Randomised clinical trial: mesalazine and/or probiotics in maintaining remission of symptomatic uncomplicated diverticular disease – a double-blind, randomised, placebo-controlled study

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SUMMARY

Background

Placebo-controlled studies in maintaining remission of symptomatic uncomplicated diverticular disease (SUDD) of the colon are lacking.

Aim

To assess the effectiveness of mesalazine and/or probiotics in maintaining remission in SUDD.

Methods

A multicentre, double-blind, placebo-controlled study was conducted. Two hundred and ten patients were randomly enrolled in a double-blind fashion in four groups: Group M (active mesalazine 1.6 g/day plus *Lactobacillus casei* subsp. *DG* placebo), Group L (active *Lactobacillus casei* subsp. *DG* 24 billion/day plus mesalazine placebo), Group LM (active *Lactobacillus casei* subsp. *DG* 24 billion/day plus active mesalazine), Group P (*Lactobacillus casei* subsp. *DG* placebo plus mesalazine placebo). Patients received treatment for 10 days/month for 12 months. Recurrence of SUDD was defined as the reappearance of abdominal pain during follow-up, scored as ≥5 (0: best; 10: worst) for at least 24 consecutive hours.

Results

Recurrence of SUDD occurred in no (0%) patient in group LM, in 7 (13.7%) patients in group M, in 8 (14.5%) patients in group L and in 23 (46.0%) patients in group P (LM group vs. M group, $P = 0.015$; LM group vs. L group, $P = 0.011$; LM group vs. P group, $P = 0.000$; M group vs. P group, $P = 0.000$; L group vs. P group, $P = 0.000$). Acute diverticulitis occurred in six group P cases and in one group L case ($P = 0.003$).

Conclusion

Both cyclic mesalazine and *Lactobacillus casei* subsp. *DG* treatments, particularly when given in combination, appear to be better than placebo for maintaining remission of symptomatic uncomplicated diverticular disease. (ClinicalTrials.gov: NCT01534754).

INTRODUCTION

Diverticulosis is present in approximately two thirds of the elderly population, and a large majority of those affected will remain entirely asymptomatic. However, an estimated 20% of patients may develop 'diverticular disease'.[1]

The treatment of symptomatic uncomplicated diverticular disease (SUDD) remains the subject of debate, particularly regarding both prevention and occurrence of diverticulitis. One open-label study found that 56.6% of patients receiving rifaximin treatment were asymptomatic after 12-month treatment.[2]

Recent observations suggest that low-grade inflammation may be detected in patients with diverticular disease, and that chronic inflammation and its impact on neuromuscular function in the colon may be partially responsible for SUDD.[3] In this way, mesalazine [5-aminosalicylic acid (5-ASA)] has recently shown promise to improve symptoms in patients with SUDD and to prevent recurrence of the disease.[4]

Change in the colonic microflora, resulting in a decrease in healthy flora and an increase in pathogenic bacteria, may be detected in patients with diverticular disease.[5] This may allow chronic inflammation and epithelial cell proliferation to develop in the colonic mucosa in and around the diverticula.[5] Probiotics, restoring the colonic microenvironment, have been proposed to treat those patients.[6]

Unfortunately, most of the data currently available are from open-label studies, and placebo-controlled studies are lacking so far. A recent randomised, open-label study found mesalazine and/or *Lactobacillus casei* (*L. casei*) subsp. *DG* to be effective in maintaining remission of SUDD.[7] In particular, the rationale about the use of the combination therapy with mesalazine and probiotics is that it may act against inflammation as follows: mesalazine controls inflammation, whereas probiotics restore regular colonic microenvironment[7].

As far as we know, this study is the first randomised, controlled double-blind trial aiming to confirm these preliminary results.

PATIENTS AND METHODS

Setting and participants

From January 2009 to December 2010, 250 consecutive out-patients (age >18 years) with SUDD were considered eligible for the study in the 14 participating centres. SUDD was defined as the presence of symptoms (mainly abdominal pain, but also constipation, diarrhoea and bloating) in patients with diverticulosis, in the absence of any complication (stenoses, abscesses, fistulas),[8] in whom the presence of abdominal pain was recorded in the lower left quadrant as lasting for >24 consecutive hours.[9] Although the definition of SUDD is difficult and without standards, abdominal pain is considered the most important symptom in SUDD, and its behaviour is considered the best tool to differentiate between SUDD and Irritable Bowel Syndrome (IBS): abdominal pain in SUDD generally shows a long-lasting behaviour, whereas abdominal pain in irritable bowel syndrome generally shows a short-lasting behaviour.[9] Other competing diagnoses were excluded before making diagnosis in all patients: IBS by the ROME III criteria; coeliac disease by antiendomysium and antitransglutaminase antibodies assessment; thyroid diseases by thyroid-stimulating hormone, free-thyroid hormone 3 and 4; bacterial and/or parasitic intestinal diseases by stool cultures were excluded before making diagnosis. Abdominal pain was assessed using a 10-point visual scale, assigning numerical values of 0 for absence of pain, 1–4 for mild pain, 5–7 for moderate pain and 8–10 for severe pain. SUDD was defined as the presence of an abdominal pain score ≥5 for at least 24 consecutive hours in patients with diverticulosis, in the absence of any complication (stenoses, abscesses, fistulas). The same scale was used to evaluate other symptoms assessed (diarrhoea, constipation, rectal bleeding, bloating, sensation of incomplete evacuation and mucorrhoea). At enrolment, all patients were asymptomatic.

