SY-05 Joint Session Infectious Diseases Pathology / Molecular Pathology: Cancer and the microbiota

**FUSOBACTERIUM AND COLORECTAL CANCER: fact or myth**

Paolo Nuciforo MD PhD - Principal Investigator - Molecular Oncology Group
We are our microbes...

Microbiome
the entire habitat, including the microorganisms, their genomes, and surrounding environmental conditions

Microbiota
the collection of microorganisms in a specified location or sample

1.3 bacterial cells/1 human cell

1000 bacterial species -> 2000 genes x specie -> 2,000,000 bacterial genes

Functions of the human microbiome: our second genome

- Functions of the human intestinal microbiome include:
  - Digestion of complex carbohydrates (extraction of energy from food)
  - Modulation of the immune system
  - Vitamin synthesis
  - Lipid metabolism
  - Control of blood glucose levels
  - Brain-gut axis mediation

Microbes influence our healthy status

Kinross et al, Genomica medicine 2011
The Integrative Human Microbiome Project

>42 Tb of multi-omic data!
(http://ihmpdcc.org)
Cancer and microbes

Many of the most common cancers are at least partly attributable to infection.

Percentage of new cancer cases caused by infection and total number of new cases

Liver
77% of 750,000

Stomach
90% of 870,000 Cases

Cervix
Nearly 100% of 530,000

CANCER-CAUSING PATHOGENS

HELICOBACTER PYLORI
HEPATITIS B VIRUS
HEPATITIS C VIRUS
HUMAN PAPILLOMA VIRUS

Adapted from the American Cancer Society’s Cancer Atlas.
**Helicobacter pylori** and gastric cancer

- Nobel Price “for their discovery of the HP and its role in gastritis and peptic ulcer disease.”
- Recognized as type I carcinogen by WHO in 1994.
- Most common known etiologic agent of infection-related cancers, which represent 5.5% of the global cancer burden.
- H. Pylori treatment reduces by 50% the incidence of metachronous gastric cancer.  
- “Screen and treat” strategies not yet applied in regular practice.

Bacteria and the aetiology of cancer of the large bowel

VIVIENNE ARIES, J. S. CROWTHER, B. S. DRASAR, M. J. HILL, AND R. E. O. WILLIAMS

From the Bacteriology Department, Wright-Floming Institute, St Mary's Hospital Medical School, London

Cancer of the large bowel shows marked variations in geographical distribution (Doll, 1967; Doll, Furse, and Waterhouse, 1966; Davis, Knottten, and Wilson, 1965) and, with the exception of Japan, the disease is more prevalent in developed than in underdeveloped countries. The reason for this variation is not known but epidemiological evidence suggests that environmental factors may be involved. It is claimed that immigrants from areas with a low incidence of cancer of the large bowel tend to show the same high incidence of this cancer as the local population (Haenszel and Dawson, 1965; Buell and Dunn, 1965). Changes in dietary habits may be especially important (Wynnd and Shigematsu, 1967; Buell and Dunn, 1965) and diet is known to affect the nature and distribution of bacteria in the faeces (Hoffmann, 1964; Dubos, 1965).

Among the important metabolic activities of intestinal bacteria is the degradation of bile salts (Hill and Drasar, 1968). It seems possible that some of the bacteria in the bowel could convert bile salts, or steroids in the diet, into carcinogenic, Haddow (1958) has reviewed the ways in which it is possible, in the laboratory, to convert deoxycholate into 2-hydroxymethylcholastërol, a potent carcinogen. We have, therefore, compared the bacterial flora of the faeces from people in England, an area with a high incidence of cancer of the large bowel, with that from people in Uganda, where the incidence is low. We have also compared the abilities of English and Ugandan strains of faecal bacteria to degrade bile salts and have examined the products of bile degradation in English and Ugandan faeces.

