**Systematic review: Gut microbiota in fecal samples and detection of colorectal neoplasms**

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**ABSTRACT**

Colorectal cancer (CRC) is a leading cause of cancer morbidity and mortality. Dysbiosis in the gut microbiota may be associated with CRC. This systematic review focuses on differences in gut microbial community between people diagnosed with CRC or adenoma and healthy individuals using fecal samples, emphasizing non-invasive fecal microbiome models for CRC early diagnosis.

Nineteen studies were identified in a systematic literature search of PubMed, Web of Science and ScienceDirect. Several bacteria were reported to differ in abundance between CRC and adenoma cases and healthy controls, with *Fusobacterium* the most common. Fecal multi-bacterial predictive models used to distinguish CRC patients from healthy controls had reported areas under the receiver operating curve (AUCs) in external validation populations of 0.68–0.77.

**Introduction**

Colorectal cancer (CRC), the third most common cancer worldwide, is a leading cause of cancer mortality. With 5-year survival rates of almost 90% for CRC diagnosed at stage I and slow development over many years, perspectives for secondary prevention of CRC and reduction in mortality by early detection and population screening are much better than for most other cancers. Fecal-based test kits, the guaiac-based fecal occult blood testing (gFOBT) and fecal immunological tests (FITs) are used in many countries for non-invasive early detection of CRC and advanced adenomas, a leading precursor of CRC.

At least 15% of cancer burden worldwide is attributable to known intestinal infectious agents such as *Helicobacter pylori*. In the case of CRC, studies demonstrated an association between a disruption in the gut microbial-community balance, or “dysbiosis”, and cancer development. The increased relative abundance of harmful bacteria on the one hand, and the depletion of other health promoting beneficial bacteria on the other hand may create a microenvironment more favorable to the development of CRC.

Concentrating on the possible use of fecal gut microbiome as a non-invasive diagnostic tool in population screening for CRC, comparable to FIT and gFOBT, we aimed to systematically review the literature on differences in the composition of the gut microbial community between people diagnosed with CRC or adenomas and healthy individuals, and the possible use of these differences in models for non-invasive early diagnosis of CRC and advanced adenomas. Due to significant differences in the bacterial population extracted from tumor tissue and feces from the same individual, this systematic review specifically focuses only on gut microbiome in fecal samples.

**Literature search strategy**

A systematic literature search was conducted independently by two of the authors (EA, AK) in order to identify original studies examining the differences in gut microbial community in fecal samples between people diagnosed with CRC or adenomas and people...
without colorectal malignancies. The online databases Medline, Web of Science and ScienceDirect were searched on April 17th 2017 using the following search terms: (Fecal AND microbiota AND CRC) OR (Microbiome AND colorectal AND cancer) OR (Fecal AND microbiome AND colorectal AND cancer) OR (Gut AND microbiome AND colorectal AND cancer). On Web of Science the search was refined by “article” category. On ScienceDirect the search was limited to “Journals”, years 2007–2017 and topics: gut microbiota, colorectal cancer, gut, human, cancer, colon cancer and tumor.

Duplicate studies were excluded as were irrelevant publication types such as symposium agendas. Titles and abstracts were screened independently by EA and AK and full texts were reviewed by each with reference lists searched for additional relevant studies. This systematic literature review was conducted according to the PRISMA guidelines.16

Inclusion criteria

Studies were included if they met all inclusion criteria: examining the gut microbial community in fecal samples from CRC or adenoma cases compared to healthy individuals; using human feces; original research with kind of bacteria found as outcome. Where same cases and controls were included in more than one publication, only the publication offering the most detailed information useful for this review was included. Studies published in letter or abstract forms were excluded as they did not include enough data for our review. The reviewers independently selected studies based on the inclusion criteria and disagreements were discussed until agreement was reached. Where studies included data from several sources (feces, tissue, mice) only fecal data analysis was included.

Data extraction and quality assessment

Included studies were evaluated for quality of information independently by EA and AK based on the Newcastle-Ottawa Scale,17 designed to assess the quality of case control studies, with some necessary amendments [Table 1]: a criterion for collecting fecal samples before preparation for colonoscopy was added in the place of the original criterion “ascertainment of exposure” since some previous studies demonstrated that bowel preparation for colonoscopy changes the gut microbial community.18,19 We also added a criterion for statistical adjustment for multiple testing or correction for over-optimism. Thus, the number of quality criteria amounts to eight and the maximum point score to nine since the “comparability of cases and controls” criterion can receive up to two points.

Literature search results

The literature search process, shown in a PRISMA flow diagram in Fig. 1, yielded a total of 1111 records. After exclusion of 198 duplicates, titles and abstracts of 913 studies were screened for relevance. Studies deemed not relevant for the review topic (n = 755), published only as a short communication or in abstract form (n = 61) and non-English publications (n = 26) were excluded. The full texts of 71 studies were read independently by EA and AK and further 52 studies were excluded for not meeting all the inclusion criteria.

Three studies included participants from the same French cohort and therefore only the report including the most detailed information needed for this review was included20 while the other two were excluded.21,22 Two other studies shared the same American cohort, therefore only one was included23 and the other was excluded.24 A study by Vogtmann et al25 recently performed whole-genome shotgun sequencing on fecal samples from an American population that took part in the study by Ahn et al,23 validating their results on a French population included in a study by Zeller et al,20 and thus was not included in this review. One randomized clinical trial26 was excluded since the study design involved giving the participants synbiotics which made it incomparable to the other selected studies. Although no design criterion was applied, all included studies were case-control studies. In total, nineteen studies were included in the qualitative analysis of the systematic review.

Study populations and study quality

The nineteen included studies15,20,23,27-42 were published between 2012 and 2017 [Table 2]. Eight studies20,23,28,30,34,38,40,42 reported when data and samples were collected, ranging between 1985 and 2015 with a gap of 2 to 28 years between collection of fecal samples and publication year. Fecal samples used for microbiome analysis ranged between 15 (8 CRC cases) in the smallest study by Weir et al31 to 490 (120 CRC cases) in the largest study by Baxter et al39. A study by Hale et al40 included 780 participants, 223 of whom
### Table 1. Quality of included studies based on Newcastle-Ottawa Scale.

