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Investigating the Interplay between the Gut Microbiota and Host Immunity in Gastroenteric Disorders: The Potential of Combined Drug Therapies to Restore Microbial-immune Homeostasis

Yanlin Qiao and Xiang Gao

Digestive Surgery, Songjiang Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China

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ABSTRACT

This study examines the interaction between the microbiota and the immune system in diseases of the gastrointestinal tract, with a special emphasis on the synergistic use of pharmacological agents.

This was a retrospective, observational study of 100 patients with moderate to severe gastrointestinal disorders, including irritable bowel syndrome and inflammatory bowel disease, receiving control, monotherapy, or combination therapy.

Over 12 weeks, combination therapy demonstrated superior efficacy in enhancing gut microbial diversity. Improvements were achieved in alpha diversity, and a decrease in inflammatory indices and a shift in the immune phenotype were observed. Patients experienced significant improvements in symptom severity, pain, and general health. In addition, the general health of patients also improved. Importantly, the combination therapy group had better responses compared with the other groups. With respect to the identified factors, regression analysis revealed that microbial diversity, immune system regulation, and inflammation had positive effects on disease symptom alleviation.

These findings therefore help support the perspective of combination therapy as a more comprehensive mode of approaching and treating gastroenteric diseases.

Keywords: Drug therapy; Immune system; Inflammation; Irritable bowel syndrome gastrointestinal diseases; Gastrointestinal microbiome

INTRODUCTION

The human gastrointestinal (GI) tract contains a large and diverse population of microorganisms of varying stability, referred to as the gut microbiota, which is essential for host health. This complex environment impacts various biochemical mechanisms in the body, such as digestion, metabolism, and the immune response. Recent investigations have revealed the intricate relationship between the gut microbial ecosystem and the immune system, especially as it is related to disorders of the GI tract. Appreciation of this link is important for the formulation of therapeutic management strategies that seek to reinstate a typical commensal-susceptible interaction. They are helpful in the growth of the immune system of the host organism and play important roles in its function. These factors

Corresponding Author: Xiang Gao, MM;
Digestive Surgery, Songjiang Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China. Tel: (+86 176) 2186 1966, Fax: (+86 021) 6772 2251, Email: feelthe68world@hotmail.com

impact the differentiation of immune cells, the formation of antimicrobial peptides, and the preservation of the integrity of the epithelial lining.[1] This bidirectional response of the gut microbiota and immune system helps maintain tolerance to commensal bacteria and simultaneously provides good defense against pathogens.[2] Alterations in the balance of the gut microbial community, referred to as dysbiosis, are associated with multiple gastroenteric diseases, including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and colorectal cancer (CRC).[3] New findings also associate dysbiosis with systemic diseases, such as metabolic syndrome and autoimmune diseases.[4]

It is also well known that many gastroenteric disorders are associated with an imbalance in the gut microbiota. For example, patients diagnosed with IBD have diminished microbial richness and inadequate proportions of pathogenic and commensal bacteria. The depletion of butyrate-producing bacteria and the expansion of pathobionts such as *Escherichia coli* are pervasive in IBD patients and lead to a compromised mucosal barrier and inflammation.[5] Similarly, the presence of IBS is associated with a unique microbial composition that is negatively related to the severity of symptoms and a decrease in *Lactobacillus* and *Bifidobacterium*, among other bacteria.[6] In CRC, certain taxa of bacteria, including *Fusobacterium nucleatum*, are associated with tumor initiation and progression, indicating the role of the microbiota promotes cancer.[7] Moreover, dysbiosis can distort the pathophysiology of functional dyspepsia and celiac disease because microbial metabolites affect gut motility and immune stimulation or suppression.[8]

The interactions between the immune system and the gut microbiota are consequently mutual. The balance of the microbiota contributes to immune system regulation, and an imbalance known as dysbiosis may result in specific immune responses. For example, in IBD, there is chronic intestinal inflammation due to an overactive immune response to commensal bacteria and strong T helper 17 (T_H17) cells and dysregulation of regulatory T-cells. On the other hand, in CRC, certain metabolites are influenced by the microbiota; for example, polyamines and short-chain fatty acids can manipulate immune checkpoints, thereby promoting tumor immune escape.[9] In IBS, alterations in immune activation and inflammation are associated with variations in mast cell function and cytokine content.[10] These findings stress the complexity of the interaction between the gut microbiota and the host immune system in gastroenteric pathology. Because the gut microbiota has a significant impact on the development of several gastroenteric diseases, different treatments have emerged with the purpose of altering the microbiota composition and functionality. Pre- and probiotics, dysregulated microbiota, and dietary approaches work collectively to repopulate a healthy flora balance.[11] For example, high-fiber diets can improve the composition of friendly microbes, including *Akkermansia muciniphila*, involved in gut barrier repair and immunomodulation.[12] Fecal microbiota transplantation (FMT) in treating recurrent *Clostridioides difficile* infection, and FMT is now being attempted for other diseases, including IBD and IBS.[13] These approaches provide inconsistent results because the microbiota content and the immune response of different patients vary; thus, targeted treatments are necessary.[14]

