



Method

Lactobacillus acidophilus versus placebo in the symptomatic treatment of irritable bowel syndrome: the LAPIBSS randomized trial

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Received January 23, 2017; Accepted July 13, 2017; Published September 30, 2017

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Doi: <http://dx.doi.org/10.14715/cmb/2017.63.9.21>

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Abstract: Irritable bowel syndrome is a chronic functional gastrointestinal disorder characterized by abdominal pain/discomfort and altered bowel habits. The use of *Lactobacilli* as probiotics during irritable bowel syndrome is based on their interesting mechanisms of action and their excellent safety profile but little is known about their clinical efficacy due to the lack of adequately designed clinical trials. The current clinical trial protocol aims to determine the effects of a mixture of *Lactobacillus acidophilus* NCFM and LAFTI L10 as probiotics to improve irritable bowel syndrome symptoms (LAPIBSS). Eighty patients with a positive diagnosis of irritable bowel syndrome according to Rome III criteria were recruited to a multicentre, double-blinded, in parallel groups, placebo-controlled randomized trial. Patients were provided with a daily dose of two capsules with two strains of *Lactobacilli* (5×10^9 cfu/capsule) or placebo for 8 weeks on a 1:1 ratio. The primary outcome is to obtain scores of abdominal pain/discomfort assessed with a 100-mm visual analogue scale. The secondary outcome is to obtain scores of bloating, flatus and rumbling tested with a 100-mm visual analogue scale, composite score, stool frequency and stool consistency/appearance assessed with the Bristol Stool Form scale. According to the hypothesis that abdominal pain is mainly the result of a visceral hypersensitivity, the current study protocol aims to provide high quality proof of concept data to elucidate the efficacy of a consumption of a mixture of *Lactobacillus acidophilus* probiotic strains after 8 weeks, for decreasing abdominal pain. Ethical approval was given by ethics committee French Consultative Committee for the Protection of Individuals in Biomedical Research of the South West (Number CPP08-014a) and ANSM (French National Agency for Medicines and Health Products Safety – Number B80623-40). The findings from LAPIBSS will be disseminated through peer-reviewed publications and at scientific conferences.

Trial registration: EudraCT N°2008-A00844-51

Key words: Irritable bowel syndrome; Microbiota; Probiotics; *Lactobacillus acidophilus* (*L. acidophilus*); Clinical study protocol.

Introduction

Irritable bowel syndrome (IBS) refers to a chronic functional gastrointestinal disorder currently defined by the Rome III criteria, including abdominal pain or discomfort associated with altered bowel habits without organic abnormalities (1). In the absence of a well-established therapeutic approach, approximately 15% of IBS patients consult a physician and usually seek alternative strategies such as probiotics for symptom relief (2-4). Probiotics are defined by the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO - WHO) as live micro-organisms which, when administered in adequate amounts, confer a health benefit to the host (5). Randomized clinical trials (RCT) have already shown that intake of probiotics such as lactic acid bacteria (LAB) could be of therapeutic interest by preventing infectious diarrhea, and possibly by modifying gut microbiota to improve IBS

symptoms (4, 6-12). Despite excellent safety profile of LAB and their demonstrated mechanisms of action according to *in-vitro* and *in-vivo* studies suggesting their benefits in IBS, the rationale of their use is limited by the low number of high-level quality RCT, especially in Western IBS patient's population (4, 10-24). *Lactobacilli* have "generally-regarded-as-safe" (GRAS) status (13). Their cell wall components, via TLR2/6 signaling pathway, have demonstrated immunoregulatory properties (13). *Lactobacillus acidophilus* (*L. acidophilus*) is one of the most predominant probiotic species, residing in the gastrointestinal tract (15). However, only 2 RCT using *L. acidophilus* strains have been reported in the symptomatic treatment of IBS (21, 24). The first one with a 2-strain mixture of *L. acidophilus*-SDC 2012, 2013 significantly reduced abdominal pain compared with placebo after 4 weeks in a pilot RCT (21). The second RCT using a dose-response design did not highlight significant benefits of 12 weeks of treatment