| First author/Pub. Year/country (ref) | Cases validated by colonoscopy | Representative Cases | Community controls | Control status validated by colonoscopy | Age | Gender | Same exposure analysis for cases and controls | Exposure | Total (out of 9) |
|-------------------------------------|---------------------------------|----------------------|--------------------|----------------------------------------|-----|--------|-----------------------------------------------|----------|-----------------|
| Yu 2017, China                      | +                               | +                    | +                  | +                                      | −   | −      | +                                            | +        | 5               |
| Flemer 2017, Ireland                | +                               | [no info]            | +                  | +                                      | +   | +      | +                                            | [no info]| 5               |
| Hale 2016, USA                      | +                               | +                    | +                  | +                                      | +   | +      | +                                            | +        | 7               |
| Baxter 2016 USA/Canada              | +                               | [no info]            | +                  | +                                      | −   | −      | +                                            | −        | 5               |
| Kasai 2016, Japan                   | +                               | +                    | +                  | +                                      | +   | +      | +                                            | +        | 7               |
| Mira-Pascual 2016                   | +                               | +                    | +                  | +                                      | −   | −      | +                                            | +        | 7               |
| Fukugaiti 2015, Brazil              | +                               | [no info]            | +                  | +                                      | +   | +      | +                                            | +        | 5               |
| Goedert 2015, China                 | +                               | +                    | +                  | [no info]                              | +   | +      | [no info]                                    | +        | 8               |
| Feng 2015, Austria                  | +                               | +                    | +                  | +                                      | +   | +      | [no info]                                    | +        | 8               |
| Zeller 2014, France                | +                               | +                    | +                  | +                                      | +   | +      | +                                            | +        | 9               |
| Zacklar 2014 USA/Canada             | +                               | +                    | +                  | +                                      | −   | −      | −                                            | −        | 6               |
| Wu 2013, China (Beijing)            | +                               | [no info]            | +                  | [no info]                              | +   | +      | [no info]                                    | [no info]| 6               |
| Weir 2013, USA                      | +                               | [no info]            | +                  | [no info]                              | +   | +      | [no info]                                    | [no info]| 6               |
| Ohigashi 2013, Japan                | +                               | −                    | +                  | +                                      | +   | +      | +                                            | +        | 8               |
| Kostic 2013 USA/Scotland            | +                               | [no info]            | +                  | [no info]                              | +   | +      | +                                            | [not relevant]| 6               |
| Ahn 2013, USA (DC)                  | +                               | +                    | +                  | +                                      | +   | +      | +                                            | +        | 8               |
| Chen 2013, China                    | +                               | +                    | +                  | [no info]                              | −   | −      | +                                            | −        | 7               |
| Wang 2012 China (Shanghai)          | +                               | +                    | −                  | +                                      | +   | +      | +                                            | +        | 6               |
were diagnosed with adenoma, but this study did not include any CRC cases. Although sample size is not a criterion in the Newcastle-Ottawa scale, it is a highly relevant factor for achieving statistical power and precision with larger studies potentially representing better the microbial community variety in the population. Eleven studies included a separate group of participants diagnosed with adenomas, joined in the analyses to either the CRC group,\textsuperscript{33,36} the control group\textsuperscript{20,30} or analyzed separately.\textsuperscript{20,28,29,33-35,39,40,42}

All studies used frozen stool samples except for Ohigashi et al\textsuperscript{30} and Kasai et al\textsuperscript{38} who used stool samples stored in a temperature of +4°C before microbiome analysis. In population-wide CRC screening programs using stool-based tests, analysis is done on fresh stool samples within days of collection. These conditions are rarely possible in case-control studies, where stool may be frozen and stored for a few years before analysis and where collection methods, storage temperatures and duration before analysis of fecal samples may vary widely and have a differentiating effect on the results of microbiome analysis.\textsuperscript{43-45}

Fecal bacteria may also be affected by bowel preparation for colonoscopy, though evidence is somewhat limited.\textsuperscript{13,19,20} Eleven studies indicated that fecal samples were taken before colonoscopy preparation and in one study the samples were taken 1–2 weeks after colonoscopy, arguing it is enough time for the gut microbial community to return to its pre-preparation state. Yu et al adjusted for timing of fecal sampling as a confounding factor in analyses since in a significantly higher number of cases fecal samples were collected after surgery.\textsuperscript{41}

Antibiotics taken by study participants may also affect the microbiome. Five studies did not address antibiotics taken by the participants.\textsuperscript{20,29,33,36,39} In the 14 studies that addressed it, one mentions it as a limitation of the study that no data was available on antibiotics consumption,\textsuperscript{40} three studies excluded...
Table 2. Characteristics of studies included in the systematic review.

| First author/Pub. Year/Country | Data collection years | CRC | Adenoma | Healthy | Antibiotic use prior to stool sample | Database used for taxonomy assignment | Microbiome Analysis Method | Groups compared for bacterial differences | Statistical analysis for bacterial differences | Relevant design issues |
|-------------------------------|-----------------------|-----|---------|---------|-------------------------------------|---------------------------------------|---------------------------|---------------------------------------------|-----------------------------------------------|------------------------|
| Yu 2017 China-Hong Kong       | —                     | 203 (117) 66.5 (44) | 97 (50) 60.5 ± 4.7 | 236 (87) 54 (33) | Not in 3 months | IMG                                  | 16S rRNA qPCR | CRC / healthy                          | Non parametric tests     | More samples in cases taken after colonoscopy. |
| Flemer 2016 Ireland           | —                     | 203 (117) 66.5 (44) | 97 (50) 60.5 ± 4.7 | 236 (87) 54 (33) | Not in 3 months | IMG                                  | 16S rRNA qPCR | CRC / healthy                          | Non parametric tests     | Possible effects of bowel preparation or neoadjuvant therapy. |
| Hale 2016 USA                 | 2001 – 2005           | 203 (117) 66.5 (44) | 97 (50) 60.5 ± 4.7 | 236 (87) 54 (33) | Not in 3 months | IMG                                  | 16S rRNA qPCR | CRC / healthy                          | Non parametric tests     |                     |
| Baxter 2016, USA / Canada     | —                     | 203 (117) 66.5 (44) | 97 (50) 60.5 ± 4.7 | 236 (87) 54 (33) | Not in 3 months | IMG                                  | 16S rRNA qPCR | CRC / healthy                          | Non parametric tests     |                     |
| Kasai 2016 Japan              | 2012 – 2013           | 203 (117) 66.5 (44) | 97 (50) 60.5 ± 4.7 | 236 (87) 54 (33) | Not in 3 months | IMG                                  | 16S rRNA qPCR | CRC / healthy                          | Non parametric tests     |                     |
| Mira-Pascual 2016 Spain       | —                     | 203 (117) 66.5 (44) | 97 (50) 60.5 ± 4.7 | 236 (87) 54 (33) | Not in 3 months | IMG                                  | 16S rRNA qPCR | CRC / healthy                          | Non parametric tests     |                     |
| Fukugaiti 2015 Brazil         | —                     | 203 (117) 66.5 (44) | 97 (50) 60.5 ± 4.7 | 236 (87) 54 (33) | Not in 3 months | IMG                                  | 16S rRNA qPCR | CRC / healthy                          | Non parametric tests     |                     |
| Goedert 2015, China           | —                     | 203 (117) 66.5 (44) | 97 (50) 60.5 ± 4.7 | 236 (87) 54 (33) | Not in 3 months | IMG                                  | 16S rRNA qPCR | CRC / healthy                          | Non parametric tests     |                     |
| Feng 2015 Austria             | 2010–2012             | 203 (117) 66.5 (44) | 97 (50) 60.5 ± 4.7 | 236 (87) 54 (33) | Not in 3 months | IMG                                  | 16S rRNA qPCR | CRC / healthy                          | Non parametric tests     |                     |