New developments indicate that combination therapy that targets microbial imbalances and immune disorders at the same time may lead to enhanced strategies to renew microbial–immune harmony. The sequential and/or concomitant administration of antibiotics with immunomodulators has been used to treat IBD, and its purpose is to eliminate pathogenic bacteria as well as change the characteristics of the immune response.[15] For example, when ciprofloxacin and azathioprine were used for 2 consecutive months, a decrease in disease activity was observed in patients with Crohn's disease.[16] Furthermore, probiotic supplementation, unlike anti-inflammatory drugs, also seems to have a positive effect on IBS symptoms, including pain and gas.[17] The combined therapies that are being tested include the use of biologic agents, specifically an anti-tumor necrosis factor (TNF) agent and microbiota modulators.[18]

The effectiveness of the combined therapies is attributed to the fact that they treat both microbial and immune factors in gastroenteric ailments. Antibiotics can attack pathogenic bacteria, decrease their loads, and dissolve biofilms, which permits the reconversion of these surfaces by protective microbes.[16] Anti-inflammatory drugs, including corticosteroids and biologics, act predominantly by reducing the immune response, targeting the overactive immune system to reverse inflammation and prevent damage to tissues.[19] Probiotics may improve the mucosal barrier, act on the immune system, and promote the growth of beneficial

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bacteria such as *F prausnitzii*, which has anti-inflammatory properties.[20] Probiotics show application potential when used together with anti-inflammatory drugs to enhance clinical efficacy and address inflammation.[21] However, challenges exist in combined drug regimens, such as the development of antibiotic resistance, side effects, and differences in the microbial flora.[22] Notably, the development of microbiome-focused and immune-related biomarkers will enable the adoption of precision oncology concepts for patient stratification.[23] For example, to predict treatment response according to microbial and host immune patterns, machine learning algorithms are being designed.[24] Furthermore, new formulations that may help to increase the effectiveness and minimize adverse effects include encapsulated probiotics and precision-targeted biologics.[25] Continued research is being conducted to identify specific microbial and immune targets, such as gut-derived metabolites and cytokine networks, for better, more precise interventional tools.[26]

Therefore, the present study aimed to investigate the interplay between the gut microbiota and the host immune system in patients with gastroenteric disorders, with a specific focus on evaluating the impact of combination therapy that targets both the microbial composition and immune modulation. By assessing changes in microbial diversity, immune cell profiles, cytokine signaling, and clinical outcomes, this study seeks to provide insights into whether combined therapeutic strategies can more effectively restore the microbial–immune balance and improve disease management compared with monotherapy or standard treatment alone.

MATERIALS AND METHODS

This work was a prospective observational study between March 2022 and March 2024 in which the investigators aimed to assess the associations among the gut microbial profile, the host immune system, and the effects of combined drugs on the modulation of the microbial–immune balance in patients with gastroenteric disorders. The participants were advised to maintain their usual diet during the study period. No specific or uniform dietary intervention was imposed. Approval from the Institutional Review Board was sought, and participants' consent was solicited and obtained before participation.

Study Participants

The sample comprised 100 patients aged 18–65 years with moderate to severe gastroenteric diseases, including IBS, Crohn's disease, and ulcerative colitis, who were diagnosed on the basis of clinical criteria and endoscopic and histopathological examinations. The participants were recruited from an outpatient gastroenterology clinic. The specific exclusion criteria were antibiotic intake in the previous 3 months, ongoing immunosuppressive therapy, pregnancy, and other systemic inflammatory or autoimmune diseases.

Sample Collection

Fecal samples were obtained from each patient using sterile specimen containers and stored at −80 °C for subsequent determination of the gut microbial profile. Venous blood samples were collected in EDTA-containing tubes for immune cell analysis and cytokine testing. For the participants who consented to undergo endoscopic assessment, endoscopic biopsies of the gastrointestinal mucosa were taken to investigate the microbial burden and immunohistochemical features.

Intervention

The participants were categorized into 3 groups:

Control group (n=30): Standard treatment without the use of any other medications.

Monotherapy group (n=35): Standard care that was augmented with a specific agent that acts on the microbiota (probiotics) or an immunomodulatory product (corticosteroids).

Combination therapy group (n=35): Concomitant treatment with gut-targeted drugs, including the synbiotic formulation VSL#3 (containing *Streptococcus thermophilus*, *Bifidobacterium breve*, *B. longum*, *B. infantis*, *Lactobacillus acidophilus*, *L plantarum*, *L paracasei*, and *L delbrueckii* subsp. *bulgaricus*), and immune modulators, such as the anti-TNF biologic infliximab (5 mg/kg IV infusion every 8 weeks) or adalimumab (40 mg subcutaneously biweekly), depending on patient profile and physician discretion.

Patient adherence to prescribed treatments was monitored through medication diaries, pill counts, and patient interviews during follow-up visits.

Gut Microbiota Analysis

DNA from the stool samples was isolated using a QIAamp DNA Stool Mini Kit (Qiagen, Germany) according to the instructions included in the kit.

Amplicon sequencing of the V3–V4 region of the 16S rRNA gene was performed with barcoded primers. All the amplicons were sequenced on an Illumina MiSeq platform, and the sequencing was performed in paired-end mode.

The sequence data were analyzed by the QIIME2 tool. Flow reads were assigned to individual samples, low-quality sequences were removed, and the remaining sequences were binned to operational taxonomic units (OTUs) at 97% sequence identity. The classification of the identified organisms was performed using a BLAST search against 2 databases, SILVA and Greengenes.

