



LACTOBACILLUS ACIDOPHILUS VERSUS PLACEBO IN THE SYMPTOMATIC TREATMENT OF IRRITABLE BOWEL SYNDROME: THE LAPIBSS RANDOMIZED TRIAL

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Running title: Irritable bowel syndrome and probiotics

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Abstract

Background: Irritable bowel syndrome is a chronic functional gastrointestinal disorder characterized by abdominal pain/discomfort and altered bowel habits. The use of Lactobacilli as probiotics in irritable bowel syndrome is based on their interesting mechanisms of action and their excellent safety profile but little is known about their clinical efficiency 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 ATCC SD5221 and CBS 116.411 as probiotics to improve irritable bowel syndrome symptoms (LAPIBSS), especially abdominal pain/discomfort.

Abstract

Contexte: Le syndrome du côlon irritable est un trouble gastro-intestinal fonctionnel chronique caractérisé par des douleurs / gênes abdominales et une altération des habitudes intestinales. L'utilisation de Lactobacilli comme probiotiques dans le syndrome du côlon irritable est basée sur leurs mécanismes d'action

intéressants et leur excellent profil d'innocuité, mais on sait peu de choses sur leur efficacité clinique en raison du manque d'essais cliniques bien conçus. Le protocole d'essai clinique actuel vise à déterminer les effets d'un mélange de Lactobacillus acidophilus ATCC SD5221 et CBS 116.411 en tant que probiotiques pour améliorer les symptômes du syndrome du côlon irritable (LAPIBSS), en particulier les douleurs/inconforts abdominaux.

Methods / Design:

Eighty patients with a positive diagnosis of irritable bowel syndrome according to Rome III criteria will be recruited to a multicentre, double-blinded, in parallel groups, placebo-controlled, randomized trial. Patients will be provided with a daily dose of two capsules with two strains of Lactobacilli (5x10⁹cfu/capsule) or placebo for 8 weeks on a 1:1 ratio. The primary outcome is scores of abdominal pain/discomfort assessed with a 100-mm visual analogue scale. The secondary outcome is 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.

Méthodes /

conception: Quatre-vingt patients avec un diagnostic positif de syndrome du côlon irritable selon les critères de Rome III seront recrutés dans

un essai randomisé contrôlé par placebo multicentrique, en double aveugle, en groupes parallèles. Les patients recevront une dose quotidienne de deux capsules avec deux souches de Lactobacilli (5x10⁹cfu / capsule) ou un placebo pendant 8 semaines selon un rapport de 1:1. Le résultat principal est un score de douleur / inconfort abdominal évalué à l'aide d'une échelle visuelle analogique de 100 mm. Le résultat secondaire est les scores de ballonnements, flatulences et grondements testés avec une échelle analogique visuelle de 100 mm, un score composite, la fréquence des selles et la consistance / apparence des selles évaluées avec l'échelle Bristol Stool Form.

Discussion:

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 probiotics after 8 weeks, for lowering abdominal pain.

Ethics and

dissemination: 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 LAPBISS will be disseminated through peer-reviewed publications and at scientific conferences

Discussion: Selon l'hypothèse que les douleurs abdominales sont principalement le résultat d'une hypersensibilité viscérale, le protocole d'étude actuel vise à fournir des données de preuve de concept de haute qualité pour élucider l'efficacité d'une consommation d'un mélange de probiotiques *Lactobacillus acidophilus* après 8 semaines, pour abaissement des douleurs abdominales.

Éthique et diffusion: L'approbation éthique a été donnée par le comité d'éthique Comité consultatif français pour la protection des personnes dans la recherche biomédicale du Sud-Ouest (numéro CPP08-014a) et l'ANSM (Agence nationale française de sécurité du médicament et des produits de santé – numéro B80623-40) . Les résultats de LAPBISS seront diffusés par le biais de publications évaluées par des pairs et lors de conférences scientifiques

Trial registration: EudraCTN°2008-A00844-51

Background

Irritable bowel syndrome (IBS) refers to a chronic functional gastrointestinal disorder without clear case of

