Cytokine and clinical response to *Saccharomyces boulardii* therapy in diarrhea-dominant irritable bowel syndrome: a randomized trial

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Introduction **This preliminary study aimed to investigate the effects of the probiotic *Saccharomyces boulardii* on proinflammatory and anti-inflammatory cytokines in patients with diarrhea-dominant irritable bowel syndrome (IBS-D). The other objectives were to document any clinical improvement as judged by symptoms, quality of life, and histology.**

Patients and methods **This was a randomized, double blind, placebo-controlled trial in which *S. boulardii,* 750 mg/day, or placebo was administered for 6 weeks in IBS-D patients, in addition to ispaghula husk standard treatment.**

Results **Thirty-seven patients received *S. boulardii* and 35 patients received the placebo. As compared with placebo, the *S. boulardii* group showed a significant decrease in blood and tissue levels of proinflammatory cytokines interleukin-8 (IL-8) and tumor necrosis factor-α ($P<0.001$) and an increase in anti-inflammatory IL-10 levels, as well as an increase in the tissue IL-10/IL-12 ratio ($P<0.001$). No significant change in the blood and tissue levels of cytokines was found in the placebo group. Bowel-related IBS-D symptoms reported in the patients' daily diary improved in both groups. However, overall improvement in the quality of life was more marked in the *S. boulardii* group. Although baseline histological findings were mild, an improvement was observed in the probiotic group in the lymphocyte and neutrophil infiltrates ($P=0.017$ and 0.018), epithelial mitosis ($P=0.003$), and intraepithelial lymphocytes ($P=0.024$). No serious adverse events were found in either group.**

Conclusion ***S. boulardii* with ispaghula husk was superior to placebo with ispaghula husk in improving the cytokine profile, histology, and quality of life of patients with IBS-D. These preliminary results need to be confirmed in a well-powered trial. *Eur J Gastroenterol Hepatol* 26:630–639 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Irritable bowel syndrome (IBS) is a chronic condition characterized by intermittent abdominal pain, altered bowel habits (diarrhea and/or constipation), and other gastrointestinal symptoms, such as bloating and flatulence, all occurring in the absence of structural abnormalities in the intestine [1]. The prevalence of IBS in the general population ranges between 3 and 25%, and a significant impact on patients' quality of life (QOL) has been described [2,3].

The pathophysiology of IBS is not well understood, but the syndrome is associated with a dysregulation of the brain–gut axis, which involves abnormal function in the enteric, autonomic, and/or central nervous systems, or disturbed interplay between the two systems [4]. These alterations are considered to lead to abnormal gastrointestinal sensitivity, motility, and secretion, which in turn contribute toward the hallmark IBS symptoms. Some studies have evidenced an important role of low-grade inflammation and immunological alterations in the development of symptoms compatible with IBS [4,5]. Mild infiltration of immune cells (mainly T cells and mast cells) and increased levels of plasma/serum proinflammatory cytokines, including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), IL-6, and IL-8, have been observed in IBS patients compared with controls [5]. One of the events considered to induce the immunological response impacting the generation of IBS symptoms is alterations in the gut microflora (bacterial overgrowth) [4]. Significant differences have been described in the gut microbiota of IBS patients compared with healthy individuals [6,7] and infectious gastroenteritis has been associated with increased chances of developing IBS symptoms [8,9]. It remains unclear whether changes in the intestinal microbiota are a cause or a consequence of IBS [10].

Probiotics are defined as 'live microorganisms which when administered in adequate amount confer a health benefit on the host' [11]. *Saccharomyces boulardii* is a probiotic yeast that has been used successfully to prevent

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antibiotic-induced diarrhea, to prevent relapse of *Clostridium difficile*, and to treat acute diarrhea [12]. It ameliorates intestinal injury and inflammation caused by a wide variety of enteric pathogens [13]. It modulates host signaling pathways involved in intestinal inflammatory responses to exert an anti-inflammatory effect, especially by producing a low-molecular-weight soluble factor that blocks NF-κB activation and activation of ERK1/2 MAP kinases. As a result, there is a decreased expression of the IL-8 gene in intestinal epithelial cells and monocytes. A decrease in the secretion of proinflammatory IL-8 helps ameliorate inflammation [14–16]. However, the role of this probiotic in the treatment of IBS-D has not been investigated extensively [17,18].

This pilot study aimed to quantify the effects of *S. boulardii* on the relative production of anti-inflammatory IL-10 and proinflammatory cytokines (IL-8, IL-12, and TNF-α) in patients with IBS-D. The other objectives were to document any clinical improvement as judged by symptoms, QOL, and histology.

Methods

Design overview

A randomized, double-blind, placebo-controlled trial was conducted to compare the cytokine response, symptoms, QOL, and histology in patients with IBS-D by treatment with *S. boulardii* or a placebo. The trial was approved by the Ethics Committee of the hospital. All patients provided written informed consent before participating in the study.