Diversity Analysis

For richness at the sample level, alpha diversity (Shannon; Chao 1) was calculated. The Shannon index reflects both the richness and evenness of species diversity. The Chao1 estimator estimates species richness, accounting for unseen rare taxa in a sample. Microbial dissimilarity (Bray-Curtis dissimilarity) was used to analyze beta diversity between groups. Functional profiling of the microbiota to predict metabolic pathways was not performed in this study but is recommended for future analyses to elucidate the functional implications of the observed compositional changes.

Host Immune Analysis

Flow cytometry: Peripheral blood mononuclear cells (PBMCs) were stained with fluorochrome-isothiocyanate-conjugated monoclonal antibodies (mAbs) against human leukocyte surface markers (e.g., CD4, CD8, CD19, and CD14). Flow cytometry analysis was performed with a BD FACSCanto II. Flow cytometry data were analyzed using FlowJo software. The gating strategies involved the exclusion of debris and doublets and the identification of lymphocyte populations on the basis of forward and side scatter properties. Marker-specific gates were applied to quantify T-cell and B-cell subsets.

Cytokine profiling: Plasma levels of cytokines were analyzed using an Illumina-based multiplex immunoassay. The cytokines quantified were proinflammatory cytokines such as interleukin-6 (IL-6) and TNF-α and anti-inflammatory cytokines such as IL-10. Interassay and intra-assay variability were determined using standard controls, with coefficients of variation maintained to ensure assay reproducibility. Quality control samples were included in each batch of measurements. Plasma cytokine levels were quantified using the Human Cytokine ELISA Panel [Human IL-6 ELISA Kit (JL14113, sensitivity: 1.36 pg/mL); Human IL-10 ELISA Kit (JL19246, sensitivity: 0.32 pg/mL); and Human TNF-α ELISA Kit (JL10208, 7.65 pg/mL)]. All samples and standards were run in duplicate, and intra- and interassay coefficients of variation were kept below 10%.

Clinical Assessment

Clinical parameters were evaluated at baseline, 4 weeks, 8 weeks, and 12 weeks:

Symptom scoring: For IBS patients, the IBS severity scoring system (IBS-SSS) was used. For IBD, the Crohn's disease activity index (CDAI) or Mayo score was used.

The irritable bowel syndrome severity scale (IBS-SSS) is used for IBS patients. The IBS-SSS quantifies symptom severity on the basis of five items: abdominal pain severity, pain frequency, bloating, bowel habit dissatisfaction, and interference with daily life. Each item is scored from 0 to 100, yielding a total score ranging from 0 to 500. The scores are interpreted as follows:

- <75=no IBS
- 75–174 = mild IBS
- 175–299 = moderate IBS
- ≥300=severe IBS

Crohn's disease activity index (CDAI): In Crohn's disease patients, the CDAI is calculated using weighted scores for symptoms, including stool frequency, abdominal pain, general well-being, complications, the use of antidiarrheal medications, the presence of an abdominal mass, hematocrit, and body weight. The CDAI score is interpreted as follows:

- <150=remission
- 150–219=mild disease
- 220–450=moderate to severe disease
- ≥450=very severe disease

Mayo score (Mayo Clinic score): For ulcerative colitis assessment, the Mayo score includes four components: stool frequency, rectal bleeding, endoscopic findings, and the physician's global assessment. Each item is scored 0–3, for a total score of 0–12. The score is interpreted as follows:

- 0–2=remission
- 3–5=mild disease
- 6–10=moderate disease
- 11–12=severe disease

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Inflammatory Biomarkers: Fecal calprotectin and C-reactive protein (CRP) levels were estimated using enzyme-linked immunosorbent assays (ELISAs) [Human Fecal Calprotectin ELISA Detection Kit (JL54967, sensitivity: 6.1 ng/mL); Human CRP ELISA Detection Kit (JL13865, sensitivity: 15.9 pg/mL)].

Quality of Life: Quality of life (QoL) was assessed using data derived from the Short Inflammatory Bowel Disease Questionnaire (SIBDQ) or an analogous patient-reported outcome instrument.

Statistical Analyses

The statistical analyses have been conducted with SPSS software version 26.0 (IBM Corp., Armonk, NY) which used to determine associations among the gut microbiota, immune biomarkers, and clinical parameters. The beta diversity of bacteria, fungi, and communities was compared using permutational multivariate analysis of variance (PERMANOVA), and the differential abundance of bacterial and fungal taxa was determined using the Mann–Whitney U test. Group differences in immune markers and clinical data were analyzed using one-way ANOVA for parametric data and the Kruskal–Wallis test for nonparametric data. Where necessary, post hoc tests with Bonferroni or Dunn's corrections were conducted to examine differences between the groups. When significant group differences were identified, post hoc analyses were performed using the Bonferroni correction for ANOVA or Dunn's test for nonparametric comparisons. In addition, given the large number of statistical tests, false discovery rate correction (Benjamini–Hochberg procedure) was applied where appropriate to control for Type I error. Spearman rank correlation analysis was used to analyze microbial diversity on the basis of nonparametric data, whereas Pearson correlation analysis was used on the basis of parametric data. For regression analyses evaluating symptom severity outcomes, models were adjusted for potential confounding variables, including baseline disease severity, age, sex, and disease type (IBS, Crohn's disease, or ulcerative colitis). This adjustment was performed to mitigate confounding and ensure a more accurate estimation of associations. Statistical significance was set at $p<0.05$ for all hypothesis testing, except where adjusted thresholds were determined by multiple comparison corrections. Thus, p values were considered statistically significant at $p<0.05$ for all the tested hypotheses.

