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Treatment of Relapsing Mild-to-Moderate Ulcerative Colitis With the Probiotic VSL#3 as Adjunctive to a Standard Pharmaceutical Treatment: A Double-Blind, Randomized, Placebo-Controlled Study

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OBJECTIVES: **VSL#3 is a high-potency probiotic mixture that has been used successfully in the treatment of pouchitis. The primary end point of the study was to assess the effects of supplementation with VSL#3 in patients affected by relapsing ulcerative colitis (UC) who are already under treatment with 5-aminosalicylic acid (ASA) and/or immunosuppressants at stable doses.**

METHODS: **A total of 144 consecutive patients were randomly treated for 8 weeks with VSL#3 at a dose of 3,600 billion CFU/day (71 patients) or with placebo (73 patients).**

RESULTS: **In all, 65 patients in the VSL#3 group and 66 patients in the placebo group completed the study. The decrease in ulcerative colitis disease activity index (UCDAI) scores of 50% or more was higher in the VSL#3 group than in the placebo group (63.1 vs. 40.8; per protocol (PP) $P=0.010$, confidence interval $(CI)_{95\%}$ 0.51–0.74; intention to treat (ITT) $P=0.031$, $CI_{95\%}$ 0.47–0.69). Significant results with VSL#3 were recorded in an improvement of three points or more in the UCDAI score (60.5% vs. 41.4%; PP $P=0.017$, $CI_{95\%}$ 0.51–0.74; ITT $P=0.046$, $CI_{95\%}$ 0.47–0.69) and in rectal bleeding (PP $P=0.014$, $CI_{95\%}$ 0.46–0.70; ITT $P=0.036$, $CI_{95\%}$ 0.41–0.65), whereas stool frequency (PP $P=0.202$, $CI_{95\%}$ 0.39–0.63; ITT $P=0.229$, $CI_{95\%}$ 0.35–0.57), physician's rate of disease activity (PP $P=0.088$, $CI_{95\%}$ 0.34–0.58; ITT $P=0.168$, $CI_{95\%}$ 0.31–0.53), and endoscopic scores (PP $P=0.086$, $CI_{95\%}$ 0.74–0.92; ITT $P=0.366$, $CI_{95\%}$ 0.66–0.86) did not show statistical differences. Remission was higher in the VSL#3 group than in the placebo group (47.7% vs. 32.4%; PP $P=0.069$, $CI_{95\%}$ 0.36–0.60; ITT $P=0.132$, $CI_{95\%}$ 0.33–0.56). Eight patients on VSL#3 (11.2%) and nine patients on placebo (12.3%) reported mild side effects.**

CONCLUSIONS: **VSL#3 supplementation is safe and able to reduce UCDAI scores in patients affected by relapsing mild-to-moderate UC who are under treatment with 5-ASA and/or immunosuppressants. Moreover, VSL#3 improves rectal bleeding and seems to reinduce remission in relapsing UC patients after 8 weeks of treatment, although these parameters do not reach statistical significance.**

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INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disease of the colon characterized by bloody diarrhea and abdominal pain. Despite recent advances in the understanding of the genetics, immune and inflammatory mechanisms, as well as potential environmental factors that contribute to the disease, an exact pathogenesis remains elusive. Hence, the treatment is aimed at modifying the pathogenic mechanisms involved, mostly by using anti-inflammatory drugs such as mesalazine, corticosteroids, immunosuppressant agents, or biologics (1).

Recently, modulation of the gut flora has been suggested as an approach to manage UC. The role of microbiome in inflammatory bowel disease is clearly supported by many experimental observations. Gut flora can be modified either by antibiotics or by probiotics. Antibiotics are not good candidates for patients with chronic disorders because of antibiotic resistance, potential side effects, and ecological concerns.

Probiotics have proven to be effective in the management of pouchitis (3,4), and preliminary data are available for the treatment of UC (5,6), but strong data are still lacking in both UC and Crohn's disease. In particular, there is limited evidence that probiotics, in addition to standard therapy, may provide benefits in terms of reduction of disease activity in patients with mild to moderately active UC because of a lack of well-designed, large, randomized, placebo-controlled trials (7).

The present study has been conducted with VSL#3, a product that has proven to be effective for the treatment and prevention of pouchitis (3). The aim of this investigation was to assess whether, by adding VSL#3 to the current standard treatment of patients with mild-to-moderate UC, it would be possible to decrease the ulcerative colitis disease activity index (UCDAI) score by at least 50% and improve some of the symptoms associated with UC. Positive results would encourage a new approach in managing UC patients to avoid or delay step-up therapies with drugs burdened by potentially serious side effects.

METHODS

A multicenter, double-blind, randomized, placebo-controlled, parallel study was conducted in a population of UC patients with relapsing disease of mild-to-moderate severity.

We defined "relapsing mild-to-moderate UC" as a disease showing symptomatic recurrence after at least 6 months of remission (8), with a new increase in UCDAI (see **Table 1**) of at least three points (between three and eight) (9).

The protocol was approved by the Investigational Review Board of each center. All patients gave written informed consent for their participation.