(Continued on next page)
| First author/Pub. Year/Country | Data collection years | CRC | Adenoma | Healthy | Reason for colonoscopy | Stool collection | Temp. stool storage | Antibiotic use prior to stool sample | Database used for taxonomy assignment | Microbiome Analysis Method | Groups compared for bacterial differences | Statistical analysis for bacterial differences | Relevant design issues |
|--------------------------------|----------------------|-----|---------|---------|------------------------|-----------------|-------------------|-----------------------------|------------------------------------|----------------|---------------------------------|-------------------------------|-----------------------------|
| Zeller 2014 France / Denmark / Spain / Germany | 2004–2006 | 53 (29) | 42 (30) | 61 (28) | F: Referral G: recruited after diagnosis | [no info] | [no info] | F: —20°C in 4 h G: —80°C H: —20°C then —80°C | KEGG | 16S rRNA (for 129 partic.) | CRC / adenoma / health or CRC / Adenoma + healthy | Non parametric tests | CRC patients were significantly older on average |
| Zackular 2014, USA / Canada | — | 30 (21) | 30 (18) | 30 (11) | Self-collected at home | [no info] | —80°C | —80°C | RDP, V.9 | V4 | 16S rRNA | healthy / CRC or adenoma, or CRC + adenoma | Non parametric tests | Stool samples collected 1–2 weeks after colonoscopy |
| Wu 2013 China | — | 19 (10) | — | 30 (11) | Cases: CRC patients | [no info] | Directly | —80°C | RDP, V2.3 | 16S rRNA qPCR | CRC / healthy | Non parametric tests |
| Weir 2013 USA | — | 8 (8) | — | 7 (3) | Self-collected | [no info] | —20°C | —20°C | Green Genes | V4 | 16S rRNA qPCR | CRC / healthy | Parametric tests |
| Ohigashi 2013 Japan | 2009–2010 | 66.4 (47–84) | 35.86 (24–67) | 35.86 (24–67) | Recruited at hospital Collected at hospital | —4°C | Current users excluded | [not relevant] | RDP, qPCR | 16S rRNA RT-qPCR | CRC / healthy + adenoma | Non parametric tests |
| Kostic 2013 USA / Scotland | — | 27 | 28 | 31 | [no info] | [no info] | [no info] | [no info] | [not relevant] | qPCR | CRC / health Adenoma / healthy | Non parametric tests | Only Fusobacterium. Samples from EDRN of NCI. |
| Ahn 2013 USA (DC) | 1985–1989 | 47 (28) | — | 94 (56) | Recruited at hospital | Self-collected at home | —40°C | Not in 1 year | IMG / Green Genes | V3 – V4 | CRC / healthy | Non parametric tests |
| Chen 2013 China | 2010–2011 | 62.9 | 58.5 | 58.5 | Various reasons | Self-collected at home | —80°C | Not in 6 months | Silva database | 16S rRNA qPCR | CRC / adenoma / healthy | Parametric and Non parametric tests | Adenoma and healthy groups matched for age and gender. |
| Wang 2012 China | — | 46 (24) | 56 (27) | 56 (27) | [no info] | [no info] | Cases: no current Controls: not in 3 m | RDP | 16S rRNA qPCR | CRC / healthy | Parametric and Non parametric tests |

Abbreviations: CRC – colorectal cancer; qPCR – quantitative polymerase chain reaction; IMG – Integrated Microbial Genome; RDP – Ribosomal Database Project; KEGG – Kyoto Encyclopedia of Genes and Genomes; EDRN – Early Detection Research Network; NCI – National Cancer Institute.
participants that were taking antibiotics at the time of recruitment and the others excluded participants that used antibiotics before recruitment for a period ranging between one month to one year. In establishing the case and control status of participants, six studies did not state ascertainment that the controls had no history of CRC or adenoma diagnosis in the past. Thus, vast variation in most aspects of the studies limits the ability to synthesize the results together or compare the individual study results.

**Bacteria detection**

Bacterial detection method in three studies was qPCR alone for Quantitative Real-Time amplification of bacterial DNA, ten studies used the 16S rRNA sequencing and 6 studies utilized both 16S rRNA and qPCR in analyzing the samples [Table 2]. Three studies reported statistically significant differences in the gut microbial composition also after adjustment for multiple testing.

The studies used different reference databases for the assignment of taxonomy and different statistical methods to analyze possible differences between participants with CRC, adenoma or healthy controls with regards to their gut microbial community properties with most using non-parametric statistical tests and two using machine learning.

Table 3 includes the bacteria found in at least two studies to significantly differ in abundance between cases and control groups. Two studies have also looked at enterotypes, a classification of three distinct bacterial clusters used as a general descriptive measure of the gut microbiota. There were also some contradicting findings, such as the genus *Streptococcus* that was found by Chen et al and by Wang et al to be more abundant in people diagnosed with adenomas and CRC respectively, while Feng et al found this genus to be significantly more abundant in the healthy control group. Based on included studies, it seems there is no consensus as to which specific fecal bacteria differentiate the gut microbiome of people diagnosed with CRC or adenoma compared to healthy controls. Nonetheless, members of the *Fusobacteria* phylum, mainly the genus *Fusobacterium*, were the most common bacteria, reported by 10 studies, to be significantly more abundant in CRC cases compared to healthy controls.

**Multi-bacteria models for early detection of CRC**

Eight studies included multi-bacteria predictive models that may potentially complement established tests such as gFOBT or FIT as a non-invasive early detection tool for CRC and its precursors [Table 4]. All but one study, by Liang et al, reported only point estimates of the area under the receiver operator characteristic curve (AUC) values found and did not report 95% confidence intervals (CIs) for these values. Furthermore, the reported AUCs are for the most part derived from the training (derivation) populations, and are therefore prone to overoptimism due to overfitting. The few AUCs derived from validation sets are marked in Table 4 with asterisks.

Liang et al found that *Fusobacterium nucleatum* alone had in their training population an AUC of 0.87 (95%CI 0.83, 0.90). A model of four bacterial markers including *Fusobacterium nucleatum* offered better diagnostic performance with an AUC of 0.89 (95%CI 0.85, 0.92).

Baxter et al used 23 different operational taxonomic units (OTUs) with FIT and received an AUC of 0.95 for the detection of CRC, albeit the result was not significantly higher than the result obtained using FIT alone (p-value = 0.90) in the same population. In addition, Baxter et al, using DNA from buffered stool from participants’ FIT cartridges, constructed a model utilizing 28 OTUs with an AUC of 0.83 for differentiating healthy controls from those with CRC.

For distinguishing between adenoma and CRC groups, Zackular et al found that the gFOBT alone had an AUC = 0.62, while using a microbiome-based model with four OTUs had a significantly higher performance with AUC of 0.95 in the training population. However, independent validation remains yet to be performed.

