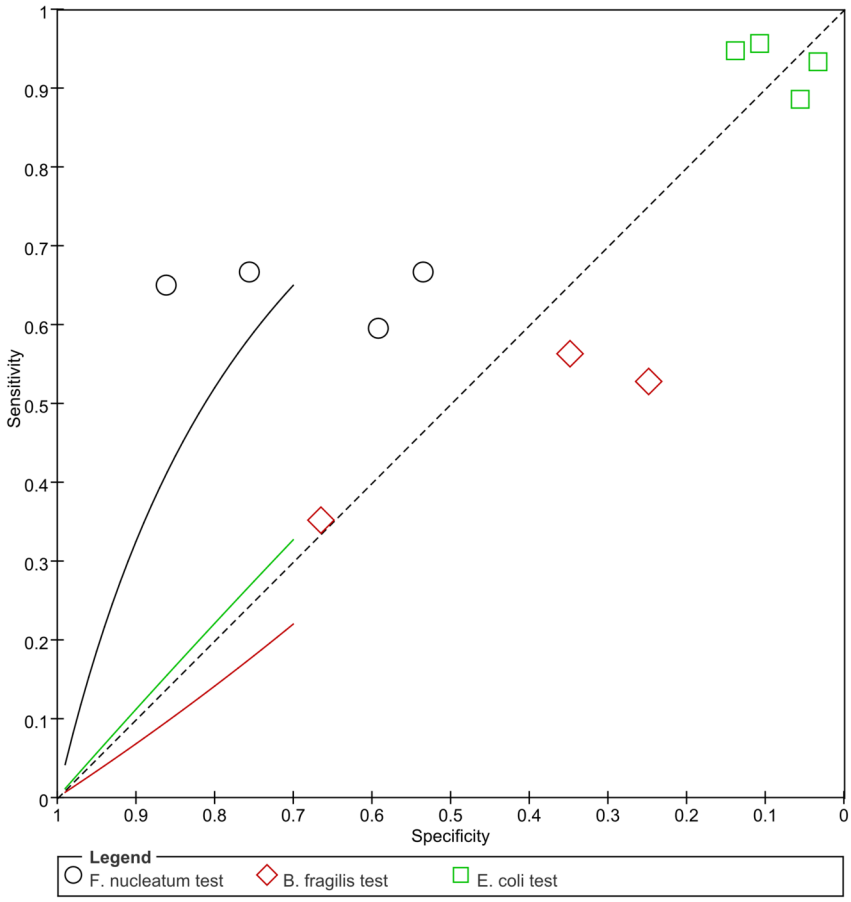


Figure 3. SROC assessment of diagnostic performance of *F. nucleatum* (black circle), *B. fragilis* (red rhombus) and *E. coli* (green square) for 5-FU resistance of colorectal cancer. SROC distributed advisably (AUC: 0.79, 95% CI: 0.74-0.81), which indicated no statistical significance ($P = 0.83$). SROC: Summary receiver operator characteristic curve.

F. nucleatum test

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
G. Serna et al. 2020	13	9	7	56	0.65 [0.41, 0.85]	0.86 [0.75, 0.93]
Sheng Zhang et al. 2019	34	20	17	23	0.67 [0.52, 0.79]	0.53 [0.38, 0.69]
Tachung Yu et al. 2017	58	21	29	65	0.67 [0.56, 0.76]	0.76 [0.65, 0.84]
Yanglong Chen et al. 2019	25	20	17	29	0.60 [0.43, 0.74]	0.59 [0.44, 0.73]

B. fragilis test

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Fakhri Haghi et al. 2019	22	13	17	7	0.56 [0.40, 0.72]	0.35 [0.15, 0.59]
Rachel Purcell et al. 2017	54	36	48	12	0.53 [0.43, 0.63]	0.25 [0.14, 0.40]
Ulger Torprak et al. 2005	6	13	11	26	0.35 [0.14, 0.62]	0.67 [0.50, 0.81]