with *L. acidophilus* NCFM strain compared with placebo to alleviate IBS symptoms (24). Abdominal pain/discomfort was decreased by the probiotic only in a subgroup of patients suffering from moderate to severe pain (24). Although modest, this latter study is consistent with data in human HT-29 epithelial cells and in a rat model of chronic colonic hypersensitivity which showed that a direct contact of *L. acidophilus* NCFM modulates and restores a normal perception of visceral pain by inducing analgesic μ -opioid receptor 1 (MOR1) and cannabinoid receptor 2 (CB2) cellular expression through the NF- κ B pathway (14). Preclinical studies with proven probiotic efficacy and known mechanisms of action should be important for the choice of probiotic strains. Taking into account the positive results of one pilot RCT performed with two strains of the same species, i.e. *L. acidophilus*-SDC 2012, 2013, a second *L. acidophilus* probiotic strain was added in our study protocol. This latter strain *L. acidophilus* LAFTI L10 exhibits gut immunoregulatory properties and proved survival in human gastrointestinal tract as well as an improvement of symptoms in healthy subjects similar to those observed in IBS (25-27). Both strains are also probiotics with approved safety and fermentative properties (27-29). Thus, the aim of the current study is to test with the highest methodology the hypothesis that a consumption for 8 weeks of a mixture of two *L. acidophilus* probiotic strains, selected for their strain-specific properties, could result in an improvement of abdominal pain/discomfort as well as other IBS symptoms such as bloating, flatus, rumbling and bowel habits, including stool frequency and consistency/appearance.

Materials and Methods

Trial design

The design of this protocol refers to a multicentre, double-blind, placebo-controlled, two-armed, parallel design, individually randomized trial, comparing probiotics with placebo in patients with IBS. Participants were included in the trial with a positive diagnosis of IBS according to Rome III criteria (1). These criteria are presented in Figure 1. The trial was performed for a maximum of 9 weeks with 4 visits planned (at points corresponding to screening, baseline, 2 control visits after 4 and 8 weeks of treatment). During the screening visit, investigators checked the eligibility of participants and obtained their informed consent. If the eligibility was confirmed within a maximum of 7 days from the date of the first visit, they were randomized to receive probiotics or placebo for 8 weeks, stratified by center. The study design was also adaptive with an interim analysis after the first sixty patients were included (75% of the overall participants), providing enough accuracy according to the positive results of a previous RCT using probiotics in 60 IBS patients (4). The study flowchart is shown in Figure 2.

Trial objectives

Primary objective

The main objective of the trial is to assess the efficacy of the probiotic mixture to relieve abdominal pain/discomfort symptom in patients suffering from IBS, in comparison with a placebo group after 8 weeks of prod-

uct consumption.

Secondary objectives

The secondary objectives are to assess the effect of the probiotic mixture:

- To relieve bloating, flatus/gas and rumbling symptoms
- On the composite score
- On the stool frequency and consistency.

An assessment of safety and tolerability was also performed throughout the course of the study. This assessment was carried out by collecting patient-reported adverse events (AE) or found during the clinical examination by the investigator and recorded on the case report forms (CRF).

Regulatory and ethics approvals

The study protocol was conducted in France in accordance with the International Council for Harmonization Guidance on Good Clinical Practice Guidelines and the Declaration of Helsinki (2008) (30). Regulatory approval was obtained on September 11, 2008, from the ANSM (French National Agency for Medicines and Health Products Safety – Number B80623-40) and ethics approval of all procedures of the study was obtained on September 15, 2008, from the French Consultative Committee for the Protection of Individuals in Biomedical Research of the South West (Number CPP08-014a). Written informed consent was obtained from each participant. The present trial was registered on July 1, 2008, under EudraCT number 2008-A00844-51 (European Union Drug Regulating Authorities Clinical Trial)

Diagnostic criteria* for irritable bowel syndrome :

Abdominal pain or discomfort** at least 3 days per month in the last 3 months associated with 2 or more of the following :

- a. Improvement with defecation
- b. Onset associated with a change in frequency of stool
- c. Onset associated with a change in form (appearance) of stool

No evidence of organic abnormalities that explain symptoms

* Criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis.

** Discomfort means an uncomfortable sensation not described as pain.

Figure 1. Rome III criteria for irritable bowel syndrome. Adapted from Longstreth GF et al. (2006) (1).

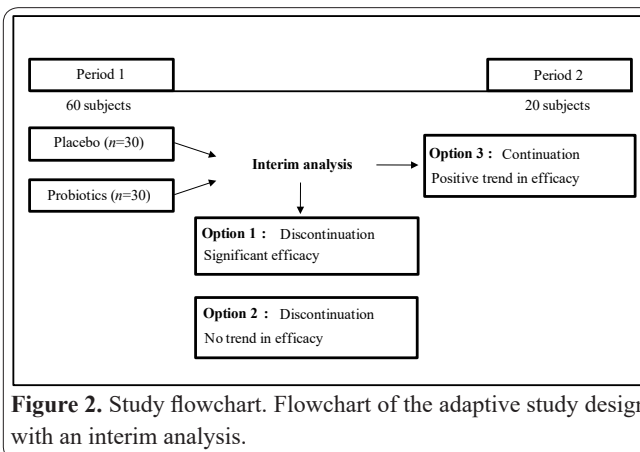


Figure 2. Study flowchart. Flowchart of the adaptive study design with an interim analysis.

in accordance with Directive 2001/20/EC.