idiopathic cause. It is currently defined by the Rome III criteria, including abdominal pain or discomfort, bloating, flatus and altered bowel habits, especially constipation, diarrhoea or alternation of both of them [1]. In the absence of a well-established therapeutic approach, approximately 15% of IBS patients consult a physician and usually seek alternative strategies as probiotics for symptom relief [2-4]. Probiotics are defined by Food and Agriculture Organization of the United Nations and World Health Organization (FAO – WHO) as live micro-organisms which, when administered in adequate amounts, confer a health benefit to the host [5]. Some randomized clinical trials (RCT) have already showed that intake of probiotics, especially lactic acid bacteria (LAB), could be of therapeutic interest, possibly by preventing infectious diarrhoea, and 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 and 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 and 2013 reduced significantly abdominal pain compared with placebo after 4 weeks in a pilot RCT [21]. The second RCT using a dose-response RCT didn't highlight significant benefits of 12 weeks of treatment with *L. acidophilus* NCFM strain compared with placebo to alleviate IBS symptoms. Abdominal pain/discomfort was decreased by the probiotic in a subgroup of patients suffering from moderate to severe pain [24]. Although modest, this latter result 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 account of positive results of the pilot RCT performed with two strains of the same species, a second *L. acidophilus* strain registered under the number CBS 116.411 was added in the current study protocol. This last strain presents gut immunoregulatory properties and proved survival in human gastrointestinal tract as well as an improvement of symptoms equivalent to IBS symptoms in healthy subjects [25-27]. Both strains are also probiotics with approved safety and fermentative properties [27-29]. Thus, the preparation of the current study protocol is to test with the highest methodology the hypothesis that a consumption for 8 weeks of a mixture of two *L. acidophilus* probiotics strains, selected especially 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 are included in the trial with a positive diagnosis of IBS according to Rome III criteria [1]. These criteria are presented in Table 1. The current trial is 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 will check the eligibility of participants and obtain their informed consent. If the eligibility is confirmed within a maximum of 7 days from the date of the first visit, they will be randomized to receive probiotics or placebo for 8 weeks, stratified by care centre. The study design is also adaptive, into two periods, with an interim analysis after the first sixty patients 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 flow chart is shown in Figure 1.

Trial objectives

Primary objective

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

product consumption.

Secondary objectives

The secondary objectives are to assess the effect of this probiotics mixture:

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

Safety and tolerability are also performed throughout the course of the study. Assessment is 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 is 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 has been obtained from the ANSM (French National Agency for Medicines and Health Products Safety – Number B80623-40) and ethics approval of all procedures of the present study has been obtained from the French Consultative Committee for the Protection of Individuals in Biomedical Research of

the South West (Number CPP08-014a). Written informed consent is obtained from each participant. The present trial has been registered in July 2008 under EudraCT number 2008-A00844-51 (European Union Drug Regulating Authorities Clinical Trial) in accordance with Directive 2001/20/EC.

Recruitment of patients

A total of 80 patients who fulfil the screening criteria are 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 are screened among patients already diagnosed with IBS. The current trial has been completed in April 2012.

Inclusion criteria

Patients are eligible for the trial if they provide written informed consent and if they meet 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 Romell 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 read and write) prior to any procedure related to the trial
- Subject affiliated with the French health care system

Exclusion criteria

Patients are not eligible for the trial if they meet 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
- Changes 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 analgic and antispasmodic medication
- 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 enable 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 is 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 probiotics strains provides for each $2,5 \times 10^9$ colony-forming unit (cfu) for a total of 5×10^9 cfu per capsule. The control product consists of a vegetable capsule indistinguishable in colour, shape, size, smell and weight from the test product but contains no bacteria. Both products use 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 will be randomized to receive either the study product or a placebo for 8 weeks. The type of randomization is the block randomization method. The trial dose will be 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 will provide for each included participant a capsule box containing 120 capsules to ensure 8 weeks of consecutive supplementation (56 ± 2 days). Eligible participants will also receive also a diary to report their bowel habits daily and their IBS symptoms weekly for 8 weeks. The diary provides also an IBS-specific product questionnaire to answer at the end of study. The study schedule is shown in Table 2.

Blinding and randomisation

The present study is a double-blind trial. Neither investigators, patients, nor research team members are aware of product allocation until all analysis is completed. An 88-case randomisation arrangement is performed by an

independent statistician according to the sequence generated with SAS® software (SAS® 8.2 software (SAS®, Cary, NC, USA)). The randomization is stratified by care center with participants randomly allocated (1:1 basis) to either probiotics or placebo. The generated sequence provides serial number lists from 001 to 88 corresponding to consecutive allocation and each care centre receives consecutively coded drugs. Tested products provided by the sponsor are numbered with a label according to the randomisation schedule. A sealed code break envelope will be held by the site of the Pharmacy Department at the university hospital, and by the investigator. This envelope contains the randomisation list and information related to the randomly allocated product. Code breaking should only occur in a case of serious adverse event or a medical emergency where knowledge of the treatment allocation is required for an appropriate curative action. If unblinding occurs, the investigator should immediately inform the sponsor. Usually, the investigator should report reasons of these unblinding on the CRF and on the envelope, together with the date and the investigator's stamp. All this information will be transmitted by the sponsor of the study to ANSM.

Outcomes assessment

Primary outcome

Score of abdominal pain/discomfort will be assessed with a 100-mm visual analogue scale (VAS; 0: none; 100: very severe) [31].