Patient selection

Patients eligible for inclusion in the study had a well-established diagnosis of IBS-D. The inclusion criteria were as follows: age between 18 and 60 years and fulfilling the Rome III criteria for IBS-D [19]. Exclusion criteria were pregnancy and breast feeding, inflammatory bowel disease, celiac disease, lactose intolerance, protozoal or worm infestation, other systemic diseases, concurrent treatment with any medications that could influence gut motility or absorptive function, preparations that could alter the enteric flora, including antibiotics and commercially available probiotics within 4 weeks before study entry, presence of on-going infections, allergy to *S. boulardii* components, and central nervous catheterization. Patients could be withdrawn from the study in case of intake of antifungal agents and probiotics other than *S. boulardii*, insufficient compliance to treatment, serious side effects, and withdrawal of patient consent.

Study design

Randomization (1 : 1 allocation) was computer-generated and the investigators were provided with sealed envelopes containing the treatment code to administer, in increasing number, according to chronological inclusion in the study. Investigators, patients, and the research team remained blinded to treatment allocation until all analyses had been completed. *S. boulardii* and placebo were provided by Biocodex (Beauvais, France) in sealed bottles. The placebo had the same aspect, taste, and smell as the test treatment. The individual in charge of manufacturing the study drugs was neither involved in the product administration nor in the evaluation of their effects. After a 2-week run-in period (week 0–week 2), eligible patients were randomized to receive probiotic treatment, *S. boulardii* 750 mg/day, or a matching placebo for 6 weeks (week 3–week 8). The Ethics Committee requested that all patients receive daily ispaghula husk (1 tablespoonful, after dinner) as the standard treatment of fiber supplementation in IBS. At the screening visit, physicians recorded patient age, sex, and medical history. Clinical evaluations included physical examination and stool microscopy to rule out protozoal and worm infestation. Colonoscopy was performed to exclude any macroscopic disease and upper rectum biopsies were taken for histopathology analysis and determination of cytokine levels. The presence of associated celiac disease was excluded through the search of tissue antitransglutaminase antibodies [immunoglobulin A and G (IgA and IgG)] and lactose intolerance through the breath hydrogen test. Routine hematological and biochemical analyses were carried out at the screening visit and were repeated at the end of the 6-week treatment period.

Throughout the entire study, patients visited on a weekly basis to evaluate safety, treatment compliance (number of capsules returned), and symptom improvement (on the basis of data reported in the daily symptom diary). Within the first week after treatment completion, patients underwent sigmoidoscopy and rectal biopsies for histopathology analyses and determination of cytokine level.

The study criteria were changes from baseline to end of treatment in blood and tissue IL-8, IL-12, and TNF-α levels, and in the IL-10/IL-12 ratio; changes in symptoms: number of bowel habits, urgency, straining, sense of incomplete evacuation, stool form (Bristol stool form scale) [20], abdominal pain, and bloating/flatulence; changes in histology; QOL; and safety.

Isolation of peripheral blood mononuclear cells and cytokine analysis

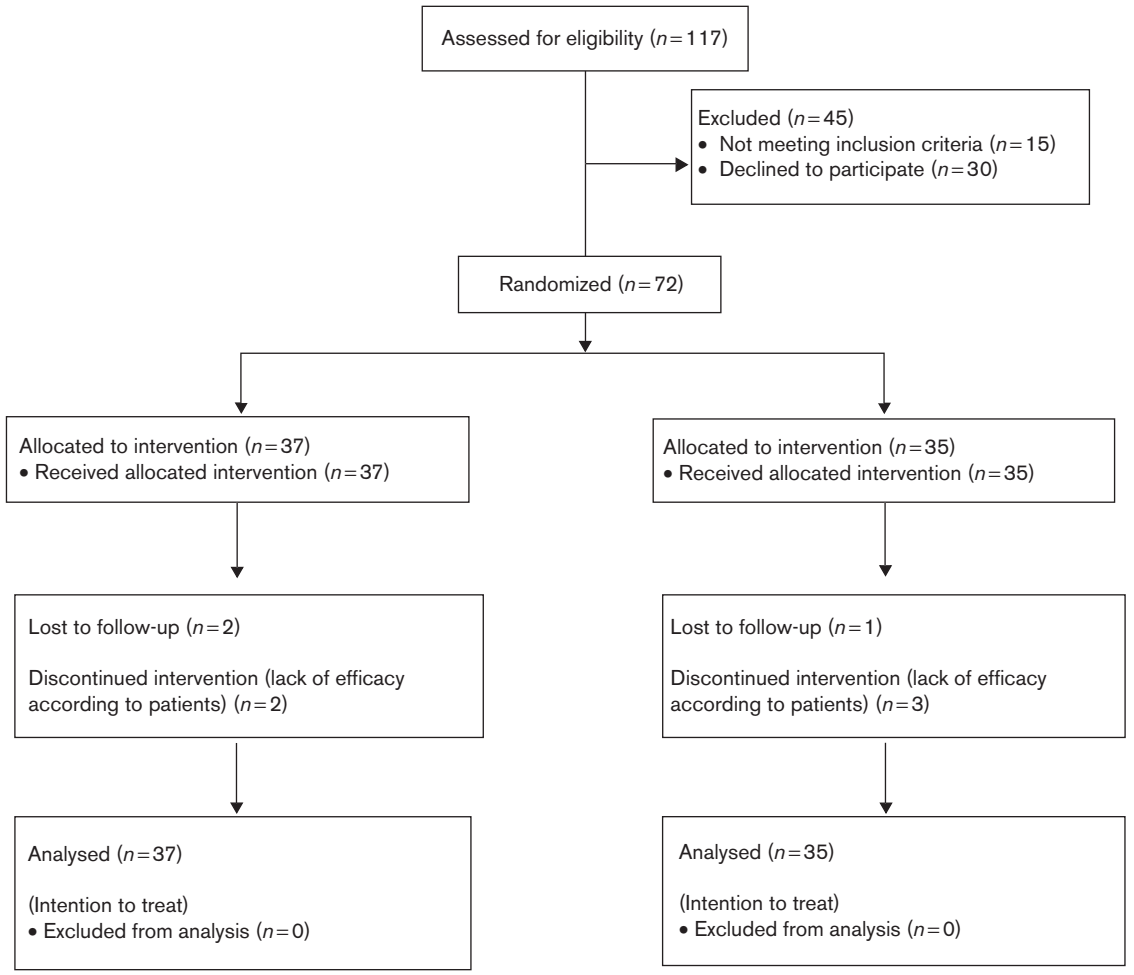
Blood samples from patients with IBS (∼4 ml) were collected in sterile EDTA-containing tubes. Peripheral blood mononuclear cells (PBMCs) were isolated using Histopaque (Sigma-Aldrich, St Louis, Missouri, USA) according to the density gradient centrifugation method [21]. The PBMCs collected were washed twice with Roswell Park Memorial Institute (RPMI 1640) culture medium. Cell suspension (0.5 ml) was added to wells of a 24-well tissue culture plate at a concentration of 10^6/ml in culture medium containing RPMI supplemented with 10% heat-deactivated fetal bovine serum, 100 U/ml penicillin–streptomycin, and 2.5 μg/ml of fungizone at 37°C in a CO_2 incubator containing 5% CO_2, 95% air, and 100% humidity. The supernatant was removed after 3 days and stored at −70°C until further testing.