Recruitment of patients

A total of 80 patients who fulfilled the screening criteria were recruited by 10 general practitioners located in medical offices based in Toulouse, Paris, Marseille, Montpellier and Poitiers, and by a gastroenterologist (Dr Jacques Moreau) of Rangueil University Hospital of Toulouse, France. In each center, eligible participants were screened among patients already diagnosed with IBS. The trial was completed on May 19, 2012.

Inclusion criteria

Patients were eligible for the trial if they provided written informed consent and if they met all of the following criteria:

- Male or female subject aged between 30 and 60 years old
- Ambulatory subject
- Subject presenting a normal clinical examination
- Subject meeting Rome III criteria (1) for a diagnosis of IBS:
 - Symptoms being present for > 6 months
 - Abdominal pain or discomfort at least 3 days/month in the last 3 months associated with 2 or more of the following:
 - 1) Improvement with defecation
 - 2) Onset associated with a change of stool frequency
 - 3) Onset associated with a stool consistency/appearance.
- Subject presenting with a negative coprological analysis for over 6 months
- Subject presenting with a negative inflammatory balance (negative CRP blood test) for over 6 months
- Subject easily reachable and cooperating enough to comply with the requirements of the study
- Subject having given his/her written consent (subject knowing how to read and to write) prior to any procedure related to the trial
- Subject affiliated with the French health care system.

Exclusion criteria

Patients were not eligible for the trial if they met any of the following criteria:

- Presence of an organic intestinal disease
- Severe or active disease with multiple treatments (psychiatric, cardio-pulmonary, kidney, haematological, neoplastic, antimicrobial or metabolic)
 - Intestinal parasitic infection in the last 6 months
 - Inflammatory intestinal disease (Crohn's disease, ulcerative colitis)
 - A history of previous abdominal surgery (except appendectomy, caesarean birth, tubal ligation, hernia)
 - Any untoward medical occurrence identified during the screening visit, according to the investigator, that could affect the safely conducting of the trial
 - Change in medication in the last 2 months
 - Intake of probiotics in the last 2 months
 - Antibiotic therapy in the last 30 days
 - Current antidepressant or antipsychotic treatment
 - Antimycotic and antiseptic treatment or treatment affecting gastrointestinal transit
 - Chronic use of antalgic and antispasmodic medica-

tion

- Subject with a known allergy to tested products, or to one of its constituents
- Regular alcohol consumption > 14 units per week
- Regular use of narcotic and psychotropic substances
 - Change in diet, start of a weight-loss program or diet in progress
 - Subject who participated in a trial during the preceding month or at the time of screening visit
 - Subject who according to the investigator, is unlikely to comply with the instructions of the protocol and / or to be non-observing to the dietary supplementation
 - Subject unable to understand and/or sign the informed consent due to linguistic or psychological limitation
 - Subject being deprived of liberty by administrative or judicial decision, or being under guardianship
 - For non-menopausal women:
 - Absence of effective contraception (oral contraceptive, intra-uterine device, tubal ligation, surgery)
 - Pregnant or breastfeeding

Test products

The study product was provided in the form of vegetable capsule containing a blend of two viable lyophilized *L. acidophilus* strains: NCFM (FDA GRAS Notice 000357, strain number ATCC SD5221, Danisco Inc. Madison, Wisconsin, United States) and LAFTI L10 (strain number CBS 116.411, DSM Food Specialties, Moorebank, Australia). This mixture of two probiotic strains provides for each 2.5×10^9 colony-forming unit (cfu) for a total of 5×10^9 cfu per capsule. The control product consisted of a vegetable capsule indistinguishable in colour, shape, size, smell and weight from the test product but contained no bacteria. Both products used the same excipient namely maltodextrin, magnesium stearate and colloidal silicon dioxide. Test products were specially manufactured for the study and provided by Laboratoire Denel-Codifra (Le Chesnay, France).