Secondary outcomes will be assessed by:

- Scores of bloating, flatus/gas and rumbling assessed with a 100-mm VAS
- Composite score comprised of the sums of the 4 cardinal symptoms VAS scores (pain/discomfort, bloating, flatus/gas and rumbling) calculated for each patient
- Mean stool frequency per week (calculated from the number of bowel movements per day)
- 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 are recorded before inclusion. Physical examination are performed at each visit and vital signs (blood pressure, heart rate, body weight) are also monitored both at screening and final visits.

Data-management

Case report forms

(CRF) are used by investigators to record data for all participants. CRF are completed by the clinical research associate of the study who sends 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 will be monitored throughout the study through regular face-to-face visits and phone calls between visits. The participants are requested to report any AE to the research staff spontaneously. All AE and as far as possible, 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. It will be stated whether or not an AE is considered as a serious adverse event (SAE). The frequency of the AE will be 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 will follow up SAE until resolution or the event is 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

show probiotics are safe and any SAE that might be possibly, probably or definitely related to the study product will 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 is necessary to include 40 patients per group [18]. Given the risk of failure at the selection, it is planned to include 10% of subjects more than necessary, hence a maximum of 88 subjects to obtain 80 assessable subjects will be recruited. This statistical sample size calculation is consistent with the EMEA guidelines [34].

Statistical analysis

Statistical analysis will be conducted based 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 is 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 (R-ANOVA) to compare differences between groups and to assess the evolution of outcomes in each group over the time. Multiple comparisons at each time will be conducted using a Tukey's multiple comparison adjustment method. The primary analysis will be also performed on PPS and the analysis of tolerability will

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 will assess the evolution of the primary outcomes and of the stool frequency and consistency over the time using R-ANOVA. In case of discontinuation, the type-1-error correction will 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 regulations

- Insufficient products safety

- Lack of efficiency or significant efficiency according to the results of the interim analysis

- Any new information that could affect the safety (Serious Adverse Events, SAE) 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 investigators will monitor the conduct and progress of the

project at each site. The trial coordinator will visit each study site to make sure that all trial procedures are compliant with the trial protocol. The principal investigators and the research team will have 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 due to their long history in food fermentation, human consumption and known preclinical strain specific probiotic effect, 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 [36]. The health claims are reviewed by the scientific committee NDA from EFSA and if a positive opinion is issued by EFSA, the EC commission approves the health claim for the human food [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* probiotics during 8 weeks for lowering 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. The Jadad score (ranging from 0 to 5) could be used to assess the methodological quality of clinical trials [39]. The Jadad score as a quality scale was based on descriptive criteria including randomization, double-blinding, withdrawals and dropouts. Methodological score ≤ 2 are considered as low quality whereas score ≥ 3 as high quality. 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 strains of probiotics, i.e. *Lactobacillus plantarum* 299v (*L. plantarum* 299v) and *L. acidophilus*-SDC 2012 and 2013, and in Asia with Asian patients possibly different from European patients [21, 22]. Lifestyle and genetic patterns are known risk factors for IBS [41, 42]. As shown in Table 3, 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 several types of strains of *Lactobacillus* and *Bifidobacterium* probiotic species 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 10⁹ or 10¹⁰ cfu per

day, did not demonstrate a significant improvement of IBS Symptom Severity Score 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, showed a significant but moderate result between active groups combined and placebo (n=99; P=0.046) [24]. To the best of our knowledge, the only other RCT performed with *L. acidophilus* species in 40 IBS patients, *L. acidophilus*-SDC 2012 and 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 scores 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* CBS 116.411 has been able to improve symptoms equivalent 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 gut and immune function published in 2011, abdominal pain or discomfort could occur both in healthy people and IBS patients, however a higher frequency and a greater severity of symptoms were found in IBS patients [36]. Further preclinical studies have then demonstrated that *L. acidophilus* CBS 116.411 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 were able to modulate the inflammation observed 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 account of results from previous studies investigating the effects of different strains of *Lactobacillus* genus in patients with IBS, especially both performed with *L. acidophilus* species, a particular attention has been paid to the daily dosage of 5×10^9 cfu/capsule twice a

day [19, 21, 24]. Furthermore, in order to assess the kinetic effect of the probiotic mixture, one intermediate visit at 4 weeks permits us to perform though a R-ANOVA analysis and 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 1. In summary, according to the hypothesis that abdominal pain/discomfort results from a visceral hypersensitivity in IBS, we expect that our study will showed that the present mixture of probiotics strains of *L. acidophilus* will significantly reduce abdominal pain and improve others secondary endpoints such as 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]. Our study protocol is intended to reach the highest Jadad score of 5/5 [39]. A longer period of analysis could be necessary to substantiate the clinical effects over time. Regarding to the 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 IBS global symptoms score [18, 24]. Some etiologic factors such as diet or psychical 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 are 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 efficiency and safety of *L. acidophilus* probiotics strains for IBS symptoms relief, especially abdominal pain/discomfort.

Trial status

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

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