The presence of colonic diverticula was evaluated by colonoscopy. The extent of diverticulosis was assessed by subdividing the colon into four segments (ascending colon, transverse colon, descending colon and sigmoid colon), and assessed according to the number of segments involved with a graded scale from 1 (one segment involved) to 4 (entire colon). The severity of diverticulosis was evaluated using the following arbitrary scale: mild (<5 diverticula per segment), moderate (5–10 diverticula per segment) and severe (>10 diverticula per segment).

A multicentre, randomised, double-blind, double-dummy, parallel groups, placebo-controlled trial was conducted to investigate the most appropriate treatment for prevention of SUDD relapse. The study was conducted in 3 University Hospitals, 10 General Hospitals, 1 Ambulatory Service.

The protocol was approved by the Ethics Committee of each recruiting centre. The study was conducted in accordance with Good Clinical Practice (GCP) and with the Declaration of Helsinki. All patients gave written informed consent for their participation. All authors had

access to the study data and had reviewed and approved the final manuscript.

This trial is registered with www.ClinicalTrials.gov, number NCT01534754.

Sample size

A minimum of 50 patients was required for each group. This calculation was based on the assumption that a continuity-corrected chi-square test with a type I error of 0.01 and a type II error of 0.05 for multiple comparisons was expected to detect a difference between a placebo group success rate of 48%[10] and mesalazine group success rate of 85%.[7]

Study procedures

The study procedures were conducted for each patient enrolled in the study. At the screening visit, demographic characteristics, medical history and current medications were recorded for each patient. A pregnancy test was also performed in women of child-bearing age to exclude pregnancy.

All adverse events, as well as symptoms, were documented, classified and graded. Study participants were supplied with diary cards to assess and record their symptoms at entry, and during the follow-up. The patients were followed during the study and reassessed at 1, 2, 6, 9 and 12 months after starting treatment. The duration of the study was 12 months.

All study medications, including all empty packages and empty blisters, were required to be returned to the Centre to allow a drug accountability check before all residual medication was destroyed. In accordance with GCP guidelines for clinical studies, any surplus medication and all packages were returned to the Centre even in the case of withdrawal or discontinuation of the study.

Concomitant treatments

Concomitant medications were permitted during the course of the study, if used at a constant dosage and if they had been started at least 1 month before the baseline visit. All concomitant medications were recorded in the case report form (CRF) with the dosage and the trade or generic name. Use of usual laxatives, only if absolutely necessary, was permitted, but lactulose was not allowed during the study period. The investigator was permitted to treat the patients with any supportive therapy considered necessary for the patient's health. These concomitant therapies were also recorded on the CRF. A regular diet avoiding a high-fibre diet during the study was advised, as high-fibre diet may be a confounding factor for response to treatment.

Randomisation

Each centre enrolled patients according to the randomisation list. Eligible patients were randomly assigned to receive active drugs or placebo using one computer-generated randomisation list.

Randomisation was carried out in a double-blind manner in blocks of four subjects using 1:1:1:1 allocation to the four groups.

Assessment of compliance

The number of medication boxes dispensed and returned throughout the study was used to measure patient compliance (at least 80% of the prescribed drugs must be taken). This was analysed descriptively to compare treatment groups during the study.

Inclusion criteria

Subjects were required to meet all the following inclusion criteria to be eligible for participation:

(i) males and females aged >18 years;

(ii) diverticulosis showed by colonoscopy no more than 6 months prior to study entry;

(iii) symptomatic episode of uncomplicated diverticular disease no more than 4 weeks prior to study entry;

(iv) patients who have given their free and informed consent.

A negative pregnancy test at the screening visit, agreement to use a valid contraceptive method for the duration of the study, patients not requiring hospitalisation, and patients willing and able to provide written informed consent were also considered inclusion criteria.

