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***Fusobacterium nucleatum* in the colorectum, and its association with cancer risk and survival: a systematic review and meta-analysis**

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ABSTRACT

Objective: 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 of published studies to assess the prevalence of *F. nucleatum* in colorectal tumours, and evaluate the association between *F. nucleatum* and colorectal cancer development and prognosis.

Design: Medline, Embase and Web of Science databases were systematically searched for studies published until October 2017. Random effects meta-analyses were used to assess the prevalence of *F. nucleatum* in colorectal cancer patients or tissues relative to controls and survival in *F. nucleatum* positive versus negative patients.

Results: Thirty relevant articles were identified. Meta-analyses indicated higher odds of *F. nucleatum* being present in colorectal tissue samples from colorectal cancer patients (n=4 studies, pooled OR=8.32, 95% CI: 2.87-24.14) and individuals with colorectal polyps (n=5 studies, pooled OR=1.83, 95% CI: 1.07-3.16) compared to healthy controls. Similar results were apparent in faecal samples, and when comparing tumour to adjacent normal tissue. Meta-analyses indicated an 87% increased risk of death in colorectal cancer patients with high compared with 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 faecal samples compared with controls was apparent. High abundance of *F. nucleatum* in colorectal tumours was also associated with poorer overall survival. *F. nucleatum* could be useful as a diagnostic and prognostic marker for colorectal cancer or as a treatment target.

Keywords: Colorectal cancer; Microbiome; *Fusobacterium nucleatum*; Survival; Prevalence

SUMMARY

- What is already known about this subject?
 - Microorganisms may play a role in cancer development and progression
 - *F. nucleatum* has been demonstrated to adhere to colorectal tumour cells, promote tumour growth and inhibit anti-tumour immune responses in animal models.
- What are the new findings?
 - *F. nucleatum* is more prevalent in colorectal tumour tissue than in healthy tissue or colorectal polyp tissue.
 - *F. nucleatum* is more prevalent in faecal samples from individuals with colorectal cancer compared to healthy controls.
 - Higher *F. nucleatum* in colorectal tumour tissue is associated with poorer overall survival.
- How might it impact on clinical practice in the foreseeable future?
 - *F. nucleatum* DNA in colorectal tumour tissue could be used as a prognostic marker.
 - *F. nucleatum* DNA in faecal samples could be used to aid current faecal screening tools.
 - This comprehensive assessment of the literature could provide insights into treatments targeted at *F. nucleatum* to reduce colorectal cancer development or progression.

INTRODUCTION

The human gut has been estimated to contain approximately 100 trillion microbial cells, which is over 10 times the number of eukaryotic cells that comprise the human body, and may include as many as a 1000 distinct bacterial species.[1]

In recent years, there has been an increased understanding of the functional role of gut microbiota in the initiation of colorectal cancer. [1] 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.[2] 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.[2] A number of bacterial species have been implicated with colorectal cancer onset including; *Streptococcus bovis*, *Helicobacter pylori*, *Escherichia coli*, *Bacteroides fragilis*, *Salmonella enterica*, and *Fusobacterium nucleatum* (*F. nucleatum*).[2]

F. nucleatum is a species of anaerobic gram-negative, non-spore forming, non-motile bacteria of the genus *Fusobacterium*. [3] *F. nucleatum* is an opportunistic commensal pathogen normally resident in the oral cavity,[3] but may use saliva to survive passage through the stomach and into the lower gastrointestinal tract.[4] In support of this, a recent study demonstrated that colorectal cancer patients have identical *F. nucleatum* strains in their oral cavity and tumours.[11] There is increasing evidence that *F. nucleatum* can adhere to and invade colorectal tumour tissue [5,6] and may create a tumour permissive, pro-inflammatory microenvironment (TME). [6–10]

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 had three main aims:

1. To assess the prevalence/abundance of *F. nucleatum* infection in biological samples from individuals with colorectal cancer or colorectal polyps compared to healthy controls or adjacent, normal tissue.
2. To assess the association of *F. nucleatum* with subsequent colorectal cancer development in individuals who were initially cancer-free.
3. To assess the association between *F. nucleatum* and survival in individuals with colorectal cancer.

METHODS

This review has been reported in accordance with PRISMA guidelines.[14]

Data sources

A systematic search of the literature was performed using Embase, Medline, and Web of Science from date of commencement to week 1, October 2017 to identify all primary studies relating to the prevalence of *F. nucleatum* infection 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 tumour(s) OR colon tumour(s) OR rectal tumour(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. Review articles and individual case studies were removed, however, non-English papers were included.

Inclusion/exclusion criteria

Study selection was carried out using two levels of study screening.

For the prevalence/abundance studies (aim 1) participants had to be aged over 16 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 faecal, tissue or dental): from individuals with colorectal cancer compared to individuals with colorectal polyp or healthy controls; from colorectal tumour tissue compared to adjacent normal tissue or; from individuals with colorectal polyps compared to healthy controls.

For the colorectal cancer development studies (aim 2) participants had to be aged 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 aged 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 focused on authors; year of publication; study location; study design; age of patients, *F. nucleatum* detection method; sample type (tissue, faecal 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 & 3) and adjustment for potential confounders.

Statistical analysis

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

- Individuals with colorectal cancer compared to healthy controls
- Individuals with colorectal cancer compared to individuals with colorectal polyps
- Individuals with colorectal polyps compared to healthy controls
- Colorectal tumour tissue compared to 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 in colorectal cancer patients (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.[15] Heterogeneity was considered high if I^2 statistic was above 50%.[15] Due to low study numbers identified for each comparison, publication bias could not be formally assessed.[16] Studies only reporting results for sub-groups within cohorts were not included in meta-analyses due to the potential for reporting bias. Stata 14 software (Stata Corporation, College Station, Texas, USA) was used for data analysis.

RESULTS

As shown in Figure 1, 985 unique titles and abstracts were screened for potential eligibility, from which 108 full-texts were screened for eligibility. Of these, 30 relevant articles were identified, [12,17–45] some of which addressed multiple aims of the systematic review.

F. *nucleatum* prevalence or abundance in biological samples

Colorectal tissue samples from separate individuals

Seven studies assessed the prevalence or abundance of *F. nucleatum* in colorectal tissue samples from separate individuals with colorectal cancer, colorectal polyp or healthy colorectal tissue (Table 1).

