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Original article

The association between *Fusobacterium nucleatum* and cancer colorectal: A systematic review and meta-analysis☆

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ABSTRACT

Introduction: The etiological factors of colorectal cancer (CRC) are not precisely known, although genetic and environmental factors have been implicated. A possible association with *Fusobacterium nucleatum* may provide opportunities for an early diagnosis.
Objective: To review studies that address the association between *F. nucleatum* and CRC.
Methods: The MEDLINE PubMed database was searched using the terms «colorectal cancer» and "*Fusobacterium nucleatum*", retrieving publications published up to January 1 2020. Stata software was used for a meta-analysis.
Results: The systematic review included 57 articles. Meta-analysis results indicated a more frequent presence of *F. nucleatum* in CRC tumour tissue samples in comparison to control samples of healthy tissue, with an odds ratio of 4.558 (95% CI: 3.312−6.272), and in comparison, to control samples of colorectal adenomas, with an odds ratio of 3.244 (95 % CI: 2.359−4.462).
Conclusion: There is a more frequent resence of *F. nucleatum* in the CRC. However, further studies are needed to verify this relationship.

La asociación entre *Fusobacterium nucleatum* y el cáncer colorrectal: una revisión sistemática y metaanálisis

Palabras clave:
Cáncer colorrectal
Fusobacterium nucleatum
Microbiota
Disbiosis
Inflamación crónica

RESUMEN

Introducción: Se desconocen los factores etiológicos exactos del cáncer colorrectal (CCR), aunque se ha intentado relacionar con factores genéticos y ambientales. La posible asociación con *Fusobacterium nucleatum* podría abrir posibilidades en el diagnóstico precoz.
Objetivo: Revisar los estudios que analizan la asociación entre *F. nucleatum* y el CCR.
Métodos: Se utilizaron las publicaciones disponibles en la base de datos MEDLINE PubMed hasta el día 1 de enero de 2020, que incluían los términos «cáncer colorrectal» y «*Fusobacterium nucleatum*». Se realizó un metaanálisis con el software Stata.
Resultados: Un total de 57 artículos fueron incluidos en la revisión sistemática. El metaanálisis indicó una mayor presencia de *F. nucleatum* en muestras de tejido tumoral de CCR, con respecto a muestras control de tejido sano, con una odds ratio de 4,558 (IC 95%: 3,312–6,272), y cuando se utilizaron muestras control de adenomas colorrectales, con una odds ratio de 3,244 (IC 95%: 2,359–4,462).

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Conclusión: Hay una mayor presencia de *F. nucleatum* en el CCR. Sin embargo, se necesitan estudios que demuestren esta relación.

Introduction

The aetiology of colorectal cancer (CRC) is multifactorial and includes genetic and epigenetic abnormalities[1]. Extrinsic factors such as intestinal dysbiosis might also play a role[2]; however, interindividual variability exists due to multiple genetic and environmental factors[3]. Hence, it is of special interest to investigate the possibility of a common microbial denominator.*Fusobacterium nucleatum* (FN) is among the most extensively studied bacteria[4–6]. It is a Gram-negative anaerobic bacterium that resides in the oral cavity as commensal microbiota, but it is also an opportunistic pathogen in periodontal diseases, primarily in gingivitis and periodontitis[7]. Its pathogenic power lies in its virulence factors, including: the presence of fimbriae, lipopolysaccharides, factors inhibiting chemotaxis of polymorphonuclear leukocytes and the production of toxic tissue metabolites[8]. However, its most significant virulence factor is FadA adhesin. This has been shown to be the greatest stimulant of inflammation as it creates a chronic proinflammatory environment that activates oncogenic signalling, thus stimulating epithelial cells[9,10]. Recent studies have demonstrated an increase in FadA in neoplastic CRC tissue, along with other proinflammatory markers such as COX-2, IL-8, IL-6, IL1ß and TNF-α[11].

The possibility that FN is involved in the process of carcinogenesis could open up new avenues for early diagnosis. This justifies a comprehensive analysis of the current evidence on the relationship between FN and CRC. The objective of this study was to integrate the information available on the relationship between FN and CRC in a meta-analysis.

Material and methods

The meta-analysis had a qualitative component (systematic review) and a quantitative component. The systematic review consisted of a description of the published studies, considering individual studies to be study "subjects". A systematic search was conducted of all articles published in English or Spanish in journals indexed in MEDLINE, ISI Web of Knowledge and the Cochrane Library. The search terms used were "colorectal cancer" and "*Fusobacterium nucleatum*". The selection criteria were the time limit (the search was restricted to all articles published in hardcopy and/or electronic format prior to January 2020), study type (research studies were selected) and language (the search was confined to articles written in English or Spanish). The following were excluded: studies on the relationship between FN and diseases other than CRC, studies on the relationship between CRC and bacteria other than FN, studies on the isolation of FN in species other than human beings, and reviews. The studies had to include patients with CRC, and they had to have the objective of directly or indirectly looking for FN and attempting to establish a possible association between FN infection and CRC. An internal quality system was used; this verified the reliability of the chosen method above, based on thorough review of each publication with tracing of the references in the publications to avoid missed studies.