Interventions

Patients were randomized to receive either the study product or a placebo for 8 weeks. The type of randomization was the block randomization method. The trial dose was 2 capsules/day taken orally either in the morning or the evening, with a full glass of water half an hour before eating. At the baseline visit, investigators provided for each included participant a capsule box containing 120 capsules to ensure 8 weeks of consecutive supplementation (56 ± 2 days). Eligible participants also received a diary to report their bowel habits daily and their IBS symptoms weekly for 8 weeks. The diary also included an IBS-specific product questionnaire to answer at the end of study. The schedule of visits is shown in Table 1.

Blinding and randomisation

The study was a double-blind trial. Neither investigators nor patients were aware of product allocation until the end of the trial. An 88-case randomisation arrangement was performed by an independent statistician according to the sequence generated with SAS®

Table 1. Schedule of visits. After the enrolment and allocation, patients will receive either probiotics or placebo for 8 weeks. The time-points of assessment are shown in the schedule.

Visit Time point	Study period			
	Enrolment Visit 1 Day -7 to Day -3	Allocation Visit 2 Day 0	Follow-up Visit 3 Day 28 ± 2	End of trial Visit 4 Day 56 ± 2
Enrolment				
Information and consent form	X			
Inclusion / Exclusion criteria	X	X		
Physical examination	X ^a	X	X	X ^a
IBS symptomatic treatment of the last 15 days	X			
IBS treatment since the last visit		X	X	X
Concomitant medications	X	X	X	X
Safety assessment		X	X	X
Adverse events		X	X	X
Clinical examination				
Urine biological test ^b	X			
Urine pregnancy test ^c	X			
Random products allocation		X		
Compliance			X	X
Assessment				
Diagnosis of IBS ^d	X			
Intestinal symptoms diary ^e		X	X	X
Visual analogue scale for IBS symptoms ^f		X	X	X
Product satisfaction questionnaire				X

a. Clinical examination completed with medical history and vital signs (blood pressure, heart rate, body weight). **b.** Urine biological test (Multistix[®] 10SG test strips) for glucose, protein, ketones, bilirubin, urobilinogen, nitrite, pH, specific gravity, leukocytes and blood. **c.** Non-menopausal women. **d.** Diagnosis of irritable bowel syndrome (IBS) according to Rome III criteria. **e.** Delivery of the patient diary to report their bowel habits (frequency and consistency) daily and IBS symptoms weekly for 8 weeks and monitored by the investigator at baseline visit (Visit 2), follow-up visit (Visit 3) and at the end of trial (Visit 4). **f.** IBS symptoms: pain/discomfort, bloating, flatus/gas and rumbling.

software (SAS[®] 8.2 software (SAS[®], Cary, NC, USA)). The randomization was stratified by care center with participants randomly allocated (1:1 basis) to either probiotics or placebo. The generated sequence provides serial number lists from 01 to 88 corresponding to consecutive allocation and each care centre received consecutively coded drugs. Tested products provided by the sponsor were numbered with a label according to the randomisation schedule. A sealed code break envelope was held by the site of the Pharmacy Department at the university hospital and by investigators. This envelope contained the randomisation list and information related to the randomly allocated product. Code breaking was allowed only in the case of serious adverse event (SAE) or a medical emergency, where knowledge of the treatment allocation was required for an appropriate treatment action. The investigator was asked to immediately inform the sponsor if unblinding occurred. The investigator was indicated to report reasons of the unblinding on the CRF and on the envelope, together with the date and the investigator's stamp. All this information was transmitted by the sponsor of the study to ANSM.

Outcomes assessment

Primary outcome

The score of abdominal pain/discomfort was assessed with a 100-mm visual analogue scale (VAS; 0: none; 100: very severe) (31).

Secondary outcomes were assessed by:

- The scores of bloating, flatus/gas and rumbling assessed with a 100-mm VAS
- The composite score consisting of the sum of the 4 VAS scores (pain/discomfort, bloating, flatus/gas and rumbling) calculated for each patient
- The mean stool frequency per week (calculated from the number of bowel movements per day)
- The mean score of Bristol Stool Form scale per week to assess stool consistency/appearance using the absolute difference from normal, i.e., score 4 (32).

Clinical and safety outcomes

A medical history, including the presence of chronic diseases and regular medication were recorded before inclusion. A physical examination was performed at each visit and vital signs (blood pressure, heart rate, body weight) were also monitored both at screening and final visits.

Data management

Case report forms (CRF) were used by investigators to record data for all participants. CRF were completed by the clinical research associate of the study who sent them to the data administrator to enter the data into the electronic data base using Capture System software (Clinsight[®], Cenon, France) according to the specifications for the present study (33).

