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A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED, SINGLE-CENTER, PILOT STUDY EVALUATING THE ROLE OF A *L. RHAMNOSUS* GG-BASED SYMBIOTIC FORMULATION IN PATIENTS WITH MILD-TO-MODERATE UC IN CLINICAL AND ENDOSCOPIC REMISSION

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Abstract – *Objective:* Intestinal dysbiosis is considered a key factor in the pathogenesis and disease progression of ulcerative colitis. Probiotics, prebiotics, and symbiotics are promising strategies for microbiota modulation and could play a role as "add-on" therapies to available drugs.

The aim of the study was to characterize the ability of a symbiotic containing Boswellia, Quercetina, Bromelina, Vit. D3, Lactobacillus Rhamnosus GG, Inulin, FOS, and Tryptophan in modulating microbiota composition, in maintaining disease remission, and in controlling the quality of life in patients with Ulcerative Colitis (UC) in clinical and endoscopic remission.

Patients and Methods: In our randomized double-blind, placebo-controlled, single-center proof-of-concept study, 26 UC patients in clinical and endoscopic remission were enrolled. One group was treated with 5-ASA + placebo, and one with the symbiotic + 5-ASA for a period of 6 months. Disease activity was assessed through the clinical and endoscopic Mayo Scores, while quality of life was evaluated using the Psychological General Well-being Index and the Gastrointestinal Symptom Rating Scale scores. 16S rRNA microbial gene sequencing was used.

Results: At the microbial analyses, the Mann-Whitney test applied to the Shannon-Wiener, Simpson, and Chao1 indices did not reveal any statistically significant differences after 6 months compared with the baseline. The PERMANOVA test did not show statistically significant dissimilarities between the two time points.
At clinical and endoscopic disease evaluation at baseline and after 6 months, no significant differences were registered between the two groups. At quality-of-life evaluation, patients treated with the symbiotic experienced a significant improvement in both the psychological/general well-being and gastrointestinal symptoms scores compared with baseline and the placebo-treated group.
Conclusions: Our proof-of-concept study demonstrates that treatment with the symbiotic formulation, in addition to 5-ASA, is effective in improving both psychological and gastrointestinal symptoms in patients with UC, while maintaining their clinical and endoscopic remission. Further investigations are underway to confirm these findings.

Keywords: IBD, Ulcerative colitis, Gut microbiota, Quality of life, Symbiotic, *Lactobacillus Rhamnosus* GG.

INTRODUCTION

Inflammatory Bowel Disease (IBD), such as Crohn's disease (CD) and ulcerative colitis (UC), are chronic, relapsing inflammatory disorders of the gastrointestinal (GI) tract, resulting from dysregulated immune responses toward environmental factors in genetically predisposed individuals. Their incidence is constantly increasing, with a significant and dramatic impact on the quality of life of affected patients[1].

Despite the range of available treatments, significant unmet therapeutic needs remain in this field. In UC, 5-aminosalicylates (5-ASAs), corticosteroids, and biologics are primarily used to induce remission based on disease severity. One of the main challenges remains designing the best strategies to maintain remission, while minimizing significant side effects and promoting continuous improvement in quality of life[2-4]. Indeed, managing the disease involves not only controlling clinical symptoms but also improving the psychological and social aspects of the patient's life. The constant concern about unpredictable symptoms can lead to stress, anxiety, and depression, negatively affecting daily activities, personal relationships, and work productivity. Therefore, an increasing interest is developing in therapeutic approaches that consider quality of life, in addition to achieving and maintaining both clinical and endoscopic remission.

In this context, the intestinal microbiota represents a significant actor implicated in IBD pathogenesis, and consequent therapeutic experimental approaches have shown promising results, especially in UC[5]. Dysbiosis, characterized by qualitative and quantitative alterations in gut microbiota structure and function, is considered a key contributor to disease progression and relapse in patients with UC, as well as a significant modulator of gut-brain axis functions[5]. Major reports suggest that subjects with UC share specific common gut microbiota features that are correlated to the alteration of crucial functions, such as the metabolism of short-chain fatty acids (SCFAs) and amino acids, the control of oxidative stress, and the biosynthesis of toxins, that could potentially contribute to the development and progression of the disease[6,7]. Thus, enthusiasm for innovative therapeutic strategies targeting gut microbiota modulation as a potential treatment for UC, both among patients and physicians, has increased in recent years. However, this concept is still hardly translated into clinical practice, as to date, only a few microbiota-oriented approaches have shown some effectiveness in inducing or maintaining remission in patients with UC[4]. In particular, studies focused on probiotics present a mixed picture regarding their efficacy in managing mild to moderate UC, with some showing benefits while others do not demonstrate significant effects compared to standard treatments. Besides this, an increasing interest has involved the combination of probiotics with pre- and post-biotics that could play a synergistic effect with the end goal of improving therapeutic outcome.