As shown in Figure 2, meta-analysis indicated that the odds of *F. nucleatum* DNA being detected were higher in colorectal tumour tissue compared to healthy tissue from controls (n=4 studies, pooled OR 8.32, 95% CI: 2.87-24.14, $I^2=0.0\%$). Smaller increased odds of *F. nucleatum* DNA being detected were apparent in colorectal tumour tissue compared to 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 to healthy tissue from controls (n=3 studies, pooled OR=2.51, 95% CI: 1.20-5.27, $I^2=0.0\%$). The high heterogeneity ($I^2=66.9\%$) when comparing colorectal tumour tissue samples to polyp tissue from controls was reduced after exclusion of a study by Ito *et al* (2015), though the association was no longer statistically significant (n=4 studies, pooled OR=1.41, 95% CI: 0.96-2.07, $I^2=0.0\%$).

Only one study by Mira-Pascual *et al.* [22] assessed the abundance of *F. nucleatum* DNA in colorectal tumour tissue compared to healthy colorectal tissue from controls, and found a higher abundance in colorectal tumour tissue. Only one study by Flanagan *et al.* [40] assessed the abundance of *F. nucleatum* in colorectal tumour tissue compared to colorectal polyp tissue, and found a higher abundance in colorectal tumour tissue. Similarly, only one study by McCoy *et al.* [21] assessed the abundance of *F. nucleatum* in colorectal polyp tissue compared to healthy colorectal tissue from controls, and found a higher abundance in colorectal polyp tissue.

Adjacent colorectal tissue samples

Eight studies assessed the prevalence and/or abundance of *F. nucleatum* in colorectal cancer tissue or colorectal polyp tissue compared to healthy adjacent tissues (Table 2). As shown in Figure 3, Meta-analyses indicated that the odds of *F. nucleatum* being detected were higher in colorectal cancer tissue compared to adjacent, normal tissue (n=5 studies, pooled OR 2.83, 95% CI: 1.90-4.20, $I^2=43.1\%$). Only one study, by Flanagan *et al.* [40] assessed *F. nucleatum* positivity in colorectal polyp tissue compared to adjacent, normal colorectal tissue, but did not find a statistically significant difference.

Table 1. The prevalence and abundance of *F. nucleatum* in tissue samples from colorectal cancer tumours, colorectal adenomas, or healthy colorectal tissue from separate individuals

Author (year)	Location	Colorectal cancer tissue			Colorectal adenoma tissue			Healthy, colorectal tissue		
		Total n	FN +	Abundance ¹	Total n	FN +	Abundance ²	Total n	FN +	Abundance
McCoy (2013) [21]	USA	-	-	-	48	-	Mean log copy number 8.44±0.22 ($p=0.01$)	67	-	Mean log copy number 7.40±0.22
Mira-Pascual (2014) [22]	Spain	7	2 (27%)	4.55 (4.40-5.80)	-	-	-	5	0 (0%)	none
Flanagan (2014) [40]	Czech Republic Germany Ireland	122	70 (57%)	RQ=2 ⁻¹⁰ CRC v CRA: $p=0.001$	52	12 (23%)	RQ=2 ⁻²⁹	-	-	-
Ito (2015) [44]	Japan	286	286 (56%)	-	456	142 (31%)	-	20	3/20 (6%)	-
Fu (2015)[41]	China	47	7 (15%) ³	-	98	15 (15%) ³	-	-	-	-
Yu (2016) [35]	China	93	62 (66%)	-	112	47 (42%)	-	20	4 (20%)	-
Yoon (2017)[34]	S.Korea	6	0 (0%)	-	12	1 (8%)	-	6	0 (0%)	-

¹Comparisons and p-values are for colorectal cancer tissue versus healthy tissue unless specified otherwise.

² Comparisons and p-values are for colorectal adenoma tissue versus healthy tissue.

³ Detected using Fluorescence In Situ Hybridization, all others detected using quantitative Real Time-Polymerase Chain Reaction

Abbreviations: CRA=colorectal adenoma; CRC=colorectal cancer; FN=*Fusobacterium nucleatum*; n=number of participants; RQ= Relative quantification.

Table 2. The prevalence and abundance of *F. nucleatum* in adjacent tissue samples from colorectal cancer tumours, colorectal adenomas, or healthy colorectal tissue.

Author (year)	Location	Colorectal cancer tissue			Colorectal adenoma tissue			Healthy, colorectal tissue		
		Total n	FN +*	Abundance ¹	Total n	FN +*	Abundance ²	Total n	FN +*	Abundance
Castellarin (2011) [28]	Canada	11	11 (100%)	($p < 0.0001$)	-	-	-	11	8 (73%)	-
Kostic (2012)[38]	Spain	9	-	LDA score: 4.2	-	-	-	9	-	-
Flanagan (2014) [40]	Czech Republic Germany Ireland	122	70 (57%)	RQ=2 ⁻¹⁰ ($p < 0.0001$) CRC v CRA: $p = 0.001$	52	12 (23%)	RQ=2 ⁻²⁹	Adjacent to CRC: 122 Adjacent to CRA: 52	Adjacent to CRC: 26 (21%) Adjacent to CRA: 7 (13%)	RQ=2 ⁻¹⁹
Tahara (2014) [26]	USA, Japan	149	78 (52%)	($p = 0.0001$)	-	-	-	89	27 (30%)	-
Li (2016) [18]	China	101	-	Median copy number: 0.24 (0.18-0.28)	-	-	-	101	-	Median copy number: 0.05 (0.02 to 0.07)
Sun (2016) [25]	China	152	118 (78%)	Median abundance=87 ($p < 0.001$)	-	-	-	152	87 (57%)	Median abundance=37
Kinross (2017) [45]	UK	18	-	FN Ct-Generic Ct: 4.2 ($p < 0.05$)	-	-	-	18	-	FN Ct-Generic Ct: 7.4
Yamaoka (2017) [32]	Japan	100	75 (75%)	Median copy number: 1.6 ($p < 0.005$)	-	-	-	72	46 (64%)	Median copy number: 0.4

¹Comparisons and p-values are for colorectal cancer tissue versus healthy tissue unless specified otherwise.

² Comparisons and p-values are for colorectal adenoma tissue versus healthy tissue.

³ Detected using Fluorescence In Situ Hybridization, all others detected using quantitative Real Time-Polymerase Chain Reaction

Abbreviations: CRA=colorectal adenoma; CRC=colorectal cancer; CT=Cycle threshold; FN=*Fusobacterium nucleatum*; n=number of participants; RQ= Relative quantification

A higher *F. nucleatum* abundance was observed in colorectal tumour tissue compared to adjacent normal tissue in all eight studies reporting this comparison; however, the use of different detection methods prevented amalgamation using meta-analyses. Only one study, by Flanagan *et al.* [40] assessed *F. nucleatum* abundance in colorectal polyp tissue compared to adjacent, normal colorectal tissue but did not find a statistically significant difference.