Exclusion criteria

Subjects who met any of the following exclusion criteria were not enrolled in this study:

(i) acute diverticulitis (both complicated and uncomplicated);

(ii) diverticular colitis;

(iii) active or recent peptic ulcer;

(iv) chronic renal insufficiency;

(v) allergy to salicylates;

(vi) patients with intended or ascertained pregnancy, lactation;

(vii) women of child-bearing age not using contraceptives;

(viii) lactulose-lactitol use in the 2 weeks before the enrolment and during the study;

(ix) presence of diverticulitis complications (fistulas, abscesses and/or stenoses);

(x) use of probiotic preparations either prescribed or over the counter within 2 weeks prior to study entry;

(xi) inability to give a valid informed consent or to properly follow the protocol;

(xii) patients with active malignancy of any type, or history of a malignancy (patients with a history of other malignancies that have been surgically removed and who have no evidence of recurrence for at least 5 years before study enrolment were also acceptable);

(xiii) recent history or suspicion of alcohol abuse or drug addiction;

(xiv) any severe pathology that can interfere with the treatment or the clinical or instrumental tests of the trial;

(xv) use of nonsteroidal anti-inflammatory drugs for 1 week before and throughout the study period (only paracetamol was permitted).

Significant hepatic, renal, endocrine, respiratory, neurological or cardiovascular diseases, as determined by the investigator, were also considered exclusion criteria. Other exclusion criteria were as follows: history of severe adverse reactions or known hypersensitivity to maltose and/or silicon dioxide; patients requiring hospitalisation; use of any investigational drug and/or participation in any clinical trial within 3 months prior to entering this study; inability to give valid written informed consent or to properly follow the protocol.

Treatment

Eligible patients were randomly assigned to one of the following treatment groups:

(i) **Group M**. Active Pentacol 800, 2 tablets/day for 10 days/month plus Enterolactis Plus placebo, 1 sachet/day for 10 days/month;

(ii) **Group L**. Active Enterolactis Plus, 1 sachet/day for 10 days/month plus Pentacol 800 placebo, 2 tablets/day for 10 days/month;

(iii) **Group LM**. Active Pentacol 800, 2 tablets/day plus Active Enterolactis Plus, 1 sachet/day for 10 days/month;

(iv) **Group P**. Pentacol 800 placebo, 2 tablets/day and Enterolactis Plus placebo, 1 sachet/day for 10 days/month.

Pentacol 800 comprises tablets containing 800 mg of mesalazine, administered at a dose of 1600 mg/day for 10 days/month; the placebo was tablets identical to those containing active mesalazine. Enterolactis Plus comprises sachets, each containing 24 billion viable lyophilised bacteria containing *L. casei subsp. DG,* administered as a single sachet per day for 10 days/month; the placebo was sachets identical to those containing active Lactobacilli.

In group LM, mesalazine and probiotics were administered simultaneously for 10 days/month. To simplify the assumption of the drugs, we suggested to take the treatment at the beginning of every month (taking together mesalazine and probiotics).

Pentacol 800 and Enterolactis Plus, as well as the placebos, were supplied by the manufacturing company (Sofar S.p.A., Trezzano Rosa (MI), Italy) for the entire duration of the trial. The supply of the experimental drugs was the only involvement of Sofar S.p.A. in this study.

Primary endpoint

The primary endpoint was the proportion of patients maintaining remission after a previous episode of SUDD. Maintenance of remission was defined as the absence of recurring abdominal pain scored ≥ 5 for at least 24 consecutive hours and recorded at any time during the follow-up. If recurrence occurred, the patient was excluded from the study.

Computerised tomography was performed in case of suspected acute diverticulitis symptoms (e.g. abdominal pain associated with fever).

Secondary end-points

Secondary endpoints included:

(i) influence of the extent of diverticulosis on maintaining remission;

(ii) influence of the severity of diverticulosis on maintaining remission;

(iii) influence of the type of the drugs used to obtain the remission on maintaining remission;

(iv) influence of comorbidities with the relative Charlson's score[11, 12] on maintaining remission;

(v) influence of acetyl salicylic acid intake on maintaining remission;

(vi) influence of concomitant diseases on maintaining remission;

(vii) influence of concomitant drugs on maintaining remission;

(viii) the prevention of acute diverticulitis of the colon occurrence.

Statistical methods

Categorical variables were expressed as absolute values and percentages in the text and tables, while continuous variables were expressed as median and range. Statistical analysis was performed using Fisher's exact test and chi-square for categorical data, and the Kruskall–Wallis test for continuous data. We analysed data using an intention-to-treat analysis, detecting the probability of persistence of clinical remission using the Kaplan–Meier method. Differences between curves were evaluated using the log-rank test. To assess the predictive value of clinical remission persistence among clinical baseline characteristics, univariate analysis with the log-rank test was used. Multivariate analysis using the Cox regression model was performed to investigate factors independently associated with clinical remission. The results are presented as the hazard ratio (HR) and 95% confidence interval (95% CI). Repeated measures analysis of variance (MANOVA) was used to compare associated symptoms (rectal bleeding, meteorism, sensation of incomplete evacuation and mucorrhoea) over time with respect to group allocation. The relative risk (RR) and 95% CI and number needed to treat (NNT) were calculated for the four study groups.