Lactobacillus rhamnosus is one of the most extensively used probiotic in gastroenterology and is represented in the bowel of healthy individuals[8]. In this context, *Lactobacillus rhamnosus* GG strain (LGG) is known for its anti-inflammatory effects. It can modulate the immune system by promoting the production of anti-inflammatory cytokines and inhibiting pro-inflammatory ones. It is able to enhance the intestinal barrier function by increasing the production of mucins and tight junction proteins, thereby reducing gut permeability. Its combination with agents that have a direct positive effect on the gut barrier could offer a synergistic approach to managing intestinal inflammation and enhancing the integrity of the intestinal barrier.

In this study, we explored the effect of a symbiotic formulation based on LGG with Boswellia, Quercetin, Bromelain, Vitamin D3, Inulin, Fructooligosaccharides (FOS) and Tryptophan in modulating the fecal microbiota biodiversity in patients with UC in clinical and endoscopic remission. In addition to the primary objective, we aimed to address its role in maintaining clinical and endoscopic remission, and we characterized the impact on the quality of life of patients.

MATERIALS AND METHODS

Study Design and Participants

We performed a double-blind, randomized, placebo-controlled, single-center, proof-of-concept pilot study.

This study was approved by the Local Ethic Committee of Fondazione Policlinico "A. Gemelli" IRCCS (number of protocol approval: 0039306/20; date of approval release: 24-Sep-2020) and conducted in compliance with the Institutional Review Board/Human Subjects Research Committee requirements, as well as the Declaration of Helsinki and the Guidelines for Good Clinical Practice criteria. Before the study began, the enrolled patients or their legal guardians signed an informed consent form.

Formulation of Symbiotic

The composition of the symbiotic (PROLACTIS® GG plus, Omega Pharma, Italy) was: Boswellia 100 mg; Quercetin 200 mg; Bromelain 2,500 GDU/g 100 mg; Vitamin D3 50 μg (2,000 IU); LGG 2×10^9 CFU; Inulin 2,000 mg; FOS 892 mg; Tryptophan 150 mg. The symbiotic was administered orally, 1 sachet per day for 6 months.

Patients' Characteristics

Twenty-six patients were enrolled (n = 13 females, mean age: 44.5 years; range: 20-75 years) at the IBD Unit of our Digestive Disease Center. The median disease duration was 13.5 years (range, 1-37.4 years). Seven patients had a history of left-sided colitis (E2), while nineteen patients suffered from pancolitis (E3). Patients enrolled were randomly assigned to two treatment arms. No significant differences for these variables were registered between the two arms.

The first treatment arm consisted of a combination of 5-ASA 2.4 g per day and symbiotic 1 sachet per day for 6 months. The second treatment arm consisted of a combination of 5-ASA 2.4 g per day and placebo 1 sachet per day for 6 months. At the end of the treatment, patients were followed up for another 2 months.

Among the twenty-six patients enrolled, seven dropped out during the study. Of these subjects, five were lost at follow-up, while one subject dropped out due to difficulties in assuming the symbiotic formulation, and one under placebo treatment for disease relapse after 3 months from the baseline visit. Nineteen patients completed the study.

Population Inclusion and Exclusion Criteria

In this pilot study, we included subjects aged 18 years or older with a confirmed diagnosis of UC and a disease duration of at least three months, regardless of sex. At the time of enrollment, patients were required to be in remission with a Full Mayo Score (FMS) <2 with no individual subscore >1 and a rectal bleed subscore of 0. Flexible sigmoidoscopy during the screening period with a Mayo endoscopic subscore (MES) ≤1 was needed. Moreover, a history of previous mild-to-moderate active disease beyond the rectum (>15 cm from the anal verge), and a maintenance treatment with oral mesalamine (<2.5 g/day) or sulfasalazine (<3.0 g/day) on a stable dose for at least 2 weeks before randomization was required.

On the other side, exclusion criteria were: diagnosis of indeterminate colitis or Crohn's disease; colonic dysplasia or neoplasia; history of colonic or small bowel obstruction or resection (except for appendectomy); use of rectal 5-aminosalicylic acid or systemic or rectal corticosteroids within 30 days before baseline, or use of immunosuppressive agents within 12 weeks before baseline; repeated use of non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin or ibuprofen (except for prophylactic use of a stable dose of aspirin up to 325 mg/day for cardiac disease); intake of probiotics or antibiotics for any condition within 30 days before baseline; any major illness/condition or evidence of an unstable clinical condition; pregnancy or lactation.

All subjects participated in the following visits: screening, baseline/randomization, and 6-month evaluation with the cessation of the drug under investigation. After 8 months, a telephone contact was conducted. Subject participation in this study spanned approximately 10 months, which included an 8-week screening period, a 6-month treatment period, and a 2-month follow-up period. During the screening visit, demographic data were collected. At each time-point, FMS (or partial Mayo Score during the screening visit and at month 8) was evaluated. During the screening period and for 6 months afterward, flexible sigmoidoscopy was performed, and fecal samples were collected.

