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AIRC

BLOOD BACTERIAL DNA IN RELATION TO ADENOMA AND COLORECTAL CANCER RISK

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AIM OF THE STUDY

To **test** the hypothesis of bacterial translocation from gastrointestinal tract to the bloodstream in intestinal adenoma (IA) and/or colorectal cancer (CRC) using an innovative metagenomic approach on blood samples

To **identify** potential microbial biomarkers in blood for CRC early detection

BACKGROUND

Inflammation and the immunity system are inextricably linked to all phases of CRC development.

Gut inflammation leads to loss of epithelial barrier function, possibly driving to the bacterial translocation from the gastrointestinal tract to the bloodstream.

An overrepresentation of bacterial cells in blood has been proposed as an indicator or a predictor of IA and/or CRC

No previous study investigated this issue

METHODS

DATA COLLECTION (2017-2019)

A **case-control study** including **100** incident cases of histological confirmed colorectal cancer **(CRC)**, **100** intestinal adenomas **(IAs), 100** healthy **controls**

Healthy controls and IAs are matched with CRC cases (1:1 by age, ± 5 years and sex) and grouped into triplets.

Settlement of 2 Recruitment Centres in Milan (Italy).

Participants 20-85 years old, enrolled among outpatients or inpatients with a scheduled colonoscopy appointment.

Exclusion Criteria: previous cancer, inflammatory bowel disease, liver or kidney failure, antibiotic/chemotherapy, blood transfusion.

A face to face interview carried out by trained interviewers through a valid food frequency questionnaire collecting dietary and life-style habits, socio-demographic information and anthropometric measures.

Blood samples collected by a nurse before colonoscopy (using the same venous access used for colonoscopy preparation).

ANALYSIS

DNA extraction, qPCR quantification and taxonomic profiling by Illumina MiSeq sequencing of the 16S rRNA gene copies from blood samples. Samples were analyzed at the same time (with the same reagents batches and manipulator), to optimize the signal to noise ratio and to reduce technical variability.

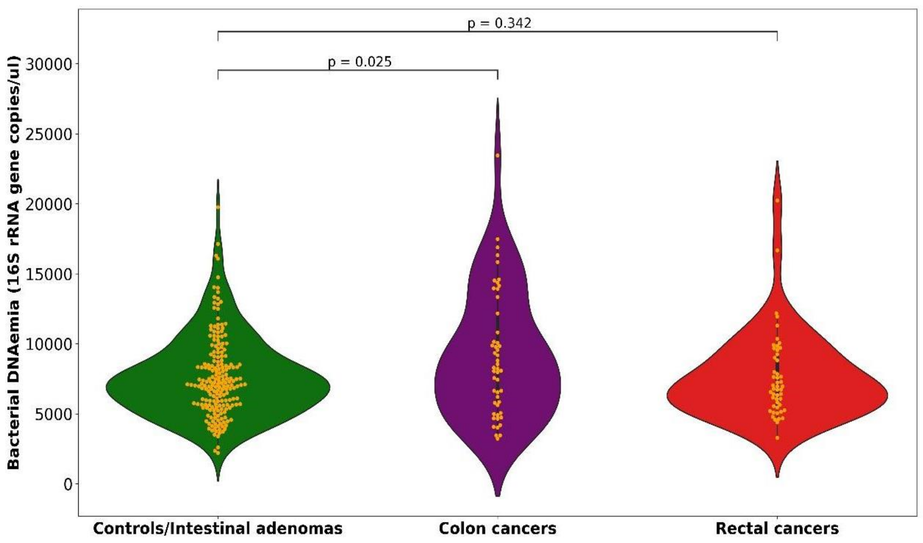
Bacterial load was analysed using multiple conditional logistic regression. Differences in terms of abundance of bacteria between groups were estimated through analysis based on negative binomial distribution normalization. Random Forest was applied to predict the group assignment. Mann-Whitney tests were used to determine differences in terms of alpha-diversity. For beta-diversity, Permutational Multivariate Analysis of Variance Suing Distance Matrices (PERMANOVA) based on the UniFrac distances, and Principal Coordinates Analysis (PCoA) were applied.

Study population

Characteristic	Controls	IA	CRC
Sex			
Male	62 (62%)	62 (62%)	62 (62%)
Female	38 (38%)	38 (38%)	38 (38%)
Age group (yr)			
<50	7 (7%)	4 (4%)	10 (10%)
50-59	23 (23%)	20 (20%)	19 (19%)
60-69	26 (26%)	36 (36%)	29 (29%)
70-79	33 (33%)	29 (29%)	31 (42%)
≥80	11 (11%)	11 (11%)	11 (11%)
χ^2 test, p=0.76			
Centre			
Niguarda	65 (65%)	65 (65%)	65 (65%)
Policlinico	35 (35%)	35 (35%)	35 (35%)

RESULTS

Figure. Distribution of 16S rRNA gene copies per µl of whole blood among controls/intestinal adenomas, colon and rectal cancers.



