Fusobacterium nucleatum in the Colorectum and Its Association with Cancer Risk and Survival: A Systematic Review and Meta-analysis

Christian Gethings-Behncke[1], Helen G. Coleman[1,2], Haydee W.T. Jordao[1], Daniel B. Longley[2], Nyree Crawford[2], Liam J. Murray[1], and Andrew T. Kunzmann[1]

ABSTRACT

Background: The gut microbiome, in particular *Fusobacterium nucleatum*, has been reported to play a role in colorectal cancer development and in patient prognosis. We aimed to perform a systematic review and meta-analysis of published studies to assess the prevalence of *F. nucleatum* in colorectal tumors and evaluate the association between *F. nucleatum* and colorectal cancer development and prognosis.

Methods: MEDLINE, EMBASE, and Web of Science databases were systematically searched for studies published until January 2019. Random effects meta-analyses were used to assess the prevalence of *F. nucleatum* in patients with colorectal cancer or tissues relative to controls and survival in *F. nucleatum*–positive versus –negative patients.

Results: Forty-five relevant articles were identified. Meta-analyses indicated higher odds of *F. nucleatum* being present in colorectal tissue samples from patients with colorectal cancer [$n = 6$ studies, pooled OR = 10.06; 95% confidence intervals (CI), 4.48–22.58] and individuals with colorectal polyps ($n = 5$ studies, pooled OR = 1.83; 95% CI, 1.07–3.16) compared with healthy controls. Similar results were apparent in fecal samples, and when comparing tumor with adjacent normal tissue. Meta-analyses indicated poorer survival in patients with colorectal cancer with high versus low *F. nucleatum* abundance ($n = 5$ studies, pooled HR = 1.87; 95% CI, 1.12–3.11).

Conclusions: A consistent increase in the prevalence and/or abundance of *F. nucleatum* in colorectal cancer tissue and fecal samples compared with controls was apparent. High abundance of *F. nucleatum* in colorectal tumors was also associated with poorer overall survival.

Impact: *F. nucleatum* could be useful as a diagnostic and prognostic marker for colorectal cancer or as a treatment target.

Introduction

The human gut has been estimated to contain over 3 trillion microbial cells which is approximately as many as the number of eukaryotic cells that comprise the human body (1), and may include as many as a 1,000 distinct bacterial species (2).

In recent years, there has been an increased understanding of the functional role of gut microbiota in the initiation of colorectal cancer (2). Cancer incidence in the large intestine is estimated to be 12-fold higher than that in the small intestine, which has been partially attributed to a larger bacterial density in the large intestine (3). Evidence suggests that an imbalance of normal intestinal microbiota can exacerbate chronic inflammatory conditions that lead to the production of carcinogenic metabolites, which promote the onset of colorectal cancer (3). A number of bacterial species have been implicated with colorectal cancer onset, including *Helicobacter pylori, Escherichia coli, Bacteroides fragilis, Salmonella enterica*, and *Fusobacterium nucleatum* (3).

F. nucleatum is a species of anaerobic gram-negative, non–spore forming, nonmotile bacteria of the genus *Fusobacterium* (4). *F. nucleatum* is an opportunistic commensal pathogen normally resident in the oral cavity (4), but may use saliva to survive passage through the stomach and into the lower gastrointestinal tract (5). In support of this, a recent study demonstrated that patients with colorectal cancer have identical *F. nucleatum* strains in their oral cavity and tumors (6). There is increasing evidence that *F. nucleatum* can adhere to and invade colorectal tumor tissue (7, 8) and may create a tumor permissive, proinflammatory microenvironment (TME; refs. 8–11).

Despite increasing evidence suggesting a potential role for *F. nucleatum* in colorectal cancer development and prognosis (12), to our knowledge, no systematic reviews with meta-analyses have fully evaluated the existing evidence. An existing systematic review (13) added to our understanding but did not conduct a meta-analyses to allow us to quantify the true association after accounting for differences in study results. This systematic review of the published scientific literature aimed to assess: (i) the prevalence/abundance of *F. nucleatum* colonization in biological samples from individuals with colorectal cancer or colorectal polyps compared with healthy controls or adjacent, normal tissue; (ii) the association of *F. nucleatum* with subsequent colorectal cancer development in individuals who were initially cancer-free; and (iii) the association between *F. nucleatum* and survival in individuals with colorectal cancer. To investigate these in sufficient depth, we were unable to review other bacterial species potentially linked with colorectal cancer.

Materials and Methods

Data sources

An *a priori* systematic search of the literature was performed in accordance with PRISMA guidelines (Supplementary Table S1; ref. 14)

[1]Centre for Public Health, Queen's University Belfast, Belfast, United Kingdom. [2]Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, United Kingdom.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Corresponding Author: Andrew T. Kunzmann, Centre for Public Health, Queen's University Belfast, Institute of Clinical Sciences-B, Royal Victoria Hospital Site, Grosvenor Road, Belfast, Northern Ireland, BT12 6BJ, UK. E-mail: a.kunzmann@qub.ac.uk

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using Medline (1947–), EMBASE (1974–), and Web of Science (1980–), from date of database inception to January 21, 2019 (after an update from October 2017), to identify all primary studies relating to the prevalence of *F. nucleatum* colonization and colorectal cancer risk and survival. A specific search strategy was devised to include at least one keyword or Medical Subject Heading (MeSH) term from each of the following: (i) colorectal cancer(s) or colon cancer(s) or rectal cancer(s) or cancer of the colorectum or cancer of the colon or cancer of the rectum or colorectal neoplasm(s) or colon neoplasm(s) or rectal neoplasm(s) or colorectal tumor(s) or colon tumor(s) or rectal tumor(s) or colorectal carcinoma(s) or colon carcinoma(s) or rectal carcinoma(s); and (ii) *fusobacterium nucleatum* or fusobacteria or fusobacterium or microbiome.