RESULTS

Gut Microbial Diversity

As shown in Table 1, the control, monotherapy, and combination therapy had temporal effects on the relative abundance of the gut microbial units, and a noticeable improvement in the diversity of the gut microbial units was obtained from the combination therapy. At baseline, the p value of the Shannon index was 0.92, whereas that of the Chao1 index was 0.87, indicating no statistically significant differences between the groups. By 4 weeks, a notable increase in both metrics was observed, particularly in the combination therapy group (Shannon index: 3.12 ± 0.28, Chao1: 175 ± 18) ($p<0.001$). This trend continued at 8 weeks and then again at 12 weeks. This study provides evidence that combination therapy improves microbial density and evenness compared with monotherapy or no therapy, indicating the value of this approach in reconstructing a beneficial gut microbiota profile.

Inflammatory Biomarkers

The findings summarized in Table 2 also demonstrate a progressive decline in inflammatory parameters, such as serum CRP and fecal calprotectin levels, with both single and combination therapy. At the outset, there were no significant differences in the measurements of serum CRP and fecal calprotectin levels among the control, monotherapy, and combination therapy groups ($p>0.05$). This finding indicates that there are no significant initial differences in systemic or gut inflammation. By week 4, the combination therapy group showed a significant reduction in serum CRP (4.9 ± 1.4 mg/L) and fecal calprotectin (90 ± 25 μg/g) levels, outperforming both the monotherapy group and the control group ($p<0.001$). These trends persisted and intensified over the 8- and 12-week periods, with the combination therapy group achieving the lowest biomarker levels at 12 weeks (CRP: 3.2 ± 1.3 mg/L; calprotectin: 55 ± 20 μg/g). Taken together, these studies indicate that combining drugs significantly reduces systemic and intestinal inflammation compared with either monotherapy or no treatment

Table 1. Gut microbial diversity (alpha diversity—Shannon index and Chao1)

Time point	Diversity metric	Control group (mean ± SD)	Monotherapy group (mean ± SD)	Combination therapy group (Mean ± SD)	*p* (ANOVA)
Baseline	Shannon Index	2.85 ± 0.30	2.87 ± 0.28	2.84 ± 0.29	0.92
	Chao1	145 ± 15	148 ± 18	146 ± 16	0.87
4 weeks	Shannon Index	2.89 ± 0.33	3.05 ± 0.30	3.12 ± 0.28	<0.001
	Chao1	150 ± 16	165 ± 20	175 ± 18	<0.001
8 weeks	Shannon Index	2.91 ± 0.32	3.15 ± 0.25	3.35 ± 0.30	<0.001
	Chao1	152 ± 14	170 ± 18	185 ± 20	<0.001
12 weeks	Shannon Index	2.93 ± 0.34	3.18 ± 0.27	3.45 ± 0.33	<0.001
	Chao1	155 ± 15	175 ± 19	190 ± 18	<0.001

ANOVA: analysis of variance; SD: standard deviation.

Table 2. Inflammatory biomarkers (serum CRP levels and fecal calprotectin levels)

Time point	Biomarker	Control group (mean ± SD)	Monotherapy group (mean ± SD)	Combination therapy group (Mean ± SD)	*p* (ANOVA)
Baseline	Serum CRP (mg/L)	8.5 ± 2.0	8.6 ± 1.9	8.4 ± 2.1	0.88
	Fecal Calprotectin (μg/g)	150 ± 35	152 ± 38	148 ± 40	0.79
4 weeks	Serum CRP (mg/L)	8.4 ± 1.9	6.2 ± 1.5	4.9 ± 1.4	<0.001
	Fecal Calprotectin (μg/g)	148 ± 30	120 ± 28	90 ± 25	<0.001
8 weeks	Serum CRP (mg/L)	8.3 ± 2.1	5.8 ± 1.7	3.8 ± 1.2	<0.001
	Fecal Calprotectin (μg/g)	145 ± 32	105 ± 30	70 ± 22	<0.001
12 weeks	Serum CRP (mg/L)	8.3 ± 2.0	5.6 ± 1.6	3.2 ± 1.3	<0.001
	Fecal Calprotectin (μg/g)	142 ± 28	98 ± 25	55 ± 20	<0.001

ANOVA: analysis of variance; CRP: C-reactive protein; SD: standard deviation.

Cytokine Levels

The data presented in Table 3 highlight the fluctuations in the investigated cytokines (IL-6, TNF-α, and IL-10) over the period of the study, and the greatest changes were observed with combination therapy. At baseline, the cytokine counts in the control, monotherapy, and combination therapy groups did not differ significantly, suggesting that the groups' starting conditions were similar ($p>0.05$). During the long study period, the combination therapy led to a reduction in the levels of IL-6 and TNF-α, which are short-term inflammatory markers, and an increase in the level of IL-10, an anti-inflammatory indicator. At 12 weeks, the combination therapy group presented the lowest IL-6 (4.5 ± 1.7 pg/mL) and TNF-α (5.9 ± 2.3 pg/mL) levels, which were significantly greater than those of the monotherapy and control groups ($p<0.001$). Similarly, the IL-10 levels at 12 weeks significantly increased in the combination therapy group to 12.5 ± 2.2 pg/mL. These results suggest that combination therapy has enhanced benefits in reducing inflammation and promoting anti-inflammatory activities. Thus, combination therapy represents a promising therapeutic approach for treating cytokine-induced inflammation-related diseases.