The qualitative component referred to the statistical grouping of the results, with individual studies acting as research subjects, yielding, within and across publications, the 95% confidence interval, the odds ratio (OR) and the weights of the studies. The weighted mean was estimated by means of the random-effects model using DerSimonian and Laird's method[12], which is affected to a lesser extent by heterogeneity between studies. The inverse-variance test was used to assess heterogeneity. In addition, Higgins's I^2 inconsistency index was calculated; the value of this index indicates the percentage of variability due to heterogeneity between studies. Values over 75% indicate a strong heterogeneity level and would suggest a need to conduct further studies. Begg's[13] and Egger's[14] tests were used to detect publication bias. Data analysis was performed with the Stata 14 software programme.

Results

Systematic review

The article search collected 199 publications; of them, 57 studies whose online version at least was published between 2011 and 2019 were selected (Table 1 and Fig. 1). Non-tumour area control samples were called Controls-1 (40 studies — 70.2%: 15 samples of adjacent non-tumour tissue, four faecal samples, two blood samples, one saliva sample, 18 non-tumour tissue samples). Adenoma samples were called Controls-2 (16%–28.1%). The Test-1 column indicates the test described by the authors to detect the microorganism. In addition, some of the studies included the use of additional laboratory tests, called Test-2. Finally, the most salient conclusion of the research articles was also collected ("Article comments" column).

An increase in the number of studies published over the years was confirmed, but only in 2017 did this research significantly increase. The sex of the cases was reported in 20 (35.1%) studies overall, 11 (19.3%) Controls-1 studies and five (31.2%) Controls-2 studies. Until 2015, no studies reported the sex of the study participants. The most recent studies have tended to specify the sex of the study population. Analysis of the distribution of the studies with quantitative data found that such data were indicated in 40 (70.2%) Cases articles, 22 (38.6%) Controls-1 articles and 10 (25%) Controls-2 articles.

Quantitative analysis

Regarding the number of positive results in the CRC samples, the highest rates of positive results were found in the studies by Castellarin et al.[15], Kostic et al.[4], Fukugaiti et al.[23] and Guevara et al.[6], in which all CRC samples analysed yielded a positive result in laboratory tests. By contrast, the lowest rate of positive results was found in a study by Nosho et al.[24], in which just 8.6% of samples had a positive result. The number of samples for the Cases in each study varied widely. The lowest number was found in studies by Mira-Pascual et al.[19] and Fukugaiti et al.[23], with a total of seven cases analysed in each study. The largest sample size for the Cases corresponded to a study by Liu et al.[42], with an analysis of 2759 samples. It should be noted that just four (7%) studies had more than 1000 individuals with CRC, and that they were conducted between 2017 and 2018.

Table 2 shows the studies (18 out of 40, 45%) that compared, in Cases and Controls-1, positivity for FN infection markers with quantitative data from samples of colon tissue using molecular biology tests. The studies by Mima et al.[28], Flanagan et al.[18] and Tahara et al.[20] were notable for their weight. All of them had a weight in excess of 10%. The overall OR estimate for the individual results obtained in these studies yielded a result of 4.558 (95% CI: 3.312–6.272), leading to the conclusion that there was a significant association ($p < 0.001$) between FN and CRC when Controls-1

Table 1
Systematic review of the literature published in the MEDLINE database and accessed via the PubMed interface until 1 January 2020 on the relationship between *Fusobacterium nucleatum* and colorectal cancer.