Sample size

The sample size was based on a power of 80% and a statistical significance (α) of 95% ($P=0.05$). This calculation was based on the assumption that a response to treatment at 8 weeks, such as with oral 5-aminosalicylic acid (ASA) preparations, was expected to occur in 71% of patients treated with VSL#3 compared with a 40% expected response for patients treated with placebo. This assumed that the probiotic is as effective as oral 5-aminosalycilic acid. Therefore, 59 patients were required in each group, with an additional 15% for dropouts and 5% for patients failing to undergo final endoscopic assessment; hence a total of 144 patients were planned for the trial.

Table 1. Ulcerative colitis (UC) disease activity index

1. Stool frequency	
Normal	0
1–2 Stools/day > normal	1
3–4 Stools/day > normal	2
>4 Stools/day > normal	3
2. Rectal bleeding	
None	0
Streaks of blood	1
Obvious blood	2
Mostly blood	3
3. Mucosal appearance	
Normal	0
Mild friability	1
Moderate friability	2
Exudation, spontaneous bleeding	3
4. Physician's rating of disease activity	
Normal	0
Mild	1
Moderate	2
Severe	3

The index assesses four variables, which include stool frequency, severity of bleeding, colonic mucosal appearance, and the physician's overall assessment of disease activity.
Each variable is scored from 0–3 so that the total index score ranges from 0–12; 0–2: remission; 3–6: mild; 7–10: moderate; >10: severe UC.

Study procedures

The study procedures were conducted for each patient enrolled in the study.

At the screening visit, each patient's demographic characteristics, medical history, and current medications were recorded. β-Chorionic gonadotropin hormone was also assessed in women of childbearing age and was collected and analyzed to exclude pregnancy.

Eligible patients were randomly assigned to receive either VSL#3 or placebo twice daily for 8 weeks. The study product, VSL#3, was provided in plastic sealed individual dose sachets. Placebo was supplied in identical sachets. Patients were asked to take the contents of the sachets in the morning and evening. Individual disease activity quantified by the patient's UCDAI was calculated. The UCDAI was calculated by the investigator, who added the individual scores of the four parameters (bowel frequency, rectal bleeding, endoscopic score, and physician's rating of severity). At each visit, a detailed physical

examination and history were performed. All adverse events were documented, classified, and graded. Study participants were supplied with diary cards to assess and record their symptoms (stool frequency, bleeding, and abdominal pain) on a daily basis. Participants' compliance was assessed by the investigators, who counted the unused sachets that the patients were requested to bring back at week 8.

Inclusion criteria

Patients had to meet all the inclusion criteria described in **Table 2** to be eligible for participation. Moreover, women who had a negative pregnancy test at the screening visit and agreed to use a valid contraceptive method for the duration of the study, as well as patients not requiring hospitalization and patients willing and able to provide written informed consent, were considered eligible for inclusion in the study.

Exclusion criteria

Patients who met any of the exclusion criteria as described in **Table 3** were not enrolled in this study.

Significant hepatic, renal, endocrine, respiratory, neurological, or cardiovascular diseases, as determined by the investigator, were also considered as exclusion criteria. Other exclusion criteria that were also taken into consideration included the following:

- a history of severe adverse reaction or known hypersensitivity to maltose and/or silicon dioxide;
- patients requiring hospitalization;
- use of any investigational drug and/or participation in any clinical trial within 3 months before entering this study;
- inability to give a valid written informed consent or to properly follow the protocol.

Treatment

Patients meeting the inclusion criteria were randomly assigned to one of the two groups of treatment and received the product for 8 weeks in addition to their standard pharmaceutical therapy (5-ASA and/or immunosuppressant). VSL#3 consists of sachets, each containing 900 billion viable lyophilized bacteria, comprising four strains of lactobacilli (*L. paracasei*, *L. plantarum*, *L. acidophilus*, and *L. delbrueckii* subsp *bulgaricus*), three strains of bifidobacteria (*B. longum*, *B. breve*, and *B. infantis*), and one strain of *Streptococcus thermophilus* (VSL Pharmaceuticals, MD). The daily dose was two sachets twice a day taken orally (3,600 billion bacteria per day). The patient was asked to mix the contents of the sachets in a glass of cold water or in yogurt. Hot beverages were excluded, as an elevated temperature may inactivate the bacteria. The placebo was in the form of identical sachets that did not contain any lyophilized bacteria.

Concomitant treatments

Patients who were taking maintenance oral 5-ASA and/or azathioprine or 6-mercapropurine continued to do so at stable doses. The 5-ASA doses had to be fixed for 4 weeks and azathioprine or 6-mercaptopurine doses were fixed for at least 3 months before study entry, and had to be maintained at the same dose throughout the study. Any change in dosing of oral 5-ASA or in dosing of oral 6-mercaptopurine and azathioprine drugs throughout the 8-week study period was considered as a protocol violation.

Rectally administered medications, steroids, antibiotics, probiotics, and antidiarrheal drugs were not allowed, nor were any fruits, vegetables, milk, or fresh milk by-products.

VSL#3 supplementation had to be interrupted for a minimum of 14 days before inclusion in the study.

Primary end point

The primary end point was the evaluation of the beneficial effects of food supplementation with VSL#3 in relapsing mild-to-moderate UC patients, assessed by a decrease in the UCDAI of 50% or more, from baseline to week 8.