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Table 3. Cytokine levels

Time point	Cytokine	Control group (mean ± SD)	Monotherapy group (mean ± SD)	Combination therapy group (mean ± SD)	*p* (ANOVA)
Baseline	IL-6 (pg/mL)	12.5 ± 3.0	12.3 ± 3.2	12.6 ± 3.1	0.91
	TNF-α (pg/mL)	15.2 ± 4.1	15.5 ± 3.9	15.4 ± 4.0	0.88
	IL-10 (pg/mL)	5.5 ± 1.5	5.7 ± 1.6	5.6 ± 1.4	0.90
4 weeks	IL-6 (pg/mL)	12.4 ± 2.9	9.8 ± 2.2	7.5 ± 2.0	<0.001
	TNF-α (pg/mL)	15.0 ± 4.0	12.2 ± 3.5	9.8 ± 2.9	<0.001
	IL-10 (pg/mL)	5.6 ± 1.4	7.5 ± 1.8	8.9 ± 1.7	<0.001
8 weeks	IL-6 (pg/mL)	12.2 ± 3.1	8.2 ± 1.9	5.5 ± 1.8	<0.001
	TNF-α (pg/mL)	14.8 ± 3.8	10.5 ± 2.8	7.2 ± 2.5	<0.001
	IL-10 (pg/mL)	5.8 ± 1.5	8.5 ± 1.6	10.5 ± 2.0	<0.001
12 weeks	IL-6 (pg/mL)	12.1 ± 3.0	7.8 ± 2.0	4.5 ± 1.7	<0.001
	TNF-α (pg/mL)	14.7 ± 3.9	9.8 ± 2.7	5.9 ± 2.3	<0.001
	IL-10 (pg/mL)	6.0 ± 1.6	9.5 ± 1.7	12.5 ± 2.2	<0.001

ANOVA: analysis of variance; IL: interleukin; SD: standard deviation; TNF: tumor necrosis factor.

Symptom Severity Scores

Although all groups had significant improvements in their symptom severity scores (IBS-SSS or CDAI) or pain intensity, as indicated by the absolute values of changes shown in Table 4, the combination therapy group demonstrated the greatest benefits. The preliminary values of symptom severity and pain intensity in the control, monotherapy, and combination therapy groups did not differ significantly ($p>0.05$), suggesting that the baseline values were consistent across all the groups. By 4 weeks, symptom severity scores and pain intensity began to decrease across all groups, with the combination therapy group achieving the most substantial reductions. These trends persisted up to the 8- and 12-week time points in terms of symptom severity scores and pain intensity. These findings confirm that, compared with monotherapy or no treatment, combination therapy significantly reduces symptom severity and pain intensity and thus results in better symptom control.

Quality of Life Scores

Table 5 shows that the combination therapy group was capable of enhancing the factor's quality of life within the months indicated in the study in terms of the SIBDQ scores and reducing the percentage of work productivity loss over time. At baseline, the SIBDQ scores and work productivity loss scores of the control, monotherapy, and combination therapy groups were comparable ($p>0.05$). By 4 weeks, the combination therapy group demonstrated a notable increase in SIBDQ scores (58 ± 12) and a sharp decrease in work productivity loss (30 ± 10%). These improvements continued through 8 and 12 weeks, with the combination therapy group achieving the highest SIBDQ scores (65 ± 10) and the lowest work productivity loss (15 ± 7%) at 12 weeks. These outcomes show that combination therapy is much more effective than single-therapy regimens in ameliorating quality of life and minimizing its negative effect on the economic productivity of the nation.

Table 4. Symptom severity scores (IBS-SSS or CDAI) and pain intensity scores

Time point	Parameter	Control group (mean ± SD)	Monotherapy group (mean ± SD)	Combination therapy group (Mean ± SD)	*p* (ANOVA)
Baseline	Symptom Severity Score	280 ± 50	285 ± 48	283 ± 49	0.89
	Pain Intensity (0–10)	7.5 ± 1.5	7.8 ± 1.6	7.7 ± 1.4	0.85
4 weeks	Symptom Severity Score	275 ± 45	210 ± 40	150 ± 30	<0.001
	Pain Intensity (0–10)	7.4 ± 1.6	5.5 ± 1.2	3.5 ± 1.0	<0.001
8 weeks	Symptom Severity Score	270 ± 50	180 ± 35	120 ± 25	<0.001
	Pain Intensity (0–10)	7.3 ± 1.5	4.8 ± 1.0	2.5 ± 0.9	<0.001
12 weeks	Symptom Severity Score	268 ± 48	170 ± 33	110 ± 22	<0.001
	Pain Intensity (0–10)	7.2 ± 1.4	4.5 ± 1.1	2.0 ± 0.8	<0.001

ANOVA: analysis of variance; CDAI: Crohn's disease activity index; IBS-SSS: irritable bowel syndrome severity scoring system; SD: standard deviation.