Faecal samples from separate individuals

Twelve studies assessed the prevalence or abundance of *F. nucleatum* in faecal samples from individuals with colorectal cancer, colorectal polyps or healthy controls (Table 3).

As shown in Figure 4, meta-analyses indicated that the odds of *F. nucleatum* DNA being detected were higher in faecal samples from colorectal cancer patients compared to 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 colorectal cancer patients compared to 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 to healthy controls (n=3 studies, pooled OR=1.41, 95% CI: 0.53-3.74, $I^2=81.7\%$). The high heterogeneity was reduced for all three comparisons after exclusion of individual studies by Amitay *et al* [17] (n=6 studies, pooled OR=11.59, 95% CI: 6.39-21.01, $I^2=7.1\%$); Eklof *et al* [39] (n=2 studies, pooled OR=2.09, 95% CI: 1.25-3.50, $I^2=0.0\%$); Suehiro *et al* [24] (n=2 studies, pooled OR=0.82, 95% CI: 0.56-1.20, $I^2=0.0\%$), respectively.

In studies that compared the abundance of *F. nucleatum* DNA in faecal samples, eight out of nine studies found a higher abundance in samples from colorectal cancer patients compared to healthy controls; colorectal cancer patients compared to polyp patients in one of two studies, and; colorectal polyp patients compared to healthy controls in two out of three studies

Dental samples from separate individuals

One study by Rajendran *et al.*[23] found a similar abundance of *F. nucleatum* DNA in dental plaque samples from colorectal cancer patients compared to healthy controls (p=0.86).

***F. nucleatum* prevalence and colorectal cancer risk**

A single study by Mai *et al.*[20] examined the association between *F. nucleatum* DNA measured prior to colorectal cancer onset and subsequent risk of colorectal cancer (Table 4). No association was found between *F. nucleatum* and colorectal cancer risk (HR= 0.78, 95% CI 0.18-3.43) in an analysis of plaque samples from 1,252 individuals.

Table 3. Prevalence and abundance of *F. nucleatum* DNA in faecal samples from individuals with colorectal cancer, colorectal adenomas, and healthy controls

Author (year)	Location	Colorectal cancer			Colorectal adenoma			Healthy controls		
		Total n	FN +	Abundance ¹	Total n	FN +	Abundance ²	Total n	FN +	Abundance
Wu (2013) [31]	China	19	13 (68%)	-	-	-	-	20	0 (0%)	-
Flanagan (2014) [40]	Ireland	7	-	RQ=2 ⁻¹⁵ (<i>p</i> <0.05)	24	-	RQ=2 ⁻²¹	25	-	RQ=2 ⁻²¹
Mira-Pascual (2014) [22]	Spain	7	6 (86%)	Log copies/mg: 4.70 (IQR 3.85-5.15)	-	-	-	9	2 (22%)	Log copies/mg: 4.16 (IQR 3.47-4.85)
Fukugaiti (2015) [42]	Brazil	7	7 (100%)	Log copies/mg: 6.2 (SD 1.5) (<i>p</i> <0.05)	-	-	-	10	9 (90%)	Log copies/mg: 4.0 (SD 1.5)
Rajendran (2015) [23]	India	-	-	mean rank: 13.67 (<i>p</i> <0.05)	-	-	-	-	-	mean rank: 4.33
Wong (2016) [30]	China	-	-	-	Advanced adenoma: 103	-	3.8-fold higher, (<i>p</i> <0.05)	102	-	-
Yu (2017) [36]	China & Denmark	74	39 (53%)	Mean rank: 78.66 (<i>p</i> =0.0015)	-	-	-	54	2 (4%)	Mean rank: 45.09
Eklof (2017) [39]	Sweden	39	27 (69%)	Log(2 ^{-ΔCT})=-7 (<i>p</i> <0.05) (CRC v CRA: <i>p</i> <0.05)	Dysplasia: 134	27 (20%)	Log(2 ^{-ΔCT})=-10.2 (<i>p</i> >0.05)	65	15 (23%)	Log(2 ^{-ΔCT})=-10
Liang (2017) [19]	China	170	-	2 ^{-ΔCT} *10e7=10 ⁵ (<i>p</i> <0.05)	-	-	-	200	-	2 ^{-ΔCT} *10e7=10 ²
		33		2 ^{-ΔCT} *10e7=10 ³ (<i>p</i> <0.05)				36		2 ^{-ΔCT} *10e7=10 ²
Amitay (2017) [17]	Germany	44	20 (45%)	-	193	50 (26%)	-	193 ³	58 (30%)	-
Vogtmann (2017) [27]	USA	52	-	0.00076 (<i>p</i> <0.05)	-	-	-	52	-	0.000027
	France	53		0.00089 (<i>p</i> <0.05)				53 ³		0.000013
Suehiro (2017) [24]	Japan	158	85 (54%)	Median copy number: 317 (<i>p</i> <0.001)	30	12 (40%)	Median copy number: Advanced:122 (<i>p</i> <0.05) Non-advanced: 311 (<i>p</i> <0.05)	60	6 (10%)	Median copy number: 17.5

¹Comparisons and p-values are for colorectal cancer tissue versus healthy tissue unless specified otherwise.

² Comparisons and p-values are for colorectal adenoma tissue versus healthy tissue.

³ Control samples may include individuals with hyperplastic polyps at endoscopy.

Abbreviations: CRA=colorectal adenoma; CRC=colorectal cancer; CT=Cycle threshold; FN=*Fusobacterium nucleatum*; n=number of participants; RQ= Relative quantification.

Table 4: Characteristics of studies relating to *F. nucleatum* DNA in association with subsequent colorectal cancer risk

Author (year)	Location	Sample type(s)	Detection method	Cohort size	Proportion Fn positive samples from CRC cases	Proportion Fn positive samples from non-cases	CRC risk (Positive versus negative FN) HR (95% CI)	Mean follow-up years
Mai (2016) [20]	USA	Subgingival plaque samples	Indirect immunofluorescence	1252	2/17 (11.8%)	173/1235 (14%)	0.78 (0.18 to 3.43)*	11.8 (SD 3.8)

* Age, BMI, smoking, second hand smoking, education, family history of cancer, combined hormone use, frequency of teeth flossing, effects of alcohol, physical activity, NSAID use, dietary variables, history of diabetes.