The search strategy was limited to studies carried out on humans. Both published full texts and conference abstracts were included. Review articles and individual case studies were removed as they would not provide new information to assess prevalence or abundance; however, to ensure relevance internationally, we did not restrict to English language papers.

Inclusion/exclusion criteria

Study selection was carried out independently, in duplicate, by two reviewers (from C. Gethings-Behncke H.G. Coleman, H.W. Jordao, L.J. Murray, and A.T. Kunzmann, with discrepancies settled by a third reviewer, using two levels of study screening.

For the prevalence/abundance studies (aim 1), participants had to be ages over 16 (to focus on adults but reduce risk of excluding population-based studies) and the study had to report the prevalence (proportion of samples with *F. nucleatum* DNA above detection threshold) or abundance (amount of *F. nucleatum* DNA in sample) of *F. nucleatum* status in any biological sample (including fecal, tissue, or dental): from individuals with colorectal cancer compared with individuals with colorectal polyp or healthy controls; from colorectal tumor tissue compared with adjacent normal tissue or; from individuals with colorectal polyps compared with healthy controls.

For the colorectal cancer development studies (aim 2), participants had to be ages over 16 and free from colorectal cancer at baseline and had to report the association between a measure of *F. nucleatum* status in any biological sample and subsequent colorectal cancer development.

For survival studies (aim 3), participants had to be had to be ages over 16 and diagnosed with colorectal cancer and the study had to report the association between a measure of *F. nucleatum* status in any biological sample and a measure of survival of the individuals with colorectal cancer.

Data extraction

Data extraction was conducted independently, in duplicate, by two reviewers (from C. Gethings-Behncke and A.T. Kunzmann), with discrepancies settled by a third reviewer (H.G. Coleman and L.J. Murray), and focused on authors; year of publication; study location; study design; *F. nucleatum* detection method; sample type (tissue, fecal, or dental); participant status (colorectal cancer, colorectal polyps, or healthy control); number of samples; prevalence of *F. nucleatum* in each sample/participant type; comparison reported; measure of colorectal cancer risk (aim 2) or prognosis (overall survival, colorectal cancer–specific survival or disease-free survival; aim 3); follow up years (aims 2 and 3); and adjustment for potential confounders. Study quality was assessed using the Newcastle Ottawa scale (15).

Statistical analysis

For prevalence of *F. nucleatum* (aim 1), random-effects meta-analyses were used to examine the pooled odds ratios (OR) and corresponding 95% confidence intervals (CI) of *F. nucleatum* prevalence in tissue and fecal samples, respectively, using published ORs, proportions, or numbers from:

(i) Individuals with colorectal cancer compared with healthy controls.

(ii) Individuals with colorectal cancer compared with individuals with colorectal polyps.

(iii) Individuals with colorectal polyps compared with healthy controls.

(iv) Colorectal tumor tissue compared with adjacent, normal tissue.

Too few studies had assessed the association between *F. nucleatum* prevalence or abundance and colorectal cancer development for a meta-analysis to be conducted (aim 2). Random-effects meta-analyses, with continuity correction for zero values, were used to examine the pooled hazard ratios (HR) and corresponding 95% CI for the association between the presence of *F. nucleatum* DNA and overall survival and disease-free survival (separately) in patients with colorectal cancer (aim 3). For all sets of meta-analyses, a X^2 test for heterogeneity was calculated and the I^2 statistic determined to estimate the proportion of variation between study results attributable to heterogeneity rather than chance (16). Heterogeneity was considered high if I^2 statistic was above 50% (16). Sensitivity analyses to examine the causes of heterogeneity were carried out by removing each study individually in turn. Because of low study numbers identified for each comparison, publication bias could not be formally assessed (17). Studies only reporting results for subgroups within cohorts were not included in meta-analyses due to the potential for reporting bias. Stata 14 software (Stata Corporation) was used for data analysis.

Results

As shown in **Fig. 1**, 2,063 unique titles and abstracts were screened for potential eligibility, from which 184 full-texts were screened for eligibility. Of these, 45 relevant articles were identified (13, 18–62), some of which addressed multiple aims of the systematic review.

F. nucleatum prevalence or abundance

Colorectal tissue samples from separate individuals

Eleven studies assessed the prevalence and/or abundance of *F. nucleatum* in colorectal tissue samples from separate individuals (Supplementary Table S2) with colorectal cancer compared with healthy colorectal tissue (six prevalence and four abundance studies) or colorectal adenoma (five prevalence and three abundance studies), and/or colorectal adenoma compared with healthy colorectal tissue (three prevalence and abundance studies).

As shown in **Fig. 2**, a meta-analysis assessing *F. nucleatum* prevalence indicated that the odds of *F. nucleatum* DNA being detected were higher in colorectal tumor tissue compared with healthy tissue from controls ($n = 6$ studies, pooled OR = 10.06; 95% CI, 4.48–22.58; $I^2 = 0.0\%$). Smaller increased odds of *F. nucleatum* DNA being detected were apparent in colorectal tumor tissue compared with polyp tissue ($n = 5$ studies, pooled OR = 1.83; 95% CI, 1.07–3.16; $I^2 = 66.9\%$) and in colorectal polyp tissue compared with healthy tissue from controls ($n = 3$ studies, pooled OR = 2.51; 95% CI, 1.20–5.27;