Analysis of day 3 PBMCs culture supernatant provided accurate information for the detection of cytokine profiles of cell-mediated immune responses. Many cytokines are known to increase continuously, but TNF-α reaches a peak at day 3 [22]. Cell culture supernatants were harvested and analyzed for cytokines IL-8, IL-10, IL-12, and TNF-α in duplicate using enzyme-linked immunosorbent assay techniques with commercially available kits (BD OptEIA; Becton Dickinson, Franklin Lakes, New Jersey, USA) or were frozen for later analysis. All cytokine assays were calibrated against the WHO international standards by the kit manufacturer. The lower limits of detection for the individual assays are as follows: IL-8, 0.8 pg/ml; IL-10, 2 pg/ml; IL-12, 4 pg/ml; and TNF-α, 2 pg/ml.

Biopsy cytokines using real-time quantitative PCR with SYBR Green

Intestinal biopsy specimens obtained for RNA extraction were collected in an eppendorf containing TRIzol Reagent (Invitrogen Corporation, Carlsbad, California, USA) and stored in a liquid nitrogen container for transport to the laboratory and stored at −70°C until further use. Total RNA was extracted from endoscopic biopsy samples of colonic mucosa using the TRIzol method described previously. Reverse transcription of the extracted RNA was performed using RNase H-deficient reverse transcriptase (Superscript II; Life Technologies, Foster City, California, USA) and oligo (dT) primers (Life Technologies). Aliquots (2 µl) of reverse transcription reaction mixture (20 µl) were used for the quantification of IL-12, IL-10, IL-8 TNF-α, and GAPDH gene expression by real-time PCR assays [23]. The SABG QRT PCR was used to quantify IL-12, IL-10, IL-8, TNF-α, and GAPDH gene expression (PE Applied Biosystems, Foster City, California, USA). The PCR reactions were performed using the SBG PCR kit (PE Applied Biosystems) as described previously [23]. After activation for 10 min at 95°C, 40 cycles of 15 s at 95°C and 1 min at 62°C were carried out in an iCycler (Bio-Rad, Berkeley, California, USA). Real-time

Fig. 1



CONSORT diagram of the study population.

Table 1 Baseline characteristics of IBS patients

	Saccharomyces boulardii (*N*=37)	Placebo (*N*=35)	*P* value
Sex (M/F)	27/10	26/9	0.899
Age (years)	37.7±11.6	33.0±12.0	0.093
Hemoglobin (g/dl)	13.7±1.5	14.0±1.6	0.391
TLC ($\times 10^9$/l)	9.5±11.8	8.5±7.0	0.657
Platelets ($\times 10^9$/l)	246±60	221±58	0.076
Creatinine (mg/dl)	0.95±0.17	0.91±0.17	0.286
IgA (g/l)	2.6±1.9	3.4±4.7	0.346
IgG (g/l)	7.8±5.3	7.6±4.9	0.833
Positivity to breath hydrogen test (*N*)	2	0	0.493
Histopathology			
Lymphocyte infiltrate: mild vs. moderate	28/9	27/8	0.553
Plasma cell infiltrate: mild vs. moderate	30/07	29/06	0.545
Neutrophil infiltrate (mild)	20	21	0.714
Eosinophil infiltrate (mild)	18	14	0.308
Flattening of cells	02	02	0.671
Epithelial mitosis (mild)	16/21	06/29	**0.022**
Goblet cell depletion	16	14	0.484
Intraepithelial lymphocytes	10	05	0.249
Paneth cell hyperplasia	01	00	1.000
Blood cytokines (pg/ml)			
IL-8	13.02±4.27	12.13±3.69	0.345
IL-10	15.55±1.95	15.97±2.09	0.381
IL-12	5.37±1.17	5.16±1.14	0.447
TNF-α	8.62±4.12	8.50±4.05	0.901
IL-10/IL-12 ratio	3.01±0.68	3.27±0.92	0.185
Tissue cytokines (pg/ml)			
IL-8	0.467±0.660	0.462±1.687	0.985
IL-10	1.289±2.559	2.372±4.546	0.222
IL-12	0.452±0.551	0.550±0.398	0.392
TNF-α	0.604±0.461	0.738±0.207	0.115
IL-10/IL-12 ratio	14.660±56.742	6.374±16.485	0.409

