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Fusobacterium nucleatum Abundance in Tumor Tissue and Stool Samples as a Prognostic Marker in Patients with Colorectal Carcinoma: A Review

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Abstract

Fusobacterium nucleatum is an oral anaerobe that turns up, unexpectedly and repeatedly, inside colorectal tumours. Whether measuring it is clinically useful depends on where it is measured, and the tissue and stool literatures have been answering different questions while using the same organism as their subject.

Objectives: To review the published evidence on *F. nucleatum* abundance in colorectal tumour tissue and in stool: what each has been shown to predict, how the two compartments differ in what they can detect, how reproducible the underlying measurements are, and what mechanism has been established.

Material and Methods: Narrative review of discovery studies, cohort analyses, diagnostic accuracy studies, meta-analyses and mechanistic work indexed in PubMed and the major oncology, gastroenterology and microbiology journals. Studies were eligible if they reported *F. nucleatum* abundance or presence in colorectal tissue or stool with a defined comparator, outcomes stratified by that measure, or mechanism bearing on a clinical observation. No new patient data were generated; every frequency, accuracy estimate and effect size quoted is one published by the original investigators.

Results: Over-representation of *F. nucleatum* in tumour relative to adjacent normal tissue was first reported in 2012 by two groups independently. A systematic review of 45 articles found substantially higher odds of the organism being present in colorectal tumour tissue than in control tissue, with a pooled odds ratio of 10.06, and an association between tumour positivity and worse overall survival, with a pooled hazard ratio of 1.87 across five studies which rose to 2.25 and lost all heterogeneity when the largest study was excluded. In stool, faecal *F. nucleatum* alone distinguished colorectal cancer from controls with 80.2 per cent sensitivity and 80.7 per cent specificity, rising to 92.8 per cent sensitivity when combined with faecal immunochemical testing and three further bacterial markers. Faecal abundance was 132 times higher in cancer than in controls but only 3.8 times higher in advanced adenoma. Mechanistically, the organism promotes chemoresistance through Toll-like receptor signalling and autophagy activation, and persists in matched liver metastases.

Conclusion: Tissue abundance carries a reproducible but moderate prognostic signal whose pooled estimate rests on a small number of studies with substantial heterogeneity. Stool abundance performs respectably for detecting established cancer and poorly for the precursor lesions that screening exists to find. Neither measurement is yet ready to alter management, and the more immediately actionable finding is mechanistic rather than prognostic.

Abbreviations: CRC – Colorectal Cancer, Fn – *Fusobacterium nucleatum*, FIT – Faecal Immunochemical Test, qPCR – Quantitative Polymerase Chain Reaction, ddPCR – Droplet Digital Polymerase Chain Reaction, MSI – Microsatellite Instability, dMMR – Deficient Mismatch Repair, CIMP – CpG Island Methylator Phenotype, OS – Overall Survival, HR – Hazard Ratio, OR – Odds Ratio, TLR – Toll-Like Receptor.

Keywords: *Fusobacterium nucleatum*; Colorectal Carcinoma; Gut Microbiome; Faecal Biomarkers; Chemoresistance

Introduction

In 2012 two groups reported, independently and in the same issue of the same journal, that *Fusobacterium nucleatum* sequences were markedly over-represented in colorectal carcinoma relative to matched normal tissue [1,2]. The finding was unexpected. *F. nucleatum* is a Gram-negative anaerobe associated with the oral

cavity and with periodontitis, and it is a rare constituent of the normal faecal microbiota [1,3]. The oral origin is not incidental to the epidemiology: periodontal disease and tooth loss have been associated with colorectal cancer risk in prospective cohort data [32].

What followed was a decade of work establishing that the association is real, that it is not uniform across tumours, and that it has functional consequences. The organism is enriched in proximal

tumours and in those showing deficient mismatch repair or microsatellite instability, the CpG island methylator phenotype, and BRAF mutation [4,5,6,7]. It adheres to host epithelium through the FadA adhesin, modulating E-cadherin and beta-catenin signalling [8], and localises to tumour tissue through recognition of host Gal-GalNAc by the fusobacterial lectin Fap2 [9].

Two clinical questions follow, and they are not the same question. The first is prognostic: does the amount of *F. nucleatum* in a resected or biopsied tumour predict how the patient will do. The second is diagnostic: can the organism be measured in stool to detect colorectal neoplasia non-invasively. These have generated largely separate literatures, and the distinction between them is often blurred in summary [10,11].

There is also a reproducibility question that this field has confronted more directly than most. An independent replication of the original prevalence finding failed to detect *F. nucleatum* in most samples and did not reproduce the statistically significant difference between tumour and adjacent normal tissue [12]. That result does not overturn the body of work that followed, but it does bear on how much weight any single abundance measurement can carry.