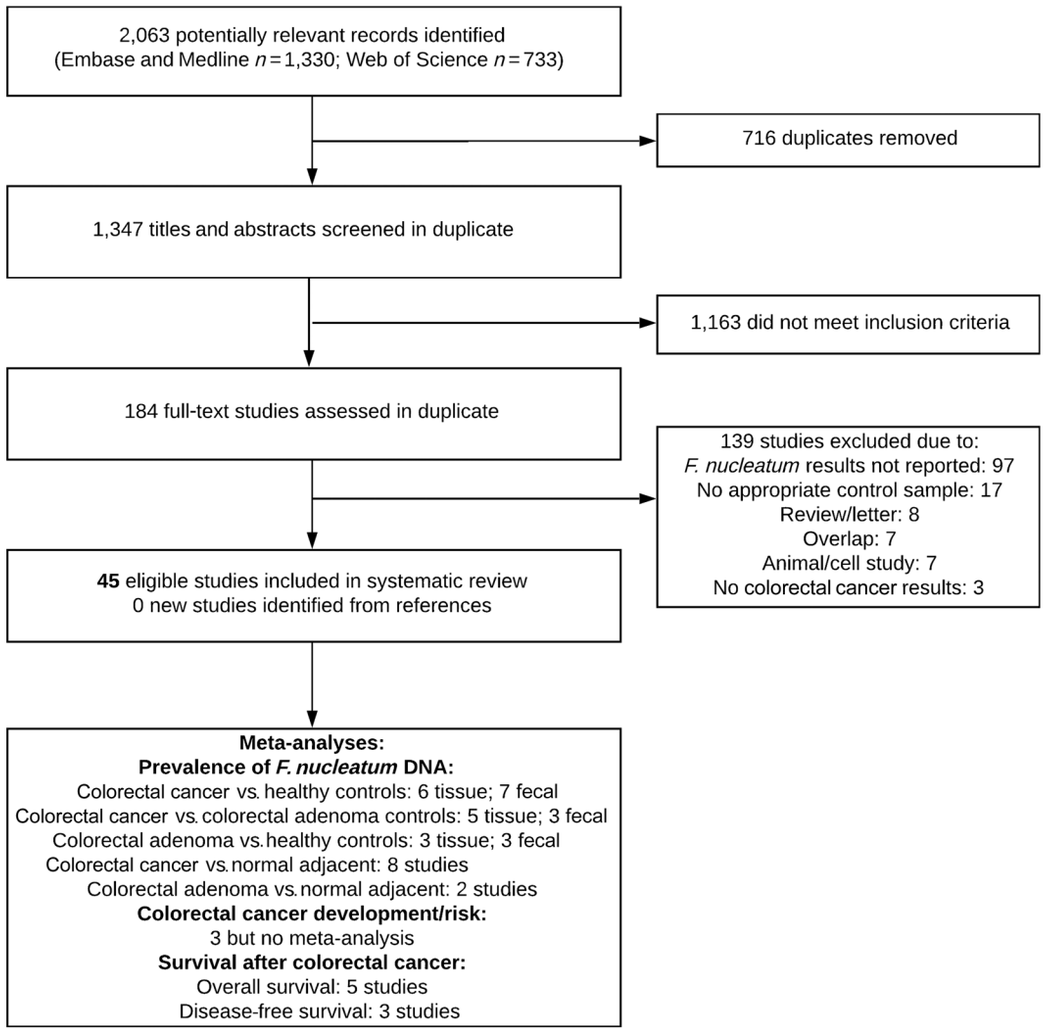

Figure 1.
Study selection. A total of 45 eligible studies were included from the 2,063 potentially relevant studies identified and reviewed.

$I^2 = 0.0\%$). When limited to studies rated as ≥4 on the Newcastle–Ottawa scale (Supplementary Table S3), similar results were apparent for colorectal cancer compared with healthy tissue ($n = 4$ studies; HR = 9.73; 95% CI, 4.02–23.54) or colorectal polyp tissue ($n = 3$ studies, no exclusions), and/or colorectal polyp tissue compared with healthy tissue ($n = 2$ studies, HR = 2.56; 95% CI, 1.20–5.48).

The high heterogeneity ($I^2 = 66.9\%$) when comparing colorectal tumor tissue samples to polyp tissue from controls was reduced after exclusion of a study by Ito and colleagues (2015), although the association was no longer statistically significant ($n = 4$ studies, pooled OR = 1.41; 95% CI, 0.96–2.07; $I^2 = 0.0\%$). The association between *F. nucleatum* in colorectal tumor tissue compared with colorectal adenoma tissue remained in a *post hoc* sensitivity analysis excluding a study by Fu and colleagues (55) and by Yu and colleagues (35), which used FISH, rather than qRT-PCR to detect *F. nucleatum* ($n = 4$ studies, pooled OR = 2.86; 95% CI, 2.22–3.69; $I^2 = 0.0\%$). Neither study using FISH found a difference in *F. nucleatum* in colorectal tumor tissue compared with colorectal adenoma tissue.

The variation in method of assessment for abundance prevented pooling of study results, although each study indicated a higher abundance in tissue at a more advanced diseased stage. All four studies assessing the abundance of *F. nucleatum* DNA in colorectal tumor tissue compared with healthy colorectal tissue from controls, found a higher abundance in colorectal tumor tissue (4, 23, 40, 42). Both studies comparing the abundance of *F. nucleatum* in colorectal tumor tissue compared with colorectal polyp tissue, and found a higher abundance in colorectal tumor tissue (42, 54). Similarly, both studies comparing the abundance of *F. nucleatum* in colorectal polyp tissue compared with healthy colorectal tissue from controls, and found a higher abundance in colorectal polyp tissue (22, 42).

Adjacent colorectal tissue samples

Thirteen studies assessed the prevalence and/or abundance of *F. nucleatum* in colorectal cancer tissue compared with healthy adjacent colorectal tissue (7 prevalence and 13 abundance) and/or colorectal adenoma tissue compared with healthy adjacent tissues (two prevalence and two abundance; Supplementary Table S4).