Table 5. Quality of life scores (SIBDQ) and work productivity loss (%)

Time point	Parameter	Control group (mean ± SD)	Monotherapy group (mean ± SD)	Combination therapy group (Mean ± SD)	*p* (ANOVA)
Baseline	SIBDQ Score	45 ± 10	44 ± 12	46 ± 11	0.78
	Work Productivity Loss (%)	50 ± 12	52 ± 14	51 ± 13	0.82
4 weeks	SIBDQ Score	46 ± 9	52 ± 11	58 ± 12	<0.001
	Work Productivity Loss (%)	48 ± 10	40 ± 11	30 ± 10	<0.001
8 weeks	SIBDQ Score	47 ± 8	55 ± 10	62 ± 11	<0.001
	Work Productivity Loss (%)	46 ± 9	35 ± 9	20 ± 8	<0.001
12 weeks	SIBDQ Score	48 ± 9	56 ± 11	65 ± 10	<0.001
	Work Productivity Loss (%)	45 ± 8	32 ± 8	15 ± 7	<0.001

ANOVA: analysis of variance; SD: standard deviation; SIBDQ: Short Inflammatory Bowel Disease Questionnaire.

Immune Cell Populations

Table 6 presents the dynamics of immune cells, as the greatest effect was shown for the patients who received the combination therapy. At baseline, the counts of immune cells, such as $CD4^+$ T cells, $CD8^+$ T cells, regulatory T cells, $CD19^+$ B cells, and $CD14^+$ monocytes, were similar between the control, monotherapy, and combination therapy groups ($p>0.05$). Furthermore, at 4 weeks, combination therapy increased the percentage of $CD4^+$ T cells (42 ± 6%), regulatory T cells (12 ± 2%), $CD19^+$ B cells (22 ± 5%), and $CD14^+$ monocytes (18 ± 4%) and decreased the percentage of $CD8^+$ T cells (14 ± 3%) compared with those in the other groups ($p<0.001$). These trends were even more evident at 8 and 12 weeks, and the combination therapy group presented the highest percentages of $CD4^+$ T cells (48 ± 6%), T cells (16 ± 3%), $CD19^+$ B cells (30 ± 5%), and $CD14^+$ monocytes (23 ± 5%) at 12 weeks, with the lowest percentage of $CD8^+$ T cells (10 ± 2%). Specifically, the results of the present study revealed that combination intervention restored immune homeostasis via increased numbers of posterior regulatory and effector immune cells compared with increased numbers of pathogenic $CD8^+$ T cells. This immune profile suggests that combination therapy could be effective in the treatment of several immune-related disorders.

Table 6. Immune cell populations ($CD4^+$ T cells, $CD8^+$ T cells, regulatory T cells, $CD19^+$ B cells, and $CD14^+$ monocytes, % of PBMCs)

Time point	Parameter	Control group (Mean ± SD)	Monotherapy group (Mean ± SD)	Combination therapy group (Mean ± SD)	*P* (ANOVA)
Baseline	$CD4^+$ T Cells (%)	32 ± 5	33 ± 6	34 ± 5	0.83
	$CD8^+$ T Cells (%)	18 ± 4	17 ± 5	19 ± 4	0.75
	Regulatory T Cells (%)	8 ± 2	8 ± 2	8 ± 2	0.89
	$CD19^+$ B Cells (%)	12 ± 3	13 ± 4	13 ± 3	0.81
	$CD14^+$ Monocytes (%)	10 ± 2	11 ± 3	10 ± 3	0.77
4 weeks	$CD4^+$ T Cells (%)	33 ± 6	38 ± 7	42 ± 6	<0.001
	$CD8^+$ T Cells (%)	18 ± 5	15 ± 4	14 ± 3	<0.001
	Regulatory T Cells (%)	8 ± 2	10 ± 3	12 ± 2	<0.001
	$CD19^+$ B Cells (%)	13 ± 3	18 ± 4	22 ± 5	<0.001
	$CD14^+$ Monocytes (%)	11 ± 3	15 ± 3	18 ± 4	<0.001
8 weeks	$CD4^+$ T Cells (%)	34 ± 5	40 ± 6	46 ± 5	<0.001
	$CD8^+$ T Cells (%)	17 ± 4	13 ± 3	12 ± 2	<0.001
	Regulatory T Cells (%)	9 ± 2	12 ± 2	14 ± 3	<0.001
	$CD19^+$ B Cells (%)	14 ± 3	21 ± 5	27 ± 4	<0.001
	$CD14^+$ Monocytes (%)	12 ± 3	16 ± 3	20 ± 4	<0.001
12 weeks	$CD4^+$ T Cells (%)	34 ± 6	41 ± 7	48 ± 6	<0.001
	$CD8^+$ T Cells (%)	17 ± 4	12 ± 3	10 ± 2	<0.001
	Regulatory T Cells (%)	9 ± 2	13 ± 3	16 ± 3	<0.001
	$CD19^+$ B Cells (%)	15 ± 3	23 ± 4	30 ± 5	<0.001
	$CD14^+$ Monocytes (%)	13 ± 3	18 ± 4	23 ± 5	<0.001

ANOVA: analysis of variance; PBMCs: peripheral blood mononuclear cells; SD: standard deviation.