Author	Year Physical publication	Total sex (f:m)	Cases CRC	Sex Cases CRC (f:m)	Control-1: No CRC (n)	Sex Control-1	Description Group Control-1	Control-2: Adenomas (n)	Sex Control-2	What was analysed?	Test 1	Test 2	FN+/total Cases	FN+/total Control-1	FN+/total Control-2	Article comments
Castellarin et al.[15]	2012	NI	11	NI	99	NI	Adjacent non-tumour tissue	NI	NI	RNA	PCR	NI	11/11	9/11	NI	Higher rate of FN in CRC than in normal tissue
Rubinstein et al.[16]	2013	NI	51	NI	14	NI	Tissue from tumour-free subjects	NI	NI	mRNA	PCR	NI	NI	NI	NI	Higher rate of FadA in CRC than in normal tissue
Kostic et al.[4]	2013	NI	27	NI	31	NI	Tissue from tumour-free subjects	28	NI	DNA and RNA	PCR	FISH	27/27	15/31	24/28	FN creates a proinflammatory state that promotes progression to CRC
Warren et al.[17]	2013	NI	65	NI	65	NI	Adjacent non-tumour tissue	NI	NI	rRNA	Metatranscriptomics	NI	NI	NI	NI	Higher rate of FN in CRC than in adjacent healthy tissue
Flanagan et al.[18]	2014	NI	122	NI	174	NI	Adjacent non-tumour tissue	52	NI	DNA	PCR	NI	70/122	33/174	12/52	FN can be used as a marker for CRC
Mira-Pascual et al.[19]	2014	NI	7	NI	5	NI	Tissue from tumour-free subjects	NI	NI	DNA and RNA	PCR	Pyrosequencing	2/7	0/5	NI	The increase in the levels of the bacteria studies was correlated with CRC
Tahara et al.[20]	2014	NI	149	NI	89	NI	Adjacent non-tumour tissue	NI	NI	DNA	PCR	NI	78/149	27/89	NI	Higher rate of FN in CRC than in controls
Ito et al.[21]	2015	NI	511	225:286	20	NI	Tissue from tumour-free subjects	456	39:59	DNA	PCR	NI	286/511	3/20	142/456	Higher rate of FN in CRC than in controls
Mima et al.[22]	2015	NI	598	NI	558	NI	Adjacent non-tumour tissue	NI	NI	DNA	PCR	NI	76/598	19/558	NI	FN creates a proinflammatory state that promotes progression to CRC
Yu et al.[10]	2015	NI	42	NI	52	NI	Adjacent non-tumour tissue	47	NI	DNA	PCR	NI	NI	NI	NI	Higher rate of FN in CRC than in controls
Fukugaiti et al.[23]	2015	4:13	7	2:5	10	2:8	Faeces	NI	NI	DNA	PCR	FISH	7/7	9/10	NI	Higher rate of FN in CRC than in controls
Nosho et al.[24]	2016	NI	511	225:286	NI	NI	No control tissue	NI	NI	DNA	PCR	NI	44/511	NI	NI	Lower rate of positive results for FN in the Japanese cohort
Repass et al.[25]	2016	NI	40	NI	40	NI	Adjacent non-tumour tissue	NI	NI	DNA	PCR	NI	NI	NI	NI	Higher rate of FN in CRC than in normal tissue
Li et al.[26]	2016	46:55	101	46:55	101	46:55	Adjacent non-tumour tissue	NI	NI	DNA	PCR	FISH	88/101	NI	NI	Larger amount of FN in CRC is associated with lower survival time
Yu et al.[27]	2016	NI	93	NI	20	NI	Tissue from tumour-free subjects	112	NI	DNA	PCR	NI	62/93	4/20	47/112	FN might play a role in the serrated pathway of carcinogenesis for CRC
Mima et al.[28]	2016	NI	1,102	NI	NI	NI	No control tissue	NI	NI	DNA	PCR	NI	138/1,102	NI	NI	Increases the rate of FN from CRC of the rectum to caecal carcinoma

Table 1 (*Continued*)

Author	Year Physical publication	Total sex (f:m)	Cases CRC	Sex Cases CRC (f:m)	Control-1: No CRC (n)	Sex Control-1	Description Group Control-1	Control-2: Adenomas (n)	Sex Control-2	What was analysed?	Test 1	Test 2	FN+/total Cases	FN+/total Control-1	FN+/total Control-2	Article comments
Yu et al.[5]	2017	NI	74	NI	54	NI	Tissue from tumour-free subjects	NI	NI	DNA	PCR	NI	39/74	2/54	NI	FN promotes CRC progression through modulation of autophagy pathways
Suehiro et al.[29]	2017	NI	158	NI	60	NI	Tissue from tumour-free subjects	19	NI	DNA	PCR	NI	NI	NI	NI	Mean copies of FN: 17.5 in the control group, 122 in the adenoma group, 317 in the CRC group
Liang et al.[30]	2017	NI	203	NI	236	NI	Tissue from tumour-free subjects	NI	NI	DNA	PCR	Meta-genomic sequenc-ing	NI	NI	NI	Higher rate of FN in CRC than in controls
Wong et al.[31]	2017	NI	104	NI	102	NI	Tissue from tumour-free subjects	103	NI	DNA	PCR	NI	NI	NI	NI	Larger amount of FN in adenomas compared to controls p = 0.022
Mehta et al.[32]	2017	NI	1,019	NI	NI	NI	No control tissue	NI	NI	DNA	PCR	NI	125/1,019	NI	NI	The association between diet and CRC is modified by FN
Chen et al.[33]	2017	NI	98	40:58	NI	NI	No control tissue	NI	NI	DNA	FISH	NI	61/98	NI	NI	Invasive FN activates the beta-catenin signalling pathway
Ye et al.[34]	2017	NI	25	NI	25	NI	Adjacent non-tumour tissue	NI	NI	DNA	PCR	NI	4/25	2/25	NI	Higher rate of FN in CRC than in controls
Amitay et al.[35]	2017	220:280	46	15:31	231	121:110	Tissue from tumour-free subjects	223	84:139	DNA	PCR	NI	25/46	58/231	53/223	Higher rate of FN in CRC than in controls
Park et al.[36]	2017	82:78	160	82:78	NI	NI	No control tissue	NI	NI	DNA	PCR	NI	107/160	NI	NI	The larger amount of FN in tumour samples is correlated with the presence of intratumoural macrophages
Eklöf et al.[37]	2017	NI	39	19:20	65	30:35	Tissue from tumour-free subjects	NI	NI	DNA	PCR	NI	27/39	15/65	NI	Larger amount of FN in CRC than in controls
Bullman et al.[38]	2017	NI	77	NI	NI	NI	No control tissue	NI	NI	RNA	PCR	FISH	45/77	NI	NI	The reduction in FN with antibiotics causes less cancer proliferation
Drewes et al.[39]	2017	NI	58	NI	34	NI	Tissue from tumour-free subjects	NI	NI	RNA	PCR	FISH	17/58	1/34	NI	Larger amount of bacteria in CRC than in healthy tissue
Yamaoka et al.[40]	2018	NI	100	NI	72	NI	Adjacent non-tumour tissue	NI	NI	DNA	PCR		75/100	46/72	NI	Larger amount of FN in CRC than in controls
Guevara et al.[6]	2018	NI	37	NI	37	NI	Tissue from tumour-free subjects	NI	NI	Anti-FN antibod-ies	ELISA	NI	37/37	12/37	NI	The FN Fap2 protein is antigenic
Dai et al.[41]	2018	206:320	255	92:163	271	114:157	Faeces	NI	NI	DNA	Metagenomics	NI	NI	NI	NI	Higher rate of FN in CRC than in controls