Safety assessment

An adverse event (AE) is defined as any adverse change of the patient condition, which means all unfavourable signs, symptoms or progressions of an associated disease already diagnosed at screening visit, whether or not considered related to the study product. This includes unintended signs, symptoms, diseases and initial biological parameters significant deviations from initial biological parameters. All biological parameters for which abnormal results are recorded after the start of the study product intake must be repeated until resolution or results are considered stable. Abnormal results are defined as those which go beyond the limits fixed by pre-determined organic standard and which are clinically significant. AE were monitored throughout the study through regular face-to-face visits and phone calls between visits. The participants were requested to report any AE to the research staff spontaneously. In all the cases of AE, their aetiology must be identified and it must be notified to the sponsor. Any AE recorded on CRF will describe nature (diagnosis, signs and symptoms), severity, starting date, termination date, actions undertaken and relation with the study product according to the investigator. The investigator was requested to state whether or not an AE was considered as a serious adverse event (SAE). The frequency of the AE was reported for each group during the trial according the MedDRA classification.

Serious adverse events

SAE includes, but is not limited to, any event that is fatal (for whatever reason), life-threatening, a persistent or considerable disability, or results in hospitalization or prolonged hospital stay, or results in malformation. Any SAE occurring during the trial observed by the investigator or noted above, whether or not attributed to study product, must be reported on the CRF, one being informed of the event. The investigator shall inform sponsor representatives and the ANSM within 24 hours. The investigator was requested to follow up SAE until resolution or the event was considered stable. A procedure for a re-examination of informed consent and any SAE occurring 1 month after the last intake of the study product or at the end of the study must be notified to the sponsor. Previous studies have shown that probiotics are safe and any SAE that might be possibly, probably or definitely related to the study product should be regarded as unexpected (27, 29).

Sample size calculation

According to the data from a previous trial using probiotics to improve IBS symptoms, a difference of 10 points for the primary endpoint of abdominal pain between the two groups of the trial is expected (4). Assuming a standard deviation (s.d.) of 10 points, the sample size needs to be 23 patients in each group to see these differences with α error = 0.05 and at a statistical power of 90%. By considering the risk that 40% of the patients included could not be assessed at the end of the study, it was necessary to include 40 patients per group (18). Given the risk of failure at the selection, it was planned to include 10% of subjects more than necessary, hence a maximum of 88 subjects to obtain 80 assessable subjects was recruited. This statistical sample size calculation

is consistent with the EMA (European Medicines Agency) guidelines (34).

Statistical analysis

Statistical analysis will be conducted on the intention to-treat (ITT) population which includes every subject who is randomized according to randomized treatment assignment since RCT often suffer from non compliance and missing outcomes limitations. All treated subjects without any major protocol deviations comprise the per-protocol set (PPS). Statistical analysis will be performed using the SAS[®] 8.2 software (SAS[®], Cary, NC, USA) and the statistical significance will be defined as a two-sided P-value < 0.05. Descriptive analysis will be performed with standard indicators. Quantitative data will be presented as means \pm s.d., n, min, max and median and qualitative data as frequency and percentage. The Shapiro-Wilk test as well as measures of Skewness and Kurtosis indicators will be performed in order to check the normal distribution of quantitative data. If the assumption to normality is violated, a non-parametric test will be used. Baseline demographic and biological data will be compared between groups using χ^2 test or Fisher's exact and Student's *t*-test or Mann-Whitney-Wilcoxon test, when appropriate. Primary and secondary outcomes will be assessed using repeated measures analysis of variance (RANOVA) to compare differences between groups and to assess the evolution of outcomes in each group over time. *Post-hoc* multiple comparisons at each time will be conducted using a Tukey's procedure. The primary analysis will be also performed on PPS and the analysis of tolerability will be conducted on the ITT population. Missing data are not replaced. The group sequential design of the current protocol is based on an interim analysis performed with the first 60 subjects allowing for prematurely stopping the trial due to futility or efficacy without options of additional adaptations based on results of interim analysis. Standard statistical methods for group sequential design may not be appropriate to control the overall type-I-error at the desired level of 5% if there is a shift in the target population (35). The interim analysis was used to assess the evolution of the primary outcomes and of the stool frequency and consistency over time using RANOVA. In case of discontinuation, the type-I-error correction should be performed to consider the shift in sample size.