Regression Analysis for Factors Associated with Improvement in Symptom Severity

Table 7 presents the results of the regression analysis, which reveals the factors that are most closely connected with the improvement in symptom severity, as reflected in the change in the score. A highly statistically significant negative beta coefficient was found for changes in the Shannon index (0.35; $p<0.001$). Here, the greater the microbial diversity, the greater the degree of symptom improvement. Similarly, we observed an inverse relationship between $CD4^+$ T-cell changes and changes in symptom severity scores ($\beta=-0.28$, $p<0.001$), thereby implying that an increase in adaptive immunity was beneficial. On the other hand, negative correlations with proinflammatory cytokines were evident, and significant changes in the levels of IL-6 ($\beta=0.45$, $p<0.001$) and TNF-α ($\beta=0.38$, $p<0.001$) were detected. These findings suggested that a decrease in inflammation was associated with symptom resolution. Most significantly, the combination of treatments presented the largest coefficient ($\beta=-0.50$, $p<0.001$), suggesting that the combination is the most effective for reducing symptom severity compared with the other treatments. According to the proposed model, microbial diversification, immune system regulation, and inflammation reduction are crucial for determining symptom severity, with combination therapy leading to better results.

Table 7. Regression analysis for factors associated with improvement in symptom severity (dependent variable: change in symptom score)

Predictor variable	Beta coefficient (β)	Standard error (SE)	t-value	*p*	95% confidence interval (CI)
Change in Shannon Index	-0.35	0.08	-4.38	<0.001*	-0.51 to -0.19
Change in IL-6 (pg/mL)	0.45	0.12	3.75	<0.001*	0.21 to 0.69
Change in TNF-α (pg/mL)	0.38	0.10	3.80	<0.001*	0.18 to 0.58
$CD4^+$ T Cell Increase (%)	-0.28	0.07	-4.00	<0.001*	-0.42 to -0.14
Combination Therapy (Yes=1)	-0.50	0.09	-5.56	<0.001*	-0.68 to -0.32

CI: confidence interval; SE: standard error.

DISCUSSION

The observed significant enhancements in gut microbial alpha diversities correspond to earlier experimental findings that demonstrated the effects of interventions addressing the gut microbiota on microbial richness and diversity. The baseline Shannon index and Chao1 values across all groups were comparable, reflecting a balanced starting point. By the end of the study, the combination therapy group achieved the highest diversity compared with the monotherapy and control groups. These findings are similar to those of Sanders et al, who reported that the administration of probiotics and synbiotics enhanced the quality of the microbiota of IBS patients.[11] According to Zhao et al, high-fiber dietary interventions accompanied by probiotics led to significant increases in alpha diversity parameters and, therefore, improved the gut microbiota.[12] Nevertheless, the absolute improvement in this study is greater than the improvement estimated in dietary- or probiotic-focused studies, which underlines the concept of add-on therapy here.

The present data also revealed lower serum CRP and fecal calprotectin levels in the combination therapy group, indicating effective anti-inflammatory activity. At enrollment, no statistically significant differences in inflammatory markers were noted between the groups. Over the course of treatment, however, the combination therapy group achieved the greatest reductions in these markers.

These observations confirm the reduction in the CRP level[15] when antibiotics are used in conjunction with immunomodulators for patients with IBD. In the same study, Ford et al reported that the use of synbiotics, including both probiotics and anti-inflammatory substances, in IBS patients also lowered the level of fecal calprotectin, suggesting decreased inflammation in the intestines.[17] Nevertheless, the results of the present

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work demonstrate a more robust effect, which may largely be due to the multilevel targeting of both microbial imbalance and immunomodulation. Combination therapy had the highest efficiency for cytokine regulation. Combination therapy demonstrated superior effects in regulating cytokines, leading to reduced levels of proinflammatory mediators and increased levels of anti-inflammatory signals. These results are consistent with those of Neurath, who reported that immunomodulatory therapies were helpful, as they lowered the IL-6 and TNF-α concentrations in patients with IBD.[28] Furthermore, studies have shown that the use of probiotics enhances the production of IL-10, which promotes anti-inflammatory action.[19] Our results further demonstrated that combination therapy outperformed monotherapy. This aligns well with the findings of Ungaro et al, who stressed the goal of enabling broader immune modulation through biologics in conjunction with microbiota manipulation.[18] The dual-targeted interaction of the gut microbiota and immune system in this study has several advantages over monotherapy or dietary practices. Previous works applied single compounds, either probiotics or anti-inflammatory substances, which offered reasonable improvements.[10] In contrast, better outcomes were observed when multiple mechanisms were used simultaneously in combination therapy because improvements were observed for all the measures used in the study.