A two-tailed P value 0.01 was considered statistically significant.

The collection and analysis of data were performed using SPSS Release 13.0 (SPSS, Inc., Chicago, IL, USA). Analysis was performed by M.P., MD, and F.A., PhD.

RESULTS

Patient flow is shown in Figure 1, with patient demographics and clinical characteristics reported in Table 1; no significant between-group differences were observed.

During the follow-up, 13 (24.1%) patients in group LM, 8 (14.5%) patients in group L, 5 (9.8%) patients in group M and 2 (4.0%) patients in group P were with a 0 pain score during the entire follow-up (LM group vs. P group $P = 0.009$, chi-square test).

Primary endpoint

Recurrence of SUDD occurred in no (0%) patient in group LM, in 7 (13.7%) patients in group M, in 8 (14.5%) patients in group L and in 23 (46.0%) patients in
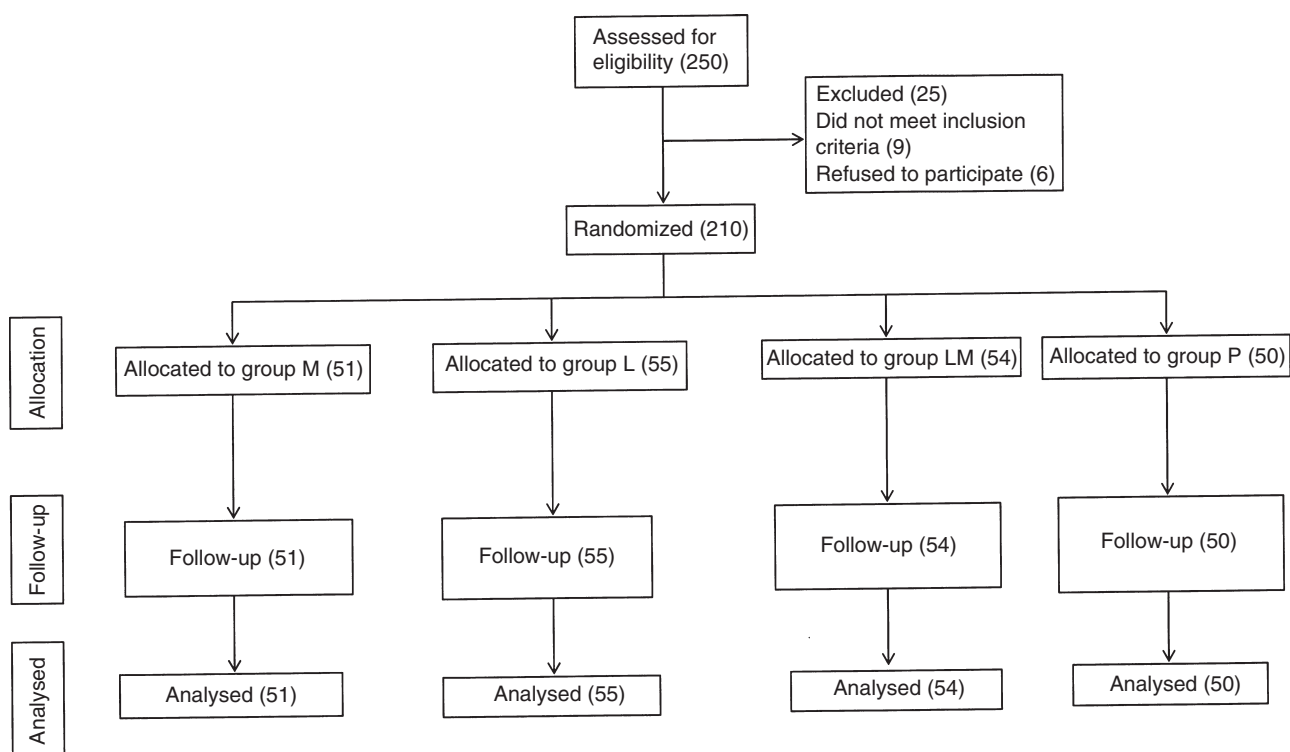
Figure 1 | CONSORT flow-chart for the study. Group M: active mesalazine + *Lactobacillus casei* placebo; group L: active *Lactobacillus casei* + mesalazine placebo; group LM: active mesalazine + active *Lactobacillus casei*; group P: mesalazine placebo + *Lactobacillus casei* placebo.

