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Randomised double blind placebo controlled trial on *Lactobacillus reuteri* DSM 17938: improvement in symptoms and bowel habit in functional constipation

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RESEARCH ARTICLE

Abstract

Dysbiosis may contribute to constipation and its symptoms, therefore probiotic administration could improve significantly gut health and functions. The aim of the study was to investigate the effects of a long-lasting administration of *Lactobacillus reuteri* DSM 17938 (LR DSM 17938) on symptoms and quality of life (QoL) score in patients with functional constipation (FC). 56 FC patients with normal colonic transit time and without anorectal disorders and pelvic floor dysfunctions completed the study. LR DSM 17938 was administered for 105 days in a randomised double-blind clinical trial (28 patients per arm). Individual and cumulative scores including the Constipaq, a modified Constipation Scoring System (CSS) that considers the patient assessment of constipation-QoL (PAC-QoL), were calculated during the preliminary visit (V0), at day 15 (end of the induction period with a LR DSM 17938 double dosage, 4×10^8 cfu), day 60 (intermediate evaluation) and day 105 (V4) after a standard dosage (2×10^8 cfu). At the end of treatment, the beneficial effect of LR DSM 17938 compared to placebo was significantly evident for symptoms related to gas content and dysbiosis (abdominal discomfort, pain and bloating), incomplete defecation and helps for defecation ($P<0.05$). At the end of the whole LR DSM 17938 treatment, a marked and positive effect on both the CSS single and the cumulative items was evident with the exception of unfruitful attempt and Bristol score. Present findings indicate that LR DSM 17938 has an effect on symptoms different from stool consistency, and they suggest that this probiotic can effectively be used in association therapy rather than as single-drug therapy in the management of FC.

Keywords: Constipaq, constipation, *Lactobacillus reuteri* DSM 17938, probiotics, symptoms

1. Introduction

Functional constipation (FC) is a disease characterized by less than 3 evacuations per week, straining, hard stools, incomplete evacuation and/or inability to pass stool. In Western countries, the prevalence fluctuates between 17% (Europe) (Peppas *et al.*, 2008) and 28% (North America) (Higgins and Johanson, 2004) and may differ according to the criteria used to define constipation. Besides, prevalence increases with age and is more common in women than men (Lovell and Ford, 2012). These differences are probably due to the fact that constipation is a symptom rather than a disease, susceptible to different and subjective interpretations of a real or imagined disturbance of bowel function. This generates many different definitions, some focusing on the number of weekly defecations and others reflecting the sensation of difficult defecation or incomplete bowel movements (Garrigues *et al.*, 2004). Additionally, other symptoms, such as bloating and discomfort, that may be reported by patients have rarely been used in clinical practice (Ashraf *et al.*, 1996). The Rome criteria (Rome I-IV) (Drossman and Hasler, 2016) have been developed and proposed to obtain a standardised definition of FC, but they are currently used only for research purposes, mainly in clinical trials. Another important issue associated with this condition is the assessment of the quality of life (QoL). Available reports have shown that the severity of symptoms correlates negatively with patient's QoL and this is especially true in constipated women who may suffer from anxiety, depression, and somatization (Koloski *et al.*, 2013).

From a diagnostic point of view, increasing attention has been paid in the last years to a combined evaluation of patients' symptoms and QoL items in an attempt to measure objectively the real effectiveness of specific treatments. Altogether, these parameters have been recognised as valid and may allow an objective measure of the effectiveness of specific treatments. Different scales for clinical evaluation have been developed such as the Constipation Scoring System (CSS), Constipation Assessment Scale, Bowel Symptom Questionnaire for the Elderly, as well as others aimed at evaluating incontinence together with constipation (Agachan *et al.*, 1996; McMillan and Williams, 1989; O'Keefe *et al.*, 1992; Osterberg *et al.*, 1996).

Constipaq is a modified CSS (Agachan *et al.*, 1996) that takes into account the patient's constipation-related PAC-QoL (Marquis *et al.*, 2005). Constipaq has been proven to be reliable for evaluating the actual conditions of and the therapeutic outcomes in these patients (Closa-Monasterolo *et al.*, 2017).