Participants' withdrawal and dropouts

Participants may withdraw from the trial for any reason at any time, but must inform the investigator. In any case, the investigator shall review the participants who are withdrawn as soon as reasonably possible for a full assessment in order to:

- Report the reason on the CRF
- Assess the clinical status of the participant
- Take appropriate therapeutic measures if necessary

Premature termination of the study

The sponsor can stop the trial at any time, for the following reasons:

- Inability of research centers to include participants
- Deviation from Good Clinical Practices and/or clinical regulations
- Insufficient product safety

- Lack of efficacy or significant efficacy according to the results of the interim analysis
- Any new information (SAE) that could affect the safety of participants

The trial can also be stopped by the French competent authority (ANSM) if there is a doubt about the safety or the scientific validity of the study.

Definition of a protocol deviation

Will be considered as major deviation (participant excluded from per-protocol analysis) misdiagnosis (inclusion and exclusion criteria not met), premature termination of the trial or inability to follow-up, taking prohibited medications, lack of data related to the primary outcome. All other cases will be considered as minor deviation and in any cases, investigators shall report reasons of dropouts on the CRF.

Monitoring

The principal investigator monitored the conduct and progress of the project at each site. The trial coordinator visited each study site to make sure that all trial procedures were compliant with the trial protocol. The principal investigator and the research team had regular teleconferences to ensure efficient study execution and ongoing monitoring of the study progress, with summary documents circulated after each meeting.

Discussion

Although the GRAS status of *Lactobacilli* in the food industry is well documented due to their long history in food fermentation, human consumption and known preclinical strain-specific probiotic effects, it is necessary to obtain recognized health claims by providing high quality proof of concept data such as RCT (13, 36). Such health claims for foods are now regulated in the European Community (EC) (36). The EC health claims are reviewed by the scientific committee on dietetic products, nutrition and allergies (NDA) from European Food Safety Authority (EFSA) and if a positive opinion is issued by EFSA, the EC commission approves the health claim for the human food consumption (36-38). The objective of the current clinical study protocol is firstly to provide high quality proof of concept data and secondly to elucidate the requirements for efficacy of a daily consumption of a mixture of *L. acidophilus* probiotic strains during 8 weeks for decreasing abdominal pain/discomfort from patients with IBS. Previous RCT with IBS patients tried to prove the benefits of *Lactobacilli* in IBS symptoms management but most suffered from methodological limitations (4, 17-24). Indeed, among the 9 RCT to improve IBS symptoms in adults patients with *Lactobacilli* probiotics, only 4 have enrolled patients according with Rome III criteria, while previous RCT selected participants diagnosed with IBS according to either author-defined IBS criteria or Rome I and II (4, 17-24). Rome III criteria are the standard today for RCT performed among IBS patients (1). We used the Jadad score and a standard method of ensuring allocation concealment to characterize their quality in terms of clinical methodology (39). The Jadad score (ranging from 0 to 5) could be used to assess the methodological quality of clinical trials. The Jadad score as a

quality scale was based on descriptive criteria including randomization, double-blinding, withdrawals and drop-outs. Methodological score ≤ 2 are considered as low quality whereas score ≥ 3 as high quality. Our study protocol is intended to reach the highest Jadad score of 5/5. However, the Jadad score did not take into account allocation concealment, viewed by The Cochrane Collaboration to limit bias (40). We used a standard method of ensuring allocation concealment by including sequentially numbered, opaque, sealed envelopes (SNOSE); sequentially numbered containers; pharmacy controlled randomization; and central randomization to improve the methodological quality. This allocation concealment method has been included in our RCT's protocol. Among the 9 previous RCT, only 2 of them reported a significant effect compared with placebo on abdominal pain assessed as primary endpoint (21, 22). The significance is also limited by the facts that they were performed with different probiotic strains, i.e. *Lactobacillus plantarum* 299v (*L. plantarum* 299v) and *L. acidophilus*-SDC 2012, 2013, and in Asia with Asian patients with potential differences from European patients (21, 22). Lifestyle and genetic patterns are known risk factors for IBS (41, 42). As shown in Table 2, only 4 RCT performed with *L. plantarum* 299v, *Lactobacillus reuteri* ATCC 55730 (*L. reuteri* ATCC 55730), *Lactobacillus casei rhamnosus* Lcr35, and *L. acidophilus* NCFM have been reported in a multicentre design whereas the 5 others are monocentric trials possibly with less methodological quality (4, 17-24). Two previous trials using *L. plantarum* 299v did not include a provision to clarify whether other IBS medication were permitted (4, 20, 43). This chronic functional gastrointestinal disorder, qualified in medical practice as functional colopathy, is frequent in the western general population with a prevalence of 10 to 15% in the general adult population (44, 45). Even if the mechanisms involved in these disorders are not clearly identified, several factors as neurological and immunological factors associated with a psychological component might contribute to IBS pathophysiology (46, 47). Abdominal pain is considered as an essential component of the diagnostic criteria in IBS and could be the consequence of a visceral hypersensitivity (1, 48). A preclinical study, performed with different probiotic strains belonging to the genera *Lactobacillus* and *Bifidobacterium*, has demonstrated that only *L. acidophilus* NCFM is able to induce, *in-vitro*, the expression of both analgesic receptors MOR1 and CB2 expression in human epithelial cells (14). Besides confirming that probiotics ability to regulate visceral sensitivity are strain-specific, this study has also provided evidence for a beneficial physiological role of CB2 in the control of visceral pain, even if the mechanisms underlying the anti-nociceptive effect remain unclear (14). Recently, the high-quality methodology and dose-response RCT ($n=391$) performed by Lyra *et al.* (2016) with *L. acidophilus* NCFM alone, at the daily dosage of 10^9 or 10^{10} cfu per day, did not demonstrate a significant improvement of IBS Symptom Severity Score (IBS-SSS) as primary endpoint compared with placebo over 12 weeks of treatment (24). However, and consistent with preclinical data, a post hoc analyses of patients with moderate to severe abdominal pain at baseline, i.e. with a IBS-SSS pain score VAS $> 35/100$,