E. coli test

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Damien Dubois et al. 2010	42	147	3	5	0.93 [0.82, 0.99]	0.03 [0.01, 0.08]
Emmanuel Buc et al. 2013	31	68	4	4	0.89 [0.73, 0.97]	0.06 [0.02, 0.14]
Jennerfer Raisch et al. 2014	44	25	2	3	0.96 [0.85, 0.99]	0.11 [0.02, 0.28]
Mathilde Bonnet et al. 2013	18	25	1	4	0.95 [0.74, 1.00]	0.14 [0.04, 0.32]

Figure 4. Forest plot of the pooled diagnostic sensitivity and specificity of *F. nucleatum* , *B. fragilis* and *E. coli* for 5-FU resistance of colorectal cancer. CI: Confidence interval. TP: True Positive; FN: False Negative; FP: False Positive; TN: True Negative; *F. nucleatum* : *Fusobacterium nucleatum* ; *B. fragilis* : *Bacteroides fragilis* ; *E. coli* :*Escherichia coli* .

References

1. Vodenkova, S., et al., 5-fluorouracil and other fluoropyrimidines in colorectal cancer: Past, present and future. Pharmacol Ther, 2020.**206** : p. 107447.

2. Wolpin, B.M. and R.J. Mayer, Systemic treatment of colorectal cancer. Gastroenterology, 2008. **134** (5): p. 1296-310.

3. van der Velden, D.L., F.L. Opdam and E.E. Voest, TAS-102 for Treatment of Advanced Colorectal Cancers That Are No Longer Responding to Other Therapies. Clin Cancer Res, 2016. **22** (12): p. 2835-9.

4. Casado, E., et al., UFT (tegafur-uracil) in rectal cancer. Ann Oncol, 2008.**19** (8): p. 1371-1378.

5. Dahan, L., et al., Modulation of cellular redox state underlies antagonism between oxaliplatin and cetuximab in human colorectal cancer cell lines. Br J Pharmacol, 2009. **158** (2): p. 610-20.

6. Zou, W., J.D. Wolchok and L. Chen, PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: Mechanisms, response biomarkers, and combinations. Sci Transl Med, 2016. **8** (328): p. 328rv4.

7. Fiorentini, C., et al., Gut Microbiota and Colon Cancer: A Role for Bacterial Protein Toxins? Int J Mol Sci, 2020. **21** (17).

8. Huus, K.E., et al., Commensal Bacteria Modulate Immunoglobulin A Binding in Response to Host Nutrition. Cell Host Microbe, 2020. **27** (6): p. 909-921.e5.

9. Hayden, H.S., et al., Fecal dysbiosis in infants with cystic fibrosis is associated with early linear growth failure. Nat Med, 2020.**26** (2): p. 215-221.

10. Dubinsky, V., et al., Predominantly Antibiotic-resistant Intestinal Microbiome Persists in Patients with Pouchitis Who Respond to Antibiotic Therapy. Gastroenterology, 2020. **158** (3): p. 610-624.e13.

11. Brennan, C.A. and W.S. Garrett, Fusobacterium nucleatum - symbiont, opportunist and oncobacterium. Nat Rev Microbiol, 2019. **17** (3): p. 156-166.

12. Komiya, Y., et al., Patients with colorectal cancer have identical strains of Fusobacterium nucleatum in their colorectal cancer and oral cavity. Gut, 2019. **68** (7): p. 1335-1337.

13. Kwong, T., et al., Association Between Bacteremia From Specific Microbes and Subsequent Diagnosis of Colorectal Cancer. Gastroenterology, 2018.**155** (2): p. 383-390.e8.

14. Yu, T., et al., Fusobacterium nucleatum Promotes Chemoresistance to Colorectal Cancer by Modulating Autophagy. Cell, 2017. **170** (3): p. 548-563.e16.

15. Zhang, S., et al., Fusobacterium nucleatum promotes chemoresistance to 5-fluorouracil by upregulation of BIRC3 expression in colorectal cancer. J Exp Clin Cancer Res, 2019. **38** (1): p. 14.

16. Sears, C.L., A.L. Geis and F. Housseau, Bacteroides fragilis subverts mucosal biology: from symbiont to colon carcinogenesis. J Clin Invest, 2014. **124** (10): p. 4166-72.