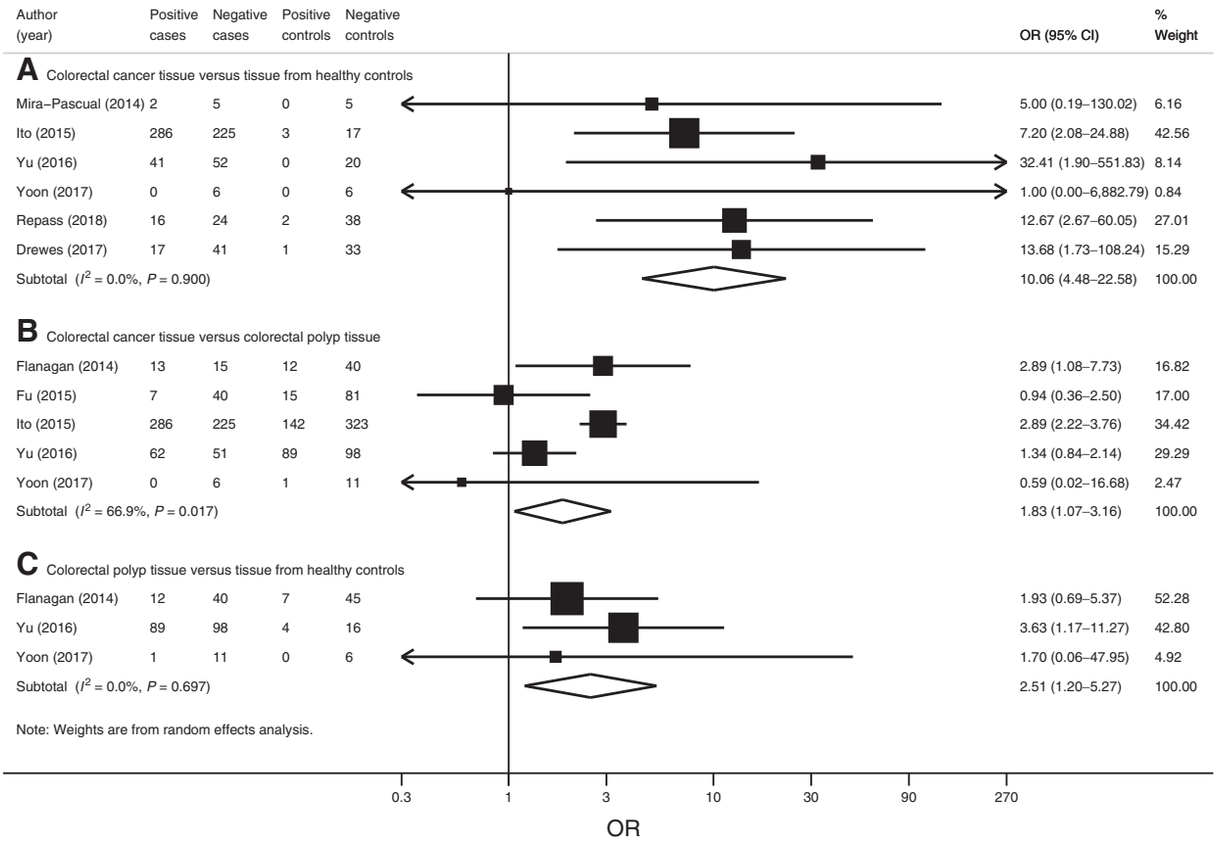

Figure 2.

Forest plot pooling the odds ratio of *F. nucleatum* positivity in colorectal tissue samples in separate individuals. The pooled odds of *F. nucleatum* positivity were higher in colorectal tumor tissue samples than normal colorectal tissue from controls (**A**); higher in colorectal tumor tissue than colorectal polyp tissue from controls (**B**); and higher in colorectal polyp tissue than in normal colorectal tissue from controls (**C**).

As shown in **Fig. 3**, meta-analyses assessing *F. nucleatum* prevalence indicated that the odds of *F. nucleatum* being detected were higher in colorectal cancer tissue compared with adjacent, normal tissue ($n = 7$ studies, pooled OR = 2.42; 95% CI, 1.62–3.61; $I^2 = 50.0\%$), with all studies were rated as ≥4 on the Newcastle–Ottawa scale. Neither of two studies (4, 54) found differences in *F. nucleatum* positivity in colorectal polyp tissue compared with adjacent, normal colorectal tissue.

A higher *F. nucleatum* abundance was observed in colorectal tumor tissue compared with adjacent normal tissue in 11 of the 12 studies reporting this comparison; however, the use of different detection methods prevented amalgamation using meta-analyses. Only two studies, assessed *F. nucleatum* abundance in colorectal polyp tissue compared with adjacent, normal colorectal tissue but neither Flanagan and colleagues (54) or Proenca and colleagues (4), identified a statistically significant difference.

Fecal samples from separate individuals

Seventeen studies assessed the prevalence and/or abundance of *F. nucleatum* in fecal samples (Supplementary Table S5) from separate individuals with colorectal cancer compared with healthy colorectum (7 prevalence and 11 abundance studies); colorectal cancer compared with colorectal adenoma (three prevalence and four abundance studies); and/or colorectal adenoma compared with healthy colorectal tissue (three prevalence and seven abundance studies).