Table 2. Characteristics of randomized clinical trials performed among irritable bowel syndrome (IBS) patients investigating benefits of *Lactobacillus* strains with probiotic properties.

Trial	Diagnostic criteria and design	Size (n)	Probiotics	Daily dosage and duration	Jadad score
Nobaek et al. (2000) (4)	- Author-defined IBS criteria - Monocentric study	60	<i>Lactobacillus plantarum</i> 299v	- 2 x 10 ¹⁰ cfu - 4 weeks	4
Niedzielin et al. (2001) (17)	- Rome I - Monocentric study	40	<i>Lactobacillus plantarum</i> 299v	- 2 x 10 ¹⁰ cfu - 4 weeks	3
Niv et al. (2005) (18)	- Rome II - Multicentric study (2 centers)	54	<i>Lactobacillus reuteri</i> ATCC 55730	- 2 x 10 ⁸ cfu - 6 months	4
O'Mahony et al. (2005) (19)	- Rome II - Monocentric study	77	<i>Lactobacillus salivarius</i> UCC4331	- 1 x 10 ¹⁰ cfu - 8 weeks	5
Simrén et al. (2006) (20)	- Rome II - Monocentric study	40	<i>Lactobacillus plantarum</i> 299v	- 5 x 10 ⁷ cfu - 6 weeks	3
Sinn et al. (2008) (21)	- Rome III - Monocentric study	40	<i>Lactobacillus acidophilus</i> -SDC 2012, 2013	- 2 x 10 ⁹ cfu - 4 weeks	5
Ducrotté et al. (2012) (22)	- Rome III - Multicentric study (4 centers)	214	<i>Lactobacillus plantarum</i> 299v	- 1x 10 ¹⁰ cfu - 4weeks	4
Dapoigny et al. (2012) (23)	- Rome III - Multicentric study (4 centers)	50	<i>Lactobacillus casei rhamnosus</i> Lcr35	- 6 x 10 ⁸ cfu - 4 weeks	4
Lyra et al. (2016) (24)	- Rome III - Multicentric study (2 centers)	391	<i>Lactobacillus acidophilus</i> NCFM	- 1 x 10 ⁹ or10 ¹⁰ cfu - 12 weeks	5

cfu : colony-forming unit.