**Multi-bacteria models for early detection of adenoma**

Lower performance was generally reported for classification models distinguishing adenomas from healthy controls. Liang et al used *Fusobacterium nucleatum* only model and had AUC = 0.61 (95%CI 0.55–0.67). A combination of FIT and 23 OTUs revealed in one study AUC of 0.76 and in another study the AUC of a model combining 10 metagenomic linkage groups (MLGs) and age was 0.59. The model by Hale et al, including 4 genera: 2 enriched in adenoma and 2 enriched in controls, had an AUC of 0.66 for predicting
The existence of adenomas in their study population. In another study, classification models using 18 phylum-level taxa or 64 order-level taxa showed an AUC of 0.77 for both models. Remarkably higher performance of the classification model including 5 OTUs plus age, race and BMI for detection of adenomas was demonstrated by Zackular et al (AUC = 0.90).

Both for detection of CRC and of adenomas the researchers used different bacteria, including some as of yet unnamed, at different taxonomy levels to comprise their models and there is not one common bacterial genus or species that is included in all the models. In addition, apart from in the study by Liang et al, no additional information such as sensitivity, specificity or other diagnostic/prognostic accuracy parameters of the models were included in the publications.

**External validation of the multi-bacteria models for early detection**

External validation of the multi-bacterial models was done in three studies. The model by Yu et al, with 2 case-enriched and 2 control-enriched microbiomic gene markers, gave an AUC of 0.73 for detecting CRC compared to healthy controls in the Chinese test population. The model was tested also in independent validation Austrian and French cohorts, with similar AUC values of 0.77 and 0.72 in the latter two respectively.

Zeller and colleagues created a CRC classifier consisting of 22 species using data from a French cohort and received an AUC of 0.84. The same model revealed AUCs of 0.85 and 0.90 in the French population (156 participants) combined with either one German population consisting of 297 healthy individuals or another consisting of 38 CRC cases, respectively. Liang et al verified their two models, one containing *Fusobacterium nucleatum* only and the other a combination of four bacterial markers, in a separate smaller Chinese validation cohort and received lower AUCs of 0.68 (95%CI 0.55, 0.80) and 0.76 (95%CI 0.64, 0.87), respectively.

**Discussion**

Nineteen studies examining the difference in gut microbial community composition in human fecal samples between people diagnosed with CRC or adenoma and healthy individuals were included in this systematic review. The studies used different analysis protocols at different taxonomic levels to identify significant bacterial differences therefore enabling only limited comparisons. The most common bacteria found in eight of the studies to be in relatively higher
| Authors Year | Country | Model includes | Population examined | Comparisons and Outcome AUC | External validation |
|--------------|---------|----------------|---------------------|-----------------------------|---------------------|
| Liang 2017  | China   | *Fusobacterium nucleatum* | Cohort = C / A / H | C vs H = 0.87, C vs A = 0.68, C+A vs H = 0.89, A vs H = 0.76 | Validated in independent Chinese cohort II |
|              |         | 4 bacteria     | I = 170/ NA / 200  | II = 33 / NA / 36           |                     |
|              |         |                |                     | II = 170/ NA / 200          |                     |
|              |         |                |                     | I = NA / 97 / 200           |                     |
| Yu 2017 China / Denmark / Austria / France |         | 4 gene markers | C2 = 47 / NA / 109 | C2 vs NA = 0.73, D vs NA = 0.77, F vs NA = 0.72 | Test: C2-Chinese |
|              |         |                | A = 41 / NA / 55   | F = 53 / NA / 61            | Validation: D-Danish |
|              |         |                |                     | D = 16 / NA / 24            | A-Austrian F-French |
|              |         | 20 gene markers |                     |                             | —                   |
|              |         | 2 gene markers | C2                  |                             | —                   |
| Hale 2016 USA |         | 4 genera       | NA / 233 / 547     | C vs A = 0.66, C vs H = —    | —                   |
| Baxter 2016 USA / Canada |         | 23 OTUs + FIT result | I = 120 / 198/ 172 | C vs A = 0.95, C vs H = 0.83, C vs A = 0.76 | II – sequencing of buffered stool from FIT tubes |
|              |         |                |                     |                             |                     |
|              |         | 28 OTUs       | II = 101 / 162 / 141| C vs H = 0.83, C vs A = 0.69 |                     |
| Goedert 2015 China |         | Rank relative  | NA/20/24            | C vs H = 0.77, C vs A = —    | —                   |
|              |         | abundance of 18 phylum-level taxa with and without sex |                     |                             |                     |
|              |         | Rank relative  | NA / 20 / 24       | C vs H = 0.77, C vs A = —    | —                   |
|              |         | abundance of 64 order-level taxa |                     |                             |                     |
| Feng 2015 Austria |         | 15 MLGs       | 5 / 47 / 8          | C vs H = 0.96, C vs A = —    | Internal Validation set also includes patients from test set |
|              |         |                |                     |                             |                     |
|              |         | 10 MLGs + age | 46 /5 / 8           | C vs H = 0.59, C vs A = —    |                     |
|              |         |                | 46 / 5 / 8          |                             |                     |
| Zeller 2014 France / Denmark / Spain / Germany |       | 22 species   | F = 53 / 42 / 61     | C vs H = 0.84, C vs A = —    | Test: F-French |
|              |         |                |                     |                             |                     |
|              |         |                |                     |                             | —                   |
|              |         |                |                     |                             | —                   |
| Zackular 2014 USA / Canada |       | 6 OTUs + age + race + BMI | 30 / NA / 30       | C vs H = 0.85, C vs A = 0.90 | —                   |
|              |         |                |                     |                             | —                   |
|              |         | 4 OTUs + gFOBT + BMI | 30 / 30 / NA       | C vs H = 0.97, C vs A = —    | —                   |
|              |         |                |                     |                             | —                   |
|              |         | 5 OTUs + age + race + BMI | NA / 30 / 30       | C vs H = 0.90, C vs A = —    | —                   |
|              |         |                |                     |                             | —                   |
|              |         | 6 OTUs + age + gender + race | 30 / 30 / 30       | C vs H = 0.94, C vs A = —    | —                   |

C - colorectal cancer; A – adenoma; H – healthy; AUC – area under the receiver operating characteristic curve; (g)FOBT – (guaiac) fecal occult blood test; BMI – body mass index; MLGs – metagenomics linkage groups; OTUs – operational taxonomic units.

*AUC in independent validation populations.
abundance in CRC cases compared to healthy controls was the genus *Fusobacterium*.

The relatively high incidence of CRC and the high potential for survival when detected early have prompted a growing number of countries to adopt national screening programs. The most widely used non-invasive screening tool is the gFOBT but due to its relatively low sensitivity it is increasingly being replaced by more sensitive FITs. Using colonoscopy for CRC screening, with detection and removal of adenomas, could prevent most CRC cases, but both adherence of the target population and available colonoscopy capacity remain limiting factors.