Abbreviations: CRC=colorectal cancer, Fn=*Fusobacterium nucleatum*, HR=hazard ratio, NSAID=nonsteroidal anti-inflammatory drugs, SD=standard deviation, USA=United States of America

***F. nucleatum* in tumour tissue and survival in colorectal cancer patients**

Seven studies assessed the association between *F. nucleatum* DNA in tumour tissue and colorectal cancer survival (Table 5).

Overall survival

As shown in Figure 5, a meta-analysis found that *F. nucleatum* positivity in tumour tissue was associated with poorer overall survival amongst colorectal cancer patients (n=5 studies, HR=1.87, 95% CI 1.12-3.11, $I^2=60.6\%$). The high heterogeneity ($I^2=60.6\%$) was reduced after exclusion of the largest study by Mima *et al* [12] and the association remained statistically significant (n=4 studies, HR=2.25, 95% CI: 1.50-3.37, $I^2=0.0\%$).

The study by Huanlong *et al.* [43] also reported worse survival in colorectal cancer patients with a high abundance of *F. nucleatum* amongst a sub-group of patients with a high abundance of *Pseudomonas fragi*, but did not report results for the entire cohort and therefore was not included in the meta-analysis.

Colorectal-cancer specific survival

Mima *et al.* [12] was the only study to report cancer-specific survival outcomes, and found an association between *F. nucleatum* positivity and colorectal cancer-related survival (HR=1.58, 95% CI: 1.04-2.39).

Disease-free survival

Wei *et al.* [29] found an association between *F. nucleatum* positivity in colorectal cancer tumour tissue and disease-free survival (HR=1.83, 95% CI: 1.00-1.35), as did Yu *et al* [37] in two further separate cohorts (HR=3.78, 95% CI: 1.89-7.58; HR=3.67, 95% CI: 2.34-5.76).

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 faecal and tissue samples from colorectal cancer patients compared to adjacent tissue, or samples from comparative healthy controls or individuals with colorectal polyps. The presence of *F. nucleatum* in colorectal tumour tissue was also associated with worse overall survival in colorectal cancer patients.

The substantial evidence that *F. nucleatum* is more likely to be present in colorectal cancer samples than healthy samples may indicate a potential causal role in colorectal cancer development. *F. nucleatum* has been demonstrated to adhere to and invade colorectal tumour cells, using specific surface proteins to bind to tumours: Fap2 binds tumour Gal-GalNAc; and the adhesin FadA binds E-cadherin which may stimulate expression of inflammatory genes, a known risk factor for colorectal cancer.[5,6]. *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.[6–10] Therefore, there is emerging evidence that *F. nucleatum* infection creates a tumour permissive, pro-inflammatory microenvironment (TME).[10,46,47] However, it is also possible that *F. nucleatum* may merely exploit and replicate effectively in the hypoxic tumour microenvironment [48], or that its presence may reflect pro-carcinogenic diets.[49] The increased prevalence seems robust to methodological changes; whilst most studies used quantitative real time-polymerase chain, one study found similar results when using 16S rRNA fluorescence *in situ* hybridization (FISH).[35]

The higher prevalence in faecal samples from colorectal cancer patients indicates *F. nucleatum* DNA could potentially be useful as an adjunct biomarker in colorectal cancer screening. A recent smaller systematic review found that measuring *F. nucleatum* DNA in faecal samples could add predictive value for the presence of colorectal cancer.[50] Similar results have also been observed for serum samples.[51] However, the utility of biomarkers for colorectal screening may also rely on detecting pre-malignant polyps. Our meta-analysis of three studies found no difference in the odds of *F. nucleatum* positivity between faecal 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 didn't allow for meta-analyses of abundance to be conducted. Therefore, further studies should attempt to investigate whether measurement of faecal

or serum *F. nucleatum* prevalence or abundance adds predictive value to current faecal screening methods.

Despite the substantial evidence regarding an increased prevalence of *F. nucleatum*, only one study by Mima *et al.*[12] assessed the association between the presence of *F. nucleatum* and future risk of colorectal cancer. The lack of an association reported in that study could be attributable to the measurement of *F. nucleatum* in dental samples that may not accurately reflect the faecal microbiome.[23] Interestingly, a recent study, published after our systematic search, found a higher subsequent risk of colorectal cancer in individuals previously diagnosed with bacteremia from *F. nucleatum*. [52] Further population-based cohort or nested case-control studies measuring the faecal microbiome and subsequent colorectal cancer risk would be useful for examining 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. Whilst the strength of the association between *F. nucleatum* and overall survival was modest, which may limit the clinical utility as a prognostic biomarker, Mima *et al.*[12] found a stronger association with cancer-specific survival. Recent evidence also hints at a similarity in the microbiome between primary colorectal lesions and metastatic lesions,[53] 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.

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. However, a limitation is that a formal assessment of publication/reporting bias was not possible due to low study numbers.[16] 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. Similarly, some studies didn't report both the prevalence and abundance of *F. nucleatum* which may give further potential for 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

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

Contributors

All authors contributed to review and revision.

LM and HC developed the main concept and designed the study.

ATK performed data analysis and interpretation.

CGB and ATK drafted the manuscript.

LM, HTJ, DL, NC, HC contributed editing and critical revision for important intellectual contents.

All authors have given approval for the final version.

Competing interests

The authors do not have conflicting interests to report.

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Table 1. The prevalence and abundance of *F. nucleatum* in tissue samples from colorectal cancer tumours, colorectal adenomas, or healthy colorectal tissue from separate individuals

Author (year)	Location	Colorectal cancer tissue			Colorectal adenoma tissue			Healthy, colorectal tissue		
		Total n	FN +	Abundance ¹	Total n	FN +	Abundance ²	Total n	FN +	Abundance
McCoy (2013) [21]	USA	-	-	-	48	-	Mean log copy number 8.44±0.22 ($p=0.01$)	67	-	Mean log copy number 7.40±0.22
Mira-Pascual (2014) [22]	Spain	7	2 (27%)	4.55 (4.40-5.80)	-	-	-	5	0 (0%)	none
Flanagan (2014) [40]	Czech Republic Germany Ireland	122	70 (57%)	RQ=2 ⁻¹⁰ CRC v CRA: $p=0.001$	52	12 (23%)	RQ=2 ⁻²⁹	-	-	-
Ito (2015) [44]	Japan	286	286 (56%)	-	456	142 (31%)	-	20	3/20 (6%)	-
Fu (2015)[41]	China	47	7 (15%) ³	-	98	15 (15%) ³	-	-	-	-
Yu (2016) [35]	China	93	62 (66%)	-	112	47 (42%)	-	20	4 (20%)	-
Yoon (2017)[34]	S.Korea	6	0 (0%)	-	12	1 (8%)	-	6	0 (0%)	-

¹Comparisons and p-values are for colorectal cancer tissue versus healthy tissue unless specified otherwise.