After completion of the screening phase, at the baseline visit, eligible patients were randomized to either mesalamine plus symbiotic formulation or mesalamine plus placebo treatment arms.

Endpoints

The primary endpoint was to characterize the effect of the symbiotic formulation in modulating the fecal microbiota biodiversity after 6 months of therapy in patients with UC in remission.

The secondary endpoints were to characterize the efficacy of the symbiotic formulation in maintaining clinical and endoscopic remission at 6 months in patients with UC in remission; to evaluate the time to relapse in the two arms; to characterize its efficacy in modulating the patients reported outcomes (PROs) at 6 and 8 months through the evaluation of the psychological status and the global gastrointestinal symptoms.

Questionnaires

Two patients reported outcomes, as the Psychological General Well-Being Index (PGWBI) was administered to assess psychological status, and the Gastrointestinal Symptom Rating Scale (GSRS) was administered to assess global gastrointestinal symptoms.

The PGWBI is a 22-item questionnaire assessing health-related quality of life. It measures the psychological and general well-being of patients. It consists of 22 items rated on a 6-point scale, which estimates well-being of patients in 6 areas: anxiety, depression, positive well-being, self-control, general health, and vitality. Each area included 3-5 items. Each item has a score from 0 to 5 (best value score). The PGWBI global score is obtained with the addition of the scores for all the areas, from 0 to 110, which represents the greatest psychological well-being status.

The GSRS is a 15-item questionnaire assessing gastrointestinal symptoms. It is used to evaluate common symptoms of gastrointestinal disorders. It consists of 15 items, rated on a 7-point scale, from the absence of discomfort to very severe discomfort. The GSRS is divided into five areas that cover the gastrointestinal system: abdominal pain, reflux syndrome, diarrhea syndrome, indigestion syndrome, and constipation syndrome. The GSRS global score is obtained with the addition of the scores for all the areas, from 0 to 90. A higher score indicates worse control of global gastrointestinal symptoms.

Library Preparation and 16S rRNA Gene Sequencing

Thirty-six fecal samples were collected to analyze gut microbiota composition and modulation throughout the study. Samples were treated by mechanical lysis using glass beads with a diameter of 425-600 µm (Sigma-Aldrich, Saint Louis, MO, USA) and thermal lysis (20 minutes, 65°C) according to the manufacturer's instructions. The QIAmp Fast DNA Stool Mini Kit (Qiagen, Germany) was used to extract bacterial DNA from each fecal sample. Next, the 16S rRNA V3-V4 variable region (~460 bp) was amplified using the primer pair 16S_F 5′-(TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG)-3′ and 16S_R 5′-(GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C)-3′, as described in the MiSeq rRNA Amplicon Sequencing protocol (Illumina, San Diego, CA, USA). Furthermore, the PCR reaction was prepared using the 2× KAPA Hifi HotStart Ready Mix Kit (KAPA Biosystems Inc., Wilmington, MA, USA) following the manufacturer's protocol. Subsequently, the successful amplification was verified by electrophoresis on an agarose gel (Lonza FlashGel DNA Cassette, 2.2%). DNA amplicons were purified using magnetic beads from the CleanNGS kit (CleanNA, Coenecoop 75, PH Waddinxveen, The Netherlands). A unique combination of indexed primers (Nextera® XT Index Kit v2 Set A, 96 indexes-192 samples) was incorporated into the amplicons through a second amplification step, verified by gel electrophoresis, for each of the 36 samples. For one sample, the amplification product was weak, so the PCR Indexing was repeated using indices other than the previous ones. The final library, consisting of 37 samples, was purified, quantified with the Quant-iTTM PicoGreen® dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA), and normalized to 4 nM. To generate 250 × 2-bp length paired-end reads, normalized libraries were pooled into a single sample and loaded onto the Illumina MiSeqTM platform, according to the manufacturer's specifications.

Bioinformatics Pipeline

72 fastq files, obtained by sequencing the V3-V4 16S rRNA hypervariable regions, were processed using QIIME2 v2023.2. With the q2-dada2 plugin, the Quality Check (QC) was performed by filtering the reads with a Phred Score > 20, and forward and reverse paired-end merging was applied with an overlapping of 12 nt. 2,965 Amplicon Sequence Variants (ASVs) were produced with an average length of 424.22 nt and with a minimum, maximum and average frequency of 1,884 (minimum), 69,672 (maximum), and 34,607 (average) ASVs. Taxonomic assignment was performed with the q2-greengenes2 plugin using the Greengenes v2022.2 database. The construction of the rooted phylogenetic tree was carried out using the q2-phylogeny align-to-tree-mafft-fasttree plugin. The ecological analyses of alpha and beta diversity were applied on the normalized count table with the rarefaction method, considering the minimum sample size. The univariate (Linear Discriminant Analysis Effect Size, LEfSe) and multivariate (Partial Least Square Discriminant Analysis, PLS-DA, and Principal Component Analysis, PCA) models were applied on the relative abundances normalized with the CSS (Cumulative Sum Scaling) method. Sequences present in fewer than 25% of the samples and with relative abundance <0.01 were filtered. FDR values were considered only in the univariate analysis.