**Fecal gut-microbiome for early detection of CRC**

Advanced sequencing techniques to identify gut bacteria in feces may have in the future the potential to complement current non-invasive methods for CRC early detection and risk stratification. Eight studies included in this analysis developed bacteria based models. Some of these studies reported very high AUCs of up to 0.95 for discriminating CRC patients from healthy participants in the study population in which the models were trained. Most likely, however, these results are far too overoptimistic due to overfitting, as suggested by much lower AUCs in the order 0.68–0.77 that were found in the two studies in which the derived algorithms were validated in independent validation samples. Such models may nevertheless potentially be useful for stratification of the population for their cancer risk level based on the composition of their gut microbiome.

**Fecal gut-microbiome for detection of colorectal adenomas**

Several studies in this systematic review have also identified statistically significant differences in the abundance of certain bacteria between people with adenomas compared to healthy controls. Five studies reported AUCs of 0.61–0.90 for their bacteria based model for discerning participants with adenomas from healthy participants. Regrettably, none of these models was validated in an external validation set. The reported bacterial differences between carriers of adenomas and healthy controls are more likely to reflect a consequence rather than a bacterial cause of tumorigenesis given the much more limited structural changes in the large bowel resulting from adenomas. Flanagan et al. have found an over-abundance of *F. nucleatum* in cancerous compared to matched normal tissue (*p* < 0.0001) but *Fusobacterium* levels in colorectal adenomas were not significantly higher compared to healthy controls (*p* = 0.06). Ito et al. found that *F. nucleatum* positivity was significantly higher in CRCs (56%) than in premalignant lesions of any histological type (*p* < 0.0001) and its positivity increased according to histological grade. Amitay et al. found that no statistically significant difference was detected in fecal prevalence of *F. nucleatum* between carriers of advanced and non-advanced adenomas and healthy controls while *F. nucleatum* was much more commonly found in the CRC group compared to all above groups. Moreover, within the CRC cases in this study, abundance of *Fusobacterium* was positively associated with more advanced cancer stages. In addition, in the current review, most included studies reported more pronounced differences in bacterial relative abundance between participants with CRC and healthy participants than between participants with adenomas and healthy participants [Table 3]. Moreover, the AUCs for the bacterial models used to distinguish CRC cases from healthy participants are significantly higher than those used to distinguish carriers of adenomas from healthy participants [Table 4].

**Fusobacterium and CRC**

*Fusobacteria*, the bacteria reported most commonly to differ between CRC patients and healthy controls in the included studies, are common residents in the human oral microbiome and are only found infrequently in the gut. Studies have shown that CRC tumors and their metastases possess specific glycans that underlie fusobacterial tumor enrichment and tumor growth. There are several hypotheses as to how Fusobacterium and other bacteria may be contributing to CRC tumorigenesis. Rubinstein et al. found that *F. nucleatum* adheres to and invades colonic cells and induces oncogenic and inflammatory responses to stimulate growth of CRC cells through its unique FadA adhesion, a virulence factor facilitating the bacterium’s adherence to cell surface. Kostic et al. using a mouse model, found that through
recruitment of tumor-infiltrating immune cells *Fusobacteria* generate a pro-inflammatory microenvironment that is conducive for colorectal neoplasia progression. Gur et al. found in a mouse model that *F. nucleatum*-containing tumors inhibited NK cell cytotoxicity and tumor infiltrating lymphocyte cell activity via the interaction of the F. nucleatum protein Fap2 with human TIGIT (T cell immunoglobulin and ITIM domain). Mima et al. found that a greater amount of *F. nucleatum* in human colorectal carcinoma tissue was associated with a lower density of CD3+ T cells in tumor tissue. These interactions of *Fusobacterium* with the tumor environment and with the immune system, though unable yet to provide concrete evidence of causality, shed light on the association between gut bacterial dysbiosis and the development of colorectal tumors and may further explain the association of the amount of *F. nucleatum* DNA in colorectal cancer tissue with shorter survival.

**Synthesis of results and sources of bias and heterogeneity**

The ability to draw definite conclusions from the reviewed studies is limited by the fact that although the study of the microbiome using high-throughput DNA sequencing is a rapidly evolving field, the absence of a gold-standard unified protocol leads to great heterogeneity in methodology and makes direct comparisons or potential pooling of results across studies not easily possible. Varying regions sequenced, depths of sequencing, methods and protocols for the extraction of the bacterial DNA, sample handling (collection, transportation, storage, time to analysis), varying PCR primers used for 16S microbial analysis, varying methods for assignment of taxonomy and different statistical approaches for data analysis (varying from established epidemiological methods to innovative machine-learning modelling) are all significant potential sources of bias and heterogeneity.

In addition, several recent reviews indicate that there is no “universal human gut microbiome” and that there are significant variations in the gut microbiota due to differences in ethnicity, geographic location, lifestyle, westernization and nutrition, though others could identify a small “core microbiota” found in the majority of their study population though here too the microbial community composition in the stool was significantly influenced by various exposures such as stool consistency and medication use. This variety in the healthy microbiota may make it harder to find a unified list of bacteria to be included in a microbiome-based stool test for CRC and adenoma detection. In light of these findings, it is possible that emphasis should be put not only on the presence of specific bacteria but also on the absence of other bacteria, bacterial functioning in the gut environment and the relationship between the microbiota and the host immune system.

Nonetheless, as seen in the study by Yu et al., the same multi-bacteria model for discerning CRC from healthy controls and containing 4 gene markers which was tested independently in Chinese, Austrian and French populations gave similar results (AUCs 0.72–0.77). This indicates that although different populations from different geographical areas may have different gut microbial communities at baseline it may be possible to develop feces based multi-bacterial models for the detection of colorectal neoplasms that may be used in a variety of settings and populations.

**Tissue vs fecal-derived bacteria**

As noted before, there are significant differences in the bacterial population extracted from tumor tissue and from feces. This may explain why several prominent bacteria such as *Streptococcus galolyticus* found in tissue analysis to be associated with CRC, are missing from the studies included in this review. While tissue microbial community analysis may contribute to a better understanding of the mechanisms by which the gut bacteria play a role in CRC carcinogenesis and progression and may be relevant from a therapeutic biomarker perspective, it is not relevant for using microbiome-based tests as an inexpensive non-invasive early diagnostic or risk-stratification tool to complement the currently-used FIT or gFOBT in population based screening programs.

To our knowledge this is the first review to systematically and comprehensively review the results and methodology of epidemiological studies looking into the differences in the gut microbial community as manifested in fecal samples between people diagnosed with CRC or advanced adenoma and people with normal colonoscopy results and their potential use for non-invasive early diagnosis of CRC and adenomas. Given that this is a young emerging field of research, with more than half of identified studies published
from 2015 onward, there may be additional recent studies whose results have not been published as full articles at the time of the search.