As regards etiopathogenesis, FC has been classified into two distinct types according to the time of colonic transit: normal (NTC) and slow transit constipation (STC) (Preston and Lennard-Jones, 1986). The latter, rather than an idiopathic, functional disease, has to be considered as the result of impaired gastrointestinal (GI) motility, autonomic neuropathy, disorders of the enteric nervous system (Altomare *et al.*, 1999; Bassotti *et al.*, 2013; Lyford *et al.*, 2002), and/or alterations in the profile of circulating GI peptides (Van der Sijp *et al.*, 1998). As concerns NTC, more recently, dysbiosis has been hypothesised to contribute to its onset and clinical manifestation and some probiotics have been shown to affect gut transit and also alleviate constipation (Dimidi *et al.*, 2014). Therefore, the probiotic administration could improve significantly gut health and functions in these patients and, even, in the general population (Zhao and Yu, 2016).

By definition, probiotics are live microorganisms that confer a health benefit on the host when administered in adequate dosages (Hill *et al.*, 2014). Among them, *Lactobacillus reuteri* DSM 17938 (LR DSM 17938) has already been shown to be safe and effective in treating infants and children (Urbanska and Szajewska, 2014).

There are several studies suggesting a possible benefit of probiotics on FC (Choi and Chang, 2015; Mearin *et al.*, 2016), but reports are not unequivocal. This heterogeneity in results may be due to the administration of strains with different modes of action as well as to the different lengths of the proposed treatments. Additionally, patients were often unselected and hence heterogeneous as regards bowel habits and underlying disease mechanisms. In this framework, the aim of the study was to investigate the effects of long-lasting administration (105 days) of LR DSM 17938 on CSS symptoms and PAC-QoL score (calculated as Constipaq) in adult patients with FC and normal colonic transit time (CTT).

2. Materials and methods

Patients

FC patients with normal CTT were recruited from the outpatients of the National Institute of Digestive Diseases, I.R.C.C.S. '*Saverio de Bellis*', Castellana Grotte, Bari, Italy from March 2011 to December 2014. The inclusion criteria were as follows: age ranging 19-65 years at the time of screening; fulfilment of the Rome III Criteria for FC without matching Rome criteria for IBS (i.e. reporting no abdominal pain associated with defecation or none of the additionally required symptoms) (Longstreth *et al.,* 2006); availability of the CTT calculation confirming NTC (see trial scheme); availability of physiologic tests excluding anorectal disorders and pelvic floor dysfunctions as well as GI imaging study (colonoscopy, sigmoidoscopy, abdominal ultrasound, barium enema) during the last 3 years.

The exclusion criteria were: organic constipation, intake of drugs, metabolic diseases, GI diseases, diseases of the enteric nervous system/muscle, concomitant participation in other clinical trials, ingestion of probiotics/prebiotics less than two weeks before the inclusion in the study, major GI surgery, pregnancy, family history of cancer or inflammatory bowel disease, blood disorders, impaired thyroid function, and recent trips to countries with endemic parasitic diseases. Written informed consent was obtained from all participants.

Trial scheme

The study design (Figure 1) provided a preliminary visit (visit 0 – V0) to sign the informed consent and to receive the symptom diary to be completed at home. The same day, the patient data were collected, the questionnaire for diagnosis of FC was administered and the Constipaq and Bristol score was calculated. Furthermore, a clinical and physical examination was performed. By the Constipaq score, constipation is classified as 'mild' (CSS score 6-10), 'moderate' (CSS score 11-15) and 'severe' (CSS score >15) (Table 1). Patient assessment of constipation-QoL (PAC-QoL) consists of 28 items classified as absent (score=0), mild (score=1), moderate (score=2), severe (score=3) and retained forming four subscales (worries and concerns, physical discomfort, psychosocial discomfort and satisfaction) and an overall scale. PAC-QoL scale scores were significantly associated with abdominal pain and constipation severity (Marquis *et al.*, 2005).