RESULTS

Gut Microbiota Modulation

We first evaluated the fecal microbiota biodiversity at baseline and after 6 months of therapy in both treatment arms in order to define the role of the symbiotic preparation in modulating gut microbiota in our cohort of UC patients.

Univariate and multivariate analyses showed homogeneity between subjects on treatment and those not on treatment at baseline, as well as after 6 months of therapy. Indeed, univariate LEfSe analysis did not reveal different microbial markers between the two cohorts. The supervised PLS-DA multivariate analysis could not identify bacterial genera able to predict and characterize the two groups of treatments, with the first two principal components not able to explain the bacterial variability. The multivariate unsupervised PCA analysis showed a notable overlap between the populations, confirming the homogeneity between the two cohorts.

Moreover, the Mann-Whitney test applied to the alpha diversity indexes, Shannon-Weiner, Simpson, and Chao1 test Figure 1A-C did not reveal any statistically significant change, indicating a lack of difference in species richness between the subjects in treatment and those not in treatment. Finally, the PERMANOVA test applied on the distance matrix calculated with the Bray Curtis and Unweighted Unifrac algorithms also did not show statistically significant dissimilarities Figure 1D-E.

Overall, the ecological analysis did not show any difference in the fecal microbiota profiles between UC patients treated with the symbiotic formulation and those treated with placebo.

Clinical and Endoscopic Outcomes

Then, we evaluated the efficacy of the symbiotic formulation in maintaining clinical and endoscopic remission after 6 months of treatment (Figure 2). After treatment was stopped, we also assessed the clinical portrait after another 2 months of follow-up. At the baseline, three patients, two in the placebo cohort and one in the treatment arm, showed an FMS of 1, while all the others had an FMS of 0. After 6 months of treatment, a disease flare (FMS ≥ 2) was reported in two patients for each group (18% in the placebo group *vs*. 25% in the treatment group, p=0.7) (Figure 2A). Similar results were registered for Partial Mayo Score after 6 months of treatment and then after 2 months of follow-up, with no significant differences between groups at both time points (Figure 2B). At endoscopic evaluation at baseline, two patients in the placebo arm and one in the treatment group showed a MES of 1, while all the others had a MES of 0. After 6 months of treatment, three patients in the placebo arm and two subjects in the treatment group showed a MES of 1 (27% *vs* 25%, p=0.9). No endoscopic disease recrudescence was observed in both groups (Figure 2C). While all these analyses were performed considering the cohort of patients that completed the study, the evaluation of the time to relapse in the two arms was conducted considering also one patient in the placebo group that relapsed after 3 months from baseline and dropped out of the study. In this scenario, survival curves did not show any significant difference between groups (Figure 2D). Finally, no adverse events related to the symbiotic formulation or placebo were registered.

Overall, these data suggest that the use of this symbiotic formulation as an add-on therapy to mesalazine did not change the clinical and endoscopic course of the disease in our cohort of patients.

Patients Reported Outcomes

Finally, we aimed to assess the impact of this symbiotic formulation on specific quality-of-life scores in our patient cohort. Firstly, we considered the psychological and well-being status and how this changed after 6 months of treatment. PGBWI questionnaire was used (Figure 3A). At baseline, no significant differences emerged between groups (62.77 ± 4.8 in the placebo group *vs* 62 ± 6.5 in the treatment group, p=0.9). After 6 months of treatment, the treatment group showed a significant higher score compared to controls (83.13 ± 6.7 *vs*. 67 ± 4, p=0.04) and to baseline levels (83.13 ± 6.7 *vs*. 62 ± 6.5, p=0.04), indicating an improvement of its psychological and well-being status. No significant variations were observed for controls between baseline and 6-month time-points (Figure 3A). The treatment was then stopped, and after two months, patients were reevaluated using the same score. At this time point, no significant differences emerged between groups (66.27 ± 4.2 in the placebo group *vs*. 75.13 ± 7.5 in the treatment group, p=0.3). Although an amelioration trend was observed, no significant changes were registered in the treatment group between the baseline and the 8-month time point status (62 ± 6.5 *vs*. 75.13 ± 7.5, p=0.2). No significant variations were observed for controls between 6 and 8 months (Figure 3A).