Table 1 | Baseline demographic and clinical characteristics by treatment group

	Group M	Group L	Group LM	Group P	P*
Age median (95% CI), years	60 (57–66)	64 (62–65)	64 (60–65)	62 (59–65)	0.241
Sex male, *n* (%)	21 (41.2)	27 (49.1)	31 (57.4)	30 (60.0)	0.213
First diagnosis of SUDD, *n* (%)	25 (49.1)	26 (47.2)	38 (70.1)	25 (50.0)	0.096
Symptom duration before diagnosis, months (95% CI)	2 (1.55–4.98)	1 (1.00–3.25)	1 (1.8–11.75)	2 (1.95–4.43)	0.111
Presence of co-morbidities	37 (72.5)	37 (72.7)	34 (59.3)	36 (48.0)	0.687
Charlson's score = 1, *n* (%)	11 (21.6)	12 (21.8)	8 (14.8)	12 (24.0)	0.672
Concomitant ASA	3 (5.9)	7 (12.7)	10 (18.5)	9 (18.0)	0.213
Concomitant calcium channel blockers	5 (9.8)	4 (7.3)	8 (14.8)	5 (10.0)	0.632
Concomitant antidiabetic therapy	7 (13.7)	6 (10.9)	5 (9.2)	7 (14.0)	0.854
Therapy to obtain remission, *n* (%)					
Antibiotics	14 (27.4)	23 (41.8)	25 (46.3)	22 (44.0)	0.070
Mesalazine	7 (13.7)	5 (9.0)	7 (12.9)	4 (8.0)	0.756
Probiotics	6 (11.8)	12 (21.8)	15 (27.8)	8 (16.0)	0.093
Extension, *n* (%)					
One segment	36 (70.6)	40 (72.7)	35 (64.8)	37 (74.0)	
Two segments	9 (17.6)	9 (16.4)	12 (22.2)	6 (12.0)	0.887
Three segments	4 (7.8)	2 (3.6)	2 (3.7)	3 (6.0)	
Four segments	2 (3.9)	4 (7.2)	5 (9.2)	4 (8.0)	
Severity before obtaining remission, *n* (%)					
Mild	0	2 (3,7)	0	3 (6.0)	
Moderate	25 (49.1)	26 (47.2)	29 (53.7)	26 (52.0)	0.067
Severe	26 (50.9)	27 (49.1)	25 (46.3)	21 (42.0)	
Symptoms before obtaining remission					
Constipation, *n* (%)	17 (33.3)	13 (23.60)	8 (14.8)	13 (26.0)	0.241
Diarrhoea, *n* (%)	13 (25.5)	17 (30.9)	11 (20.4)	8 (16.0)	0.137
N of evacuation/week, median (95% CI)	7 (5–8)	7 (6–8)	7 (6–8)	7 (6–8)	0.949
Rectal bleeding, median (95% CI)	0 (0–0)	0 (0–1)	0 (0–0)	0 (0–1)	0.533
Abdominal pain, median (95% CI)	3 (2–4)	3 (1–3)	2 (1–3)	3 (1–3)	0.709
Mucorrhoea, median (95% CI)	0 (0–1)	1 (0–2)	0 (0–1)	0 (0–0)	0.262
Incomplete evacuation, median (95% CI)	3 (3–4)	3 (2–3)	2 (2–3)	2 (1–3)	0.124
Meteorism, median (95% CI)	3 (3–4)	3 (3–4)	3 (3–3)	4 (3–4)	0.175

CI, confidence interval; ASA, acetyl salcylic acid.

Group M: active mesalazine + *Lactobacillus casei* placebo; group L: active *Lactobacillus casei* + mesalazine placebo; group LM: active mesalazine + active *Lactobacillus casei*; group P: mesalazine placebo + *Lactobacillus casei* placebo.

* Fisher's exact test and chi-square for categorical data; Kruskall–Wallis test for continuous data.

group P (LM group vs. M group, $P = 0.015$; LM group vs. L group, $P = 0.011$; LM group vs. P group, $P = 0.000$; M group vs. P group, $P = 0.000$; L group vs. P group, $P = 0.000$; chi-square test). Persistence of remission of SUDD during 1-year of follow-up is shown in Figure 2. There were a significant number of recurrences in group P compared with all other groups. The benefit of different treatments in terms of RR and NNT is reported in Table S1. No significant difference in relative risk of clinical remission was observed between groups M and L.

Secondary endpoints

Predictors of persistence of clinical remission. To assess whether patient baseline clinical characteristics may have influenced the likelihood of persistence of clinical remis-

sion at follow-up, univariate and multivariate analyses were performed. With exception of the type of treatment, no baseline characteristics significantly influenced the persistence of clinical remission (see Table 2).

Associated abdominal symptoms during follow-up. Numbers of patients experiencing constipation and diarrhoea during follow-up are reported in Table S2; there were no important differences between study groups for these symptoms.