Table 1 (*Continued*)

Author	Year Physical publication	Total sex (f:m)	Cases CRC	Sex Cases CRC (f:m)	Control-1: No CRC (n)	Sex Control-1	Description Group Control-1	Control-2: Adenomas (n)	Sex Control-2	What was analysed?	Test 1	Test 2	FN+/total Cases	FN+/total Control-1	FN+/total Control-2	Article comments
Liu et al.[42]	2018	NI	2,759	NI	NI	NI	No control tissue	NI	NI	DNA	PCR	NI	951/2,759	NI	NI	Positive association between the empirical dietary inflammatory pattern (EDIP) and FN + CRC
Chen et al.[43]	2018	NI	25	NI	7	NI	No control tissue	8	NI	DNA	FISH	Immuno-fluore-scence	NI	NI	NI	Greater expression of TOX and CD4+ in FN − tissue versus FN + tissue. Negative correlation between FN abundance and TOX expression
Guo et al.[44]	2018	NI	215	NI	156	NI	Tissue from tumour-free subjects	NI	NI	DNA	PCR	NI	NI	NI	NI	The FN/*Bifidobacterium* ratio had a sensitivity of 84.6% and a specificity of 92.3% in detecting CRC
Komiya et al.[45]	2018	NI	14	NI	NI	NI	No control tissue	NI	NI	DNA	PCR	NI	8/14	NI	NI	FN samples in tumour tissue and saliva
Lee et al.[46]	2018	NI	246	NI	NI	NI	No control tissue	NI	NI	DNA	PCR	NI	NI	NI	NI	High levels of FN are associated with lower survival in CRC metastases
Rezasoltani et al.[47]	2018	NI	NI	NI	31	15:16	Tissue from tumour-free subjects	87	33:54	DNA	PCR	NI	NI	NI	NI	Significant relationship between polyp size and FN measurement
Hamada et al.[48]	2018	609:432	1,041	609:432	NI	NI	No control tissue	NI	NI	DNA	PCR	NI	135/1,041	NI	NI	The association between FN and CRC varies depending on whether the CRC has an abnormal microsatellite pathway
Oh et al.[49]	2018	231:362	593	231:362	NI	NI	No control tissue	NI	NI	DNA	PCR	NI	204/593	NI	NI	The tumour prognosis in relation to FN depends on the CRC location
Proença et al.[50]	2018	NI	43	NI	70	NI	Adjacent non-tumour tissue	27	NI	DNA	PCR	NI	33/43	23/70	14/27	FN is a risk factor for CRC
Zhang et al.[51]	2019	41:53	94	41:53	NI	NI	No control tissue	NI	NI	DNA	PCR	Micro-array	21/90	NI	NI	FN induces expression of the BIRC3 gene in CRC
Saito et al.[52]	2019	30:51	24	7:17	10	7:3	Tissue from tumour-free subjects	47	16:31	rRNA	PCR	NI	14/24	NI	28/47	FN may be associated with CRC and adenomas, and is a potential diagnostic marker for both
Guo et al.[53]	2019	NI	46	NI	36	NI	Blood	NI	NI	DNA	Genomic sequencing	NI	NI	NI	NI	Greater presence of FN in tumour tissue than in blood samples
Feng et al.[54]	2019	13:12	15	8:7	10	5:5	Tissue from tumour-free subjects	NI	NI	DNA	PCR	Western blot	10/15	5/10	NI	CREB protein expression is correlated with CRC metastasis whether in FN + or in FN−
Tunsjø et al.[55]	2019	NI	25	NI	22	NI	Tissue from tumour-free subjects	25	NI	DNA	PCR	16S rRNA sequenc-ing	15/25	5/25	4/22	PCR for FN detection could be included as a CRC biomarker

Table 1 (*Continued*)