Secondly, we administered and recorded the GSRS score to evaluate the course of global gastrointestinal symptoms throughout the treatment period and at 2 months of follow-up (Figure

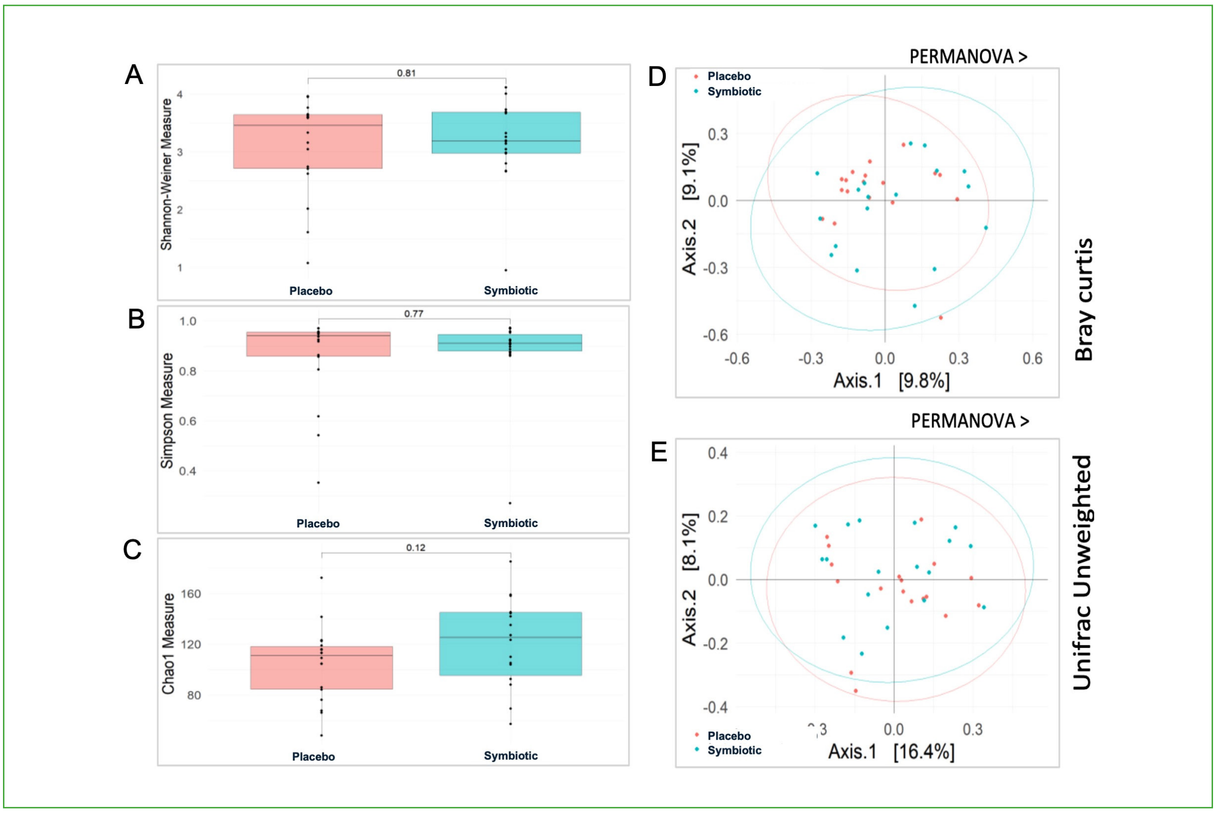

Figure 1. Gut microbiota characterization and evaluation of its modulation. **A-C,** The Mann-Withney test applied on the Shannon-Weiner, Simpson and Chao1 indices. The red box represents patients treated with placebo; the green box represents patients treated with the symbiotic. This test did not reveal any statistically significant difference between the two arms. **D, E,** The Permanova test applied on the distance matrix calculated with the Bray Curtis and Unweighted Unifrac algorithms. The red dots represent patients treated with placebo, the green dots indicate patients treated with the symbiotic. This test did not show any statistically significant difference between the two arms.

3B). At baseline, no significant differences were described between groups (25.15 ± 4.2 in the placebo group *vs* 18.46 ± 3.6 in the treatment group, *p*=0.24). After 6 months of treatment, the treatment group showed a significant lower score compared to controls (7.13 ± 2.4 *vs*. 19.27 ± 3.8, *p*=0.02) and to baseline levels (7.13 ± 2.4 *vs*. 18.46 ± 3.6, *p*=0.04), indicating an improvement of its global gastrointestinal symptoms. No significant variations were shown for controls between baseline and the 6-month time point (Figure 3B). Then, after treatment was stopped and 2 months of follow-up evaluated, although a trend was observed, no significant changes were registered between groups (19.91 ± 3.3 in the placebo group *vs*. 11 ± 2.3 in the treatment group, *p*=0.06), and in the treatment group between the baseline and the 8-month time point status (18.46 ± 3.6 *vs*. 11 ± 2.3, *p*=0.2). No significant variations were shown for controls between 6 and 8 months (Figure 3B).

Overall, these data suggest that the use of this symbiotic formulation as an add-on therapy to mesalazine improved the psychological and well-being status, as well as the overall gastrointestinal symptoms, after 6 months of treatment. This double effect on PROs was partially lost after 2 months from the end of therapy.