Notably, the outcomes also revealed the susceptibility of the gut microbiota to the regulation of systemic inflammation. These changes, including changes in the fecal calprotectin cutoff as well as serum CRP and proinflammatory cytokine levels, suggest that microbial diversity underpins immune functions.[20] Moreover, the microbial SR was positively related to both the DSS score and RQ value for IL-10, indicating enhanced immune tolerance and less inflammation. The findings of the present study regarding improved symptom severity and pain intensity are consistent with the results of prior integrated therapeutic approach studies. By the end of treatment, patients receiving combination therapy experienced the most significant improvements in symptom severity and pain relief compared with the other groups. This outcome is more notable than the decreases observed in various studies in which a combination of probiotics and anti-inflammatory agents led to meaningful but less pronounced benefits in terms of symptoms and pain for patients with IBS.[17]

We hypothesize that the greater enhancements in our study are due to the combined targeting of microbial and immune molecules. Similarly, Torres et al reported that combining immunomodulators with a microbiota approach in patients with Crohn's disease resulted in considerable symptom improvement, although the results of the present study suggest even greater effectiveness.[16] These variations may be attributed to the characteristics of patient samples and therapeutic interventions; however, they provide evidence for the value of combining treatments in the effective management of symptoms.

The increase in SIBDQ scores and decrease in work productivity loss percentage identified in the present study also substantiate the overall utility of combination therapy. The combination therapy group reported a higher SIBDQ score and work productivity loss compared with the monotherapy and control groups. These outcomes can also corroborate those of Sanders et al, who reported that dietary and probiotic interventions augmented the overall health-related quality of life score over time in patients with gastroenteric disorders.[11] However, the extent of benefit reported was less than that in our study, implying that combination therapy offers longer-term benefits. Furthermore, Cammarota et al reported that FMT translated to productivity loss in patients who had recurrent *Clostridioides difficile* infection, confirming the productive health economic outcomes of microbiota-directed therapies.[13] Thus, it can be concluded that the use of multiple treatment approaches further reduces productivity loss; the specificity of the combination therapy group therefore allows us to explore the potential of integrated treatments. In this study, the immune modularity of combination therapy was reflected in the shifts in the immune cells described above.

These results contrast with those of Miele et al, who reported that probiotics increased regulatory T cells while reducing $CD8^+$ T cells in IBD patients after 12 weeks.[19] Ungaro et al noted that biologics and microbiota-targeted therapies increased the number of $CD4^+$ T cells in Crohn's disease patients.[18] The improved outcomes reported in our study may be attributed to the synergistic action of the microbiota and immune system, , revealing pivotal facets encompassing microbiota restoration and subduing the immune-inflammatory potential. The observations derived from this study therefore establish that combination therapy is more effective than monotherapy or no treatment in

every output measure assessed. The types of approaches that were revealed in the current work, illustrating moderate evidence in the individual components of the schemes described in the work of Ford et al and Sanders et al, revealed expounding therapeutic effects when applied in the combination therapy setting.[11,17] For example, Khan et al reported that antibiotics and immunomodulators lowered CRP levels and increased the severity indices of symptoms in patients with IBD but did not affect quality of life or immunity levels to the same extent.[15] On the other hand, the present study revealed significant changes in symptom severity, health-related quality of life, immune cell recruitment, and work productivity loss after combination therapy. The findings of this work demonstrate that it is necessary to focus on both microbial and immunological interventions to obtain balanced therapeutic results. The combination of antidysbiosis and immune-modulating therapies is therefore a rational intervention target for treating gastroenteric disorders and has both biomedical and economic benefits.[29]

Significance of the Study

The results of this work demonstrate the significant potential of the combination therapy approach in the treatment of gastroenteric diseases that involve microbial imbalance and immune dysfunction. Thus, the effectiveness of therapy is multidimensional, showing an enhanced gut microbiota, increased biomarkers of inflammation, increased levels of effective immune cells, a reduction in the severity of symptoms, and an increase in quality of life. Hence, it offers sustained evidential support for the concurrent use of microbial and immune-focused strategies, as demonstrated by research that compared therapy with monotherapy or no therapy. These outcomes are not only in accordance with the prevailing modalities of treatment and management but also provide the foundation for the use of differential modalities of treatment and approaches in the face of complex GI- and immune-mediated disorders.

Limitations of the Study

Of course, there are some weaknesses in this study. In large samples where variance increases, the sample size chosen for the study may prove inadequate in some cases of analysis, even if it is suitable for basic comparison. The study may also not be long enough-12 weeks-to assess the effectiveness of combination therapy in the long term and the possible adverse effects. The immunological response and composition of the gut microbiota are not accurately described by intraindividual differences that can affect treatments. Furthermore, there is no microbiome or immune system tailoring as a result of the use of a broad approach. In conclusion, future work needs to involve larger sample sizes or more extended follow-up for confirmation of the conclusions drawn in this work, as well as stratified analysis.

The current work highlights the therapeutic potential of combination therapy to positively impact the GI microbial composition, inflammation, immune system, GI symptoms, and overall quality of life in patients with gastroenteric disorders. Consisting of microbiota- and immune-targeted strategies, this type of therapy is comprehensive and more efficient than monotherapy or no treatment. Such outcomes may be useful in providing evidence regarding various directions for the management of intricate diseases using comprehensive approaches. Furthermore, research on intensive long-term and individualized approaches should be continued to obtain high therapeutic effectiveness and long-term maintenance of the results.

STATEMENT OF ETHICS

This study was approved by the Ethics Committee of Songjiang Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (2022032).

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Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Not applicable.

DATA AVAILABILITY

Due to privacy concerns, the data that support the findings of this study are available from the corresponding author upon reasonable request.

AI ASSISTANCE DISCLOSURE

No AI assistance involved

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