17. Chung, L., et al., Bacteroides fragilis Toxin Coordinates a Pro-carcinogenic Inflammatory Cascade via Targeting of Colonic Epithelial Cells. Cell Host Microbe, 2018. **23** (2): p. 203-214.e5.

18. Multidrug-resistant bacteroides fragilis–Seattle, Washington, 2013. MMWR Morb Mortal Wkly Rep, 2013. **62** (34): p. 694-6.

19. Lieuw-a-Fa, M., et al., Sepsis from liver abscesses in metastatic colorectal carcinoma after chemoimmunotherapy. J Clin Oncol, 2008. **26** (8): p. 1381-2.

20. Butt, J., et al., Association of Pre-diagnostic Antibody Responses to Escherichia coli and Bacteroides fragilis Toxin Proteins with Colorectal Cancer in a European Cohort. Gut Microbes, 2021.**13** (1): p. 1-14.

21. Liu, Q.Q., et al., Enterotoxigenic Bacteroides fragilis induces the stemness in colorectal cancer via upregulating histone demethylase JMJD2B. Gut Microbes, 2020. **12** (1): p. 1788900.

22. Ge, W., et al., High-risk Stage III colon cancer patients identified by a novel five-gene mutational signature are characterized by upregulation of IL-23A and gut bacterial translocation of the tumor microenvironment. Int J Cancer, 2020. **146** (7): p. 2027-2035.

23. O'Leary, K., MYC inhibition, courtesy of E. coli. Nat Rev Cancer, 2021.**21** (4): p. 214-215.

24. Holmes, C.L., et al., Pathogenesis of Gram-Negative Bacteremia. Clin Microbiol Rev, 2021. **34** (2).

25. Lucas, C., et al., Autophagy of Intestinal Epithelial Cells Inhibits Colorectal Carcinogenesis Induced by Colibactin-Producing Escherichia coli in Apc(Min/+) Mice. Gastroenterology, 2020. **158** (5): p. 1373-1388.

26. Serna, G., et al., Fusobacterium nucleatum persistence and risk of recurrence after preoperative treatment in locally advanced rectal cancer. Ann Oncol, 2020. **31** (10): p. 1366-1375.

27. Chen, Y., et al., Prognostic impact of the Fusobacterium nucleatum status in colorectal cancers. Medicine (Baltimore), 2019.**98** (39): p. e17221.

28. Jing, L., et al., Composition of fecal microbiota in low-set rectal cancer patients treated with FOLFOX. Ther Adv Chronic Dis, 2020.**11** : 2040622320904293.

29. Deng, X., et al., Comparison of Microbiota in Patients Treated by Surgery or Chemotherapy by 16S rRNA Sequencing Reveals Potential Biomarkers for Colorectal Cancer Therapy. Front Microbiol, 2018. **9** : 1607.

30. Li, J., et al., Microbiome characteristics and Bifidobacterium longum in colorectal cancer patients pre- and post-chemotherapy. Translational Cancer Research, 2020. **9** (4): 2178-2190.

31. John, N., et al., Pilot trial of genetically modified, attenuated Salmonella expressing the E. coli cytosine deaminase gene in refractory cancer patients. Cancer Gene Therapy, 2003. **10** : 737-744.

32. Clemens, U., et al., Double-blind Randomised Placebo-controlled Phase III Study of an E. coli Extract plus 5-Fluorouracil versus 5-Fluorouracil in Patients with Advanced Colorectal Cancer, 2001.**51** (4): 332-8.

33. Maria, L., et al., Influence of varying doses of granulocyte-macrophage colony-stimulating factor on pharmacokinetics and antibody-dependent cellular cytotoxicity. Cancer Immunol Immunotherapy, 2008. **57** : 379–388.

34. Shapiro, J., et al., A pilot study of interferon alpha-2a, fluorouracil, and leucovorin given with granulocyte-macrophage colony stimulating factor in advanced gastrointestinal adenocarcinoma. Clin Cancer Res 1999.**5** (9).

35. Mann, R., Hewitt, C.E. & Gilbody, S.M. Assessing the quality of diagnostic studies using psychometric instruments: applying QUADAS. Soc Psychiat Epidemiol, 2009. **44** (300).