Author	Year Physical publication	Total sex (f:m)	Cases CRC	Sex Cases CRC (f:m)	Control-1: No CRC (n)	Sex Control-1	Description Group Control-1	Control-2: Adenomas (n)	Sex Control-2	What was analysed?	Test 1	Test 2	FN+/total Cases	FN+/total Control-1	FN+/total Control-2	Article comments
Bundgaard-Nielsen et al.[56]	2019	141:132	99	55:44	76	35:41	Adjacent non-tumour tissue	96	51:47	DNA	PCR	16S rRNA sequencing	23/99	NI	3/96	There is no evidence that FN plays a role in the onset of adenomas, but it might play a role in the transition from adenoma to CRC
Guven et al.[57]	2019	NI	71	NI	77	NI	Saliva from cancer-free controls	NI	NI	DNA	PCR	NI	NI	NI	NI	Larger amount of FN in saliva from patients with CRC compared to controls
Yachida et al.[58]	2019	NI	258	NI	291	NI	Faeces	67	NI	DNA	Capillary electrophoresis	Metagenomic and metabolomic study	NI	NI	NI	FN abundance is correlated with tumour progression from intramucosal carcinoma to more advanced stages
Leung et al.[59]	2019	10:9	19	10:9	19	10:9	Adjacent non-tumour tissue	NI	NI	DNA	PCR	NI	NI	NI	NI	Higher rate of FN in CRC than in normal tissue
Kunzmann et al.[60]	2019	60:130	190	60:130	190	60:130	Adjacent non-tumour tissue	NI	NI	DNA	PCR	NI	61/190	129/190	NI	FN is more abundant in tumour tissue than surrounding tissue
Butt et al.[61]	2019	NI	485	NI	485	NI	Blood	NI	NI	Anti-FN antibodies	Serology	NI	NI	NI	NI	Anti-FN antibodies have no association with the risk of developing CRC
De Carvalho et al.[62]	2019	NI	152	NI	57	NI	Adjacent non-tumour tissue	NI	NI	DNA	PCR	NI	35/152	6/57	NI	Detection of FN DNA is associated with proximal tumour location; positivity for MSI or BRAF mutation; loss of MLH1, MSH2 or PMS2; and lower survival
Chen et al.[63]	2019	36:55	91	36:55	NI	NI	No control tissue	NI	NI	DNA	PCR	NI	25/91	NI	NI	Larger amount of FN in CRC tissue is associated with lower survival time
Mima et al.[64]	2019	208:304	256	208:304	NI	NI	No control tissue	NI	NI	DNA	PCR	NI	140/256	NI	NI	Larger amounts of FN and other bacteria associated with CRC increase the risk of anastomotic rupture following surgery
Liang et al.[65]	2019	NI	274	NI	353	NI	Faeces	353	NI	DNA	PCR	Faecal immunohistochemistry test	71/274	97/385	145/353	Identification of m3 marker for CRC diagnosis
Haruki et al.[66]	2019	423:301	724	423:301	NI	NI	No control tissue	NI	NI	DNA	PCR	NI	99/724	NI	NI	Inverse association between FN amount and tumour expression of BECN1
Chen et al.[67]	2019	NI	148	NI	NI	NI	No control tissue	NI	NI	DNA and RNA	PCR	FISH	88/148	NI	NI	FN is found in larger amounts in CRC with metastases

CRC: colorectal cancer; FN: *Fusobacterium nucleatum*; NI: no information.

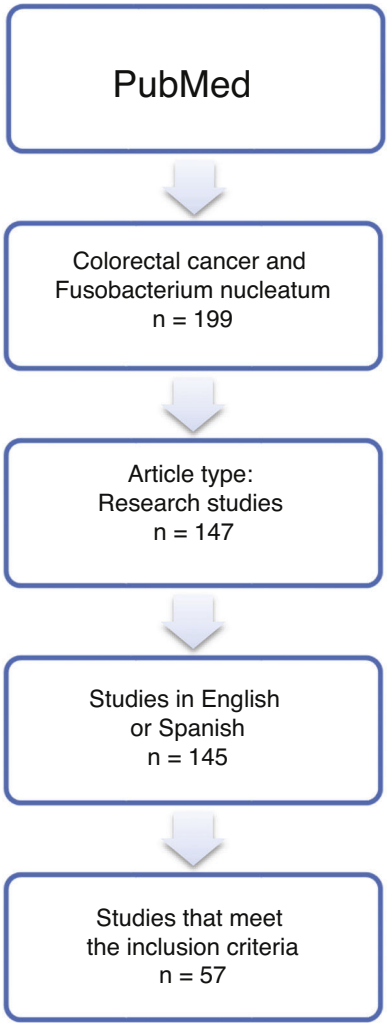

Fig. 1. Systematic review flow chart.

Table 2
List of the 18 studies that compared, in Cases and Controls-1, positivity for *Fusobacterium nucleatum* infection markers in intestinal biopsy samples using molecular biology.

Study	OR	95% CI	% weight
Castellarin et al., 2011	6.053	0.258–142.040	0.96
Kostic et al., 2013	58.548	3.282–1,044.602	1.14
Flanagan et al., 2014	5.752	3.413–9.693	10.85
Mira-Pascual et al., 2014	5.000	0.192–130.024	0.90
Tahara et al., 2014	2.523	1.449–4.393	10.46
Ito et al., 2015	7.203	2.085–24.884	4.62
Mima et al., 2015	4.130	2.463–6.926	10.91
Yu et al., 2017	28.971	6.567–127.806	3.55
Yu et al., 2016	8.000	2.465–25.968	4.96
Ye et al., 2017	2.190	0.363–13.219	2.61
Amitay et al., 2017	3.551	1.850–6.815	9.34
Yamaoka et al., 2018	1.696	0.876–3.282	9.24
Eklöf et al., 2017	7.500	3.074–18.297	6.97
Drewes et al., 2017	13.683	1.730–108.242	2.06
Proença et al., 2018	6.743	2.838–16.026	7.20
Feng et al., 2019	2.000	0.388–10.309	3.04
Tunsjø et al., 2019	6.000	1.693–21.262	4.50
De Carvalho et al., 2019	2.543	1.007–6.421	6.68
D + L pooled OR	4.558	3.312–6.272	100.00

Heterogeneity $\chi^2 = 30.54$ (gl = 17) p = 0.023.
I^2 (variation in OR that can be attributed to heterogeneity) = 44.3 %.
Estimated variance between studies tau-squared = 0.1736.
Test of OR = 1: z = 9.31, p = 0.000.