F, female; IBS, irritable bowel syndrome; IgA, immunoglobulin A; IgG, immunoglobulin G; IL, interleukin; M, male; TLC, total leucocyte count; TNF-α, tumor necrosis factor-α.
Bold indicates statistically significant values.

fluorescence measurements were recorded and the threshold cycle (C_t) value for each sample was calculated using the above sequence detector [24]. For IL-12, TNF-α, and GAPDH, standard curves of C_t values were obtained from real-time PCR of *pMFGhTNF*, *pBSKIhIL-12*, and PCRII GAPDH (reference plasmids). C_t values for IL-8, IL-10, IL-12, TNF-α, and GAPDH transcripts from clinical specimens were plotted on the standard curves, and the amounts (in pg) of each transcript were calculated. The amounts of IL-8, IL-10, IL-12, and TNF-α transcripts (pg) were expressed relative to that of GAPDH (pg). Each biopsy sample obtained from the same patient was tested in duplicate and the average of two C_t values was used in this study.

Colonic mucosal histology

The parameters evaluated were as follows: (i) infiltration of lymphocyte, plasma cell, neutrophil, and eosinophil (each evaluated independently), (ii) cell flattening, (iii) epithelial mitosis, (iv) goblet cell depletion, (v) intraepithelial lymphocytes, and (vi) Paneth cell hyperplasia. The degree of inflammation for each biopsy site was scored on a four-point scale as follows: (0) inactive/absent, (1) mild, (2) moderate, or (3) severe [25]. To prevent interobserver variation, all the biopsies were evaluated by a single expert pathologist who was blinded to the time of the biopsy and the treatment received.

Symptoms daily diaries

At baseline and every day throughout the 6-week intervention period, the participants recorded a diary the following eight IBS-related symptoms: number of bowel habits, presence/absence of urgency, straining, sense of incomplete evacuation, stool form (Bristol stool form scale [20]), abdominal pain, bloating/flatulence, and passage of mucus. The intensity of abdominal pain and bloating was measured on a four-point scale of 0–3. IBS symptom scores were expressed as weekly mean scores and symptom improvement was assessed as changes in the weekly mean scores.

Quality-of-life assessment

The IBS-QOL consists of 34 items, each with a five-point response scale [26]. The individual responses to the 34 items were summed. There were also eight subscale scores for the IBS-QOL (dysphoria, interference with activity, body image, health worry, food avoidance, social reaction, sexual, relationships). The IBS-QOL was interviewer administered. The pretreatment and post-treatment IBS-QOL scores were then compared within each treatment group. The overall scores and subscales of each IBS-QOL domain were compared.

Statistical analysis

At the time this exploratory study was planned, no previous study was available to document the effect of *S. boulardii* on cytokine levels in IBS-D. The sample size was arbitrarily fixed to 35 patients in each group. Statistical Package for Social Science, version 19.0 (IBM, New York, New York, USA) was used for data analysis. Data recorded in the symptom diary were analyzed and weekly means were determined. All analyses were carried out on the intention-to-treat population using the last observation carried forward principle. Intragroup changes for histology and cytokines were assessed from baseline to the end of the treatment (8th week) using the Wilcoxon signed rank test.

Continuous variables were represented as mean ± SD and categorical variables as numbers. χ^2-Tests and Fisher's exact test were used to determine the distribution of categorical variables between probiotic and placebo groups. To determine differences between groups for continuous normally distributed variables, Student's *t*-test was performed and non-normally distributed variables were compared using the Mann–Whitney *U*-test. Statistical significance was set up to 0.05.

Results

Patients

From January 2010 to December 2011, 117 patients were assessed for inclusion in the study; 30 declined participation