DISCUSSION

Finding new and effective therapeutic strategies is a global research priority for IBD. This includes not only the use of pharmacological treatments to control inflammation and maintain remission but also interventions able to enhance the quality of life for patients, which

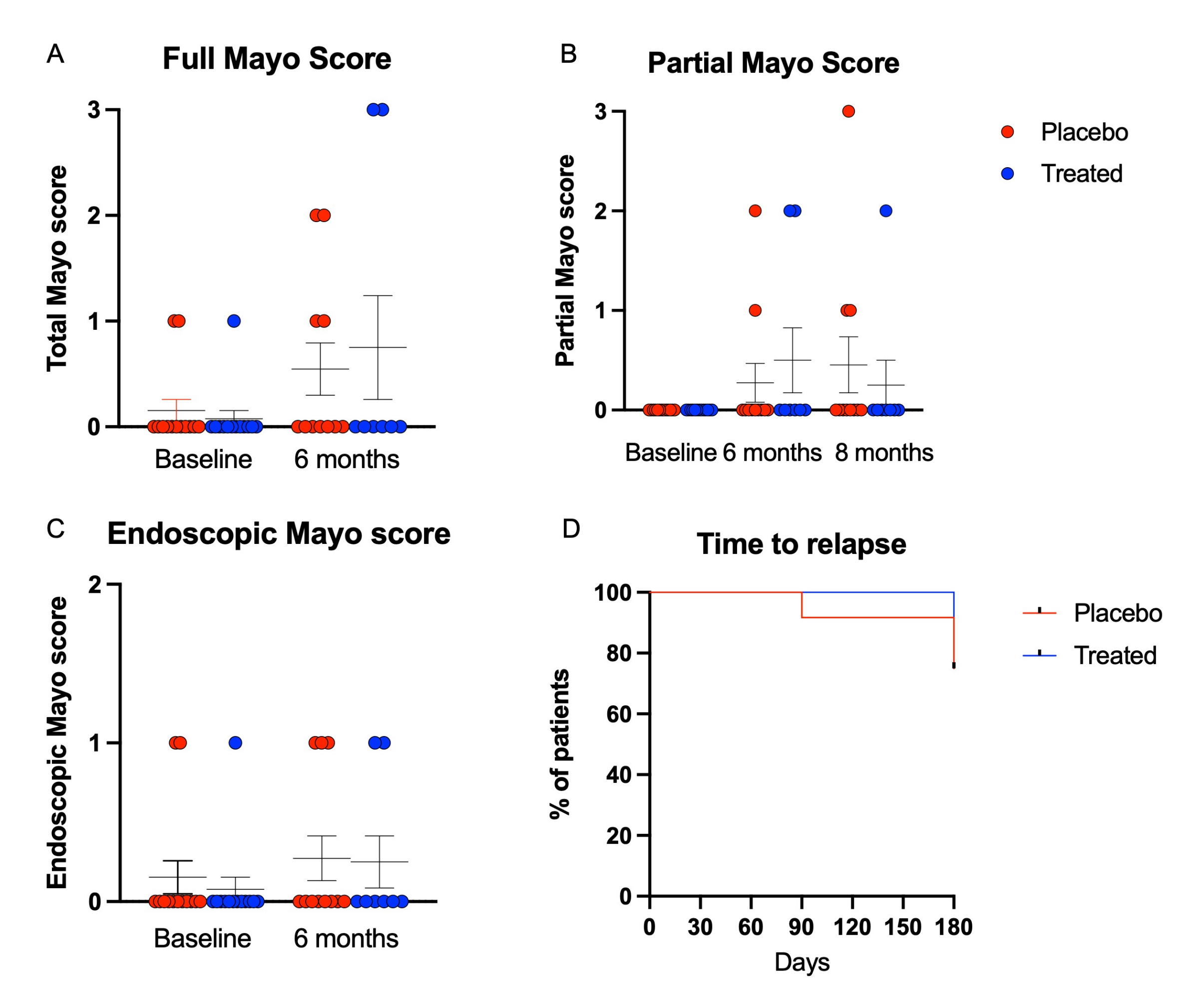

Figure 2. Clinical and endoscopic outcomes. **A,** Total Mayo Score in patients with UC treated with the symbiotic *vs.* placebo at baseline and 6 months. **B,** Partial Mayo Score in patients with UC treated with the symbiotic *vs.* placebo at baseline, 6 and 8 months. **C,** Endoscopic Mayo Score in patients with UC treated with the symbiotic *vs.* placebo at baseline and 6 months. The red dots represent the arm treated with placebo; the blue dots represent the arm treated with the symbiotic. No statistically significant differences were found between the two arms. **D,** Time to relapse in patients with UC treated with the symbiotic *vs.* placebo. The red line represents the arm treated with placebo; the blue line represents the arm treated with the symbiotic. No statistically significant differences were found between the two arms.