As shown in **Fig. 4**, meta-analyses assessing *F. nucleatum* prevalence indicated that the odds of *F. nucleatum* DNA being detected were higher in fecal samples from patients with colorectal cancer compared with healthy controls ($n = 7$ studies, pooled OR = 9.01; 95% CI, 3.39–23.95; $I^2 = 72.6\%$). Smaller increases in the odds of *F. nucleatum* DNA being detected were apparent in individuals with patients with colorectal cancer compared with individuals with colorectal polyps ($n = 3$ studies, pooled OR = 3.31; 1.29–8.45; $I^2 = 78.4\%$) and in individuals with colorectal polyps compared with healthy controls ($n = 3$ studies, pooled OR = 1.41; 95% CI, 0.53–3.74; $I^2 = 81.7\%$). When limited to studies rated as ≥4 on the Newcastle–Ottawa scale (Supplementary Table S6), similar results were apparent. The high heterogeneity was reduced for all three comparisons after exclusion of individual studies by Amitay and colleagues ($n = 6$ studies, pooled OR = 11.59; 95% CI, 6.39–21.01; $I^2 = 7.1\%$; ref. 29); Eklof and colleagues ($n = 2$ studies, pooled OR = 2.09; 95% CI, 1.25–3.50; $I^2 = 0.0\%$; ref. 48); Suehiro and colleagues ($n = 2$ studies, pooled OR = 0.82; 95% CI, 0.56–1.20; $I^2 = 0.0\%$; ref. 25), respectively.

A higher abundance of *F. nucleatum* DNA in fecal samples was found in patients with colorectal cancer compared with healthy controls in 12 of 13 studies; patients with colorectal cancer compared

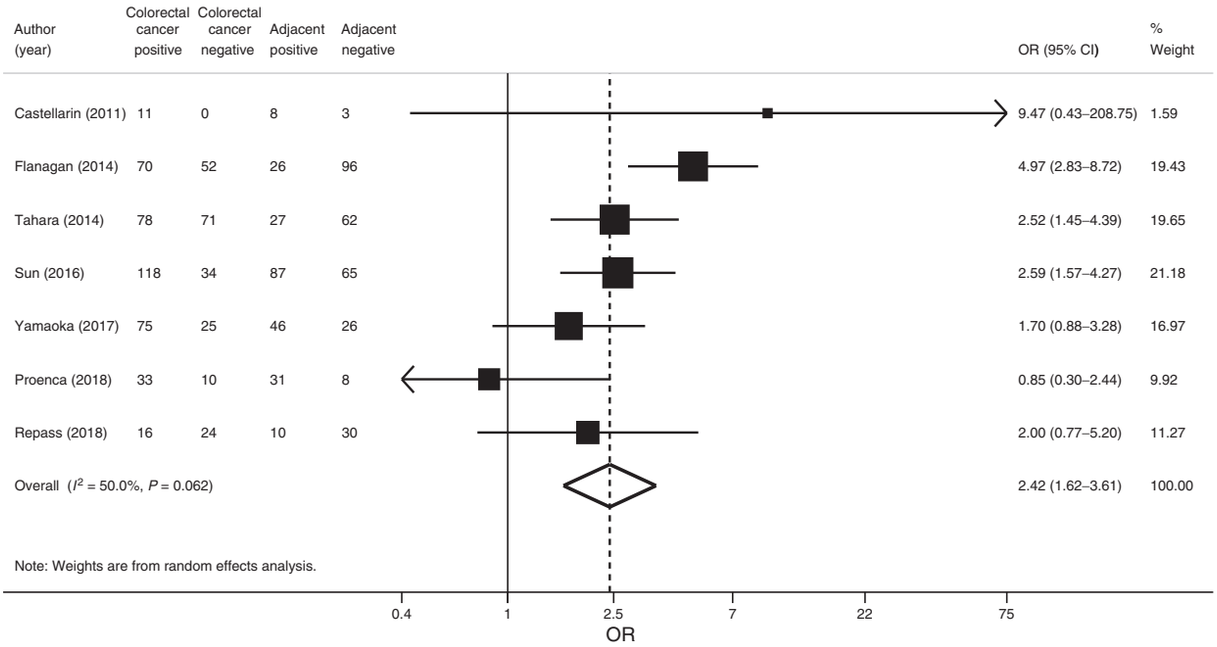

Figure 3.
Forest plot showing that the pooled odds of *F. nucleatum* positivity are higher in colorectal tissue samples compared with adjacent, normal tissue.

with polyp patients in three out of four studies, and; patients with colorectal polyp compared with healthy controls in four out of seven independent studies.

Dental samples from separate individuals

One study by Rajendran and colleagues (24) found a similar abundance of *F. nucleatum* DNA in dental plaque samples from patients with colorectal cancer compared with healthy controls ($P = 0.86$).

F. nucleatum prevalence and colorectal cancer risk

Three studies examined the association between *F. nucleatum* in biological samples and subsequent colorectal cancer development (Supplementary Table S7), although the samples used differed which meant a meta-analysis was not warranted. Mai and colleagues (21) did not find a statistically significant association between *F. nucleatum* in subgingival plaque samples and subsequent colorectal cancer risk (HR = 0.78; 95% CI, 0.18–3.43) in an analysis of a cohort of 1,252 individuals with 11.8 years of follow-up (mean). Yang and colleagues (59) found *F. nucleatum* in prediagnostic saliva samples from 99.6% of 231 individuals who subsequently developed colorectal cancer (cases) and in 99.6% of 461 controls (matched on age, ethnicity, smoking, season of enrolment, and recruitment method) who did not develop colorectal cancer, with no difference in prevalence (HR = 1.12; 95% CI, 1.70–27.90). Kwong and colleagues (49) found that 79 individuals with blood culture tests positive for *F. nucleatum* were at a higher subsequent risk of colorectal cancer development than 395 propensity matched controls (based on age, sex, and comorbidities) with blood culture tests negative for *F. nucleatum* (HR = 6.89; 95% CI, 1.70–27.90). All studies were rated as ≥4 on the Newcastle–Ottawa scale (Supplementary Table S8), although sample sizes were all small.

F. nucleatum in tumor tissue and survival in patients with colorectal cancer

Seven studies assessed the association between *F. nucleatum* DNA in tumor tissue and survival in patients with colorectal cancer (Supplementary Table S9).

