Double-blind, randomized, controlled clinical trial to evaluate the in vivo effect of traditional bread compared with modern bread in patients with quiescent Ulcerative Colitis (UC)

Protocol Code: RTC-2017-CU

Promotor: Consortium Retos - Col·laboració Grant: ELIAS FORNER, S.L. and Digestive Disease and Microbiota Group of IDIBGI

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Centre where biological samples will be processed:

Intestinal Diseases and Microbiota Group

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Duration: 20 months, Starts in December 2019

1. Background

Currently, numerous studies have shown that the intestinal microbiota is a key factor in the correct intestinal function but also in the regulation of the immune response in humans (1). Alterations in the intestinal microbiota are a determining factor in the development of serious chronic diseases. An example of this could be the case of Ulcerative Colitis (UC), where studies carried out with faecal or mucosa-associated microbial communities have shown that patients with UC have an altered microbiota that differs from those individuals who do not have the disease (2). These alterations in the microbiota cause a constant antigenic stimulation, aggravated by genetic defects of the host, which will lead to a continuous activation of its immune system and end in chronic intestinal damage (3). Thus, the intestinal microbiota is considered a direct marker of the state of intestinal inflammation (4).

Recent studies from our group show that patients with inflammatory bowel disease, such as UC, have a decrease in bacterial diversity and proportions in their gut microbiota, specifically with a significant decrease in the abundance of *Faecalibacterium prausnitzii* and an increase in *Escherichia coli*. These changes in the composition of the intestinal microbiota are called dysbiosis and several studies have confirmed that these specific bacteria are good indicators of dysbiosis in inflammatory bowel disease (2).

To try to reverse this situation of dysbiosis, one possibility would be the use of prebiotics, which have the potential to stimulate the growth of beneficial intestinal bacteria selectively (5); such as *F. prausnitzii*, which is a butyrate producer apart from being one of the three most abundant species in a healthy human intestine (6). The importance of the abundance of butyrate-producing species in the intestinal microbiota lies in the anti-inflammatory activity of this compound (7)

Among the wide variety of prebiotic foods, in this case we are interested in a common food in the Mediterranean diet, in order not to excessively change the diet of patients. Bread is a potentially prebiotic food that has a significant percentage of insoluble dietary fibre. Dietary fibre induces the production of short-chain fatty acids, basically acetic, propionic and butyric, which are important nutrients for epithelial cells and intestinal microbiota. Therefore, it has the ability to promote the growth of butyrate-producing bacteria in the intestinal microbiota (3). A quality bread, made with compact and long-fermented sourdough, could have beneficial prebiotic effects both for the general population and for those patients with UC in remission, but who continue to present symptoms compatible with Irritable Bowel Syndrome. If the prebiotic effect of these breads were confirmed, they could even be recommended by health agents to the general population as prevention or relief of symptoms caused by intestinal inflammation.

Thus, from the perspective of public health and as a personal interest of our group in collaboration with ELIAS FORNER S.L., we believe it would be interesting to better understand the impact of bread on the microbiota-inflammation relationship of the intestinal mucosa; and if the intake of breads made in a traditional way, with sourdough and long fermentation, could be an advantage for our study population.

2. HIPOTHESIS AND OBJECTIVES

2.1 Hipothesis

The intake of bread produced following traditional breadmaking techniques is beneficial for people with quiescent Ulcerative Colitis (UC), but who present symptoms compatible with Irritable Bowel Syndrome (IBS), through the modulation of its intestinal microbiota.

2.2 Objectius

To compare in vivo the influence of 2 types of bread, one long-fermented and one short-fermented, on the clinic and intestinal microbiota of patients with quiescent Ulcerative Colitis (UC), with symptoms compatible with IBS (Rome IV Criteria).

Specific objectives:

- To determine changes in the overall gut microbiota composition in patients with quiescent UC, before (V0) and after 8 weeks (VF) of the intake of traditional bread.
- To determine the correlation between the changes observed in the composition of the intestinal microbiota in V0 and VF and the clinical condition of the patient with quiescent UC.
- Assess the impact of traditional bread versus modern bread on improving the clinical response of patients with quiescent UC.

3. MATERIALS AND METHODS

3.1. Type of study

Randomized, double-blind, prospective clinical trial

3.2. Inclusion criteria

- > 18 years of age
- Patients diagnosed with ulcerative colitis according to established clinical and histological criteria as common clinical practice
- Remission of ulcerative colitis defined as a total Mayo score ≤2 and faecal calprotectin values under 250 ng/g
- Moderate-to-severe IBS-like symptomatology defined by Rome IV criteria and IBS Symptom Severity Score (IBS-SSS) > 175

3.3. Exclusion criteria

- Presence of flare-up of UC (Index de Mayo ≥2, Calprotectin ≥ 250 ug/gr)
- Coeliac disease, colectomy, or intestinal resection
- Antibiotic intake, prebiotic or probiotic treatment within 3 months before the study
- Any malignancy, pregnancy, or breastfeeding
- Intake of medication potentially influencing gastrointestinal function
- Disability to give informed consent

3.4. Interventions

The study intervention will consist of the daily consumption of 200g of one of the two types of study bread, a traditional bread (treatment) and a modern bread (control), for 8 weeks by the participants.