Table 2. Inclusion criteria

Male and female patients aged more than 18 years;
Diagnosis of UC established by previous colonoscopy, with consistent histology and clinical course;
UC involving at least the rectosigmoid region; activity confirmed by colonoscopy at the beginning of the study;
Mild-to-moderate relapsing UC, defined as a UCDAI score ranging from three to eight;
Symptoms (relapsing episodes) for less than 4 weeks before study entry;
A minimum endoscopic score of three on the UCDAI at screening (mucosal appearance);
Use of oral 5-ASA at least 4 weeks before study entry at a stable dose (mesalazine at least 1.6 g/day or balsalazide at least 4.5 g/day) and/or use of azathioprine (at least 1.5 mg/kg/day) or 6-mercaptopurine (at least 1 mg/kg/day) at least 3 months before study entry at a stable dose.

ASA, aminosalicylic acid; UC, ulcerative colitis; UCDAI, ulcerative colitis disease activity index.

Table 3. Exclusion Criteria

Crohn's disease or pouchitis;
A UCDAI score greater than eight (need for emergency surgery or the presence of severe disease);
Use of oral steroids within the last 4 weeks before study entry;
Use of antibiotics within the last 2 weeks before study entry;
Change in dose of oral 5-ASA within the last 4 weeks before study entry and throughout the 8-week study period or a change in dose of oral 6-mercaptopurine and azathioprine drugs within the last 3 months before the study;
Use of rectal 5-ASA or steroids within 1 week before entering the study or throughout the 8-week study period;
Use of probiotic preparations either prescribed or over-the-counter within 2 weeks before study entry;
Use of NSAIDs for 1 week before and throughout the 8-week study period.

ASA, aminosalicylic acid; NSAID, non-steroidal anti-inflammatory drug; UCDAI, ulcerative colitis disease activity index.

Secondary end points

Secondary end points were the possible beneficial effects of VSL#3 on the following:

- activity of relapsing UC;
- remission, considered as UCDAI ≤2, assessed at week 8;
- improvement in endoscopic scores, assessed by the endoscopic subgroup score of the UCDAI at week 8;
- change in objective symptoms (rectal bleeding and stool frequency);
- change in subjective symptoms (physician rating of disease activity);
- lack of beneficial effects, defined by the need for pharmacological treatment or inability to remain on the study regimen until week 8.

Randomization

Each center enrolled patients according to the randomization list. Patients who fulfilled the eligibility criteria specified above were randomly assigned to receive VSL#3 or placebo in a random order, using only one randomization list. The randomization number was strictly given according to the order of the patient's enrollment, assigning each patient the first available number on the randomization list. The randomization number, or the reason for not enrolling the patient, was reported for each patient in the appropriate forms. Randomization was carried out in a double-blind manner in blocks of four patients using 1:1 allocation to the two groups.

Assessment of compliance

The investigators assessed compliance by checking the number of unused sachets that the patients brought back at each visit.

Statistical assessment

Baseline characteristics of patients were compared using Student's *t*-test for independent samples or Pearson's χ^2-test as appropriate. Values of $P \geq 0.05$ were considered statistically significant. Pearson's χ^2-test was used to compare the UCDAI score at each visit with the basal visit score after adjustment of data using the last-observation-carried-forward method. Comparison of stool frequency, rectal bleeding, and mucosal appearance at each time between treatment groups and at each visit vs. the basal value was performed using Pearson's χ^2-test. The 95% confidence interval (CI) was also assessed.

A multivariate analysis was also performed. The general linear model multivariate procedure is based on a general linear model in which factors and covariates are assumed to have linear relationships to the dependent variables. As dependent variables, we chose UCDAI overall response at visit three (increase of 50% or more in the UCDAI score compared with the screening score) and disease extension at visit three (left-sided colitis, distal colitis, pancolitis). Fixed factors categorical predictors were selected as factors in the model (treatment with placebo or VLS#3, and concomitant treatment with or without the combination of 5-ASA and immunosuppressors). The general linear model multivariate procedure assumes that all model factors are fixed, i.e., they are generally thought of as variables, the values of interest of which are all represented in the data file, usually by design.

The statistical analysis of all the data sets pertaining to efficacy (specifically, primary and secondary end points) and safety (specifically, serious adverse events as defined by federal guidelines) has been independently performed by a biostatistician who is not employed by the corporate entity. The corresponding author had full access to all data and takes full responsibility for the veracity of the data and analysis.

RESULTS

Participant flow

A total of 144 patients (71 in the VSL#3 group and 73 in the placebo group) were enrolled. No patient was withdrawn before treatment assignment (see **Figure 1**).

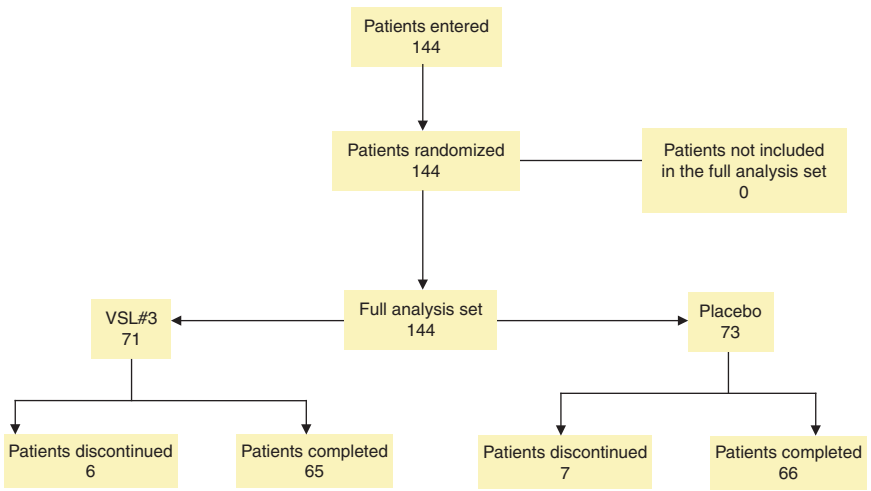

Figure 1. Patient disposition.