At 'visit 1 – V1', after a period of 7 days, during which the patients had to avoid the use of laxatives or enemas, the CCT was performed if not done previously. Sixty radio-opaque

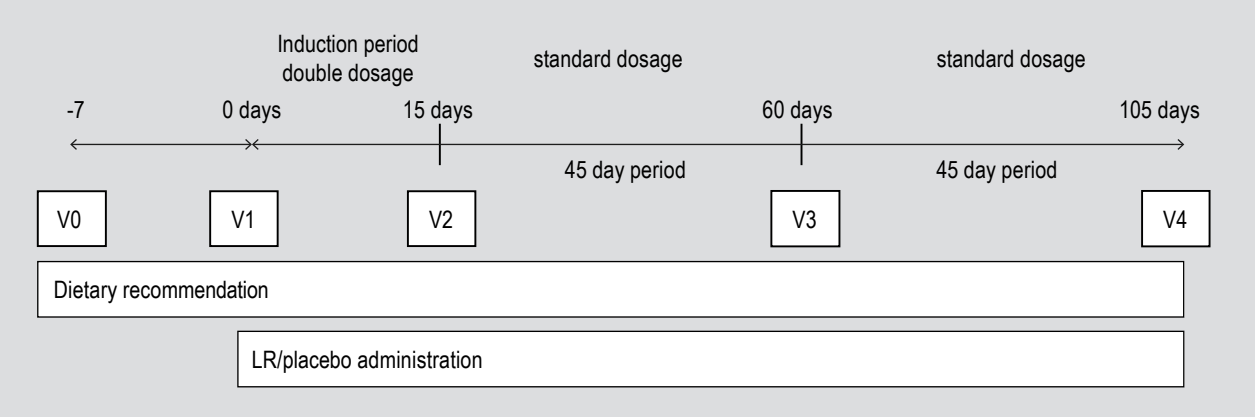

Figure 1. Study design. V0: preliminary evaluation (data collection, clinical and physical examination), questionnaire for diagnosis of functional constipation, Constipaq, Bristol score, informed consent. V1: Clinical end diagnostic evaluation, symptom diary, colonic transit time evaluation. V2: Constipaq, symptom diary, Bristol score. V3: Constipaq, symptom diary, Bristol score. V4: Constipaq, symptom diary, Bristol score. LR= *Lactobacillus reuteri* DSM 17938.

Table 1. Constipation scoring system (CSS) code and Constipaq code.[1]

C stool frequency (number of defecation)	O obstruction, pain, straining
0 >2 per week	0 never
1 2 per week	1 <1 per month
2 1 per week	2 1-4 per month
3 <1 per week	3 1-2 per week
4 <1 per month	4 >2 per week
N incomplete defecation	S abdominal discomfort, pain, bloating
0 never	0 never
1 <1 per month	1 <1 per month
2 1-4 per month	2 1-4 per month
3 1-2 per week	3 1-2 per week
4 >2 per week	4 >2 per week
T time spent in toilet (minutes)	I-P helps for defecation
0 <5 0	0 <1 per week
1 5-10	1 laxatives, suppositories (I) ≥1 per week
2 10-20	2 enema, digitation (P) ≥1 per week
3 20-30	
4 >30	
A unfruitful attempts (numbers)	Q duration of constipation (year)
0 never	0 <1
1 1-3 per day	1 1-5
2 4-6 per day	2 6-10
3 7-9 per day	3 11-20
4 >9 per day	4 >20

[1] Constipaq = CCS (sum of the item scores 0.30) + number of capital letters (0.9) + Quality of Life (sum of the item scores, 0.84).

markers had been divided into three tubes containing 20 markers each. The content of each tube was ingested with water at 12:00 pm for three consecutive days and simple abdominal radiographs were taken at 12:00 pm on days 4 and 7 (Metcalf *et al.,* 1987). The sum of radio-opaque markers was multiplied by 1.2 to obtain the value of CTT in h. According to data on CTT of Western populations, the mean CTT value is 30-40 h. Considering that women have a longer maximal CTT than males, in this study only patients with a CTT less than 40 h, were enrolled (Kim and Rhee, 2012).