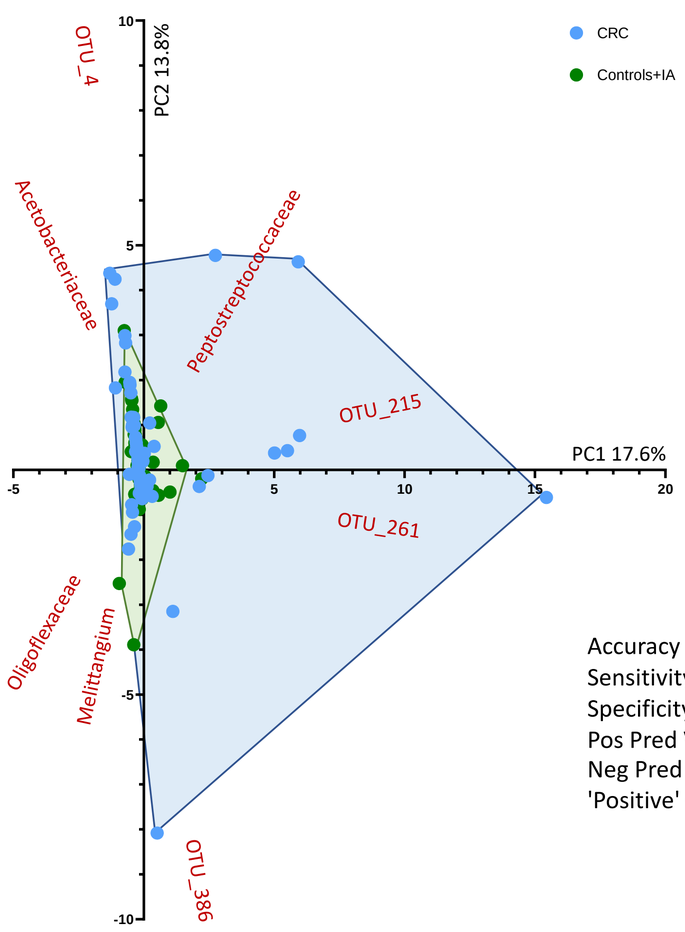
	Quintile of number of copies[a]		
	1-3[c]	4	5
Upper cutpoints[a] (n copies/µl)	7618	9707	-
Controls/IA n (%)	120 (60%)	40 (20%)	40 (20%)
Colon cancer n (%)	21 (42%)	10 (20%)	19 (38%)
OR[b]	1[c]	1.96	**2.62**
(95% CI)		(0.75-5.08)	**(1.22-5.65)**
Rectal cancer n (%)	31 (62%)	10 (20%)	9 (18%)
OR[b]	1[c]	0.73	0.81
(95% CI)		(0.29-1.84)	(0.32-2.03)
p value for interaction between colon and rectum			0.021

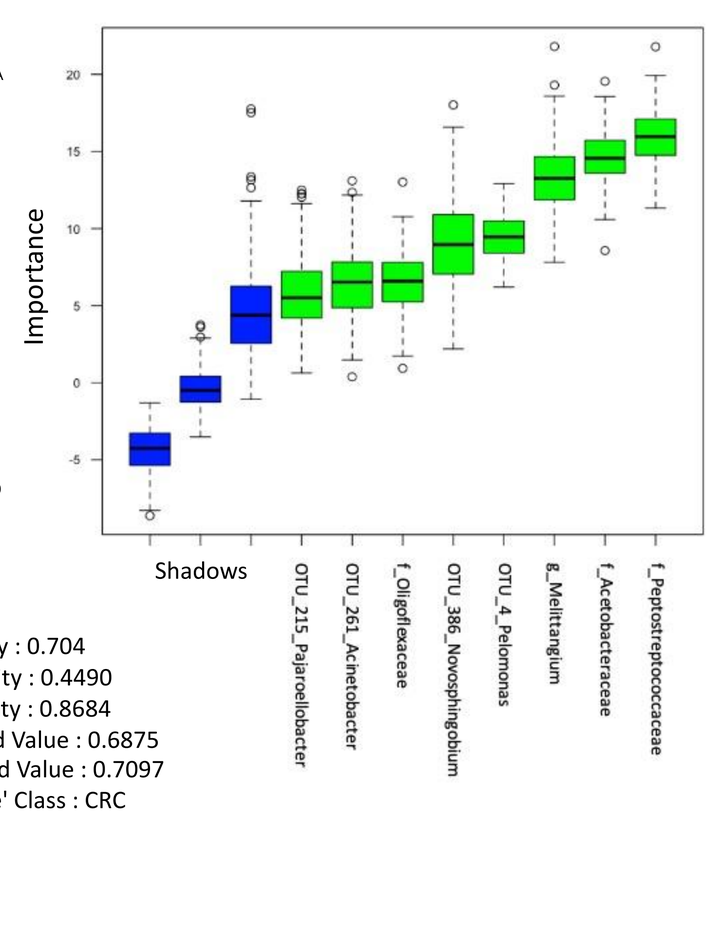
[a]computed among control and IA distribution; [b]Odds ratios (OR) estimated from logistic regression models, conditioned on age, sex, and study centre; [c]Reference category.

CONCLUSIONS

Colon cancer patients had a higher DNA bacterial load and a different bacterial profiling as compared to healthy subjects, IA and rectal cancers, indicating a higher passage of bacteria from gastrointestinal tract to bloodstream. Further studies are needed to confirm this result and exploit it to conceive new non-invasive techniques for an early diagnosis of CRC.

Figure. Machine Learning (Random Forest): CRC vs controlsi/IA





Accuracy : 0.704
Sensitivity : 0.4490
Specificity : 0.8684
Pos Pred Value : 0.6875
Neg Pred Value : 0.7097
'Positive' Class : CRC

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