Table 3
List of nine studies that compared, in Cases and Controls-2, positivity for *Fusobacterium nucleatum* infection markers in intestinal biopsy samples using molecular biology.

Study	OR	95% CI	% weight
Kostic et al., 2013	10.102	0.517–197.311	1.11
Flanagan et al., 2014	4.487	2.145–9.388	12.37
Ito et al., 2015	2.811	2.158–3.661	29.36
Yu et al., 2016	2.766	1.562–4.899	16.85
Amitay et al., 2017	3.819	1.980–7.366	14.35
Proença et al., 2018	3.064	1.089–8.623	7.54
Saito et al., 2019	0.950	0.350–2.580	7.97
Tunsjø et al., 2019	6.750	1.755–25.956	4.86
Bundgaard-Nielsen et al., 2019	9.382	2.713–32.442	5.59
D + L pooled OR	3.244	2.359–4.462	100.00

Heterogeneity $\chi^2 = 12.33$ (gl = 8) p = 0.137.
I^2 (variation in OR that can be attributed to heterogeneity) = 35.1%.
Estimated variance between studies tau-squared = 0.0719.
Test of OR = 1: z = 7.24 p = 0.000.

were used. To calculate heterogeneity, the inverse-variance test was performed, with a value of χ^2 exp. = 30.54, with 17 degrees of freedom and p = 0.023, meaning that the differences found between the studies were not due to chance. The heterogeneity study was corroborated using Higgins's I^2, with a result of $I^2 = 44, 3\%$ (Table 2 and Fig. 2). There was no obvious publication bias, as indicated in Begg's and Egger's tests, which were not significant (p = 0.767 and p = 0.210, respectively) (Fig. 3).

Table 3 shows the studies (nine out of 16, 56.25%) that compared, in Cases and Controls-2 (colorectal adenomas), positivity for genetic markers of FN infection with quantitative data from samples of colon tissue using molecular biology tests. The studies by Ito et al.[21], Flanagan et al.[18], Yu et al.[27] and Amitay et al.[35] all had notable weights, exceeding 12%. The overall OR estimate for the individual results obtained in these studies yielded a result of 3.244 (95% CI: 2.359–4.462), showing a statistically significant association between the presence of FN and CRC (p < 0.001). To analyse heterogeneity, the inverse-variance test was performed, with a value of χ^2 exp. = 12.33, with eight degrees of freedom and p = 0.137, showing that there was homogeneity between the studies compared. In addition, Higgins's I^2 showed a value of 35.1%, indicating a low level of heterogeneity (Table 3 and Fig. 4).

There was no obvious publication bias, as indicated by Begg's and Egger's tests, which were not significant (p = 0.108 and p = 0.441, respectively) (Fig. 5).

Discussion

This study found that the relationship between FN and CRC is a topic of growing interest, given the exponential rise in studies that have explored this relationship. With regard to the sample size of the studies, 31 (54.4%) had fewer than 100 case samples, and just four (7%) had more than 1000 samples. Similarly, in the studies published, just 22 (55%) had numerical data for Controls-1 and 10 (17.5%) had numerical data for Controls-2. Efforts have been made in recent years to correct this, however, and the most current studies have exhibited a trend towards publishing the corresponding values. Above all, future studies must report quantitative data for FN positivity in samples, as this constitutes solid evidence subject to statistical processing on the association thereof with CRC. Also important is the influence of sex on the results, which has scarcely been studied in this regard, given that such data have been omitted from many studies. The breakdown of this relationship by sex could shed light on whether sex could be considered a risk factor in relation to the presence of FN, since non-transmissible chronic diseases are related to lifestyle factors in men and women. In this