² Comparisons and p-values are for colorectal adenoma tissue versus healthy tissue.

³ Detected using Fluorescence In Situ Hybridization, all others detected using quantitative Real Time-Polymerase Chain Reaction

Abbreviations: CRA=colorectal adenoma; CRC=colorectal cancer; FN=*Fusobacterium nucleatum*; n=number of participants; RQ= Relative quantification.

Table 2. The prevalence and abundance of *F. nucleatum* in adjacent tissue samples from colorectal cancer tumours, colorectal adenomas, or healthy colorectal tissue.

Author (year)	Location	Colorectal cancer tissue			Colorectal adenoma tissue			Healthy, colorectal tissue		
		Total n	FN +*	Abundance ¹	Total n	FN +*	Abundance ²	Total n	FN +*	Abundance
Castellarin (2011) [28]	Canada	11	11 (100%)	($p < 0.0001$)	-	-	-	11	8 (73%)	-
Kostic (2012)[38]	Spain	9	-	LDA score: 4.2	-	-	-	9	-	-
Flanagan (2014) [40]	Czech Republic Germany Ireland	122	70 (57%)	RQ=2 ⁻¹⁰ ($p < 0.0001$) CRC v CRA: $p = 0.001$	52	12 (23%)	RQ=2 ⁻²⁹	Adjacent to CRC: 122 Adjacent to CRA: 52	Adjacent to CRC: 26 (21%) Adjacent to CRA: 7 (13%)	RQ=2 ⁻¹⁹
Tahara (2014) [26]	USA, Japan	149	78 (52%)	($p = 0.0001$)	-	-	-	89	27 (30%)	-
Li (2016) [18]	China	101	-	Median copy number: 0.24 (0.18-0.28)	-	-	-	101	-	Median copy number: 0.05 (0.02 to 0.07)
Sun (2016) [25]	China	152	118 (78%)	Median abundance=87 ($p < 0.001$)	-	-	-	152	87 (57%)	Median abundance=37
Kinross (2017) [45]	UK	18	-	FN Ct-Generic Ct: 4.2 ($p < 0.05$)	-	-	-	18	-	FN Ct-Generic Ct: 7.4
Yamaoka (2017) [32]	Japan	100	75 (75%)	Median copy number: 1.6 ($p < 0.005$)	-	-	-	72	46 (64%)	Median copy number: 0.4

¹Comparisons and p-values are for colorectal cancer tissue versus healthy tissue unless specified otherwise.

² Comparisons and p-values are for colorectal adenoma tissue versus healthy tissue.

³ Detected using Fluorescence In Situ Hybridization, all others detected using quantitative Real Time-Polymerase Chain Reaction

Abbreviations: CRA=colorectal adenoma; CRC=colorectal cancer; CT=Cycle threshold; FN=*Fusobacterium nucleatum*; n=number of participants; RQ= Relative quantification.

Table 5: Characteristics of studies relating to the association between *F. nucleatum* DNA in CRC tissue samples and survival in CRC patients

Author (year)	Location	Sample type(s)	Detection method	Number of samples	Average follow-up (years)	Overall survival (High versus low/no FN) HR (95% CI)	Colorectal-cancer specific survival HR (95% CI)	Disease-free survival HR (95% CI)
Flanagan (2014)	Czech Republic	Tissue	qRT-PCR	32	3-5 (mean)	19.96 (1.42-281.42)	-	-
Huanlong (2015)	Shanghai, China	Tissue	qRT-PCR FISH	283	-	2.91 (0.91-9.37)*	-	-
Mima (2015)	USA	Tissue	qRT-PCR	1069	10.7 (median)	1.08 (0.76-1.52)	1.58 (1.04-2.39)	-

Wei (2016)	Qingdao, China	Tissue	qRT-PCR	180	3.9 (median)	1.99 (1.02-3.88)	-	1.83 (1.00-3.35)
Sun (2016)	Beijing, China	Tissue	qRT-PCR	118	3 (median)	2.10 (1.09-4.07) ² <i>p</i> =0.032	-	-
Yamaoka (2017)	Yamaguchi, Japan	Tissue	qRT-PCR	100	-	2.46 (1.06-5.69) ³ <i>p</i> =0.027	-	-
Yu (2017)	Shanghai, China	Tissue	qRT-PCR	92 173	-	-	-	3.78 (1.89-7.58) 3.67 (2.34-5.76)

*Sub-group analysis limited to patients with over-represented *Pseudomonas fragi*

² Estimates of the hazard ratio and 95% confidence intervals were produced using the indirect methods proposed by Parmar et al., (1998) [54]

³ Estimates of the hazard ratio and 95% confidence intervals were produced using the survival curve methods proposed by Parmar et al., (1998) [54]

Abbreviations: CI=confidence interval, CRC=colorectal cancer, FISH=fluorescence in situ hybridization, Fn=*Fusobacterium nucleatum*, HR=hazard ratio, qRT-PCR= quantitative real time polymerase chain reaction.

FIGURE LEGENDS

Figure 1: Study selection

¹Studies may have been included in more than one meta-analysis

Figure 2. Forest plot comparing the odds of *F. Nucleatum* positivity in colorectal cancer tissue samples compared to normal colorectal tissue from controls; colorectal cancer tissue samples compared to colorectal polyp tissue and; colorectal polyp tissue compared to normal colorectal tissue from controls.

Figure 3. Forest plot comparing the odds of *F. nucleatum* positivity in CRC tissue samples compared to adjacent, normal tissue.

Figure 4. Forest plot comparing the odds of *F. Nucleatum* positivity in faecal samples from colorectal cancer patients compared to healthy controls; from colorectal cancer patients compared to individuals with colorectal polyps; and; from individuals with colorectal polyps compared to healthy controls.

Figure 5. Random effects meta-analysis for the association between *Fusobacterium Nucleatum* and overall survival in individuals with colorectal cancer.