Table 2 Effect of treatment on cytokine levels in blood and colonic mucosa

	Saccharomyces boulardii		Placebo		Improvement		
	Baseline (W2)	End of treatment (W8)	Baseline (W2)	End of treatment (W8)	*Saccharomyces boulardii*	Placebo	*P* value improvement
Blood							
IL-8	13.02±4.27	9.74±3.00	12.13±3.69	12.07±3.39	3.28±4.74[a]	0.06±2.22[a]	**0.000**
IL-10	15.55±1.95	17.19±1.74	15.97±2.09	16.40±1.90	1.64±1.73[b]	0.42±1.37[b]	**0.002**
IL-12	5.37±1.17	5.52±1.20	5.16±1.14	4.79±1.23	−0.16±1.48[a]	0.37±1.13[a]	0.059
TNF-α	8.62±4.12	3.68±2.56	8.50±4.05	7.84±2.51	4.94±4.33[a]	0.66±3.39[a]	**0.000**
IL-10/IL-12	2.99±0.71	3.26±0.83	3.33±0.96	3.79±1.21	0.24±0.85[b]	0.41±1.06[b]	0.600
Colonic mucosa							
IL-8	0.47±0.66	0.18±0.18	0.46±1.69	1.26±2.26	0.29±0.67[a]	−0.80±1.81[a]	**0.000**
IL-10	1.29±2.56	3.31±2.16	2.37±4.55	1.78±2.01	2.02±3.10[b]	−0.57±4.53[b]	**0.002**
IL-12	0.45±0.55	0.52±0.59	0.55±0.40	0.66±0.50	−0.07±0.26[a]	−0.12±0.40[a]	0.693
TNF-α	0.60±0.46	0.37±0.28	0.74±0.21	0.70±0.64	0.23±0.38[a]	0.03±0.70[a]	0.389
IL-10/IL-12	14.66±56.74	38.44±74.84	6.38±16.49	4.27±5.80	23.78±53.87[b]	−2.11±16.30[b]	**0.004**

Cytokine levels are expressed as mean±SD.
Saccharomyces boulardii versus placebo *P* values were calculated using the Mann–Whitney test.
IL, interleukin; TNF-α, tumor necrosis factor-α.
[a]Improvement = W2 − W8.
[b]Improvement = W8 − W2.
Bold indicates statistically significant values.

and 15 did not fulfill the inclusion criteria. Seventy-two patients were randomized and received the allocated treatment, 37 in the *S. boulardii* group and 35 in the placebo group. Sixty-four patients completed the study; three patients were lost to follow-up and five withdrew their consent (Fig. 1).

Patient characteristics

Among the 72 patients included in the study, 53 were men (74%). The mean age of the patients was 37.7 and 33 years in the *S. boulardii* and the placebo arms, respectively. No significant differences were reported between the two groups with respect to serum chemistry determinations, serum levels of IgA, and IgG tissue transglutaminase antibodies, and positivity to the breath hydrogen test (Table 1). Histopathology and blood and tissue cytokine levels were also comparable.

Response to treatment

Blood and tissue cytokine levels

The variation between baseline and post-treatment (after a 6-week treatment) blood cytokine levels as well as cytokine mRNA expression in the colonic mucosa was calculated in the two groups. The *S. boulardii* group showed a decrease in the blood and tissue levels of proinflammatory cytokines IL-8 and TNF-α and an increase in the anti-inflammatory IL-10 levels. Moreover, the tissue IL-10/IL-12 ratio was increased in the *S. boulardii* group. In the placebo group, there were no significant changes in the blood and tissue levels of cytokines, except for a slight increase in the IL-10/IL-12 blood ratio and an increase in the tissue level of IL-8 (Table 2) (Fig. 2).

Intestinal histological changes

At baseline, patients presented mild histological changes indicative of low-grade inflammation, as expected in IBS patients with a normal colonoscopy. After a 6-week treatment (at week 8), an improvement in histological changes was noted in the *S. boulardii* group with respect to the following parameters: lymphocyte infiltrate ($P = 0.017$), neutrophil infiltrate ($P = 0.018$), epithelial mitosis ($P = 0.003$), and intraepithelial lymphocytes ($P = 0.024$). No such improvements were noted in the placebo group (Table 3).

IBS-related symptoms

Symptom improvement was calculated as the difference between the weekly mean scores calculated at the beginning of the study (week 2) and those calculated at the end of the study (week 8). Symptoms improved in both groups without statistical differences, except for abdominal pain, which was less severe at the end of treatment in the placebo group ($P = 0.005$) (Table 4).