now represents a critical target in the IBD management[9]. In this scenario, the modulation of gut microbiota and the overall gut barrier has obtained preliminary and encouraging results, which, however, are not yet transferable to clinical practice[5]. Among the various strategies, probiotics, prebiotics, and symbiotics represent promising options that could serve as supportive therapy in IBD, particularly in UC. In particular, LGG has been extensively evaluated in several inflammatory conditions, yielding significant results that have opened up new and interesting potential therapeutic options, also in UC. LGG possesses a peculiar adhesive ability to the gut barrier and gut cells sustained by the presence of pili and the production of mucus-binding protein[10,11]. Furthermore, LGG is able to modulate NF-κB-mediated activities, inducing the secretion of anti-bacterial soluble proteins (i.e., p40, p75, and mucins) and creating a biofilm, thereby promoting overall barrier protection and restoration of gut permeability[12-15]. Finally, LGG displays immunomodulatory properties and can stimulate the innate response and reduce the pro-inflammatory acquired response[16,17], while controlling dysbiosis and inducing butyrate-producing bacteria[18,19]. Beside these properties, Boswellia, Querce-

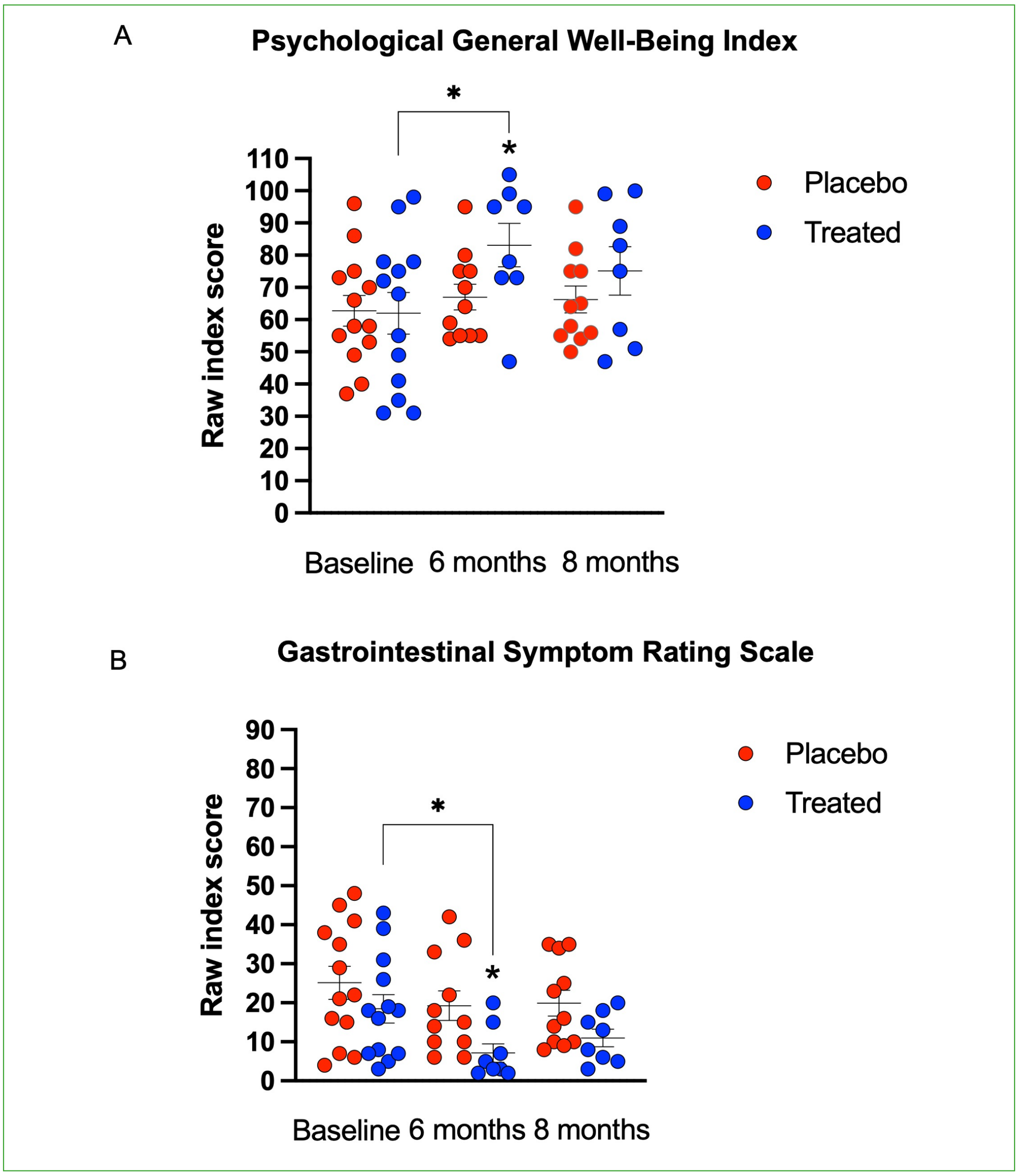

Figure 3. Patients reported outcomes. **A,** Psychological General Well Being Index in patients with UC treated with the symbiotic *vs.* placebo at baseline, 6, and 8 months. After 6 months of treatment, the treatment group showed a significant higher score compared to controls and to baseline levels. **B,** Gastrointestinal Symptom Rating Scale in patients with UC treated with the symbiotic *vs.* placebo at baseline, 6, and 8 months. After 6 months of treatment, the treatment group showed a significant lower score compared to controls and to baseline levels. * Indicates p-value < 0.05. The red dots represent the arm treated with placebo; the blue dots represent the arm treated with the symbiotic.