The aim of this review is to separate the tissue and stool strands, report what each has actually demonstrated, and set out where the measurement is and is not ready for use.

Material and Methods

Search strategy: PubMed and the archives of the major oncology, gastroenterology and microbiology journals were searched for studies of *Fusobacterium nucleatum* in colorectal neoplasia. Search terms combined *Fusobacterium nucleatum* with colorectal cancer, colorectal adenoma, tumour tissue, stool, faecal, prognosis, overall survival, chemoresistance, microsatellite instability and faecal immunochemical test. Reference lists of retrieved reviews and meta-analyses were screened by hand for further primary sources.

Eligibility: Studies were included if they reported *F. nucleatum* abundance or presence in colorectal tissue or stool against a defined comparator; clinical outcomes stratified by that measure; diagnostic accuracy for colorectal neoplasia; or mechanistic findings that bear directly on a clinical observation. Meta-analyses were included where they pooled patient-level or study-level estimates. Purely preclinical work is cited only where it explains a clinical result.

Data extraction and handling: Design, sample size, specimen type, detection method, threshold for positivity and reported effect estimates with their confidence intervals were extracted as published. Detection method is reported alongside every abundance figure, because quantitative polymerase chain reaction, droplet digital polymerase chain reaction, fluorescence in situ hybridisation and metagenomic sequencing do not yield interchangeable results. No pooling or re-analysis was performed and no patient-level data were obtained. Figures 1 to 3 and Tables 1 and 2 reproduce published values only. This is a narrative synthesis and was not registered as a systematic review.

Results

Presence in tissue and its association with outcome: The most comprehensive synthesis identified 45 relevant articles published to January 2019. Pooling six studies, the odds of *F. nucleatum* being present in colorectal tissue from patients with cancer, relative to control tissue, gave an odds ratio of 10.06. Pooling five studies reporting survival, tumour positivity was associated with worse overall survival, with a hazard ratio of 1.87 (95% CI 1.12-3.11) and substantial heterogeneity at $I^2 = 60.6$ per cent [10].

That heterogeneity is informative in itself. Excluding the largest contributing study, which analysed tissue from a large prospective cohort [13], the pooled hazard ratio rose to 2.25 (95% CI 1.50-3.37) and heterogeneity fell to zero (Figure 1) [10]. In other words, the four smaller studies agree closely with one another and disagree with the largest one, which is the pattern expected when smaller studies report larger effects. The same review found no significant association with disease-free survival [10].

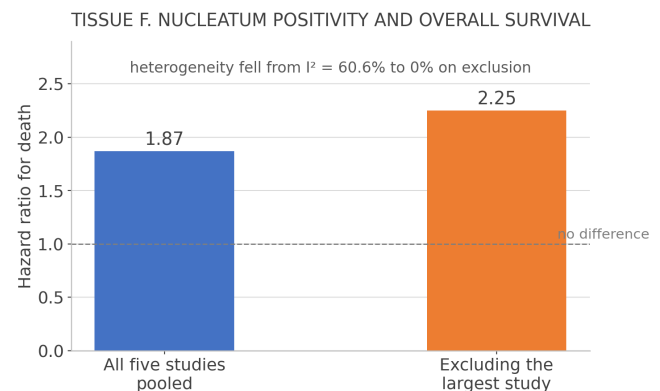


Figure 1: Pooled hazard ratio for overall survival by tumour *F. nucleatum* positivity, with and without the largest contributing study [10]

Which tumours carry it: The organism is not distributed at random. Its presence is more common in tumours arising in the proximal colon [4,14], in tumours with deficient mismatch repair or microsatellite instability [5,6], in those exhibiting the CpG island methylator phenotype [6,15], and in those carrying somatic BRAF mutations [5,6]. Invasive *F. nucleatum* has been proposed to act through the serrated neoplasia pathway in proximal colon cancer [14]. Mucosal microbial composition shifts measurably across the stages of colorectal carcinogenesis [31], and distinct microbiome patterns associate with the consensus molecular subtypes of colorectal cancer [16].

This clustering matters for interpreting the prognostic data. Proximal location, mismatch repair deficiency and BRAF mutation each carry their own prognostic weight, so an unadjusted association between *F. nucleatum* and survival may be reporting the company the organism keeps rather than an independent effect. The organism also shapes the immune environment, with reported associations between abundance and T cell density in colorectal carcinoma [17,18], and it has been linked with diminished adaptive immunity within the tumour [19].