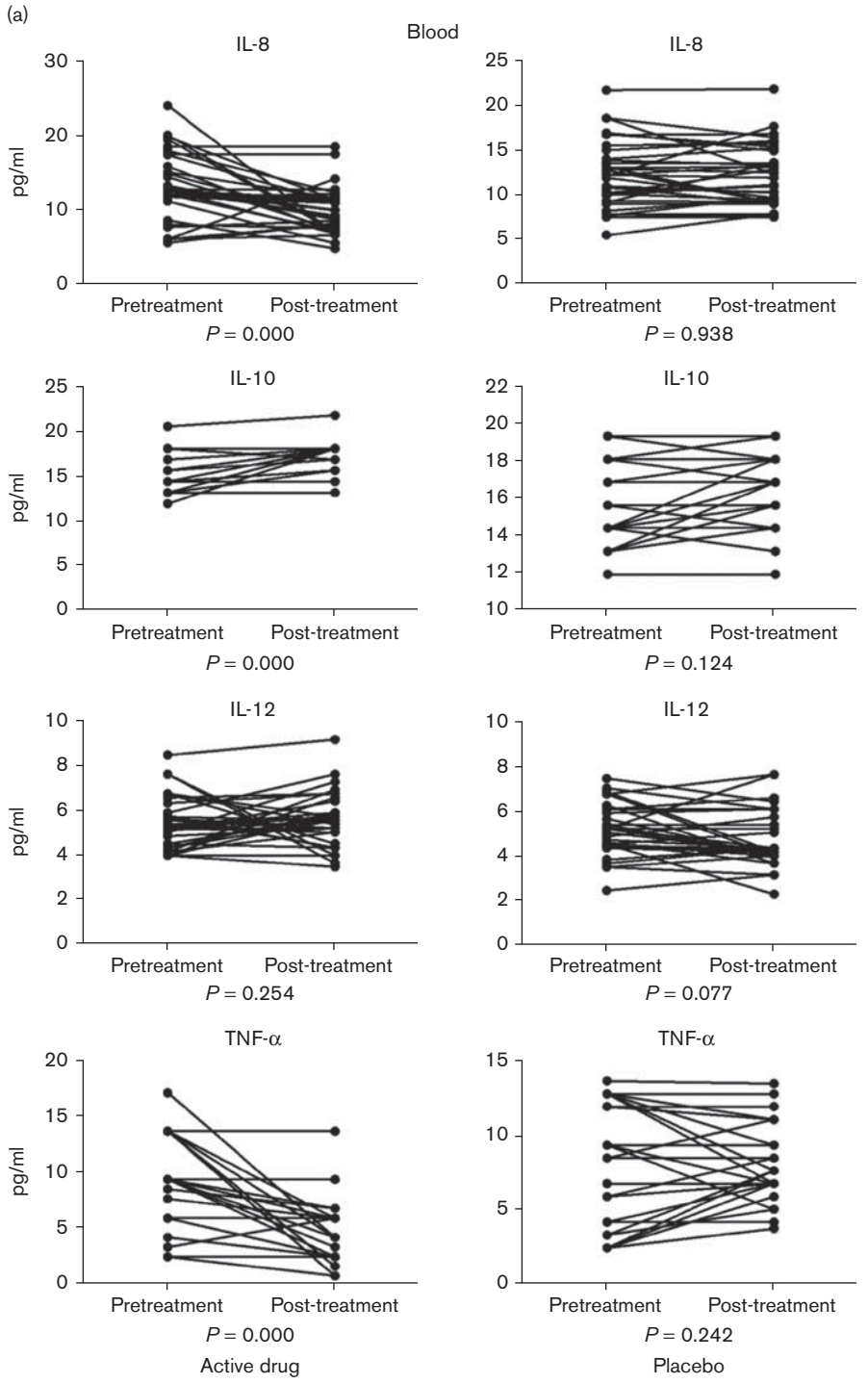
Quality of life

Pretreatment comparison of the *S. boulardii* group with the placebo group for the IBS-QOL did not show significant differences. The direct comparison of post-treatment IBS-QOL scores showed improvement in the body image ($P = 0.024$) and food avoidance ($P = 0.030$) favoring the *S. boulardii* group. In this group, there was an overall improvement in the QOL ($P = 0.002$). Moreover, of the eight domains, improvement was observed in dysphoria ($P = 0.001$), interference with activities ($P = 0.013$), health worry ($P = 0.030$), and food avoidance ($P = 0.005$). In the placebo group, there was an overall improvement ($P = 0.023$), but less marked than that in the probiotic group. There was an intragroup improvement in dysphoria ($P = 0.043$) and food avoidance ($P = 0.025$).

Safety

During the study, 19 patients in the *S. boulardii* group and 15 patients in the placebo group reported adverse