Rectal bleeding, bloating, sensation of incomplete evacuation and mucorrhoea scores at entry and during follow-up in the four study groups are reported in Table S3. On MANOVA, rectal bleeding, meteorism, sensation of incomplete evacuation and mucorrhoea did not differ significantly by
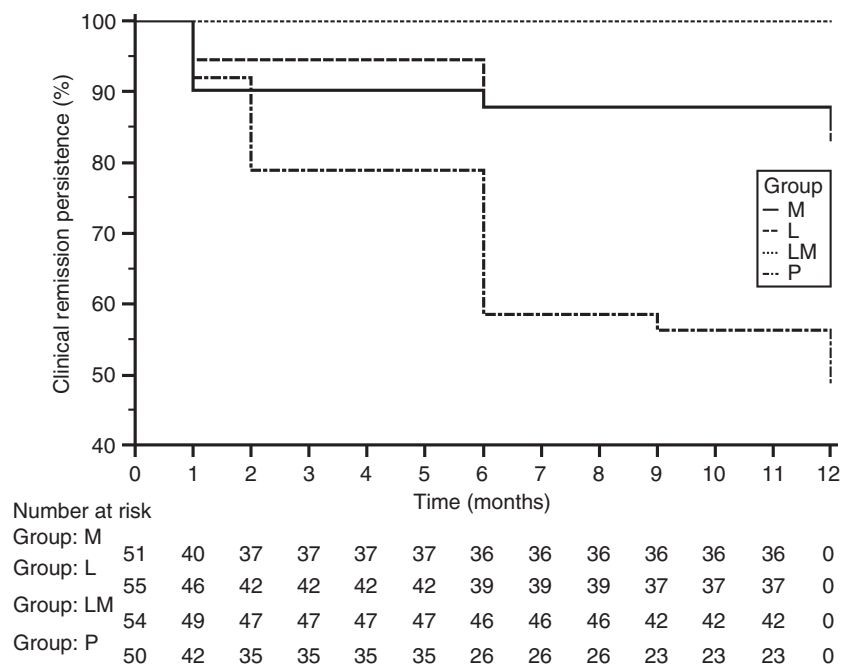
Figure 2 | Kaplan–Maier analysis of cumulative rates of persistence of clinical remission of Symptomatic Uncomplicated Diverticular Disease by study group. Clinical remission was defined as the absence of recurring abdominal pain scored ≥5 for at least 24 consecutive hours and recorded at any time during the follow-up. Group M: active mesalazine + *Lactobacillus casei* placebo; group L: active *Lactobacillus casei* + mesalazine placebo; group LM: active mesalazine + active *Lactobacillus casei*; group P: mesalazine placebo + *Lactobacillus casei* placebo. $P < 0.0001$, log-rank test.

treatment group (M group: $F = 1.165$, $P = 0.142$; L group: $F = 1.801$, $P = 0.132$; LM group $F = 0.842$, $P = 0.501$; P group $F = 1.177$, $P = 0.139$; respectively).

Occurrence of acute diverticulitis during follow-up. Acute diverticulitis occurred in 7 (3.1%) of the analysed patients during follow-up: Six patients were in group P and one patient in group L, with no cases of diverticulitis in the other two groups ($P = 0.003$, chi-square test). Acute diverticulitis was uncomplicated in five of six patients, but one patient receiving placebo underwent surgery due to free perforation. Univariate and multivariate analyses were not performed to assess the effect of baseline clinical characteristics on acute diverticulitis occurrence due to a lack of power. However, four of six patients developing acute diverticulitis had diverticulosis involving more than two segments and severe diverticulosis at baseline.

Compliance and safety. All patients took at least 80% of the prescribed drugs. No difference in compliance was found between the studied groups. No adverse events related to study drugs were reported.

DISCUSSION

The natural history of diverticular disease remains largely unknown. The lack of any placebo-controlled studies has precluded any meaningful conclusion regarding the appropriate management of this disease. For example, we know that SUDD may recur in up to 35% of cases,[13] and acute diverticulitis may occur in up to 11% of SUDD cases,[14] yet Current Practical Guidelines from the American College of Gastroenterology do not provide any advice regarding treatment for the prevention of recurrence.[15]

Changes in GI microflora are hypothesised to influence the aetiology of diverticular disease.[6, 16] It is hypothesised that microbial imbalance, associated with colonic bacterial overgrowth, may be key for the development of diverticular disease.[16–18] More recently, it has been hypothesised that chronic inflammation may play a role in the aetiology of diverticular disease.[6, 16] The association between inflammation and diverticular disease was first suggested following detection of both chronic inflammatory infiltrate and increased faecal calprotectin in patients with diverticular disease and acute diverticulitis.[19, 20]