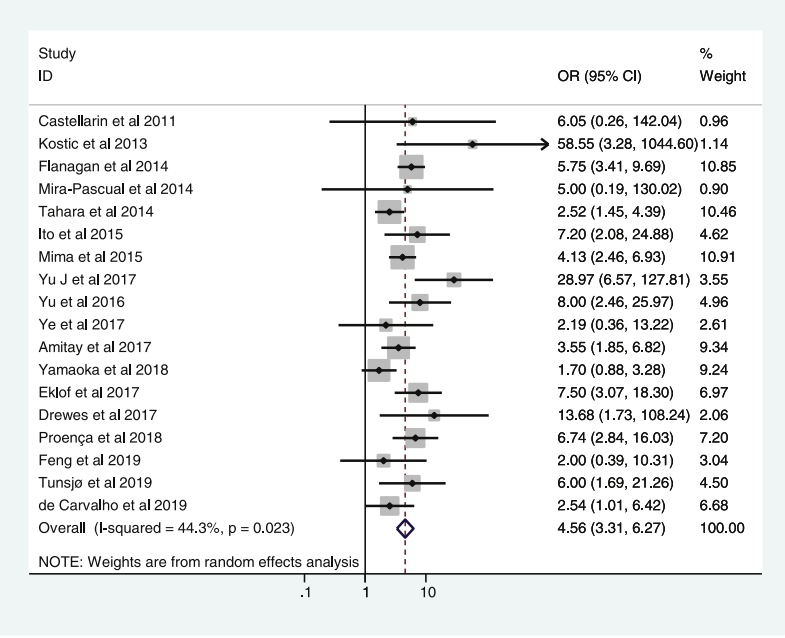

Fig. 2. Forest plot of the 18 studies that compared, in Cases and Controls-1, positivity for *Fusobacterium nucleatum* infection markers in intestinal biopsy samples using molecular biology.

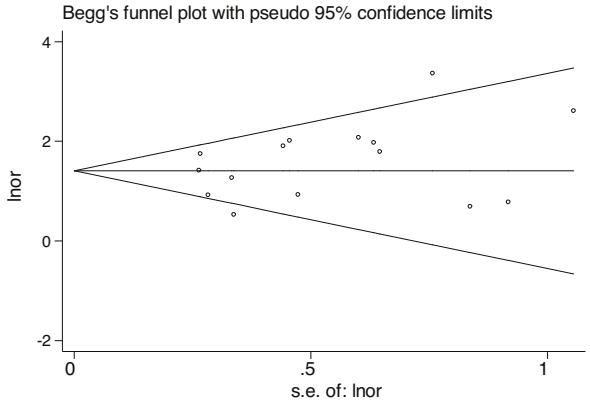

Fig. 3. Funnel plot of the 18 studies that compared, in Cases and Controls-1, positivity for *Fusobacterium nucleatum* infection markers in intestinal biopsy samples using molecular biology.

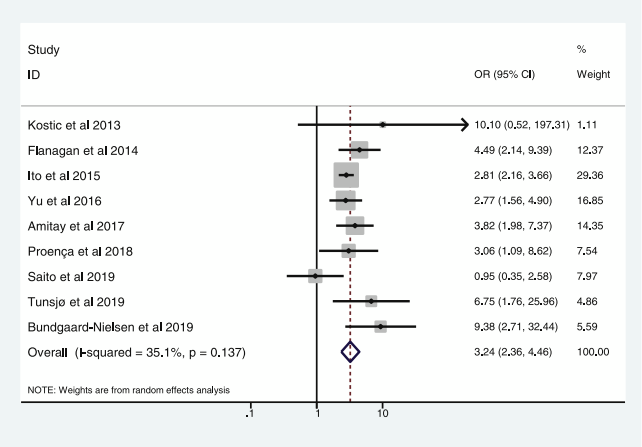

Fig. 4. Forest plot of the eight studies that compared, in Cases and Controls-2, positivity for *Fusobacterium nucleatum* infection markers in intestinal biopsy samples using molecular biology.

systematic review, the sex of the patients was only indicated in 20 (35.1%) studies out of the total of 57 articles collected.

Similarly, future studies could examine other variables, such as diet. Some studies, including the study by Hussan et al.[68], have collected data for this variable and found a fibre-rich diet to be linked to a lower risk of developing CRC despite having a larger number of FN-positive samples in the colon mucosa, which would seem contradictory.

It should also be noted that the majority of the samples analysed in the study were tissue samples. That is to say, the CRC samples were tumour tissue samples and the control samples were samples of healthy tissue, since, in theory, the study thereof should offer more complete information. From what has been published to date, it has been deduced that FN has a significant presence in CRC tissue compared to adjacent healthy tissue[15,18,20,28,69], with a gradual increase in FN from healthy mucosa to adenoma and from adenoma to adenocarcinoma. This suggests that FN may play an important role as of the earliest stages of colon cancer onset[4,10,18,70], that there is an association between the presence of FN and lymph node invasion by CRC[15,26,71] and that FN accompanies tumour cells

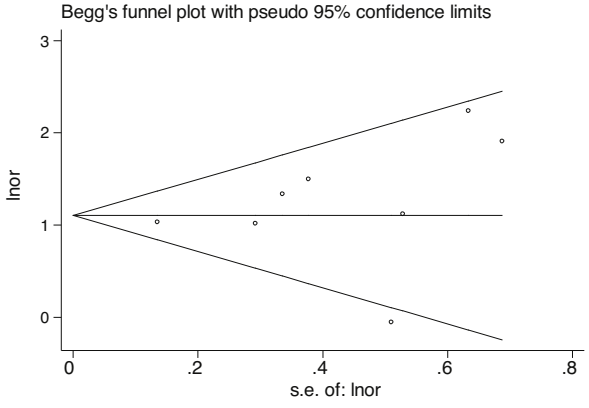

Fig. 5. Funnel plot of the eight studies that compared, in Cases and Controls-2, positivity for *Fusobacterium nucleatum* infection markers in intestinal biopsy samples using molecular biology.

in metastases[38]. Thus it has been explained that the amount of FN in CRC is linked to stage, chemotherapy resistance, higher recurrence and lower survival, with said amount acting as an independent predictive factor[10,26,28,38,70,72,73].