Overall survival

As shown in **Fig. 5**, a meta-analysis found that *F. nucleatum* positivity in tumor tissue was associated with poorer overall survival among patients with colorectal cancer ($n = 5$ studies, HR = 1.87; 95% CI, 1.12–3.11; $I^2 = 60.6\%$). All five studies included in the meta-analysis were rated as ≥4 on the Newcastle–Ottawa scale (Supplementary Table S8). The high heterogeneity ($I^2 = 60.6\%$) was reduced after exclusion of the largest study by Mima and colleagues (12) and the association remained statistically significant ($n = 4$ studies, HR = 2.25; 95% CI, 1.50–3.37; $I^2 = 0.0\%$).

Three additional studies, which were not eligible for inclusion in the meta-analysis because either they only reported subgroup results [based on *Pseudomonas fragi* positivity (57) or palliative status (51)] or did not report HRs (53), also found poorer survival in individuals with *F. nucleatum* identified in their colorectal tumor tissue.

Colorectal cancer–specific survival

Mima and colleagues (12) found an association between *F. nucleatum* positivity and colorectal cancer–related survival (HR = 1.58; 95% CI, 1.04–2.39); as did Yan and colleagues (2017; HR = 2.22; 95% CI 1.48–3.33).

Disease-free survival

As shown in **Fig. 6**, a meta-analysis found that *F. nucleatum* positivity in tumor tissue was not associated with disease-free survival among patients with colorectal cancer ($n = 3$ studies, HR = 1.48; 95%

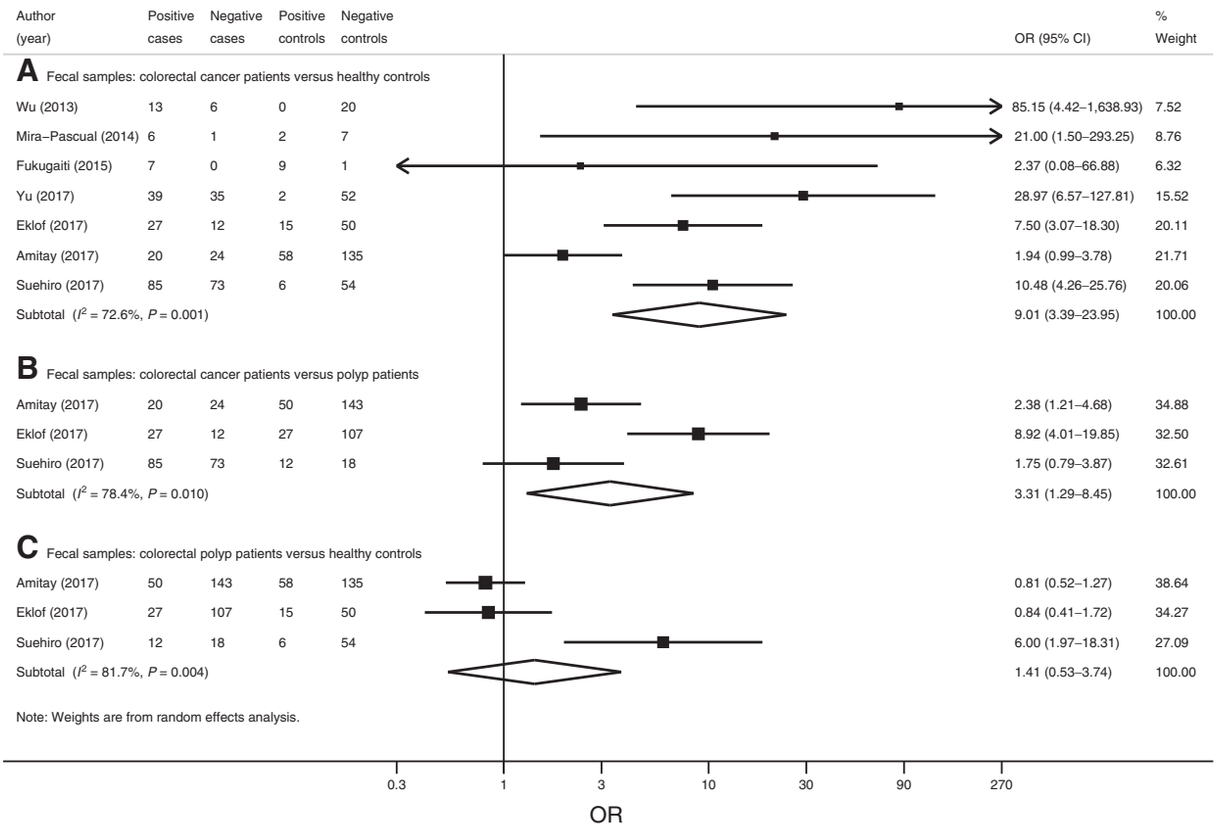

Figure 4.
Forest plot pooling the odds ratio of *F. nucleatum* positivity in fecal samples from individuals with or without colorectal neoplasms. The pooled odds of *F. nucleatum* positivity are higher in fecal samples from patients with colorectal cancer compared with healthy controls (**A**); higher in patients with colorectal cancer compared with individuals with colorectal polyps (**B**); but not from individuals with colorectal polyps compared with healthy controls (**C**).