Table 2 | Predictors of clinical remission persistence during follow-up

	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P
Group						
M	3.575	1.746–7.317	0.001	0.329	0.134–0.811	0.016
L	3.506	1.728–7.113	0.001	0.276	0.112–0.685	0.005
LM	–	–	–	–	–	–
Age	–	–	–	1.013	0.969–1.059	0.574
Sex	1.085	0.574–2.053	0.796	0.886	0.444– 1.767	0.732
First diagnosis of SUDD	1.216	0.636–2.322	0.549	0.827	0.373–1.833	0.640
Symptom duration before diagnosis	–	–	–	0.988	0.974–1.007	0.229
Presence of co-morbidities	0.915	0.463–1.806	0.789	1.300	0.589–2.867	0.516
Charlson's score	1.211	0.550–2.665	0.606	3.069	0.397–23.722	0.282
Concomitant ASA	0.877	0.357–2.149	0.778	0.870	0.310–2.437	0.719
Concomitant calcium channel blockers	1.312	0.650 to 2.435	0.615	2.185	0.856 to 18.645	0.385
Concomitant antidiabetic therapy	1.836	0.706–4.773	0.111	1.837	0.845–3.992	0.127
Extension						
2 segments	0.674	0.290–1.565	0.402	0.922	0.198–4.285	0.918
3 segments	1.267	0.340–4.718	0.687	0.992	0.175–5.632	0.993
4 segments	0.963	0.297–3.117	0.949	1.353	0.229–7.980	0.739
Severity						
Moderate	0.5720	0.208–1.571	0.182	2.201	0.651–7.446	0.204
Severe	0.4760	0.159–1.427	0.114	1.059	0.384–2.921	0.912

SUDD, symptomatic uncomplicated diverticular disease; ASA, acetyl salicylic acid.

Clinical remission was defined as the absence of recurring abdominal pain scored ≥5 for at least 24 consecutive hours and recorded at any time during the follow-up.

Group M: active mesalazine + *Lactobacillus casei* placebo; group L: active *Lactobacillus casei* + mesalazine placebo; group LM: active mesalazine + active *Lactobacillus casei*; group P: mesalazine placebo + *Lactobacillus casei* placebo.

The use of both mesalazine and probiotics was recently proposed for the treatment of SUDD and for the prevention of diverticulitis occurrence.[6] In 2006, we found that both mesalazine and *L. casei* DG appeared to be effective for the prevention of recurrence of SUDD, but their combined use showed greater promise.[7] This combination was thought to have a synergistic effect, but the small sample size, lack of a placebo group and the open-label enrolment were limiting factors in this early study.

In this way, we chose to replicate this study, using the same mesalazine and probiotic dosage but in a double-blind way and adding a placebo arm.

Recent placebo-controlled trials help further to clarify the conflicting evidence on the role of mesalazine in diverticular disease. For example, Kruis *et al.*[21] found a trend towards significance in using mesalazine granules 3 g daily rather than placebo on controlling abdominal pain in SUDD; Gaman *et al.*[22] found mesalazine granules 514.7 ± 30.5 mg daily better than placebo in preventing diverticulitis occurrence, diverticulitis flares and occurrence of surgical treatment; Smith *et al.*[23] found mesal-azine better than placebo in reducing the duration of abdominal pain in SUDD and in reducing the relative quantity of inflammatory genes expression in colonic biopsies. This study found mesalazine to be effective in preventing the recurrence of SUDD, obtaining a significantly higher rate of remission maintenance than placebo.

The rationale for using probiotics in the treatment of diverticular disease is related to the pathogenesis of the disease. Stasis of luminal contents occurs in colonic diverticula, and is probably combined with changes in the spectrum of intestinal microflora. This process may be followed by the occurrence of abnormal metabolites, causing functional changes leading to abdominal symptoms.[24] The action of probiotics includes the production of antimicrobials, competitive metabolic interactions with proinflammatory organisms, and inhibition of adherence and translocations of pathogens. Probiotics may also influence mucosal defence at the levels of immune and epithelial function, such as decreasing several proinflammatory cytokines.[25] This is the first placebo-controlled study assessing the effectiveness and safety of probiotics in preventing the recurrence of SUDD. In this study, we

used *L. casei* as a single probiotic strain. We chose this particular strain as it has previously been shown to colonise the human intestine and resist hydrochloric acid and bile salts,[26] and it appears to persist in the gastrointestinal tract for approximately 2 weeks after discontinuation of treatment.[26] This is an important point, because this persistence permits cyclic administration with adequate patient compliance, without the risk of developing bacterial resistance. Moreover, it seems to be effective against Gram-negative anaerobes.[6, 26] The results obtained confirm this hypothesis: *L. casei* is significantly better than placebo in maintaining SUDD remission.