MATERIALS AND METHODS

Samples of freshly voided faeces from 48 healthy Ugandan adults living in and around Kampala and from 40 healthy English adults living in London were examined. Specimens were preserved for transport and storage at a 10% suspension in most infusion broth containing 10% glycerol from solid carbon dioxide (Drasar, Shiner, and McDardle, 1969); the bacteria have been found to survive well under these conditions. Specimens were cultured by the methods described previously (Drasar, 1967) with minor modifications. Approximately equal numbers of English and Ugandan specimens were examined on each day of testing in order to compensate for minor fluctuations in culture media, incubation temperature, and operational techniques. Methods for investigating the degradation of bile salts are described elsewhere (Hill and Drasar, 1968; Aries, Crowther, Drasar, and Hill, 1969).

RESULTS AND DISCUSSION

Our findings are summarised in the Table. The same

<table>
<thead>
<tr>
<th>TABLE</th>
<th>BACTERIAL COUNTS OF FAECES FROM 40 ENGLISH AND 48 UGANDAN ADULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organism</td>
<td>English</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>9.8 ± 0.6</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>9.9 ± 0.9</td>
</tr>
<tr>
<td>Aerobic streptococci</td>
<td>7.0 ± 0.8</td>
</tr>
<tr>
<td>Enterococci</td>
<td>5.7 ± 1.3</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>6.0 ± 1.6</td>
</tr>
<tr>
<td>Yeasts</td>
<td>1.3 ± 1.8</td>
</tr>
<tr>
<td>Enterobacteria</td>
<td>7.5 ± 1.2</td>
</tr>
<tr>
<td>Clostridia</td>
<td>4.4 ± 1.8</td>
</tr>
<tr>
<td>Veillonella</td>
<td>4.4 ± 2.1</td>
</tr>
<tr>
<td>Filamentous fungi</td>
<td>1.4 ± 1.2</td>
</tr>
</tbody>
</table>

^t^p^t^-value from both the student t-test and the x^2^ test.

Aries et al 1969 Gut: 10. 334-335

First published report suggesting importance of microbiota in bowel cancer (St. Mary’s Hospital, London)
MICROBIOTA AND COLORECTAL CANCER (...in 2019)

How do oral bacteria make colorectal cancer more aggressive?
Medical News Today - 4 mar. 2019
... shown that around a third of people who develop colorectal cancer also have the bacterium, which has the name Fusobacterium nucleatum.

Colon Cancer Deadlier When Oral Bacterium Completes Feedback...
Genetic Engineering & Biotechnology News - 4 mar. 2019
How a common oral bacteria makes colon cancer more deadly
Medical Xpress - 4 mar. 2019

The unusual bacterial link between colorectal cancer and tooth decay
New Atlas - 4 mar. 2019
Fusobacterium nucleatum, or F. nucleatum, is a common bacteria that... has found F. nucleatum enhances colorectal cancer growth in about...

Cancer colorectal : une bactérie buccale pourrait accélérer sa ...
Pourtour Docteur ? - 5 mar. 2019
Cancer colorectal : une bactérie buccale pourrait accélérer sa ... déterminé comment une bactérie buccale appelée Fusobacterium nucleatum, ...

La clave para entender el cáncer de mama también está en los ...
Vozpúblic - 27 ago. 2019
La incidencia del cáncer de mama ha aumentado en todo el mundo a ... de Fusobacterium nucleatum se correlaciona con el cáncer de colon y ...

Bacteria din gură care accelerează creșterea celulelor canceroase
Adăvârlul - 11 mar. 2019
Un nou studiu arată că o bacterie din cavitatea bucală crește riscul de cancer colorectal. Fusobacterium nucleatum, o bactérie întâmpinată în ...

Une bactérie responsable des caries rendrait le cancer du côlon plus ...
Medisite - 5 mar. 2019
Les personnes qui souffrent d'un cancer du côlon devraient elles ... la bactérie Fusobacterium nucleatum (F. nucleatum), responsable aussi des caries dentaires.
... En suivant l'attitude de la fameuse bactérie F. nucleatum sur des ... mots-clés : Cancer du colon, Caries dentaires, Cancer du colon causes.

Les bacterias intestinales que vinculan la dieta con el cáncer colorrectal...
Clarín.com - 2 feb. 2017
Un reciente estudio demostró la fuerte participación de la bacteria Fusobacterium nucleatum en el desarrollo del cáncer de colon. Cáncer de ...