Baseline data

The clinical characteristics of patients in the two groups were comparable (**Table 4**). No significant differences were identified in terms of demographic characteristics (mean age, male–female ratio, weight, height, and mean UCDAI).

Clinical response

The main clinical outcomes of the study according to per-protocol (PP) and intention-to-treat (ITT) methods are shown in **Table 5**.

Table 4. Patient demographic and baseline characteristics

Characteristic	VSL#3	Placebo
Gender (male:female)	49:22 (69%)	44:29 (60.3%)
Age in years (mean ± s.d.)	47.7 ± 14.1	46.4 ± 14.4
Number of previous relapses (mean ± s.d.)	2.24 ± 1.05	2.37 ± 1.04
UCDAI at entry (mean ± s.d.)	5.52 ± 1.33	5.42 ± 1.43
Disease extent (number of patients) (%)		
Proctosigmoiditis	36 (50.7%)	38 (52.1%)
Left-sided colitis	24 (33.8%)	21 (28.8%)
Pancolitis	11 (15.5%)	14 (19.1%)
Concomitant medications		
Mesalamine alone (mean/median ± s.d.)	65 (91.55%) (2.08/2.4 ± 0.39)	69 (94.52%) (2.08/2.4 ± 0.40)
Balsalazide alone (mean/median ± s.d.)	2 (2.82%) (4.5/4.5)	2 (2.74%) (4.5/4.5)
Azathioprine alone (mean/median ± s.d.)	1 (1.23%) (1.62/1.5 ± 0.25)	0 (0%)
Methotrexate alone (mean/median ± s.d.)	1 (1.23%) (15 mg i.m./week)	0 (0%)
No medications	0 (0%)	0 (0%)
Combinations of drugs		
Mesalamine + azathioprine (mean/median ± s.d.)	2 (3.90%) (2.08/2.4 ± 0.39) + (1.62/1.5 ± 0.25)	2 (2.74%) (2.08/2.4 ± 0.40) + 1.75/1.75 ± 0.25
Balsalazide + azathioprine	0 (0%)	0 (0%)
Balsalazide + methotrexate	0 (0%)	0 (0%)
Total	71 (100%)	73 (100%)

i.m., intra-muscular; UCDAI, ulcerative colitis disease activity index.

Six patients in the VSL#3 group withdrew during the follow-up, two had protocol violations (these patients took beclomethasone dipropionate and prednisone), two withdrew their informed consent, and three were lost to follow-up. Among the seven patients in the placebo group who withdrew during the follow-up, five patients experienced a worsening of symptoms, one was lost to follow-up, and one withdrew informed consent (see **Table 6**).

Primary end point

Overall, VSL#3 was significantly superior to placebo in reducing the disease activity of mild-to-moderate UC. Significantly more patients in the VSL#3 group experienced an improvement in their UCDAI score of at least 50% at the end of 8 weeks than those who received the placebo (41 (63.1%) vs. 29 (40.8%), respectively; PP $P = 0.010$, $CI_{95\%}$ 0.51–0.74; ITT $P = 0.031$, $CI_{95\%}$ 0.47–0.69) (see **Figure 2**).

To evaluate a more homogeneous set of patients, we also excluded patients who were under immunosuppressive treatment from the final evaluation. However, no statistical difference was found because VSL#3 was still significantly better in improving UCDAI scores of at least 50% at the end of 8 weeks than placebo (37 (56.1%) vs. 25 (36.2%), respectively; PP $P = 0.008$; ITT $P = 0.025$).

Secondary end points

Similarly, a significantly higher number of patients in the VSL#3 group had a decrease of three or more points in their UCDAI score from baseline to week 8 than the placebo group (39 (60%) vs. 29 (43.94%), respectively; PP $P = 0.017$, $CI_{95\%}$ 0.51–0.74; ITT $P = 0.046$, $CI_{95\%}$ 0.47–0.69) (see **Figure 2**).

Regarding the induction of remission, 31 (47.7%) patients in the VLS#3 group and 23 (32.4%) patients in the placebo group experienced remission by the end of 8 weeks; although a Δ value of 15.3% was observed, this difference was not statistically significant (PP $P = 0.069$, $CI_{95\%}$ 0.36–0.60; ITT $P = 0.132$, $CI_{95\%}$ 0.33–0.56) (see **Figure 2**). None of the parameters assessed in the multivariate analysis was found to have a significant role in influencing remission.

To evaluate a more homogeneous set of patients, we also excluded patients under immunosuppressive treatment from the final evaluation. However, no difference was found because VSL#3 was still better in obtaining remission at the end of 8 weeks than placebo, and the result did not reach statistical significance (28 (42.4%) vs. 20 (29%), respectively; PP $P = 0.067$; ITT $P = 0.110$).