tin, Bromelain, Vitamin D3, Inulin, FOS, and Tryptophan have shown to act on the multiple components of the gut barrier the ability to preserve its integrity and preventing leaky gut, while reducing the oxidative stress and the release of pro-inflammatory cytokines, enhancing anti-inflammatory pathways, promoting the activity of beneficial bacteria and modulating the gut-brain axis[20].

To translate these findings into a clinical setting, the present study evaluated the effect of a symbiotic formulation based on LGG, Boswellia, Quercetin, Bromelain, Vitamin D3, Inulin, FOS, and Tryptophan in patients with UC in clinical and endoscopic remission. We found that the use of this symbiotic as an add-on therapy to mesalazine was able to improve the psychological and well-being status, as well as the global gastrointestinal symptoms, after 6 months of treatment. This double effect on PROs was partially lost after 2 months from the end of therapy indicating a direct effect of the symbiotic administration. Two well-recognized patients reported outcomes, the PGWBI and the GSRS, were used for depicting the psychological status and the global gastrointestinal symptoms, respectively. Quality of life is a determinant aspect of UC management and the use of this symbiotic appears to positively modulate it. However, this effect was not accompanied by a significant modulation of gut microbiota composition. Indeed, the ecological analysis did not show any difference in the fecal microbiota profiles between UC patients treated with the symbiotic formulation and those treated with a placebo. This could be explained by the pleiotropic effects that LGG, together with the other compounds, could have on the gut barrier and on the pathogenic pathway of UC. In particular, while maintaining the ecological structure unaltered, their beneficial effects on PROs and quality of life could be due to the positive modulation of the immune system, the gut permeability, and the gut-brain axis, as already shown in previous studies[20-22]. This is in line with other reports focusing on probiotic supplementation in UC patients, which have shown sustained remission rates, a reduction in gastrointestinal symptoms, and an amelioration in PROs[23,24] and quality of life[25]. In our cohort of patients, we did not observe a change in the clinical and endoscopic course of the disease between the arm treated with the symbiotic and mesalazine and the arm treated with mesalazine alone. This finding could be justified by the consistent and prolonged remission rates observed in both arms and is in line with the available literature[26], confirming that gut barrier-targeted therapies should represent a personalized and tailored treatment in order to maximize their utility.

Overall, the present study confirms that despite the potential benefits of probiotics and symbiotics in managing intestinal inflammation and modulating immune response, their efficacy varies significantly across different strains and administration methods. Current evidence is mixed and, following the important clues obtained with this and other reports, necessitates larger and well-designed human studies to determine the optimal dosages, treatment duration, and the efficacy of single versus multi-strain formulations. Understanding the interactions with the gut barrier represents another crucial future challenge. Additionally, a deeper understanding of the microbiome's structure and function is required. This could be achieved through advanced techniques in bioengineering, genetic engineering, systems biology, molecular biology, multi-omics, nanotechnology, and immunology. Employing these modern approaches will help clarify the therapeutic potential of gut barrier-targeted therapies in clinical practice. In this scenario, the present findings suggest a role for the LGG-based symbiotic as supportive and add-on therapy to improve the quality of life in terms of both gastrointestinal and psychological symptoms in patients with UC, opening new horizons also towards the potential use in the subcategory of patients with IBS overlapped with UC. Indeed, these patients show a significant impairment of gut-brain axis functions and may potentially further benefit from this therapeutic approach.

The strength of this study is that it is a randomized, prospective, double-blinded, placebo-controlled trial, which applied consistent and well-recognized PROs to evaluate the quality of life in UC patients. However, there are important limitations. In particular, the small sample size, the short follow-up period, and the potential for type 1 error for secondary endpoint analysis are significant aspects that will require future larger studies to confirm these findings.

CONCLUSIONS

In conclusion, LGG-based symbiotic supplementation results in a significant improvement in PROs in patients with mild-to-moderate UC in clinical and endoscopic remission and could represent an adjuvant treatment for UC patients to enhance their quality of life. Further investigations are warranted to confirm these findings.

Ethics Statement

This study was approved by the Local Ethics Committee and conducted in accordance with the requirements of the Institutional Review Board/Human Subjects Research Committee, the Declaration of Helsinki, and the Guidelines for Good Clinical Practice. Approval was obtained in April 2021.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Fundings

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Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

Not applicable.

Author's Contributions

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AI Disclosure

This manuscript was prepared without the use of AI-based tools.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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