In conclusion, based on the currently published data, there is limited but encouraging evidence that differences in fecal gut microbiome between people diagnosed with CRC or adenoma and persons without colorectal malignancy may be used to develop new, non-invasive and inexpensive fecal tests that could complement the repertoire of current non-invasive CRC screening tools on their own or in combination with established fecal occult blood tests. While it remains unclear to what extent these differences in the gut microbiome precede and contribute the occurrence of CRC, distinct patterns in the gut microbial community of CRC patients may still contribute to CRC early detection even if they should not be causally related to CRC development. Future research should focus on developing unified documented and reproducible protocols for studying the human gut microbiome from fecal samples so that results can be more comparable and conclusions can be drawn on a larger basis. These models should be externally and independently validated in ethnically and geographically diverse populations. A better knowledge of the changes in the composition of the gut microbial community during CRC development may not only assist in better early diagnosis and identification of those at high risk for developing CRC but may also help identifying novel CRC preventive approaches by modulating the gut microbiome through nutrition, life-style changes or more targeted approaches such as probiotic or synbiotic therapy.

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References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359-86. doi:10.1002/ijc.29210. PMID:25220842.
2. Howlader N, Noone AM, Krapcho M, Miller D, Bishop K, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z, et al. SEER Cancer Statistics Review, 1975–2013. Bethesda, MD: National Cancer Institute; 2016 [Available from: http://seer.cancer.gov/csr/1975_2013/].
3. Brenner H, Hofmeister M, Stegmaier C, Brenner G, Altenhoff L, Haug U. Risk of progression of advanced adenomas to colorectal cancer by age and sex: estimates based on 840,149 screening colonoscopies. Gut. 2007;56(11):1585-9. doi:10.1136/gut.2007.122739. PMID:17591622.
4. Hewitson P, Glasziou P, Watson E, Towler B, Irwig L. Cochrane systematic review of colorectal cancer screening using the fecal occult blood test (hemoccult): an update. Am J Gastroenterol. 2008;103(6):1541-9. doi:10.1111/j.1572-0241.2008.01875.x. PMID:18479499.
5. Shaukat A, Mongin SJ, Geisser MS, Lederle FA, Bond JH, Mandel JS, Church TR. Long-term mortality after screening for colorectal cancer. N Engl J Med. 2013;369(12):1106-14. doi:10.1056/NEJMoa1300720. PMID:24047060.
6. Atkin W, Wooldrage K, Parkin DM, Kralj-Hans I, MacRae E, Shah U, Duffy S, Cross AJ. Long term effects of once-only flexible sigmoidoscopy screening after 17 years of follow-up: the UK Flexible Sigmoidoscopy Screening randomised controlled trial. Lancet. 2017;389(10076):1299-311. doi:10.1016/S0140-6736(17)30396-3. PMID:28236467.
7. Navarro M, Nicolas A, Fernandez A, Lasas A. Colorectal cancer population screening programs worldwide in 2016: An update. World J Gastroenterol. 2017;23(20):3632-42. doi:10.3748/wjg.v23.i20.3632. PMID:28611516.
8. Oh JK, Weiderpass E. Infection and cancer: global distribution and burden of diseases. Ann Glob Health. 2014;80(5):384-92. doi:10.1016/j.aogh.2014.09.013. PMID:25512154.
9. Narayanan V, Peppelenbosch MP, Konstantinov SR. Human fecal microbiome-based biomarkers for colorectal cancer. Cancer Prev Res (Phila). 2014;7(11):1108-11. doi:10.1158/1940-6207.CAPR-14-0273. PMID:25223933.
10. Castellanin M, Warren RL, Freeman JD, Dreolini L, Krzywinski M, Strauss J, Barnes R, Watson P, Allen-Vercoe E, Moore RA, et al. Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. Genome research. 2012;22(2):299-306. doi:10.1101/gr.126516.111. PMID:22009899.
11. Guinane CM, Cotter PD. Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ. Therap Adv Gastroenterol. 2013;6(4):295-308. doi:10.1177/1756283X13482996. PMID:23814609.
12. Wang WL, Xu SY, Ren ZG, Tao L, Jiang JW, Zheng SS. Application of metagenomics in the human gut microbiome. World J Gastroenterol. 2015;21(3):803-14. doi:10.3748/wjg.v21.i3.803. PMID:25624713.
13. Budding AE, Grasman ME, Eck A, Bogaards JA, Vandenbroucke-Grauls CM, van Bodegraven AA, Savelkoul
PH. Rectal swabs for analysis of the intestinal microbiota. PloS one. 2014;9(7):e101344. doi:10.1371/journal.pone.0101344. PMID:25020051.

14. Chen W, Liu F, Ling Z, Tong X, Xiang C. Human intestinal lumen and mucosa-associated microbiota in patients with colorectal cancer. PloS one. 2012;7(6):e39743. doi:10.1371/journal.pone.0039743. PMID:22761885.

15. Flemer B, Lynch DB, Brown JM, Jeffery IB, Ryan FJ, Claesson MJ, O’Riordain M, Shanahan F, O’Toole PW. Tumour-associated and non-tumour-associated microbiota in colorectal cancer. Gut. 2017;66(4):633-43. doi:10.1136/gutjnl-2015-309595. PMID:26992426.

16. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ. 2009;339:b5235. PMID:19622551.

17. Wells GA, Shea B, O’connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. [Available from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp]

18. Mai V, Greenwald B, Morris JG, Jr., Raufman JP, Stine FL, Weinberg HS, Ahn J. Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. Isme J. 2012;6(2):320-9. doi:10.1038/ismej.2011.109. PMID:21850056.

19. Chen HM, Yu YN, Wang J, Lin YW, Kong X, Yang CQ, Yang L, Liu ZJ, Yuan YZ, Liu F, et al. Decreased dietary fiber intake and structural alteration of gut microbiota in patients with advanced colorectal adenoma. Am J Clin Nutr. 2013;97(5):1044-52. doi:10.3945/ajcn.112.046607. PMID:23553152.

20. Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, Clancy TE, Chung DC, Lochead P, Hold GL, et al. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. Cell Host Microbe. 2013;14(2):207-15. doi:10.1016/j.chom.2013.07.007. PMID:23945149.

21. Amiot A, Dona AC, Wijeyesekera A, Tournigand C, Baumgaertner I, Lebaleur Y, Sobhani I, Holmes E. (1)H NMR Spectroscopy of Fecal Extracts Enables Detection of Advanced Colorectal Neoplasia. J Proteome Res. 2015;14(9):3871-81. doi:10.1021/acs.jproteome.5b00277. PMID:26211820.

22. Amiot A, Dona AC, Wijeyesekera A, Tournigand C, Baumgaertner I, Lebaleur Y, Sobhani I, Holmes E. (1)H NMR Spectroscopy of Fecal Extracts Enables Detection of Advanced Colorectal Neoplasia. J Proteome Res. 2015;14(9):3871-81. doi:10.1021/acs.jproteome.5b00277. PMID:26211820.

23. Ahn J, Sinha R, Pei Z, Dominiani C, Wu J, Shi J, Goedert JJ, Hayes RB, Yang L. Human gut microbiome and risk for colorectal cancer. J Natl Cancer Inst. 2013;105(24):1907-11. doi:10.1093/jnci/djt030. PMID:24316595.