Traditional bread has the following composition: whole-grain wheat flour (stone-ground), water, freeze-dried Paris yeast (<1%), compact sourdough and salt. Fermentation is of the acetic-lactic type for more than 40 hours.

On the other hand, modern bread has the following ingredients: refined flour, water, salt, yeast. With a fast fermentation fully controlled in a maximum of 2 hours.

Elias Forner SL will supply the bread needed for participants' daily consumption at the centres, where patients will collect it 1 or 2 times a week during the 8 weeks of the intervention. All breads will be prepared with identical presentation by Elias Forner SL. All members of the research team will be blinded to intervention allocation.

No dietary or lifestyle intervention (diet, physical activity, etc.) will be carried out in any of the two experimental groups.

3.5. Randomization

Participants will be assigned to one of the two interventions using a random sequence separated by sex. It will remain double-blind throughout the study. In the case of withdrawal or abandonment of a participant, the new subject will be assigned a consecutive number and the intervention he will receive will be assigned using the same random sequence separated by sex as the rest of the participants.

3.6. Variables

a) Demographic variables

- **Age** expressed in years (y)
- **Sex** Male (M)/ Female (F)
- **Weight** expressed in IMC
- **Ethnic**: Caucasian/ African/ Asiatic/ American
- **Tobacco**: YES / NO / EX-Smoker
- **Age of UC diagnosis** expressed in years (y)
- **Familiar Antecedents** (YES/NO) which kind of disease?

b) Clinical variables:

- **Mayo Clinical Score** (*Clinical colitis activity index*) (Schroeder KW *et al.* *NEngl J Med* 1987; 317: 1625-9). Punctuation range: 0-9 punts.

- **Rome IV Criteria** (Lacy BE, Mearin F, Chang L, *et al*. Bowel disorders. Gastroenterology 2016;150:1393-407).

- **IBS- SSS** (*Irritable Bowel Syndrome – Symptom Severety Score*) (Almansa C *et al*. Rev Esp Enferm Dig 2011 Dec;103 (12):612-8.

- **HADS** (*Hospital Anxiety and Depression Scale*) (Johnston M, *et al*. J Psychosom Res 2000;48:579-84.9)

- **Escala de Likert:** Subjective valuation of improvement of symptoms after the intervention

- **Adherence to Mediterranean Diet Questionnaire** (Estruch R et al NEJM 2013).

c) Analytical parameters:

Haemoglobin: blood haemoglobin concentration (g/dl).

Albumin: blood albumin concentration (g/dl).

Triglycerides: blood triglycerides concentration (mg/dl).

Cholesterol: blood cholesterol concentration (mg/dl).

C-reactive protein: blood C-reactive protein concentration (mg/dl).

Faecal calprotectin: faecal calprotectin concentration (µg/g).

d) Variables Microbiològiques

The microbiological profile of the microbiota of these patients will be analysed through high-throughput sequencing before and after the intervention.

3.7. Methods

The proposed study will include 96 patients with UC in remission (Mayo Index ≤2 and Calprotectin ≤250ug/gr), over 18 years of age, who have signed informed consent, who meet Rome IV criteria and present a moderate-severe severity index will be included (IBS-SSS >175). These patients will be randomized to one of the two intervention arms (N=48 per group):

- Treatment group: traditional bread (200g daily).
- Control group: modern bread (200g daily).

For both intervention groups stool and blood samples will be collected before starting the intervention (Visit 0 - Basal), and then after 8 weeks (Final Visit). The monitoring period will be 8 weeks. In addition, we will collect demographic data (age, sex, smoking habit, etc.), clinical data (Mayo index, Rome IV, IBS-SSS, CVE 20, HADS, PCR Likert scale, VSG, albumin, hemoglobin, fecal calprotectin, etc.), and microbiological variables.

The intervention will last 8 weeks and the following visits will be made during the study:

1. Consent- Pre-Randomization Visit

The patient will be evaluated by a doctor from the Gastroenterology service at the University Hospital Dr. Josep Trueta who will explain the study and evaluate the inclusion and exclusion criteria. Participants will be able to ask all the questions they have about it before signing the written consent. If the potential volunteer wishes to participate, after reading the informative documentation and receiving the relevant explanations, he must carefully read the informed consent and if he agrees with it he must sign it.