Table 5. Clinical outcomes

	Per-protocol			Intention-to-treat		
	VSL#3	Placebo	*P* value	VSL#3	Placebo	*P* value
≥50% Improvement in UCDAI (week 8)	41	29	0.010	41	29	0.031
≥3 Decrease in UCDAI score (week 8)	39	29	0.017	39	28	0.046
Remission (week 8)	31	23	0.069	31	23	0.132

UCDAI, ulcerative colitis disease activity index.

Table 6. Reasons for discontinuation of treatment

	VSL#3 number of patients (%)	Placebo number of patients (%)
Lack of efficacy	0 (0.0)	5 (6.8)
Clinical episode	0 (0.0)	0 (0.0)
Abnormal laboratory result	0 (0.0)	0 (0.0)
Death	0 (0.0)	0 (0.0)
Protocol violation	2 (1.4)	0 (0.0)
Lost to follow-up	3 (4.2)	1 (1.4)
Protocol interim criteria not met	0 (0.0)	0 (0.0)
Patient's consent withdrawn	2 (2.8)	1 (1.4)

It is interesting that **Tables 7** and **8** show that none of the patients in the VSL#3 group experienced a worsening of symptoms during the follow-up, whereas several patients in the placebo group showed a worsening of symptoms, and five of them had to be withdrawn from the study.

Patients receiving VSL#3 had a significant reduction in rectal bleeding (PP $P=0.014$, $CI_{95\%}$ 0.46–0.70; ITT $P=0.036$, $CI_{95\%}$ 0.41–0.65). On the other hand, we did not find any significant difference in stool frequency (PP $P=0.202$, $CI_{95\%}$ 0.39–0.63; ITT $P=0.229$, $CI_{95\%}$ 0.35–0.57), physician's rating of disease activity (PP $P=0.088$, $CI_{95\%}$ 0.34–0.58; ITT $P=0.168$, $CI_{95\%}$ 0.31–0.53), or mean endoscopy scores (PP $P=0.086$, $CI_{95\%}$ 0.74–0.92; ITT $P=0.366$, $CI_{95\%}$ 0.66–0.86) (see **Figure 3**).

Safety and tolerability

No major adverse event was reported. Eight patients on VSL#3 (11.2%) reported mild side effects (one patient reported dizziness, one reported a flu-like syndrome, and six initially complained of abdominal bloating and discomfort), whereas nine patients on placebo (12.3%) reported mild side effects (one reported a fever, one had cystitis, three had abdominal bloating, and four patients had an unpleasant taste in their mouth).

DISCUSSION

UC is a chronic inflammatory disease of the colon involving still largely unknown interactions between genetic, environmental, and immunological factors.

UC is characterized by flare-ups of inflammation and periods of remission or quiescence that can be achieved or maintained by drugs having, as a common denominator, anti-inflammatory and/or immunosuppressive properties (5-aminosalicylates, 6-mercaptopurine, azathioprine, and anti-TNFα antibodies). If left without any maintenance drug, about 70% of patients will relapse within 12 months (2), and many patients on maintenance drugs will still require step-up therapy.

After the initial report by Gionchetti *et al.* (3) on pouchitis, followed by other confirmatory clinical studies, it is now accepted that VSL#3, a combination of probiotic bacteria, can place this disease in remission or quiescence in a large number of patients with a J-pouch, as recommended in the guidelines of international gastroenterological associations (10,11).

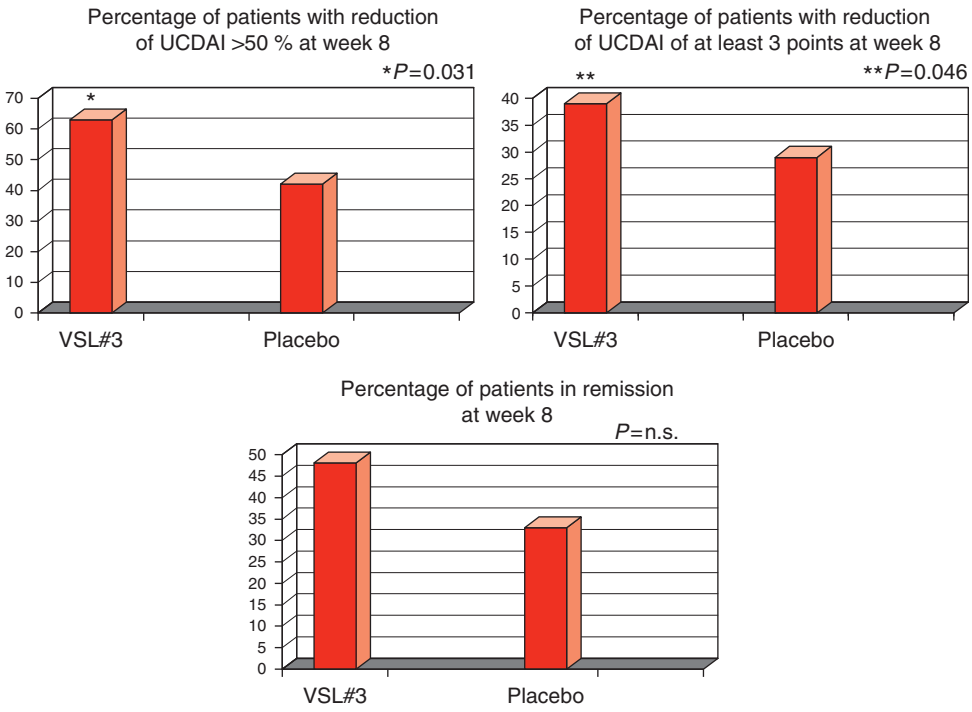

Figure 2. Percentage of patients with reduction of ulcerative colitis disease activity index (UCDAI) > 50% or of at least three points, and patients in remission at week 8 (on intention-to-treat analysis). n.s., not significant.