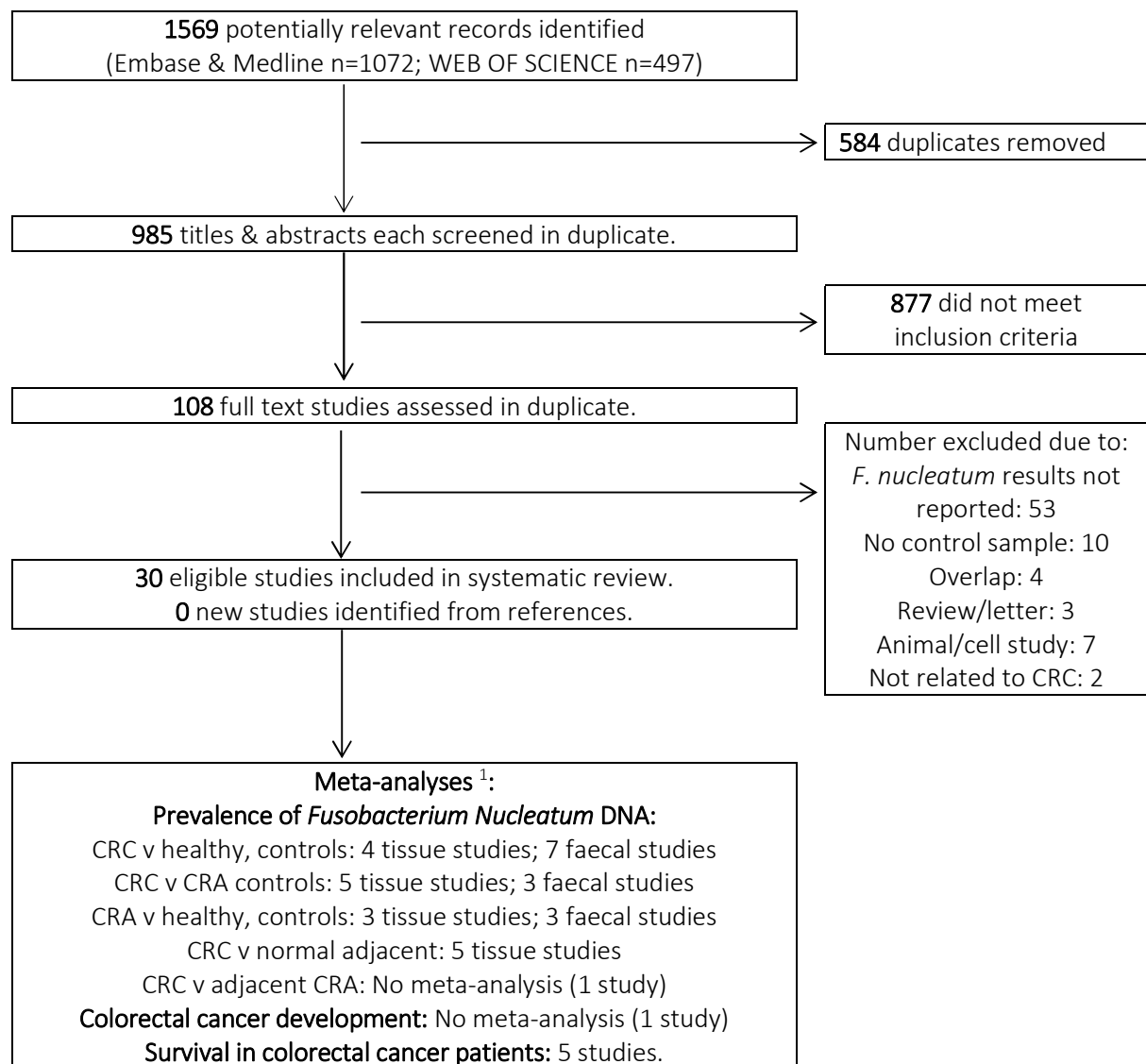


Figure 1: Study selection

¹Studies may have been included in more than one meta-analysis

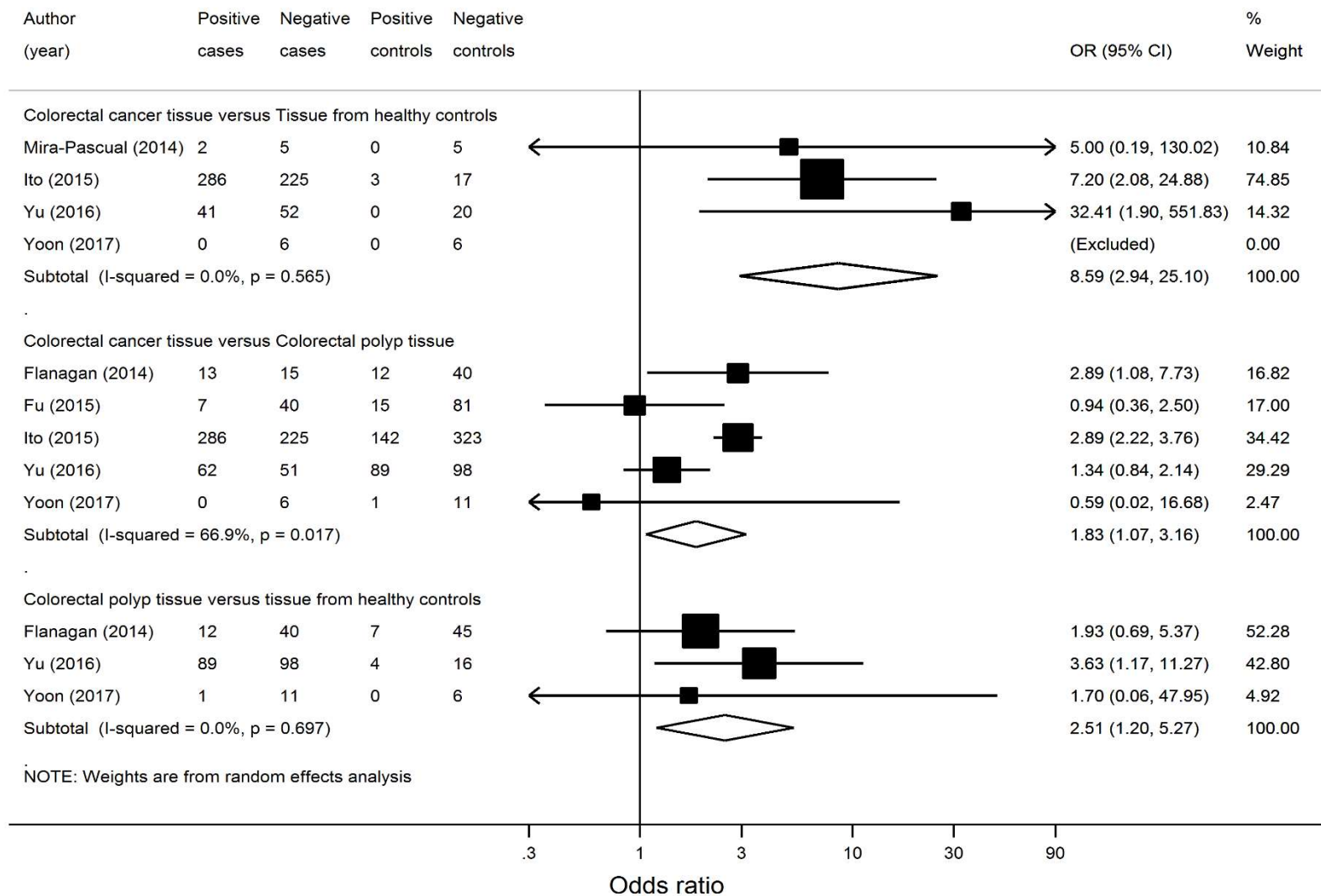


Figure 2. Forest plot comparing the odds of *F. Nucleatum* positivity in colorectal cancer tissue samples compared to normal colorectal tissue from