Backed by OrbiMed and J&J to build precision bacteria-killing tool ...
Endpoints News - 20 feb. 2019
Additionally, they are cooking up treatments for cancer and liver disease ... and Fusobacterium nucleatum to gastric and colorectal cancers has ...

Un simple antibiotico frena en ratones un cancer que mata a 800.000 ...
EL PAIS - 24 nov. 2017
La Iniciativa del cáncer de mama ha aumentado en todo el mundo a ... de Fusobacterium nucleatum se correlaciona con el cáncer de colon y ...

Las bacterias del cáncer colorectal favorecen la metástasis
Completo - ABC.es - 23 nov. 2017
Ver todos

Quel est le lien entre la carie et le cancer? Les scientifiques répondent
Sputnik France - 9 mar. 2019
... des souris, ils ont constaté que l'activité de l'annexe empêchait la bactérie fusobacterium nucleatum d'activer le développement du cancer.
**Fusobacterium nucleatum**

**Taxonomy**
- **Domain**: Bacteria
- **Phylum**: Fusobacteria
- **Class**: Fusobacteria
- **Order**: Fusobacteriia
- **Family**: Fusobacteriaceae
- **Genus**: Fusobacterium
- **Species**: Fusobacterium nucleatum
- **Subspecies**: Nucleatum, animalis, fusiforme, polymorphum, and vincentii

---

**Diseases F. nucleatum associated with.**

<table>
<thead>
<tr>
<th>Diseases</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Infections</td>
<td></td>
</tr>
<tr>
<td>Chronic Periodontitis</td>
<td>[6-11]</td>
</tr>
<tr>
<td>Aggressive Periodontitis</td>
<td>[8,11,12]</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>[8,11,13-15]</td>
</tr>
<tr>
<td>Endodontic Infections</td>
<td>[16-22]</td>
</tr>
<tr>
<td>Adverse Pregnancy Outcomes</td>
<td></td>
</tr>
<tr>
<td>Chorioamnionitis</td>
<td>[18,20-42]</td>
</tr>
<tr>
<td>Preterm Birth</td>
<td>[17,61-46,66,67]</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>[18]</td>
</tr>
<tr>
<td>Neonatal Sepsis</td>
<td>[17]</td>
</tr>
<tr>
<td>Pre eclampsia</td>
<td>[43]</td>
</tr>
<tr>
<td>GI Disorders</td>
<td></td>
</tr>
<tr>
<td>Colorectal Cancer</td>
<td>[16,212-213]</td>
</tr>
<tr>
<td>Inflammatory Bowel Disease</td>
<td>[16,27]</td>
</tr>
<tr>
<td>Appendicitis</td>
<td>[17-801]</td>
</tr>
<tr>
<td>Other Infections</td>
<td></td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td></td>
</tr>
<tr>
<td>Central Nervous System</td>
<td>[16]</td>
</tr>
<tr>
<td>Listeria’s Syndrome</td>
<td>[81,92]</td>
</tr>
<tr>
<td>Other Respiratory tract Infections</td>
<td></td>
</tr>
<tr>
<td>Organ Aneurysms</td>
<td></td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td></td>
</tr>
<tr>
<td>Alzheimer's</td>
<td></td>
</tr>
</tbody>
</table>

---

Han, Curr Opin Microbiol (2015)
Genomics, colorectal carcinoma, and *Fusobacterium nucleatum*

**Kostic et al, Genome Res (2012)**

**Castellarin et al, Genome Res (2012)**


**Associations between *Fusobacterium* and CRC**

- **Cohorts**
  - >6000 tumors
  - >50 studies
  - >1400 healthy controls

- **Specimen type**
  - 47% Fresh frozen tumor
  - 35% Stool
  - 18% FFPE tumor

- **Detection Method**
  - 16S rRNA 42%
  - FN qPCR 47%
  - Metagenomics 9%
  - FISH 2%

- **Sequencing Depth**
  - 70% Species
  - 16% Genus
  - 8% Phylum
  - 6% Subspecies

---

*Fusobacterium* genus is enriched in CRC as compared to adjacent normal/healthy tissue controls.

*Fusobacterium nucleatum* is the most prevalent species of *Fusobacterium* in CRC.