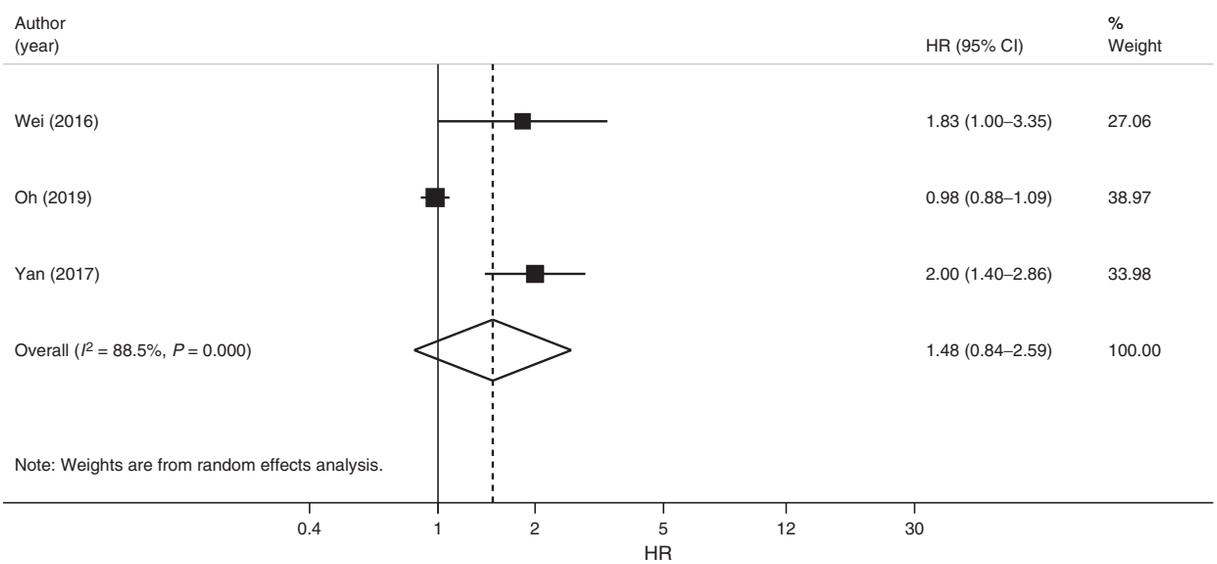

Figure 5.
Random effects meta-analysis showing that *F. nucleatum* positivity in colorectal tumor tissue was not associated with poorer disease-free survival in individuals with colorectal cancer.

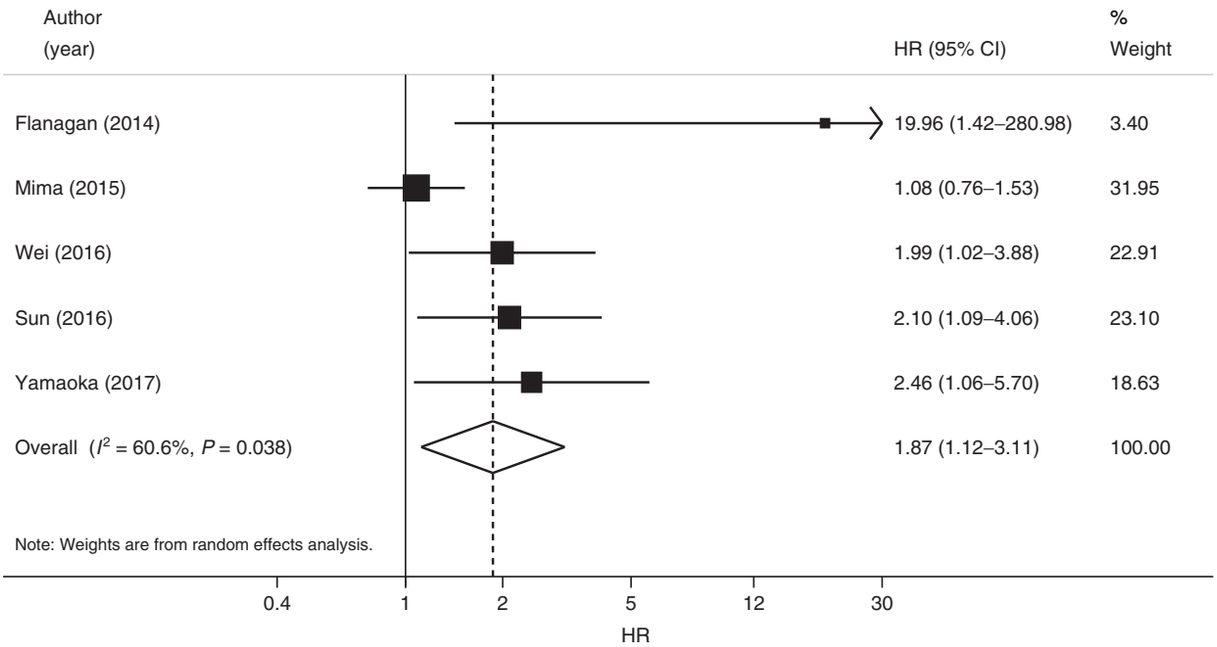

Figure 6.
Random effects meta-analysis showing that *F. nucleatum* positivity in colorectal tumor tissue is associated with poorer overall survival in individuals with colorectal cancer.

CI, 0.84–2.59; $I^2 = 88.5\%$). The high heterogeneity ($I^2 = 60.6\%$) was reduced after exclusion of the lowest quality study by Oh and colleagues (50) and the association became statistically significant ($n = 2$ studies, HR = 1.95; 95% CI, 1.44–2.66; $I^2 = 0.0\%$).

Discussion

In this first comprehensive systematic review with meta-analyses of the published literature on *F. nucleatum* in colorectal cancer, we found consistent evidence that *F. nucleatum* was more prevalent in fecal and tissue samples from patients with colorectal cancer compared with adjacent tissue, or samples from comparative healthy controls or individuals with colorectal polyps. The presence of *F. nucleatum* in colorectal tumor tissue was also associated with worse overall survival in patients with colorectal cancer.