Study	Sample	Assay	Principal reported finding
Castellarin et al. 2012 [1]	Tissue	RNA-seq	F. nucleatum over-represented in tumour against normal
Kostic et al. 2012 [2]	Tissue	NGS	Independent confirmation in the same issue
Mima et al. 2016 [13]	Tissue	qPCR	Largest cohort linking tissue abundance to prognosis
Yu et al. 2017 [20]	Tissue	NGS	Abundance higher in patients recurring after chemotherapy
Wong et al. 2017 [21]	Stool	qPCR	Faecal abundance 132-fold higher in cancer than controls
Liang et al. 2017 [22]	Stool	qPCR	Sensitivity 80.2%, rising to 92.8% with FIT and three markers
Gethings-Behncke et al. 2020 [10]	Both	Pooled	45 articles; pooled OS hazard ratio 1.87

Table 1: Principal studies reviewed, with the material examined and the finding as reported by the original investigators

The stool literature answers a different question: Faecal measurement has been evaluated for detection rather than prognosis. In a study of faecal bacterial markers, *F. nucleatum* alone distinguished colorectal cancer from controls with 80.2 per cent sensitivity and 80.7 per cent specificity. Combining it with faecal immunochemical testing and three further bacterial markers raised sensitivity to 92.8 per cent (Figure 2) [22]. A companion study reported that quantifying faecal *Fusobacterium* improved the performance of faecal immunochemical testing for advanced colorectal neoplasia [21].

The limitation appears when the target shifts from cancer to its precursors. Relative faecal abundance in the cancer group was 132 times that of controls, but only 3.8 times higher in the advanced adenoma group (Figure 3) [21]. Pooled analyses across the faecal literature show the same asymmetry, with respectable accuracy for carcinoma and poor discrimination for adenoma [11]. Since the purpose of population screening is to find and remove adenomas before they become cancer, that is the wrong way round.

Method also matters. Faecal quantification has been performed by quantitative polymerase chain reaction [21,22], by droplet digital polymerase chain reaction [23], by highly sensitive stool DNA testing [24], and by isothermal amplification targeting the *FadA* gene [25], and metagenomic profiling of the faecal microbiome has been proposed as a broader route to non-invasive markers [26]. A third compartment has also been examined: serum antibody levels against *F. nucleatum* have been assessed for diagnostic use, though with weaker performance than either tissue or stool measurement [33]. These do not produce interchangeable numbers, and cross-study comparison without reference to method is not meaningful [11,27].

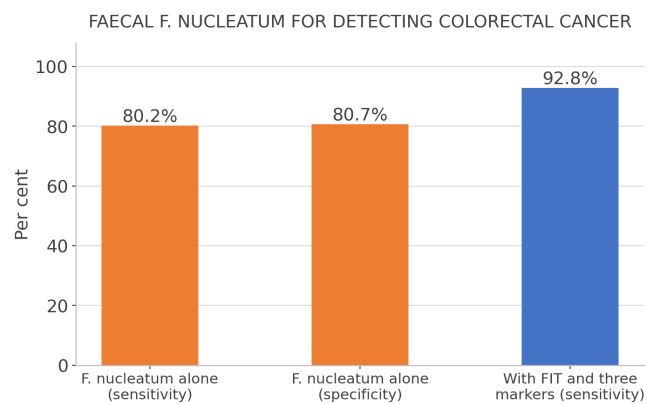


Figure 2: Reported accuracy of faecal *F. nucleatum* alone and in combination with faecal immunochemical testing and further bacterial markers [22]

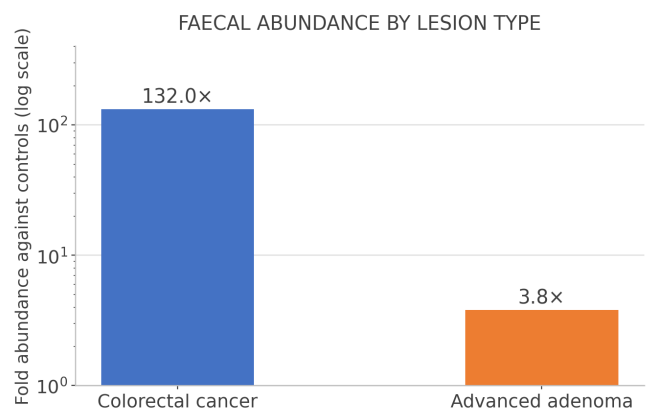


Figure 3: Relative faecal abundance of *F. nucleatum* against controls, in cancer and in advanced adenoma [21]

The mechanistic finding is the strongest one: Independent of prognosis or detection, the most clinically consequential result concerns chemotherapy. *F. nucleatum* was found to be abundant in colorectal tumour tissue from patients who recurred after chemotherapy, and functional work demonstrated that the organism promotes resistance to oxaliplatin and fluorouracil. The mechanism runs through Toll-like receptor 4 and MYD88 innate immune signalling and the loss of specific microRNAs, miR-18a* and miR-4802, activating the autophagy pathway; adding an autophagy inhibitor abolished the induced resistance in preclinical models [20,28].