The prevalence of *Fusobacterium nucleatum* varies between 8.6% and 87.1%.

Specimen types and detection methodologies used may account for highly heterogenous prevalence results.

Conflicting data re. *Fusobacterium nucleatum* and patients characteristics associations were found.

**Updated in August 2019**
Associations between *Fusobacterium* and CRC

Cohort specific noises, specimen types, and detection methodologies used may distort the structure of microbial dysbiosis in CRC and lead to inconsistent *Fusobacterium* prevalence results among studies.
Associations between \textit{Fusobacterium} and dietary characteristics

Higher long-term prudent dietary pattern scores were associated with a lower risk of F nucleatum–positive colorectal cancers but not F nucleatum–negative cancers.

Mehta et al. JAMA Oncol (2017)

### Prudent dietary pattern:
high intake of vegetables, fruits, whole grains, and legumes

### Western dietary pattern:
red and processed meats, refined grains, and desserts

#### Table. Hazard Ratios of Incident Colorectal Cancer, Overall and by \textit{Fusobacterium} nucleatum Status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>P Value</th>
<th>Trend</th>
<th>Heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall colorectal cancer</td>
<td>Person-years</td>
<td>913 569</td>
<td>907 676</td>
<td>912 395</td>
<td>909 922</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>No. of cases (n = 1039), No. (%)</td>
<td>250 (24.3)</td>
<td>240 (23.4)</td>
<td>268 (26.3)</td>
<td>253 (24.3)</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Age-adjusted HR (95% CI)$^a$</td>
<td>1 (Reference)</td>
<td>0.93 (0.77-1.11)</td>
<td>0.90 (0.75-1.08)</td>
<td>0.79 (0.65-0.95)</td>
<td>&lt;.01</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Multivariable HR (95% CI)$^a$</td>
<td>1 (Reference)</td>
<td>0.95 (0.80-1.14)</td>
<td>0.95 (0.80-1.14)</td>
<td>0.85 (0.69-1.03)</td>
<td>&lt;.01</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>\textit{F} nucleatum-positive colorectal cancer</td>
<td>No. of cases (n = 1235), No. (%)</td>
<td>41 (34.4)</td>
<td>26 (20.8)</td>
<td>34 (27.2)</td>
<td>22 (17.6)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Age-adjusted HR (95% CI)$^a$</td>
<td>1 (Reference)</td>
<td>0.54 (0.33-0.89)</td>
<td>0.67 (0.42-1.05)</td>
<td>0.40 (0.24-0.67)</td>
<td>&lt;.001</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Multivariable HR (95% CI)$^a$</td>
<td>1 (Reference)</td>
<td>0.56 (0.34-0.93)</td>
<td>0.70 (0.44-1.10)</td>
<td>0.43 (0.25-0.72)</td>
<td>&lt;.001</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>\textit{F} nucleatum-negative colorectal cancer</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

| Western Dietary Pattern | Overall colorectal cancer | Person-years | 910 036 | 913 525 | 910 465 | 911 916 | NA | NA |
| No. of cases (n = 1039), No. (%) | 252 (24.3) | 240 (23.4) | 268 (26.3) | 253 (24.3) | NA | NA |
| Age-adjusted HR (95% CI)$^a$ | 1 (Reference) | 1.24 (1.04-1.48) | 1.23 (1.00-1.44) | 1.46 (1.28-1.62) | <.001 | NA |
| Multivariable HR (95% CI)$^a$ | 1 (Reference) | 1.19 (1.00-1.43) | 1.12 (0.92-1.36) | 1.29 (1.03-1.62) | <.05 | NA |
| \textit{F} nucleatum-positive colorectal cancer | NA | NA | NA | NA | NA | NA |
| No. of cases (n = 1235), No. (%) | 35 (26.4) | 34 (26.4) | 33 (26.4) | 32 (25.2) | NA | NA |
| Age-adjusted HR (95% CI)$^a$ | 1 (Reference) | 1.42 (1.04-2.40) | 1.59 (0.94-2.69) | 1.92 (1.32-2.99) | <.01 | NA |
| Multivariable HR (95% CI)$^a$ | 1 (Reference) | 1.37 (1.01-2.21) | 1.49 (0.86-2.53) | 1.69 (1.08-2.60) | <.05 | NA |
| \textit{F} nucleatum-negative colorectal cancer | NA | NA | NA | NA | NA | .23 |
| No. of cases (n = 1039), No. (%) | 219 (24.3) | 242 (27.1) | 210 (23.3) | 223 (24.3) | NA | NA |
| Age-adjusted HR (95% CI)$^a$ | 1 (Reference) | 1.25 (1.03-1.50) | 1.16 (0.95-1.42) | 1.42 (1.13-1.78) | <.004 | NA |
| Multivariable HR (95% CI)$^a$ | 1 (Reference) | 1.20 (0.99-1.44) | 1.08 (0.80-1.43) | 1.25 (0.99-1.58) | <.01 | NA |
Associations between *Fusobacterium* and CRC anatomic location and molecular features


