Golimumab effect in the modulation of gut microbiota in Ulcerative Colitis. Pilot Study.

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Collaborating centers: Centers of Catalonia, that will wish to participate after the approval of this project

Duration: 26 months, Starts in May 2016

1. BACKGROUND

Recently, a consistent body of evidence indicates that the gut microbiota ability to modulate and direct the host immune response is considered one of the pivotal factors that modulate the delicate balance between health and disease in digestive diseases (1). In the case of inflammatory bowel disease (IBD), the gut microbiota has a central role in maintaining the microbiological homeostasis of the patient (2, 3). Recent studies describe the existence of different patterns of intestinal microbiota from patients with IBD and healthy individuals (4, 5). The alteration of the microbiological pattern not associated with disease is known as dysbiosis and is directly related to the degree of inflammation of the intestinal mucosa, and, thus, the clinic status: a greater dysbiosis, greater clinical correlation with disease state, so that the gut microbiota is a direct marker of the state of colonic inflammation (6).
The state of dysbiosis is measured through different microbiological indices of the total population of bacteria in the colon. Changes in rates of these indices are the parameters that are measured to characterize the gut microbiota of the patients. In the case of ulcerative colitis (UC) studies have shown that intestinal microbiota is a central factor in maintaining the balance between deep remission and the presence of a flare-up (7, 8). Despite advances, in-depth knowledge of how the gut microbiota interacts with the intestinal mucosa and alters the intestinal barrier and the molecular/genetic mechanism is still unknown. Some genes and associated cofactors have been identified but the exact mechanism of interaction has not yet been described. The data indicate that, presumably, the mechanism of regulation is a multifactorial process (9, 10).
In this way, a deep understanding of gut microbiota and its interactions with the host and the colon will be of great interest, in order to shape the future of IBD treatment, especially of UC. Thus, IBD patients' intestinal microbiota could be either a therapeutic target itself (fecal transplantation) or be consider as an adjustable adjuvant with immunomodulators (11, 12).
The group of anti-TNFα drugs are the most recently incorporated in the therapeutic arsenal of UC, in some cases changing the natural curse of the disease. However, it is still unknown how these drugs modulate the intestinal microbiota and how they interfere with it, although, probably,

they develop a role. Recent results from studies by our group, indicate that on entering clinical remission, gut microbiota is modified to patterns less related with dysbiosis (13, 14,15, 16). Given the increasing importance of the use of anti-TNFα drugs, it is of great interest to discriminate between the patterns associated with dysbiosis and those related with healthy mucosa, and how they are modified as a result of the use of anti-TNFα drugs. In this way, previous results of our group analyzing the changes of intestinal microbiota associated with Adalimumab anti-TNFα drug treatment have shown that during the progression of the patient into remission, the mucosal dysbiosis pattern changes (17). On the other hand, our group has also observed that after drug treatment failure, the gut microbiota returns to a pattern closer to dysbiosis. For that reason, gut microbiota could be considered as an excellent indicator of the real drug effectiveness in the patient.

Regarding Golimumab, a recently introduced anti-TNFα drug therapy in UC, it is still unknown how it is able to modulate the intestinal microbiota to remission-related patterns, since to date there are not available studies about the relationship between Golimumab and this phenomenon.

The use of prebiotics and probiotics has shown some effectiveness as adjuvants in the treatment of UC (18). For that reason, further characterization of the gut microbiota patterns is very important to develop new strategies for adjuvant ability to modulate it, especially in patients receiving anti-TNFα drugs and do not achieve complete remission. Similarly to recent studies, we suggest that the modulation of gut microbiota could optimize the response outcomes in patients treated with Golimumab.

In conclusion, based on current trends in the literature, we suggest that modulation of the intestinal microbiota and the characterization of remission-related patterns, will have a huge impact on the management of patients with UC. Moreover, the modulation of gut microbiota together with the anti-TNFα drug effectiveness could be the most promising field in the management of inflammatory bowel disease.

2. HYPOTHESIS AND OBJECTIVES

2.1 Hypothesis

1. Intestinal microbiota profile change according UC activity.

2. The Intestinal microbiota profile correlated to clinical remission is represented by stable intestinal microbiota biodiversity.

3. Determination of Intestinal Microbiota is a useful tool to measure Golimimab efectiveness in patients naïve to anti-TNF treatment and patients recurrent to anti-TNF treatment.

2.2 Objectives

1. To correlate clinical remission under Golimumab treatment with stable intestinal microbiota profile biodiversity

2. To characterize the pattern of intestinal microbiota associated with the use of Golimumab and temporal dynamics of microbial change.

3. Assess the effect of golimumab on the degree of colic dysbiosis in the treatment of CU naive to Golimumab.

3. MATERIAL AND METHODS

3.1 Type of study/design

Multicentrer transversal pilot Study

3.2 Study population

The proposed study will include 15 UC anti-TNFα naïve patients from Hospital Universitari Dr. Josep Trueta, Hospital Santa Caterina and Hospitals collaborators. We will consider remission when patients have an endoscopic Mayo score ≤1, and activity index score, Mayo= 0 points.