The organism also persists. Analysis of matched primary tumours and liver metastases showed that the same bacteria present in the primary were present in the metastasis, and that the association could be modified by antibiotic treatment in xenograft models [29]. Together these findings reframe *F. nucleatum* from a passive marker of tumour biology into a candidate therapeutic target, which is a stronger claim than anything the prognostic data support [30].

Compartment	Question it answers	Best reported performance	Principal limitation
Tumour tissue	Prognosis after diagnosis	Pooled OS hazard ratio 1.87 [10]	Five studies only; confounded by site and MSI [5,10]
Stool	Detection of existing cancer	Sensitivity 80.2%, 92.8% with FIT [22]	Poor discrimination of adenoma [11,21]
Tumour tissue	Response to chemotherapy	Abundance higher in recurrent disease [20]	No prospective validation as a predictive marker [20]

Table 2: What each compartment has been shown to measure, and the limitation reported for it

Discussion

Three conclusions follow, and they differ in strength. The weakest is the prognostic one. The pooled association between tumour *F. nucleatum* and overall survival is real but modest, rests on five studies, and shows the small-study pattern that ordinarily invites caution: excluding the largest contributing cohort raises the effect and eliminates heterogeneity entirely [10,13]. Added to that, the organism clusters with proximal location, mismatch repair deficiency and BRAF mutation, each independently prognostic, so an unadjusted association is difficult to interpret [5,6,14].

The diagnostic conclusion is stronger but points away from the application that would matter most. Faecal *F. nucleatum* detects established colorectal cancer at accuracy comparable to existing non-invasive tests and adds to faecal immunochemical testing when combined with it [21,22]. It does not detect adenomas well, and a screening test that finds cancers but misses their precursors delivers much less than one that does the reverse [11].

The strongest conclusion is mechanistic. The chemoresistance work establishes a causal pathway, identifies an intervention point in autophagy, and is supported by the demonstration that the organism travels with the tumour to distant sites [20,29]. That is a more solid foundation for clinical development than anything the abundance-as-marker literature provides.

Reproducibility and measurement

This field deserves credit for something uncommon: an independent, pre-registered attempt to replicate its founding observation. That replication detected *F. nucleatum* in 25 per cent of carcinomas, 15 per cent of adjacent normal tissue and none of the matched control tissue, and the difference between tumour and adjacent normal did not reach statistical significance in the samples where the organism was detectable [12]. The authors attributed the divergence largely to an inability to detect the organism in most samples at all.

The practical reading is not that the association is spurious, since a large subsequent literature supports it [10]. It is that abundance measurements in this organism sit close to the detection limit of the assays used, which is exactly the circumstance in which method differences produce divergent results [11,23,27]. Any future clinical use will require a standardised assay and a prespecified threshold, neither of which currently exists.

Study limitations

The limitations are those of the underlying evidence. The survival meta-analysis pooled five studies with high heterogeneity and used study-level rather than patient-level data, so adjustment for tumour site, mismatch repair status and stage could not be performed consistently [10]. Detection methods vary widely across both the

tissue and stool literatures and are not calibrated against one another [11,23,27]. Faecal studies are predominantly case-control in design, comparing patients with diagnosed cancer against healthy controls, which inflates apparent accuracy relative to a screening population [11,22]. Several of the mechanistic findings rest on cell line and xenograft work, with human correlative data that are observational [20,29].

Two gaps deserve naming. No prospective study has tested whether measuring *F. nucleatum* changes any management decision or any patient outcome, which is the standard a prognostic marker must eventually meet. And no study has evaluated faecal *F. nucleatum* in an unselected screening cohort with colonoscopy as the reference standard, which is the design that would establish whether the adenoma limitation is fatal to its use [11,21].

Conclusion

Fusobacterium nucleatum is genuinely enriched in colorectal tumours relative to normal tissue, and the association with worse overall survival is reproducible in direction while remaining modest in size and dependent on a handful of studies. In stool it detects established cancer respectably and precursor adenomas poorly, which limits its screening value. What is best established is neither prognostic nor diagnostic but mechanistic: the organism promotes resistance to standard chemotherapy through a defined pathway and persists in metastatic deposits. Until assays are standardised and thresholds prespecified, abundance is a research measurement rather than a clinical one, and the most promising translation is as a therapeutic target rather than as a marker.

Acknowledgements

The evidence assembled here was generated elsewhere, by the many groups whose cohort studies, sequencing work and replication attempts we have summarised.

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Conflict of Interest

The authors have nothing to declare as a competing interest.

Ethics Statement

Because this is a synthesis of work already in the literature, no fresh patient data were compiled and nobody was enrolled as a participant, so ethical approval did not apply.

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