Table 7. Overall UCDAI response after 8 weeks (per-protocol analysis)

UCDAI after 8 weeks	Treatment					
	VLS#3		Placebo		Overall	
	n	%	*n*	%	*n*	%
None or light (0–2)	31	47.7	23	32.4	54	39.7
Mild (3–5)	27	41.5	34	47.9	61	44.9
Moderate (6–8)	7	10.8	11	15.5	18	13.2
Severe (9–12)	0	0	3	4.2	3	2.2
Overall	65	100	71	100	136	100

UCDAI, ulcerative colitis disease activity index.

Table 8. Overall UCDAI response after 8 weeks (on intention-to-treat analysis)

UCDAI after 8 weeks	Treatment					
	VLS#3		Placebo		Overall	
	n	%	*n*	%	*n*	%
None or light (0–2)	31	43.7	23	31.5	54	37.5
Mild (3–5)	30	42.3	35	47.9	65	45.1
Moderate (6–8)	10	14.1	12	16.4	22	15.3
Severe (9–12)	0	0	3	4.1	3	2.1
Overall	71	100	73	100	144	100

UCDAI, ulcerative colitis disease activity index.

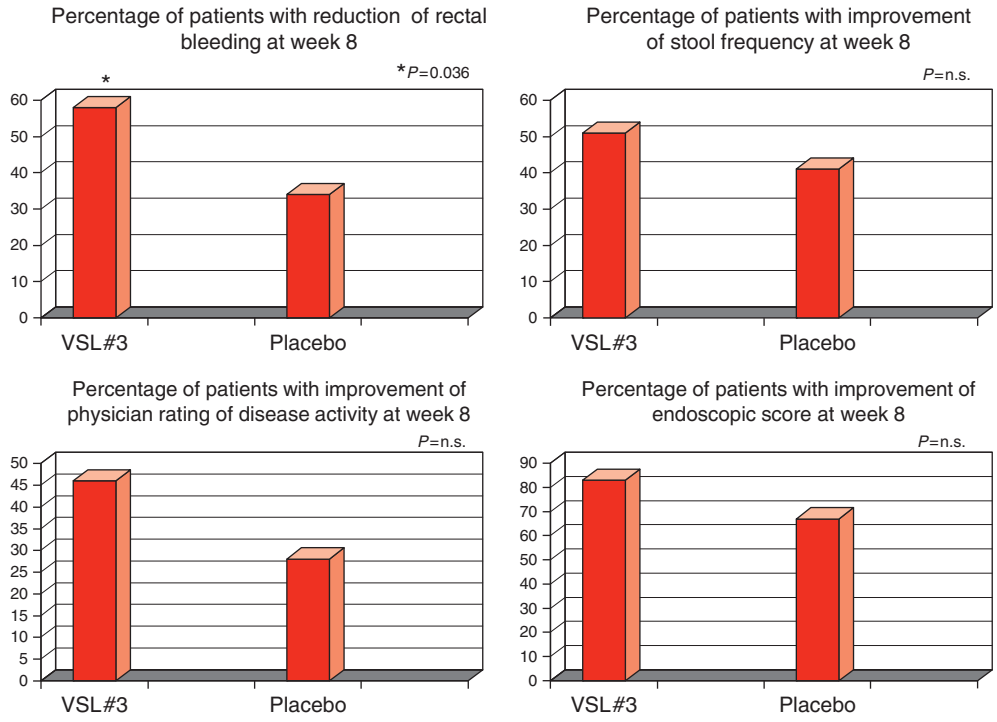

Figure 3. Percentage of patients with improvement in different subgroups of ulcerative colitis disease activity index (UCDAI; rectal bleeding, stool frequency, physician rating of disease activity, and endoscopic score) at week 8 (on intention-to-treat analysis). n.s., not significant.

We report the results of an Italian multicenter study aimed at evaluating the efficacy of the specific probiotic product, VSL#3, for the treatment of mild-to-moderate UC used in conjunction with standard treatment. Our study is a double-blind randomized placebo-controlled trial on adult patients affected by relapsing mild-to-moderate UC, in which VSL#3 or placebo was added to the standard treatment, and aimed to assess the decrease in UCDAI score of 50% or more. For ethical reasons, the "placebo" group was a group in which the patients continued to take their standard treatment (5-ASA and/or immunosuppressant), with the simple addition of a placebo.