3.2.1 Inclusion criteria

- > 18 years
- Signed the informed consent
- Anti-TNFα naïve
- Screening for opportunistic infections

3.2.2 Exclusion criteria

- Active tuberculosis or another chronic infections
- Antibiotic treatment prior 1 month
- Probiotics & Prebiotics
- Gestation and lactation
- Heart disfunction
- Colectomy

3.3 Interventions

3.3.1 Treatments

GOLIMUMAB induction with 200mg at week 0, and 100mg at week 2. Under 70kg, the follow up treatment will be 50mg/month, and 100mg/month in patients over 70kg, as clinical practice.

3.4 Variables

3.4.1 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?
- **Localization** Left (L)/ Righ (R) / extended (E)

3.4.2 Clinical variables

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

- **CRP** *(C-reactive protein):* blood C-reactive protein concentration (mg/L)

- **Faecal calprotectin**: stool sample expressed in concentration of calprotectin in ug for g of feces (ug/g)

-**Standard Analysis:**

Hemoglobin: blood hemoglobin concentration (g / dl)
Platelets: blood platelets concentration ($x10^3$/ ul)
Leucocytes: blood leucocytes concentration ($x10^3$/ ul)
Albumin: blood albumin concentration (g / dl)
Creatinine levels: blood creatinine concentration (mg/ dl)

3.4.3 Endoscopic variables

- **Mayo** (Endoscopic score of Ulcerative Colitis) (Schroeder KW et al. 1987). Mayo Endoscopic Score is based only on Endoscopic Findings. Mayo Score range from 0 to 3.

Note: We will send the image in a centralization digital platform, where we will reevaluate the Mayo score with 2 independent professionals, in order to have an objective score.

3.4.4 Microbiological variables

- Operational Taxonomic Units (OTUs)
- Abundance and bacterial load.

3.5 Methods

The proposed study will include 15 UC patients over 18 years, that have signed the informed consent, subjected to treatment with anti-TNFα by clinical practice and who have made screening for opportunistic infections. Patients will be anti-TNFα naïve patients.

Stool samples will be collected before starting Anti-TNF treatment (M0), and then every 3 months (M1, M2, M3 and M4) to complete the study. The monitoring period shall be one year. We will be also collected at M0, M1, M2, M3 & M4 demographic variables (age, sex, tobacco, age of diagnosis, localization,…), clinical data (Mayo clinical score, CRP, albumin, hemoglobin, creatinine, leucocytes, platelets…), microbiological variables, also a 4 calprotectin determinations (at baseline, at week12, at 6 months and at the end of the study as a clinical practice) and Mayo endoscopic index (at baseline and at the end of the monitoring period as a clinical practice). Follow-up visits will also take place within routine clinical practice. For better follow-up the evolution of the patient we propose an additional test as a clinical practice, but optional, a rectosigmoidoscopy at week 12 after starting Anti-TNF treatment.

We will consider remission when patients have an endoscopic Mayo score ≤1, and activity index score, Mayo clinical score =0 points.

Moreover, depending on the evolution of the patient, additional tests shall also be performed as routine clinical protocol during the monitoring period.

3.5.1 Sample processing

DNA Extraction:
Before microbiological analyses, genomic DNA of 16s RNA gene will be extracted using NucleoSpin® Soil Kit (Machery-Nagel GmbH & Co., Germany). DNA concentration will be determined with Qubit® BR (Invitrogen) Kit.

Bacterial 16S rRNA Gene Amplification by Pyrosequencing

For pyrosequencing purposes, the 16S rRNA gene was partially amplified from extracted genomic DNA using the universal bacterial primers GC-357F 5'- CGCCCG CCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCC- CCT ACG GGA GGC AGC AG-3' (341Y357) and 907R 5'-CCG TCA ATT CCT TTG AGT TT-3' (907Y926). PCR was performed with a GeneAmp PCR System ® 2700 (AppliedBiosystems)). Then we purify the PCR product with kit Ampure (Agencourt AMPure, Beckman Coulter Inc), and quantify the PCR products with Qubit® BR (Invitrogen) Kit. The pyrosequencing will be performed with a 454 Life Genome Sciences Sequencer FLX.

Sequence Editing and Analysis

High-quality consensus sequences were obtained and manually refined with the Bioedit software package. Alignments were carried out with ClustalW24 software. Consensus sequences were compared with those in GenBank and the Ribosomal Database Project by using BLASTN 2.2.10. Sequences were grouped by number of operational taxonomic units or phylotypes with the DOTUR program26 using the farthest neighbor method at a precision level of 0.01, i.e., 99% minimum similarity for any pair of sequences to belong to the same phylotype, on a distance matrix with the Jukes-Cantor correction calculated with the DNADIST program of the Phylip software package.

3.6 Statistical analysis

Statistical analysis was performed with the SPSSx version 11.0. Significance of distances between groups was checked using an analysis of variance. Pearson_s x2 test was used to compare the prevalence of genus and species.

Clinical and laboratory data will be correlated with the values of quantitative microbial indices using Receiver Operating Characteristic (ROC) curves.

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BUDGET

MATERIAL	
DNA extraction	750 €
PCR reactives	2.100 €
Pyrosequencing analysis	9.000 €
Fungible	950 €
SERVICES	
Sample transport	339 €
OTHERS	
Technical	4.800 €
Overhead IDIBGI (30%)	5096,7
Total	**23.036 €**