Just four (7%) studies tested faecal samples, one (1.8%) tested blood samples and one (1.8%) tested saliva samples, concluding that there is an overabundance of FN in the faeces of patients with CRC. FN is 132 times more abundant in the faeces of patients with CRC, and its determination has been identified as a useful marker for detecting CRC[29–31], showing a sensitivity of 80.2% and a specificity of 80.7%, with these figures increasing to 92 %–93% when it is combined with faecal occult blood detection[74]. It would be interesting to confirm the association between FN and CRC simultaneously in samples of tissue, faeces, saliva and blood. Should research be intensified and a significant association discovered, it would have a great deal of diagnostic utility for patients, since these samples are relatively easy to collect with no need for a colonoscopy to acquire them.

Based on all studies conducted to date, the involvement of FN in the mechanism of carcinogenesis in CRC could be agreed upon, since there is a statistically significant association between the presence of FN and CRC. However, in order to determine that this bacterium is the true origin of CRC, the causality criteria, primarily temporality, direction and association, must be met[75]. In this case, the temporality criterion, by which the effect must be preceded by the cause, could not be demonstrated. Most studies that have investigated the association between FN and CRC have been retrospective, meaning it could not be known whether the bacterium was present before the tumour developed or was acquired later; more evidence, then, is needed to confirm this causality.

Multiple studies, including those by Mima et al.[28], Flanagan et al.[18] and Tahara et al.[20], which are notable for their weight in the meta-analysis, confirmed that there is a relationship between FN and CRC in comparison of healthy tissue from Controls-1 and CRC Cases. However, this association must be clarified for reasons including the fact that, at present, no prospective studies have found the presence of the bacterium to precede the onset of cancer. It is now known that the aetiopathogenic substrate corresponds to the activation of a chronic inflammatory state resulting from adhesion of the bacterium through a mechanism of intestinal dysbiosis. This entire process leads to an initiation of pathways associated with colorectal carcinogenesis, notably the Wnt/beta-catenin pathway, whose dysregulation causes failures in cell growth and tumour progression[76]. In-depth examination of the activation of these proinflammatory pathways and the mechanisms by which they occur will open up multiple possibilities for diagnosis and treatment in one of the highest incidence cancers today, with substantial benefits in the course of this disease. Our meta-analysis found FN to be more abundant in colorectal cancer tissue samples compared to healthy tissue with an OR of 4.558 and a 95% confidence interval of 3.312−6.272, confirming the significant association between the presence of FN in the tissue samples with the development of CRC. However, the meaning of this relationship must be clarified with prospective studies that confirm the temporality of the association. Other meta-analysis such as Hussan et al.'s[68] have also pointed to this need.

In addition, recent studies such as Yu et al.'s[70] have described the role of FN in chemoresistance in patients with CRC, indicating that an increased amount of FN in tumour tissue is linked to a higher rate of chemoresistance. For this reason, it would be useful to conduct further studies on survival time in patients in relation to FN levels in tumour tissue, since this could signify the existence of more therapeutic targets in this disease such as bacteria-eradicating treatment.

In the case of the comparison between the Controls-2 (colorectal adenomas) and the CRC Cases, it is important to note that not the all articles used samples of the same types of colorectal adenomas; a wide variety of histological types falling under the label of "colorectal adenoma" were studied. Furthermore, at present, fewer studies have linked the presence of FN to colorectal adenomas. The scarcity of articles, coupled with the lack of uniform criteria for sample selection, indicates that this association, as well as the question of whether FN contributes less to an adenoma forming and more to it becoming malignant, should be studied in greater depth in the future.

Multiple articles, including but not limited to Mima et al.[28], Yu et al.[5] and Lee et al.[46], have found a relationship between CRC survival and the presence or absence of FN, though no studies have found FN eradication to lead to a better prognosis in cancer treatment or an improvement in chemotherapy. In addition, no studies have linked good dental hygiene to CRC. FN is a type of bacteria that colonises the oral cavity, and therefore the relationship between FN amounts in the oral cavity and in the colon mucosa could be examined.

The main limitation of this systematic review was that it included studies published in MEDLINE, accessed via PubMed, and not in other databases or doctoral dissertations on the topic. Hence we assume that published information, however limited, might have been missed; on the other hand, such information could be presumed to be of little significance. Regarding the language limitation, only studies published in English or Spanish were included, although most of the journals indexed in MEDLINE were published in English, and only two studies were excluded for this reason. Even the association of higher rates of FN in CRC samples does not imply a causality that could point to a preventive strategy. It could stem from facilitation by tumour tissue of subsequent FN infection.

Conclusions

Based on the results of this systematic review and meta-analysis, we found that there is an association between the presence of FN and CRC. Lastly, to arrive at a definitive conclusion, further comparative studies must be conducted in sufficient numbers of patients using a combination of multiple microbiological techniques for individual subjects and samples, with simultaneous analysis of neoplastic tissue and healthy tissue by means of standardised techniques with suitable sensitivity.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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