controls; colorectal cancer tissue samples compared to colorectal polyp tissue and; colorectal polyp tissue compared to normal colorectal tissue from controls.

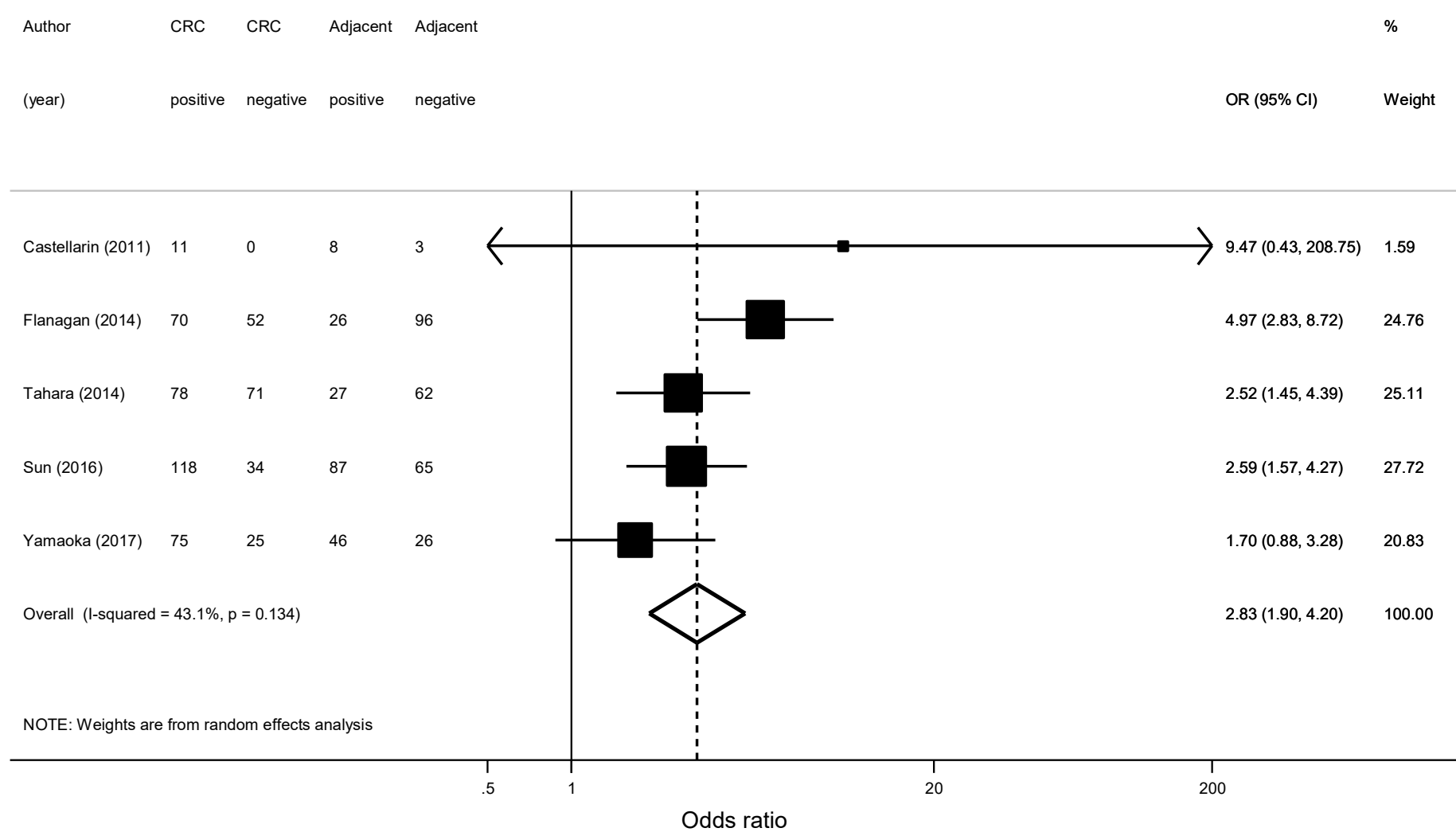


Figure 3. Forest plot comparing the odds of *F. nucleatum* positivity in CRC tissue samples compared to adjacent, normal tissue.