The remission rate in group LM is particularly impressive. The combination of mesalazine and probiotics acts against inflammation in two ways: mesalazine down-regulates the inflammatory cascade by inhibition of several proinflammatory factors, whereas *L. casei* maintains adequate and balanced colonisation of the gastrointestinal tract, inhibiting both colonic bacterial overgrowth and metabolism of pathogens, as well as reducing some proinflammatory cytokines.[25] This approach appears able to maintain an optimal colonic microenvironment, and to keep virtually all patients symptom free.

Regarding secondary endpoints, an interesting finding was that neither the presence of comorbidities nor concomitant therapies (including ASA) were a risk factor for SUDD recurrence. This study confirms recent findings from Etzioni *et al.*[27] and Humes *et al.*,[28] who did not find any increased risk of diverticulitis recurrence or diverticular perforation in patients with higher Charlson's score or taking ASA. Neither severity nor extent of diverticulosis was a risk factor for SUDD recurrence. This finding differs from that of Hall *et al.*, who found that an involved diverticular segment >5 cm in length was associated with a higher risk of diverticulitis recurrence.[29] This discrepancy may be due to differences in the severity of the disease in the patient populations in the two studies. SUDD is associated with low-grade inflammation, with lower risk of recurrence and complication, whereas diverticulitis is associated with high-grade inflammation, with risk of recurrence and complication related to the severity and the extent of the inflammation. This study showed that acute diverticulitis occurred significantly more frequently in the placebo group than in the active treatment groups. This reinforces the hypothesis that inflammation plays a key role in the pathogenesis of diverticular disease, and that only by controlling inflammation rather than controlling bacteria, we can prevent diverticular disease complica-

tions.[10, 30] On the contrary, a recent large trial of mesalazine (MMX 5-ASA) was reported negative in preventing relapses of diverticulitis.[31] However, the study focused on diverticulitis attacks rather than on abdominal symptoms. In this way, the recent DIVA trial evaluated the efficacy of mesalazine Eudragit L in reducing gastrointestinal symptoms after an acute attack of diverticulitis.[32] In this 1-year double-blind, randomised, placebo-controlled study in which patients with CT scan confirmed acute diverticulitis received placebo, mesalazine, or mesalazine + *Bifidobacterium infantis* 35 624 for 12 weeks and followed up for nine additional months, the rate of complete response by a global symptom score (GSS) of 10 symptoms (abdominal pain, abdominal tenderness, nausea/vomiting, bloating, constipation, diarrhoea, mucus, urgency, painful straining and dysuria) (GSS = 0) was significantly higher with mesalazine than placebo at weeks 6 and 52 ($P < 0.05$), and was particularly high for rectosigmoid symptoms at weeks 6, 12, 26 and 52. Surprisingly, probiotic in combination with mesalazine did not provide additional efficacy, and why this occurs is unknown.[33] We eagerly await the results of other ongoing randomised trials in preventing diverticulitis relapses.

With respect to other symptoms assessed in this study, no difference was found between the active treatment arms and placebo. In particular, the lack of effect of both mesalazine and probiotics in terms of controlling diarrhoea and constipation is surprising, and we do not currently know the reason for this. Papi *et al.* did not find any difference between rifaximin and placebo in terms of control of diarrhoea in patients complaining of SUDD[10]; therefore, further studies are needed to investigate this specific point.

This trial has some limitations. Definition of SUDD is still debatable. SUDD, also known as 'symptomatic diverticulosis',[15] is characterised by abdominal symptoms (abdominal pain, particularly in the lower left abdominal quadrant and alteration of bowel habits) without macroscopic signs of inflammation (no mucosal inflammation on colonoscopy, no increases in erythrocyte sedimentation rate or C-reactive Protein, no fever). However, we know that episodes of prolonged pain were more frequently associated with diverticular disease than irritable bowel syndrome.[9] The experience of prolonged abdominal pain in diverticular disease patients suggests that these abdominal symptoms may originate from diverticular inflammatory foci, the intensity and severity of which remain subclinical without reaching well-defined acute diverticulitis.[9]

Although the use of faecal calprotectin would overcome this limitation in terms of clinical diagnosis of SUDD,[20] this was not available for each centre when the trial started, necessitating the use of a clinical classification for defining SUDD and its recurrence.

In conclusion, this study showed that both cyclic mesalazine and *L. casei* subsp. *DG* appear to be better than placebo for maintaining remission in SUDD, especially when used in combination. Moreover, both treatments, alone or in combination, are significantly better than placebo in preventing occurrence of acute diverticulitis in SUDD patients. Further studies are warranted to investigate the best dose of these drugs for the treatment and prevention of SUDD recurrence, as well as their role in preventing diverticulitis occurrence.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Relative risk (RR) of clinical remission persistence and number needed to treat (NNT) among the study groups.

Table S2. Presence of constipation and diarrhoea during follow-up in the four study groups.

Table S3. Scores of associated symptoms during follow-up in the four study groups.

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