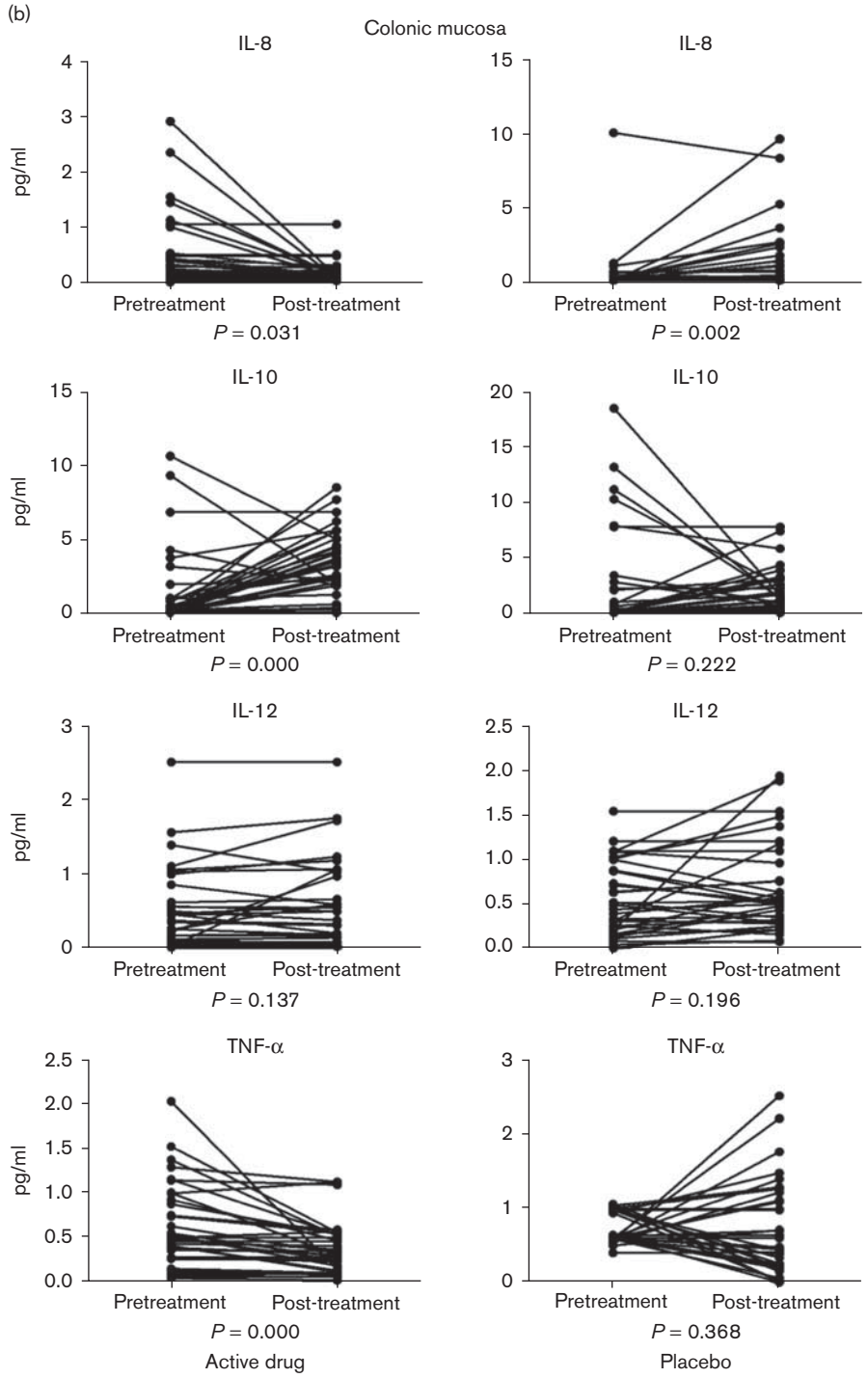
Fig. 2



(a) The effect of treatment on blood cytokine levels: active drug (*Saccharomyces boulardii*) versus placebo. *P* values by the Wilcoxon rank sum test (intention to treat). (b) The effect of treatment on tissue cytokine levels: active drug (*S. boulardii*) versus placebo. *P* values by the Wilcoxon rank sum test (intention to treat).

events (AE). The most common AEs were diarrhea, flatulence, bloating, sleepiness, abdominal pain, and heartburn. All were of mild to moderate intensity, and no significant difference was observed between the two study groups in the number and type of AEs. No clinically significant changes in full blood count, serum chemistry, or serum immunoglobulin levels were recorded in any of the patients during the study.

Fig. 2 (*Continued*)



Discussion

The therapies adopted for IBS are palliative and supportive, targeting individual symptoms in a diverse IBS population [27]. Alternative management of IBS is based on cognitive behavioral therapy, which seems to be effective and associated with improvement in IBS symptoms and QOL [28,29]. Several clinical trials have investigated the effects of probiotics on the main clinical and psychological aspects of IBS, but definitive conclusions are lacking because of the large variability among

Table 3 Effect of treatment on intestine histological changes

	Saccharomyces boulardii ($N=37$)			Placebo ($N=35$)		
Histological changes	Baseline (W2)	End of treatment (W8)	*P* value (W2 – W8)	Baseline (W2)	End of treatment (W8)	*P* value (W2 – W8)
Lymphocyte infiltrate						
Absent to mild/moderate	28/9	35/2	**0.017**	27/8	29/6	0.550
Plasma cell Infiltrate						
Absent to mild/moderate	30/07	32/5	0.754	29/6	29/6	1.000
Neutrophil infiltrate (mild)	20	10	**0.018**	21	14	0.094
Eosinophil infiltrate (mild)	18	15	0.640	14	13	0.806
Cells flattening	2	2	1.000	2	1	1.000
Epithelial mitosis (mild)	16	4	**0.003**	6	3	0.477
Goblet cell depletion	16	9	0.085	14	8	0.122
Intraepithelial lymphocytes	10	2	**0.024**	4	4	1.000
Paneth cell hyperplasia	1	0	1.000	0	0	1.000

P values by χ^2 or Fisher's exact test.
Bold indicates statistically significant values.

Table 4 Effect of treatment on IBS symptoms

	Saccharomyces boulardii ($N=37$)			Placebo ($N=35$)			
Symptom	Baseline (W2)	End of treatment (W8)	Improvement (W2 – W8)	Baseline (W2)	End of treatment (W8)	Improvement (W2 – W8)	*P* value improvement
Number of stools	2.768±1.363	2.373±1.242	0.395±1.331	3.421±1.727	2.659±1.229	0.761±1.625	0.200
Urgency	2.657±2.531	2.057±2.611	0.660±2.945	2.705±2.657	2.411±2.872	0.294±2.769	0.710
Straining	1.628±2.156	2.171±2.617	0.542±2.944	2.676±2.803	1.794±2.336	0.882±2.345	0.063
Sense of incomplete evacuation	3.714±3.204	3.285±2.706	0.428±0.890	3.352±2.509	4.352±2.942	0.305±0.526	0.056
Stool shape	3.632±1.586	3.625±1.225	0.006±1.221	4.415±1.281	3.991±1.165	0.423±1.203	0.076
Abdominal pain	1.122±1.328	1.079±1.021	0.042±0.890	0.851±0.624	0.546±0.555	0.305±0.526	0.005
Bloating	0.677±0.750	0.703±0.812	0.025±0.994	0.722±0.752	0.651±0.636	0.071±647	0.421
Passage of mucus	0.253±0.370	0.269±0.409	0.016±0.397	0.201±0.332	0.298±0.604	0.096±0.572	0.894