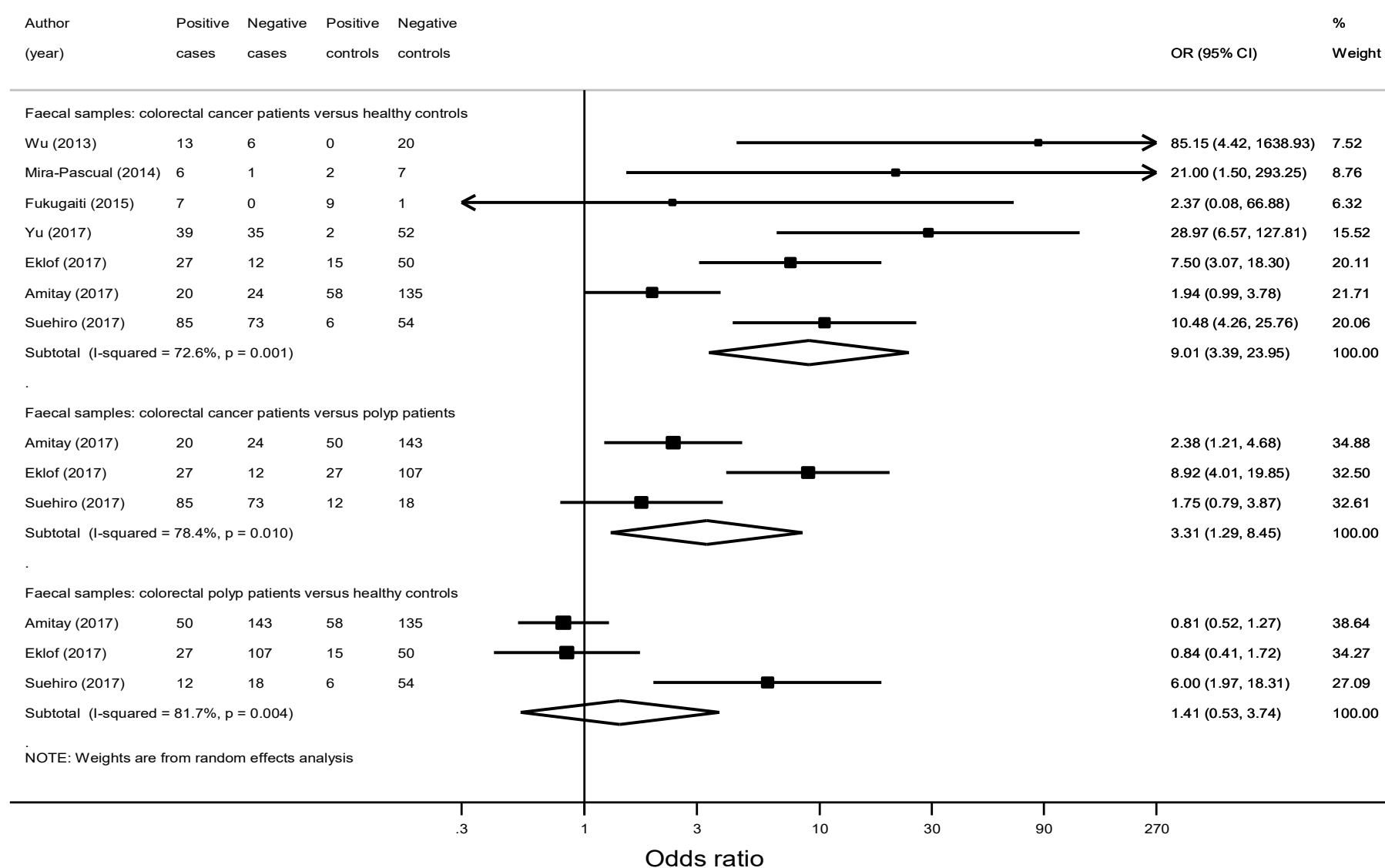


Figure 4. Forest plot comparing the odds of *F. Nucleatum* positivity in faecal samples from colorectal cancer patients compared to healthy controls; from colorectal cancer patients compared to individuals with colorectal polyps; and; from individuals with colorectal polyps compared to healthy controls.

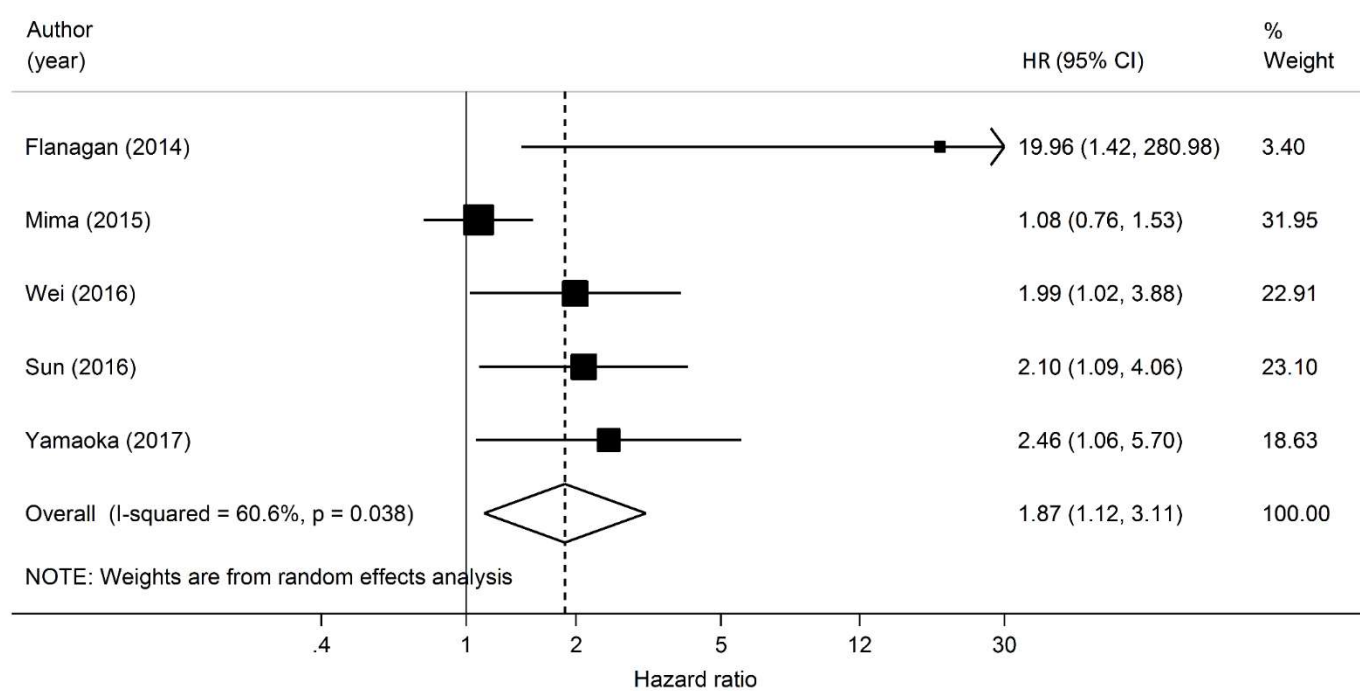


Figure 5. Random effects meta-analysis for the association between *Fusobacterium Nucleatum* and overall survival in individuals with colorectal cancer.