Overall, VSL#3 was significantly superior to the placebo in reducing the activity of mild-to-moderate UC (primary end point). A significantly higher proportion of patients in the VSL#3 group experienced an improvement in their UCDAI score of at least 50% at week 8 over those who received placebo (63.1% vs. 40.8%, $P=0.010$). As a secondary end point, 31 individuals (47.7%) in the VLS#3 group and 23 individuals (32.4%) in the placebo group experienced remission by the end of 8 weeks, reaching results that did not show a significant difference (PP $P=0.069$; ITT $P=0.132$). We believe that this might represent a type II error and that a larger study might have had enough power to detect a statistically significant difference. None of the patients in the VSL#3 group experienced any worsening of symptoms during follow-up (**Tables 6** and **7**), whereas five individuals in the placebo group showed a deterioration in their clinical status and had to be withdrawn from the study. No significant difference in stool frequency, physician rating of disease activity, and mean endoscopy scores was detected between the two groups ($P=$ n. s. (not significant)). However, VSL#3 patients had a significant reduction in rectal bleeding compared with the placebo group (PP $P=0.014$; ITT $P=0.036$). Finally, no major adverse event was reported in either group. To confirm the efficacy of VSL#3, we also considered the patients who dropped out because of clinical ineffectiveness. In the "placebo" group, five patients abandoned the study for this specific reason (7%), whereas all VSL#3 patients completed the study.

VSL#3 has proven to be effective by colonizing the host, changing the epithelial function and the immune response. Experimentally, in murine models of colitis, VSL#3 prevents redistribution and reduced expression of sealing tight-junction proteins (12) and specifically stimulates the expression of genes associated with lipid, xenobiotic, and peroxisome proliferator-activated receptor signaling (13).

The roles of probiotics in managing active UC have also been reported in literature. Studies have reported *Escherichia coli* 1917 Nissle to be as effective as low-dose mesalamine in preventing a relapse of quiescent UC (14–16), and treatment with *Saccharomyces boulardii* for 4 weeks was shown to induce clinical remission in 71% of patients with mild-to-moderate disease; however, very few patients were enrolled to draw any conclusions (17). Moreover, *S. boulardii* should be managed with caution, especially in immunocompromised patients (e.g., in patients under immunosuppressant treatment) (18).

Other studies have reported the efficacy of VSL#3 in patients affected by UC (19). An open-label study (20) showed that in 5-ASA allergic or nonresponsive UC patients, VSL#3 was able to colonize the intestine and suggested that the product may be useful in maintaining remission (15 out of 20 patients remained in remission during the 1-year study). Thereafter, an open-label study found that 77% of mild-to-moderate UC patients obtained remission with 3,600 billion CFU/day of VSL#3 at 6 weeks (6). An Italian randomized, controlled study found that VSL#3 900 billion CFU/day added to low-dose balsalazide shows better results in treating active UC than balsalazide or mesalazine alone (5). Two studies with VSL#3 in pediatric UC have recently been carried out; the first one is an open-label study showing that 56% of pediatric patients obtained remission, with a combined remission/response rate of 61% (21), and the second one is a double-blind placebo-controlled trial, showing that VSL#3 supplementation was only able to induce remission in 92.8% of UC children compared with 36.4% with steroid alone, and was effective in maintaining remission in 78.6% of patients during a 12-month follow-up compared with 26.7% in the placebo group (22).

A recent Indian multicenter placebo-controlled trial investigating VSL#3 in mild-to-moderate UC patients was published (23). Patients were given 3,600 billion CFU/day VSL#3 for 12 weeks. At week 6, the percentage of patients with an improvement in UCDAI >50% was significantly higher in the group given VSL#3 (25, 32.5%) than in the placebo group (7, 10%; $P=0.001$). At week 12, 42.9% of VSL#3 patients achieved remission, compared with 15.7% of placebo patients ($P<0.001$). Furthermore, significantly more number of patients given VSL#3 (40, 51.9%) achieved a UCDAI decrease of more than three points, compared with those given placebo (13, 18.6%; $P<0.001$).

Although the design of our study was similar, we recorded a higher placebo response compared with the Sood *et al.* (23) study (40% in our trial vs. 10% in Indian trial). The high "placebo" response rate of our study (40.8% of placebo patients had a 50% reduced UCDAI) may be easily explained by the continuous standard medical treatment provided to all the patients and allows for the statistically borderline results reached in this study for obtaining remission and mucosal healing. A possible suggestion for future studies, in addition to increasing the number of enrolled patients, may be to extend the study period to 12 weeks, expecting, as the Sood *et al.* (23) study proved, that a longer treatment with VSL#3 will offer more divergence from the placebo group. As stated by a recent review, another possible explanation for this high "placebo" response is that the country in which the study is conducted significantly influences the placebo response rate (24). In particular, studies carried out exclusively in Europe have a significantly higher placebo remission rate than studies outside Europe, ranging from 20.8% to 33.6% (24). Our placebo results are therefore in line with the literature estimates. This high percentage of placebo response may also account for some results of this study. For example, the failure to improve stool frequency vs. placebo may be very relevant to patients. We found VSL#3 better than placebo when we assessed the objective parameters (UCDAI,

rectal bleeding, remission, and mucosal healing). On the contrary, subjective parameters (stool frequency and physician rating of disease activity) do not seem to improve so significantly under VSL#3 treatment. Two reasons may explain these conflicting results. First, the "placebo" response may affect some subjective parameters (e.g., stool frequency). The second is that unchanged stool frequency may be related to overlapping irritable bowel syndrome, as this sometimes affects patients with inflammatory bowel disease (25).