showed a significant but moderate efficacy between active groups combined and placebo ($n=99$; $P=0.046$) (24). To the best of our knowledge, the only other RCT ($n=40$) performed with the species *L. acidophilus* in IBS patients, i.e., *L. acidophilus*-SDC 2012, 2013 strains, has showed a significant improvement of abdominal pain/discomfort ($P=0.003$) over 4 weeks of treatment at the daily dosage of 2.10⁹ cfu per day (21). Although methodology limitations of this pilot RCT, the reduction in abdominal pain/discomfort as primary endpoint exceeding the placebo score by more than 20% suggests an additional effect of two strains of the same species without opposite effects known (21). Moreover, mucosal immunity activation associated with a low-grade inflammation and increased intestinal permeability could be involved in visceral hypersensitivity observed in IBS patients (47-51). *L. acidophilus* LAFTI L10 has been able to improve symptoms similar to IBS symptoms in healthy subjects with gastro-intestinal disturbances (27). According to the EFSA guidance on the scientific requirements for health claims related to the immune system, the gastrointestinal tract and defence against pathogenic microorganisms published in 2016, abdominal pain or discomfort could occur both in healthy people and IBS patients, however higher frequency and greater severity of symptoms were found in IBS patients (36). Further preclinical studies have demonstrated that *L. acidophilus* LAFTI L10 is able to enhance specific gut immune responses in mice by increasing immunoglobulin A (IgA), interleukin-10 (IL-10) and interferon gamma (IFN γ) producing cells (25, 26). Therefore, we expect that these properties in a mixture of probiotics would contribute to reducing visceral hypersensitivity found in IBS patients. Furthermore as outlined by EFSA guidelines, these patients are considered as an appropriate study group to substantiate health

claims on gastro-intestinal discomfort intended for the general population (36). Taking into account the results from previous studies investigating the effects of different strains of the genus *Lactobacillus* in patients with IBS, a particular attention has been paid to the daily dosage of 5x10⁹ cfu/capsule twice a day (19, 21). Moreover, in order to assess the kinetic effect of the probiotic mixture, one intermediate visit at 4 weeks allows us to perform a RANOVA analysis in order to test the product effect (between-subject effect), the time effect (within-subject effect) as well as interactions between the two types of effects (product x time). Among RCT performed with probiotic strains of *Lactobacillus* and selecting IBS patients with Rome III criteria, only 2 of them have been performed with abdominal pain/discomfort as primary endpoint and both with a significant result over 4 weeks of treatment (21, 22). The current protocol aims to assess abdominal pain/discomfort severity using VAS score as primary endpoint for a study period two times longer. Finally, we use a two-stage adaptive design used for ethical reasons that allows adjustments after the review of the interim analysis as shown in Figure 2. In summary, according to the hypothesis that abdominal pain/discomfort results from a visceral hypersensitivity in IBS, we expect that our study demonstrates that the present mixture of probiotic strains of *L. acidophilus* significantly reduced abdominal pain and improved others secondary endpoints such as other IBS symptoms and bowel habits.

Limitations of the study

In order to confirm the efficacy of probiotics as a well-established therapeutic approach in the management of IBS symptoms, further investigations will be needed by performing other large-scale studies in accordance with EFSA guidance to evidence health-claims

related to gastrointestinal discomfort or bowel function (36). A longer period of analysis could be necessary to substantiate the clinical effects over time. Regarding to Rome III criteria and considering the episodic appearance of this chronic syndrome, an assessment of abdominal pain and other IBS symptoms for a longer period of time between 3 and 6 months could be more appropriate. However, probiotic strains of *L. acidophilus* NCFM and *L. reuteri* ATCC 55730 have been investigated respectively during 3 and 6 months giving no significant improvement of overall IBS symptoms scores (18, 24). Some etiologic factors such as diet or psychological factors may be involved in the pathogenesis of IBS (1, 46, 52-56). An assessment of psychological conditions of participants over the study time using the HRSD (Hamilton Rating Scale for Depression) could also provide further clinical data about the established relationship between emotion and gut sensitivity (23, 24, 57,58). To avoid these possible limitations, exclusion criteria in the present protocol have been established so that patients with change in their diet or undergoing antidepressant and antipsychotic treatments were ineligible. In conclusion, according to the hypothesis that abdominal pain is mainly the result of a visceral hypersensitivity in IBS patients, we expect the LAPIBSS trial design with appropriate quality level methodology providing high quality proof of concept data will bring new insights on the efficacy and safety of probiotic strains of *L. acidophilus* for IBS symptoms relief, especially abdominal pain/discomfort.

Trial status

At the time of submission of this manuscript, the trial has been completed.

Acknowledgements

We are most grateful to Dr Thierry Coste (Novastell, Étrepagny, France) for the proofing of the manuscript and Dr Christel Rousseaux (Intestinal Biotech Development, Lille, France) for helpful discussions on probiotics.

Author Contributions

The author's responsibilities were as follow: SS, BG, SM, JM, OP and JMM for the study design, SS, SRS, SM, JG and JMM in the process of statistical analysis and writing the manuscript. The study was managed and coordinated by research team members (Stéphane Sadrin, Bernard Gout and Olivier Pons). All authors participated in the study and take responsibility for the content of this report. None of the authors have any competing interests except SS who is an employee of Laboratoire Denel-Codifra.

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