The patient will be provided with the 2 containers necessary to collect the stool sample at home for the following visits

2. Baseline Visit (V_0)

The patient will come to the visit with the stool sample corresponding to the basal Visit (V0) before the intervention.

The demographic variables (age, sex, weight, ethnicity, smoking habit, family history, etc.) will be collected, the different established questionnaires and indexes will be completed (Rome IV, Mayo, IBS-SSS, CVE-20, HADS), and a blood test will be performed (PCR, Calprotectin, blood count, etc.).

The patient will be randomized and the bread will be provided according to the randomization result to intervention A or B. The patient will be told that he can collect the following portions of bread at the centre 1 or 2 times a week, for the next 8 weeks of intervention.

3. End of study Visit (EOS; W8)

The patient will come to the visit, 8 weeks later, with the stool sample corresponding to the final visit (VF) of the study.
The various established questionnaires and indexes will be performed (Rome IV, Mayo, IBS-SSS, CVE-20, HADS, Likert scale), and a blood test will be performed (PCR, Calprotectin, blood count, etc.).
The evolution of the patient's symptoms will be assessed after 8 weeks of the intervention, and a new medical assessment will be carried out.

3.8. Microbiological analysis

The stool samples collected at V0 and VF will be stored at -80ºC at the Biomedical Research Institute of Girona (IdIBGi). Before the microbiological analyses, the genomic DNA of the 16S RNA gene will be extracted using the commercial NucleoSpin® Soil Kit (Machery-Nagel GmbH & Co., Germany). The DNA concentration will be determined with the Qubit® BR kit (Invitrogen). The microbiological profile of the microbiota of these patients will be analyzed through mass sequencing by the subcontracted company StarSEQ. The region corresponding to the V3-V4 variable region of the 16S rRNA gene will be sequenced using specific primers (e.g., 515F/806R, [Caporaso et al Nat Method 2010]) and Illumina HiSeq200 technology through paired-end reads (generating 300bp sequences).

3.9. Statistical analysis

All statistical analyses will be performed using R software (2.14.0, http://www.r-project.org/). Pearson's chi-squared test will be used to determine whether there is a statistically significant difference between demographics and questionnaire data with more than one category. Normality for numerical data will be assessed through the Shapiro-Wilk test. For data with normal distribution, a paired t-test will be used to determine whether there is a statistically significant difference, whereas, for data with non-normal distribution, the Wilcoxon signed-rank exact test will be used. Significance levels will be established for $p \leq 0.05$.

Alpha diversity indices together with beta-diversity matrices will be computed by groups of intervention. Normality will be assessed through the Shapiro-Wilk test, and statistical differences in each treatment group will be analysed using Welch two sample t-test or paired t-test for data with normal distribution and Wilcoxon rank sum exact test or Exact Wilcoxon Signed-Rank test for data with non-normal distribution. For beta diversity matrices, unweighted Unifrac, weighted Unifrac and Bray-Curtis distances will be computed and plotted through principal coordinate analyses (PCoA).

4. ETHICAL CONSIDERATIONS

The research will respect the fundamental principles of the Declaration of Helsinki, in the Council of Europe convention on human rights and Biomedicine, as well as the requirements established by Spanish legislation in the field of biomedical research, data protection of a personal nature and bioethics.

Complying at all times with the general data protection regulation (No. 2016/679) of the European Parliament and the Council of April 27, 2016.

5. CHRONOGRAPH

	2020												2021											
Tasks	**G**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**G**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**
CEIC approval																								
Recruitment																								
Faecal DNA purification and quantificaiton																								
Illumina sequencing																								
Data analysisand final report																								

6. PRESSUPOST

This essay is funded by the Retos - Collaboration grant RTC-2017-6467-2 from the Ministry of Economy, Industry and Competitiveness. The Digestive Research Group budget for the clinical trial is 32.300€, which is broken down as follows:

PARTIDA	**2020**	**2021**	**TOTAL**
Fungible	3.000	7.150	10.150
NucleoSpin® Soil Kit DNA extraction kit	2.450	1.000	3.450
Quantification DNA kit Qubit® BR (Invitrogen)	1.000	200	1.200
Subcontracting of Services			
Clinical Analytics of the Clinical Laboratory Service Hospital Dr. Josep Trueta	3000	571,2	3.571,2
Faecal calprotectin - LabCo	4000	928,80	4.928,8
High throughput sequencing (StarSEQ)		12.500	12.500
		TOTAL	**32.300**

ELÍAS FORNER S.L., as promotor of the study and with the Retos-Collaboration Grant obtained, will assume the expenses generated by the supply of bread and the expenses incurred by the patients (food and travel) as a result of being part of the study.

7. References

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