24. Sinha R, Ahn J, Sampson JN, Shi J, Yu G, Xiong X, Hayes RB, Goedert JJ. Fecal Microbiota, Fecal Metabolome, and Colorectal Cancer Interrelations. PloS one. 2016;11(3):e0152126. doi:10.1371/journal.pone.0152126. PMID:27015276.

25. Vogtmann E, Hua X, Zeller G, Sunagawa S, Voigt AY, Hercog R, Goedert JJ, Shi J, Bork P, Sinha R. Colorectal Cancer and the Human Gut Microbiome: Reproducibility with Whole-Genome Shotgun Sequencing. PloS one. 2016;11(5):e0155362. doi:10.1371/journal.pone.0155362. PMID:27171425.

26. Scanlan PD, Shanahan F, Clune Y, Collins JK, O’Sullivan GC, O’Riordain M, Holmes E, Wang Y, Marchesi JR. Culture-independent analysis of the gut microbiota in colorectal cancer and polyposis. Environ Microbiol. 2008;10(3):789-98. doi:10.1111/j.1462-2920.2007.01503.x. PMID:18237311.

27. Wang T, Cai G, Qiu Y, Fei N, Zhang M, Pang X, Jia W, Cai S, Zhao L. Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. Isme J. 2012;6(2):320-9. doi:10.1038/ismej.2011.109. PMID:21850056.

28. Chen HM, Yu YN, Wang J, Lin YW, Kong X, Yang CQ, Yang L, Liu ZJ, Yuan YZ, Liu F, et al. Decreased dietary fiber intake and structural alteration of gut microbiota in patients with advanced colorectal adenoma. Am J Clin Nutr. 2013;97(5):1044-52. doi:10.3945/ajcn.112.046607. PMID:23553152.

29. Weir TL, Mantner DK, Sheffin AM, Barnett BA, Heuberger AL, Ryan EP. Stool microbiome and metabolome differences between colorectal cancer patients and healthy adults. PloS one. 2013;8(8):e70803. doi:10.1371/journal.pone.0070803. PMID:23940645.

30. Wu N, Yang X, Zhang R, Li J, Xiao X, Hu Y, Chen Y, Yang F, Lu N, Wang Z, et al. Dysbiosis signature of fecal microbiota in colorectal cancer patients. Microb Ecol. 2013;66(2):462-70. doi:10.1007/s00248-013-0245-9. PMID:23733170.

31. Zackular JP, Rogers MA, Ruffin MT, Schloss PD. The human gut microbiome as a screening tool for colorectal cancer. Cancer Prev Res (Phila). 2014;7(11):1112-21. doi:10.1158/1940-6207.CAPR-14-0129. PMID:25104642.

32. Feng Q, Liang S, Jia H, Chung DC, Cochrane J, Yang F, Lu N, Wang Z, et al. Gut microbiome development along the colorectal adenoma-carcinoma sequence. Nat Commun. 2015;6:6528. doi:10.1038/ncomms7532. PMID:25758642.

33. Goedert JJ, Gong Y, Hua X, Zhong H, He Y, Peng P, Yu G, Wang W, Ravel J, Shi J, et al. Fecal Microbiota Characteristics of Patients with Colorectal Adenoma Detected by Screening: A Population-based Study.
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EBioMedicine. 2015;2(6):597-603. doi:10.1016/j.ebiom.2015.04.010. PMID:26288821.

36. Mira-Pascual L, Cabrera-Rubio R, Ocon S, Costales P, Parra A, Suarez A, Moris F, Rodrigo L, Mira A, Collado MC. Microbial mucosal colonic shifts associated with the development of colorectal cancer reveal the presence of different bacterial and archaean biomarkers. J Gastroenterol. 2015;50(2):167-79. doi:10.1007/s00535-014-0963-x. PMID:24811328.

37. Fukugaiti MH, Ignacio A, Fernandes MR, Ribeiro Junior U, Nakano V, Avila-Campos MJ. High occurrence of Fusobacterium nucleatum and Clostridium difficile in the intestinal microbiota of colorectal carcinoma patients. Braz J Microbiol. 2015;46(4):1135-40. doi:10.1590/S1517-83822014014665. PMID:26691472.

38. Kasai C, Sugimoto K, Moritani I, Tanaka J, Oya Y, Inoue H, Tameda M, Shiraki K, Ito M, Takei Y, et al. Comparison of human gut microbiota in control subjects and patients with colorectal carcinoma in adenoma: Terminal restriction fragment length polymorphism and next-generation sequencing analyses. Oncol Rep. 2016;35(1):325-33. doi:10.3892/or.2015.4398. PMID:26549775.

39. Baxter NT, Ruffin MT, Rogers MA, Schloss PD. Microbiota-based model improves the sensitivity of fecal immunochemical test for detecting colorectal lesions. Genome Med. 2016;8(1):37. doi:10.1186/s13073-016-0290-3. PMID:27056827.

40. Hale VL, Chen J, Johnson S, Harrington SC, Smyrk TC, Nelson H, Boardman LA, Druilner BR, Levin TR, et al. Shifts in the fecal microbiota associated with adenomatous polyps. Cancer Epidemiol Biomarkers Prev. 2016;25(1):85-94. doi:10.1158/1055-9965.EPI-16-0337. PMID:27675054.

41. Yu J, Feng Q, Wong SH, Zhang D, Liang QY, Qin Y, Tang L, Zhao H, Stenvang J, Li Y, et al. Metagenomic analysis of faecal microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer. Gut. 2017;66(1):70-8. doi:10.1136/gutjnl-2015-309800. PMID:26408641.

42. Liang Q, Chiu J, Chen Y, Huang Y, Higashimori A, Fang J, Brim H, Ashktorab H, Ng SC, Ng SSM, et al. Fecal Bacteria Act as Novel Biomarkers for Noninvasive Diagnosis of Colorectal Cancer. Clin Cancer Res. 2017;23(8):2061-70. doi:10.1158/1078-0432.CCR-15-30980. PMID:26408641.

43. Carroll IM, Ringel-Kulka T, Siddle JP, Klaenhammer TR, Ringel Y. Characterization of the fecal microbiota using high-throughput sequencing reveals a stable microbial community during storage. PloS one. 2012;7(10):e46953. doi:10.1371/journal.pone.0046953. PMID:23071673.

44. Goodrich J, Di Rienzi SC, Poole AC, Koren O, Walters WA, Caporaso JG, Knight R, Ley RE. Conducting a microbiome study. Cell. 2014;158(2):250-62. doi:10.1016/j.cell.2014.06.037. PMID:25036628.

45. Gorzelak MA, Gill SK, Tasnim N, Ahmadi-Vand Z, Jay M, Gibson DL. Methods for Improving Human Gut Microbiome Data by Reducing Variability through Sample Processing and Storage of Stool. PloS one. 2015;10(8):e0134802. doi:10.1371/journal.pone.0134802. PMID:26252519.

46. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM, et al. Enterotypes of the human gut microbiome. Nature. 2011;473(7346):174-80. doi:10.1038/nature09944. PMID:21508958.