Drewes et al. npj Biofilms and Microbiomes (2017)

Purcell et al. Scientific Reports 2017
Associations between *Fusobacterium* and prognosis

![Log-rank test for trend](image1)

![Log-rank test for trend](image2)

**Number at risk**

<table>
<thead>
<tr>
<th>Year</th>
<th>Negative</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>935</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>2</td>
<td>782</td>
<td>55</td>
<td>48</td>
</tr>
<tr>
<td>4</td>
<td>670</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>6</td>
<td>546</td>
<td>34</td>
<td>36</td>
</tr>
<tr>
<td>8</td>
<td>446</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>10</td>
<td>350</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>

The amount of *Fusobacterium nucleatum* DNA in colorectal cancer tissue and patient mortality

<table>
<thead>
<tr>
<th>The amount of <em>Fusobacterium nucleatum</em> DNA</th>
<th>Colorectal cancer-specific mortality</th>
<th>Overall mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariable HR (95% CI)</td>
<td>Multivariable stage-stratified HR (95% CI)*</td>
</tr>
<tr>
<td>Negative</td>
<td>935</td>
<td>265</td>
</tr>
<tr>
<td>Low</td>
<td>67</td>
<td>24</td>
</tr>
<tr>
<td>High</td>
<td>67</td>
<td>26</td>
</tr>
</tbody>
</table>

p for trend*:

| Negative | 0.023 |
| Low      | 0.020 |
| High     | 0.50  |

*Confidence interval; HR, hazard ratio.

*Adjusted for sex, age, year of diagnosis, family history, tumour location, microsatellite instability, CpG island phenotype, KRAS, BRAF, PIK3CA mutations.

Underlying mechanism of *Fusobacterium nucleatum* pathogenesis in colorectal cancer
Adding causality to correlative data

• Can *Fusobacterium* travel to distant metastasis?

• *Fusobacterium* and antibiotics sensitivity in patient derived xenografts?
Fusobacterium’s culture and persistence in distant metastasis

Fusobacterium+ by qPCR
9/11 PT -> 8/9 cultured
7/11 DM -> 2/7 cultured

RNAseq showed similar relative abundance of Fusobacterium and overall dominant microbiome between PT and DM of the same patient Fuso+ but not in Fuso- tumors

Fusobacterium plus Culture
Spatial distribution of *Fusobacterium nucleatum* in primary/metastatic positive tumors

Fusobacterium’s sensitivity to antibiotic treatment

**A**

![Graph A](image)

**B**

![Graph B](image)

**C**

![Graph C](image)
Antibiotics inhibited the clinical benefit of immune checkpoint inhibitors in patients with advanced cancer (n=175, NSCLC, mRCC, mUC)

Antibiotics (ATB) taken 2 months before and/or 1 month after the 1st administration of anti-PD-1/PD-L1 abs (Routy et al. Science 2017)
Kill the enemy:

1. Target FN virulent genes (Fap2)
2. Fn vaccination
3. Microbial ecosystem replacement therapy
4. Phage-based therapeutics (Bacteriophage FNU1 is capable of disrupting Fusobacterium nucleatum biofilms)

OPtimistic

Opportunity To Investigate the Microbiome's Impact on Science and Treatment In Colorectal Cancer