Symptom scores are expressed as weekly mean scores±SD.
Saccharomyces boulardii versus placebo *P* values were calculated using the Mann–Whitney test.
IBS, irritable bowel syndrome.

published results [30,31]. Most of these studies showed beneficial effects of probiotics, although specific IBS symptoms were improved in some trials, but not in others, and the therapeutic gain over placebo was modest [31]. The high-grade variability among the results may be attributable to the type of probiotic or probiotic combinations used as well as methodological differences between the trials and the outcomes assessed.

Our study showed that after treatment with *S. boulardii*, IBS-D patients' blood levels of proinflammatory cytokine IL-8 and TNF-α decreased, whereas tissue levels of anti-inflammatory cytokine IL-10 and the IL-10/IL-12 ratio increased significantly. These data are in good agreement with considerable evidence showing that *S. boulardii* exerts an anti-inflammatory effect. *S. boulardii* decreased the secretion of IL-8 during an *Escherichia coli* infection [14]. This agent prevented relapse in patients with Crohn's disease currently in remission [32] and benefited patients with ulcerative colitis currently presenting with moderate symptoms [33]. *S. boulardii* has been shown to exert anti-inflammatory effects in a rat model of inflammatory bowel disease as well [34]. *S. boulardii* treatment limited the infiltration of T-helper 1 cells in the inflamed colon and the amplification of inflammation induced by proinflammatory cytokine production. A study indicated that *S. boulardii* exerted part of its anti-inflammatory potential through modulation of dendritic cell phenotype, function, and migration by inhibition of their immune response to bacterial microbial antigens such as lipopolysaccharide [35]. The culture of primary human myeloid dendritic cells in the presence of *S. boulardii* culture supernatant significantly reduced the secretion of TNF-α and IL-6, whereas the secretion of anti-inflammatory IL-10 increased [35]. It has also been shown that *S. boulardii* binds the invading microbe, for example *E. coli* and *Salmonella typhimurium*, to its cell wall by lectin receptors and prevents them from attaching to the brush border, thus aiding their elimination from the body during the bowel movement [36].

The above observations as well as our data support the hypothesis that the systemic and gastrointestinal immune activation could play a role in the pathophysiology and symptom generation of IBS-D [5] This hypothesis was derived from the observation that increased levels of proinflammatory cytokines are detected systematically in the peripheral blood of IBS-D patients [37]. Moreover, mucosal mediators released by immune cells led to increased activation of sensory pain pathways when applied to isolated intestinal preparations from rats [38]. Reduction of the inflammatory mediators should inhibit

this supposed physiological pathway, and the anti-inflammatory effects of *S. boulardii* treatment observed in our study could presumably be associated with significant improvement in IBS-D symptoms. Nevertheless, this was not the case. After a 6-week treatment period, IBS-D symptoms improved in both the *S. boulardii* and the placebo groups, without significant differences between the two groups, and abdominal pain was even more improved with the placebo as compared with the *S. boulardii* group. Even if apparently incoherent, our data are consistent with results from another clinical trial showing that *S. boulardii* failed to significantly improve IBS-D symptoms relative to placebo [39]. Despite this, there was a significant improvement in the QOL in the *S. boulardii* group compared with the placebo group. An explanation for these results might be the fact that *S. boulardii* also exerts systemic effects, such as inhibition of proinflammatory cytokines (which in turn modulate nervous system activity) or increases in tryptophan concentration, thus improving the general health state of IBS-D patients [39]. A study evaluating the effects of another probiotic, *Bifidobacterium infantis*, reported evidence of a possible antidepressant effect of this probiotic in rats with a significant reduction in proinflammatory cytokine secretion and an increase in the plasma concentration of the serotoninergic precursor tryptophan [40].

It is worth mentioning that both *S. boulardii* and placebo groups received ispaghula husk together with the investigational product. Ispaghula husk is traditionally used in IBS-D patients. It is a prebiotic with already defined anti-inflammatory properties [41–43]. This is the first trial associating *S. boulardii* and ispaghula husk in the treatment of IBS-D patients. The potential interaction between the two agents should be investigated in a three-arm study.

A strong placebo effect in our study is evident, as in all IBS-D studies. However, this was a pilot study. The sample size was not adequate and the study was apparently not powered enough to show an effect on symptoms and draw any definite conclusions. Another limitation of this study may be the methodology used to define symptom scores. Each IBS-D symptom has been defined according to a severity scale that was different, in the number and the type of points, from those used for other symptoms.

Conclusion

This exploratory trial showed that the probiotic *S. boulardii* administered in combination with ispaghula husk was more effective than ispaghula husk alone in improving the cytokine profile of patients with IBS-D, although not in terms of bowel-related symptoms. The results need to be validated in larger studies.

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Conflicts of interest

There are no conflicts of interest.

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