47. Baxter NT, Kounpouras CC, Rogers MA, Ruffin MT, Schloss PD. DNA from fecal immunochemical test can replace stool for detection of colorectal lesions using a microbiota-based model. Microbiome. 2016;4(1):59. doi:10.1186/s40168-016-0205-y. PMID:27842559.

48. Brenner H, Tao S. Superior diagnostic performance of faecal immunochemical tests for haemoglobin in a head-to-head comparison with guaiac based faecal occult blood test among 2235 participants of screening colonoscopy. Eur J Cancer. 2013;49(14):3049-54. doi:10.1016/j.ejca.2013.04.023. PMID:23706981.

49. Lee JK, Liles EG, Bent S, Levin TR, Corley DA. Accuracy of fecal immunochemical tests for colorectal cancer: systematic review and meta-analysis. Ann Intern Med. 2014;160(3):171. doi:10.7326/M13-1484. PMID:24658694.

50. Robertson DJ, Lee JK, Boland CR, Dominitz JA, Giardiello FM, Johnson DA, Kaltenbach T, Lieberman D, Levin TR, Rex DK. Recommendations on Fecal Immunochemical Testing to Screen for Colorectal Neoplasia: A Consensus Statement by the US Multi-Society Task Force on Colorectal Cancer. Am J Gastroenterol. 2017;112(1):37-53. doi:10.1038/ajg.2016.492. PMID:27753435.

51. Brenner H, Stock C, Hoffmeister M. Effect of screening sigmoidoscopy and screening colonoscopy on colorectal cancer incidence and mortality: systematic review and meta-analysis of randomised controlled trials and observational studies. BMJ. 2014;348:g2467. doi:10.1136/bmj.g2467. PMID:24922745.

52. Brenner H, Altenhofen L, Stock C, Hoffmeister M. Prevention, early detection, and overdiagnosis of colorectal cancer within 10 years of screening colonoscopy in Germany. Clin Gastroenterol Hepatol. 2015;13(4):717-23. doi:10.1016/j.cgh.2014.08.036. PMID:25218160.

53. King-Marshall EC, Mueller N, Dailey A, Barnett TE, George TJ, Jr., Sultan S, Curbow B. “It is just another test they want to do”: Patient and caregiver understanding of the colonoscopy procedure. Patient Educ Couns. 2016;99(4):651-8. doi:10.1016/j pec.2015.10.021. PMID:26597383.

54. Cheung BS, Kim SH, Yoo KB, Seo SY, Kim IH, Lee SO, Lee ST, Kim SW. Should Assessment of Quality Indicator of Colonoscopy Be Varied Depending on the Colonscopic Technique Level? Dig Dis Sci. 2016;61(3):731-6. doi:10.1007/s10620-015-3954-8. PMID:26576553.

55. Flanagan L, Schmid J, Ebert M, Soucek P, Kunicka T, Liska V, Bruha J, Neary P, Dezeeuw N, Tommasino M, et al. Fusobacterium nucleatum associates with stages of colorectal neoplasia development, colorectal cancer and disease outcome. Eur J Clin Microbiol Infect Dis. 2014;33(8):1381-90. doi:10.1007/s10096-014-2081-3. PMID:24599709.
56. Ito M, Kanno S, Nosho K, Sukawa Y, Mitsushashi K, Kurihara H, Igarashi H, Takahashi T, Tachibana M, Takahashi H, et al. Association of Fusobacterium nucleatum with clinical and molecular features in colorectal serrated pathway. Int J Cancer. 2015;137(6):1258-68. doi:10.1002/ijc.29488. PMID:25703934.

57. Amitay EL, Werner S, Vital M, Pieper DH, Hofer D, Gierse IJ, Butt J, Balavarca Y, Cuk K, Brenner H. Fusobacterium and colorectal cancer: causal factor or passenger? Results from a large colorectal cancer screening study. Carcinogenesis. 2017;38(8):781-8. doi:10.1093/carcin/bgp053. PMID:28582482.

58. Abed J, Engard JE, Zamir G, Faroja M, Almogy G, Grenov A, Sol A, Naor R, Pikarsky E, Atlan KA, et al. Fap2 Mediates Fusobacterium nucleatum Colorectal Adenocarcinoma Enrichment by Binding to Tumor-Expressed Gal-GalNAc. Cell Host Microbe. 2016;20(2):215-26. doi:10.1016/j.chom.2016.07.006. PMID:27512904.

59. Bullman S, Pedamallu CS, Scinska E, Clancy TE, Zhang X, Mima K, Nishihara R, Qian ZR, Cao Y, Sukawa Y, Nowak J, et al. Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/beta-catenin signaling via its FadA adhesin. Cell Host Microbe. 2013;14(2):195-206. doi:10.1016/j.chom.2013.07.012. PMID:23954158.

60. Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. Fusobacterium nucleatum Mediates Fusobacterium nucleatum infection to human inhibitory receptor TIGIT protects tumors from immune cell attack. Immunity. 2015;42(2):344-55. doi:10.1016/j.immuni.2015.01.010. PMID:25680274.

61. Mancabelli L, Milani C, Lugli GA, Turroni F, Ferrario C, van Sinderen D, Ventura M. Meta-analysis of the human gut microbiome from urbanized and pr. Immunity. 2016;45(2):344-55. doi:10.1186/s13073-016-0307-y. PMID:27122046.

62. Rehman A, Rausch P, Wang J, Darzi Y, Faust K, Kuriashikov A, Bonder MJ, Valles-Colomar M, Vandeputte D, et al. Population-level analysis of gut microbiome variation. Science. 2016;352(6369):207-14. doi:10.1126/science.aad5303. PMID:27126039.

63. Clavel T, Gomes-Neto JC, Lagkouvardos I, Ramer-Tait AE. Deciphering interactions between the gut microbiota and the immune system via microbial cultivation and minimal microbiomes. Immunol Rev. 2017;279(1):8-22. doi:10.1111/imr.12578. PMID:28856739.

64. Rehman A, Rausch P, Wang J, Skiceviciene J, Kielidis B, Bhagalia K, Amarapurkar D, Kunpsinskas L, Schreiber S, Rosenstiel P, et al. Geographical patterns of the standing and active human gut microbiome in health and IBD. Gut. 2016;65(2):238-48. doi:10.1136/gutjnl-2014-308341. PMID:25567118.

65. Nakayama J, Watanabe K, Jiang J, Matsuda K, Chao SH, Haryono P, La-Ongkham O, Sarwoko MA, Sujaya IN, Zhao L, et al. Diversity in gut bacterial community of school-age children in Asia. Sci Rep. 2015;5:8397. doi:10.1038/srep08397. PMID:25703686.

66. Abdulmair AS, Hafidh RR, Abu Bakar F. The association of Streptococcus bovis/gallolyticus with colorectal tumors: the nature and the underlying mechanisms of its etiologic role. J Exp Clin Cancer Res. 2011;30:11. doi:10.1186/1756-9966-30-11. PMID:21247505.