An important point of discussion to be addressed is the rationale of this study. People may argue that a higher dose of 5-ASA therapy might be just as well tolerated and may be more convenient and less expensive for obtaining remission. This may be a rational and advisable approach. However, we need a new therapeutic approach to relapsing UC, especially when the patient is already under treatment with immunosuppressors. Increased doses of mesalazine formulations may be safe and effective in obtaining remission, but the azo-bonded formulations may be compromised by secretory diarrhea at doses providing >2–2.4 g/day of mesalazine (26). Moreover, biologics are at higher risk of severe side effects and are much more expensive than a high-dose probiotic treatment in obtaining remission in relapsing UC. On the contrary, VSL#3 is classified as a food or food supplement in most countries and is characterized by a very high safety profile that has also been confirmed throughout this study. The safety of VSL#3 has also been proven in pediatric inflammatory bowel disease and intensive care unit patients (21,22,27).

Of course, once remission has been obtained, physicians also need to know how these patients should be managed in the longer term, i.e., with maintenance doses of probiotic. A clinical trial assessing the optimal dose of VSL#3 in maintaining remission of UC is needed.

Another criticism may be that the VSL#3 dose used in this study is quite high, compared with other studies reporting an effect on remission of UC or pouchitis (7). This choice was based on the assumption that a high probiotic concentration is needed to treat an extensive and active colonic disease. Of course, the optimal dose to maintain remission may be much lower (e.g., one sachet daily for the maintenance of remission in pouchitis (3)), and, as stated, a further trial assessing the optimal dose of VSL#3 in maintaining remission of UC is needed.

In this trial, probiotics and 5-ASA seem to have a synergistic activity. It is unclear how the association between probiotic and 5-ASA may take effect. It is possible that VSL#3 may function in synergy with, or perhaps increases, the anti-inflammatory action of 5-ASA compounds. 5-ASA compounds are potent inhibitors of several inflammatory mediators, such as leukotrienes, prostaglandins, and platelet-activating factor, all of which have roles in the pathogenesis of UC (28). In addition, 5-ASA compounds inhibit the production of interleukin-1 and free radicals and have an intrinsic antioxidant activity (29). Probiotics reduce inflammation by a number of mechanisms, including alteration of the mucosal immune system, competitive exclusion of proinflammatory pathogens, production of antimicrobial factors such as bacteriocins and other metabolites (28,30), and support of increased intestinal barrier function (31,32). At present, on the basis of what has recently been published for acetaminophen, we cannot exclude the possibility that gut bacteria may be the principal target for drugs, and that by manipulating the gut flora in the drug treatment, the outcome can be improved (33).

We do not know whether similar results could have been obtained only by increasing the 5-ASA daily dosage by up to 4 g, provided that the incidence of 5-ASA-related side effects remains unchanged regardless of whether the dose is set at 2 g or 4 g. However, independent of any economic considerations (VSL#3, being a probiotic, is not covered by insurance policies), we believe that the association between 5-ASA and VSL#3 should be preferred, even to a high-dose 5-ASA regimen or to the 5-ASA/immunosuppressant association for the treatment of UC patients with mild-to-moderate UC. Our opinion is based on the fact that, because the mammalian genome does not encode for all functions required for proper immunological responses, it is therefore evident that humans depend on critical interactions with their microbiome for health (34,35).

In conclusion, our study found that the addition of the high-potency probiotic mixture VSL#3 to the standard UC treatment is able to induce significant symptomatic improvement of relapsing mild-to-moderate UC compared with the placebo group on standard treatment only. This double-blind, placebo-controlled study found that VSL#3 is also able to improve the clinical picture, reduce symptoms, and improve the endoscopic appearance of the colonic mucosa. Therefore, VSL#3 may be considered as a safe and effective option for patients suffering from relapsing mild-to-moderate UC, to avoid or delay the administration of steroids, immunosuppressants, and biologics.

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CONFLICT OF INTEREST

Guarantor of the article: Antonio Tursi, MD.

Specific author contributions: Antonio Tursi conceived the study and wrote the paper. Giovanni Brandimarte, Alfredo Papa, Andrea Giglio, Walter Elisei, Gian Marco Giorgetti, Giacomo Forti, Sergio Morini, Cesare Hassan, Maria Antonietta Pistoia, Maria Ester Modeo, Stefano Rodinò, Teresa D'Amico, Ladislava Sebkova, Natale Saccà, Emilio Di Giulio, Francesco Luzza, Maria Imeneo, Tiziana Larussa, Salvatore Di Rosa, Vito Annese, Silvio Danese, and Antonio Gasbarrini conducted the study and approved the paper before submission. Walter Elisei revised the statistical analysis.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Patients with ulcerative colitis (UC) may relapse even when under treatment.
- ✓ If UC is already being treated with mesalazine and/or immunosuppressants, the next therapeutic step is represented by a further course of steroids or by the use of biologics.

WHAT IS NEW HERE

- ✓ VSL#3 probiotic mixture seems to effect a significant improvement in the clinical picture of patients with relapsing UC.
- ✓ VSL#3 also seems to improve several other parameters, e.g., remission.
- ✓ VSL#3 may be a useful tool in the treatment of relapsing UC in patients already under treatment with mesalazine and/or immunosuppressants, because humans depend on critical interactions with their microbiome for health.

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