The substantial evidence that *F. nucleatum* is more likely to be present and more abundant in colorectal cancer samples than healthy samples and increases along the normal-adenoma-carcinoma tissue pathway may indicate a potential causal role in colorectal cancer development. *F. nucleatum* has been demonstrated to adhere to and invade colorectal tumor cells, using specific surface proteins to bind to tumors: Fap2 binds tumor Gal-GalNAc; and the adhesin FadA binds E-cadherin which may stimulate expression of inflammatory genes, a known risk factor for colorectal cancer (7, 8). *F. nucleatum* has also been demonstrated to promote immune evasion in colorectal cancer: *F. nucleatum* specifically binds the inhibitory receptor TIGIT expressed on natural killer (NK) cells through its Fap2 protein, thereby inhibiting the cytotoxic activity of the NK cells (8–11). Therefore, there is emerging evidence that *F. nucleatum* colonization creates a tumor permissive, proinflammatory microenvironment (TME; refs. 10, 61). However, it is also possible that *F. nucleatum* may merely exploit and replicate effectively in the hypoxic tumor microenvironment (29), or that its presence may reflect procarcinogenic diets (37). The increased prevalence seems robust to methodologic changes; whereas most studies used qRT-PCR, one study found similar results when using 16S rRNA FISH (35).

The higher prevalence in fecal samples from patients with colorectal cancer indicates *F. nucleatum* DNA could potentially be useful as an adjunct biomarker in colorectal cancer screening. Two recent smaller systematic reviews found that measuring *F. nucleatum* DNA in fecal samples could add predictive value for the presence of colorectal cancer (62, 63). Similar results have also been observed for serum samples (64). However, the utility of biomarkers for colorectal screening may also rely on detecting premalignant polyps. Our meta-analysis of three studies found no difference in the odds of *F. nucleatum* positivity between fecal samples from individuals with colorectal polyps than from healthy controls, which casts doubt on the utility of using *F. nucleatum* in the early detection of colorectal polyps. Abundance however may give a more comprehensive picture, although studies tended to use different reporting methods which did not allow for meta-analyses of abundance to be conducted. Therefore, further studies should attempt to investigate whether measurement of fecal or serum *F. nucleatum* prevalence or abundance adds predictive value to current fecal screening methods.

Despite the substantial evidence regarding an increased prevalence of *F. nucleatum*, the evidence for an association between *F. nucleatum* and future risk of colorectal cancer is limited with only three studies eligible for inclusion in the review. Two studies (21, 59) found no association between the presence of *F. nucleatum* and subsequent colorectal cancer risk, which could be in part due to the use of oral samples that may not accurately reflect the fecal microbiome as *F. nucleatum* is commensal to the oral cavity (33). Similarly, statistical power was limited as few cases or controls (<0.4%) had no *F. nucleatum* in the study by Yang and colleagues (59) and the cohort

used by Mai and colleagues (21) was small ($n = 1,252$) with only 17 individuals developing colorectal cancer. Interestingly, a study by Kwong and colleagues found a higher subsequent risk of colorectal cancer in individuals previously diagnosed with bacteremia from *F. nucleatum* (49). However, the no lag time was built into the analysis for the association between bacteremia episode and colorectal cancer, reverse causality is possible. The results were not materially changed when excluding lower quality studies rated as $\geq$4 on the Newcastle–Ottawa scale, suggesting the results are unlikely due to low study quality. This review highlights the need for further population-based cohort or nested case–control studies measuring the fecal microbiome and subsequent colorectal cancer risk to examine whether *F. nucleatum* precedes colorectal cancer development.

The observation of an association between *F. nucleatum* DNA prevalence and overall survival could highlight a role in cancer progression or a role as a passive prognostic biomarker. Although the strength of the association between *F. nucleatum* and overall survival was modest and heterogeneous, which may limit the clinical utility as a prognostic biomarker, Mima and colleagues (12) found a stronger association with cancer-specific survival. The weaker association between *F. nucleatum* and overall survival in the study by Mima and colleagues (12), compared with other studies, could reflect the large, high-quality nature of the study design but could also reflect use of formalin-fixed paraffin-embedded tissue rather than fresh frozen tissue used for other studies reporting results for overall survival. Recent evidence also hints at a similarity in the microbiome between primary colorectal lesions and metastatic lesions (65), which may improve the prognostic benefits of targeted treatments. Therefore, studies should investigate whether *F. nucleatum* in primary or metastatic lesions could be used a prognostic indicator for cancer-specific survival to aid treatment decisions, or using mechanistic designs to assess whether *F. nucleatum* could be a therapeutic target.

The heterogeneity was high (I^2 over 50%) when comparing colorectal tumor tissue samples to polyp tissue from controls, for the analyses in fecal samples and for the overall survival analyses, which were lowered after removing individual studies. Often these studies were the larger, higher quality studies. Therefore, replication of the results in large high-quality analyses within large sample sizes may further increase confidence in future pooled estimates.

A strength of this systematic review is that it offers a comprehensive assessment of the existing literature on the role of *F. nucleatum* in colorectal cancer identification, development, and prognosis, which better quantifies the differences in prevalence and better highlights gaps in the literature than existing reviews (66). A comprehensive assessment of other bacterial species linked with colorectal cancer was not feasible within the scope of this review but future systematic reviews on these are recommended. However, a limitation is that the low study numbers prevented a formal assessment of publication/reporting bias (17), and may limit robustness of some meta-analyses with only three studies. Whole microbiome studies using shotgun metabolomics methods for assessing microbiome could help determine whether the increased prevalence in colorectal cancer tissue is unique to Fusobacteria or whether other bacterial genera are increased. However, the nature of whole microbiome studies may make reporting null results at a species level difficult which increases the risk of publication/reporting bias. Therefore, estimates should be interpreted cautiously, and future whole microbiome studies should use careful reporting of null results to reduce the potential for reporting bias.

Conclusions

There is consistent evidence indicating an increased prevalence and/or abundance of *F. nucleatum* DNA in fecal and tissue samples from patients with colorectal cancer. Further prospective studies investigating the role of fecal *F. nucleatum* as a causal factor or a predictive biomarker for colorectal cancer development and prognosis are required.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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