At the end of clinical and diagnostic evaluations to confirm the eligibility, the treatment package with placebo or LR DSM 17938 was given to patients, according to the randomisation scheme. The product was in the form of tablets contained in a sealed box showing the randomisation based on four-digit code (1001 to follow). According to the certificate of analysis provided from the manufacturer, the amount of viable cells was expressed as cfu of LR DSM 17938 per probiotic tablet after plating on agar plates. The tablet contained about 7×10^8 cfu, with an expiry date of 2 years from packaging. At the end of shelf life, 1×10^8 cfu/tablet was guaranteed. Placebo consisted of tablet with the same shape, size, colour, and flavour containing ≤2,000 LR cfu/tablet. Tablets are not commercially available and were exclusively produced for this study by BioGaia (Stockholm, Sweden). The patients were instructed to take four tablets daily between meals on the first 15 administration days. This was the 'induction period' with a double dosage compared to the following period in which patients had to take 2 tablets daily between meals. Then, Constipaq and Bristol scores were calculated again at 'visit 2 – V2' (after the 'induction period'), at 'visit 3 – V3' (after 45 days of standard treatment), and at the end of treatment ('visit 4 – V4' – after another 45 standard treatment days), with a total probiotic administration period of 105 days (Figure 1).

The induction period was utilised in order to guarantee the colonization of the gut by LR DSM 17938.

The stool consistency was investigated using the Bristol stool form chart (Lewis and Heaton, 1997). Considering the long period of administration, patients were allowed to use enema and/or laxatives for constipation only after 3 days of absence of evacuation and had to report their use in the diary.

The study was approved by the local Scientific and Ethics Committees of IRCCS '*Saverio de Bellis*', Castellana Grotte (BA), Italy and it was part of a registered research on www.clinicaltrials.gov, reg. number: NCT01244945.

Study endpoints

The primary endpoint was the reduction at the end of the study in the Constipaq score by about 20 score units compared to placebo. Secondary endpoints were constipation symptom item's scores (single score and composite score obtained as the sum of single items) such as GI symptoms, bowel habit, and PAC-QoL.

Statistical analysis

Sample size was calculated to be 28 subjects per arm in order to detect a difference based on the primary endpoint, using a two-tailed test with 80% power and alpha risk of 5%. Considering a drop-out of 20%, we planned to enrol 72 subjects.

Individual and cumulative score were analysed at specific times (V0-V4). Analysis was performed per protocol (PP), including patients entered in the study with no major protocol deviation. Besides, data on primary endpoint had to be available at least on 3 out of the 4 evaluation dates in the study and necessarily include V1 and V4.

The statistical analysis was performed using the t-test for the anthropometric data. The CSS score, PAC-QoL score and Costipaq score were analysed using the Mann-Whitney Rank Sum Test, a nonparametric test since questionnaires are calculated for discrete values. The main reasons for exclusion of patients from the PP population were: deviation on inclusion/exclusion criteria, consumption of prohibited medications, deviation on visit date and consumption of other probiotics during the study.

Linear regression analysis was performed considering the Constipaq score after 105 days of treatment as independent variable and Constipaq at V0 as dependent variable along with treatment as dummy variable included in the model. For the regression, the explained variance (adjusted R square) was determined, and tested with the F-test. T-values and their significance level were calculated to test the hypothesis whether the contribution (the regression coefficient) of an entered variable significantly differed from zero. Data were expressed as medians and the range unless otherwise specified.

3. Results

72 FC adult patients were recruited. Following the inclusion criteria, 56 FC patients with NTC and without anorectal disorders and pelvic floor dysfunctions (28 per arm) completed the study (Figure 2).

Table 2 shows the demographic data (number of men/women, average age, height, weight, and BMI of patients) for the group as a whole as well as LR DSM 17938 and placebo groups. There was a clear predominance of constipated women over men, and all the subjects were suffering from moderate-severe constipation (baseline total CSS median score: 24 (11-29)). No differences between the two groups were present concerning anthropometric data at the beginning of the study. Lastly, no difference in colonic transit time was present comparing the two groups (data not shown).

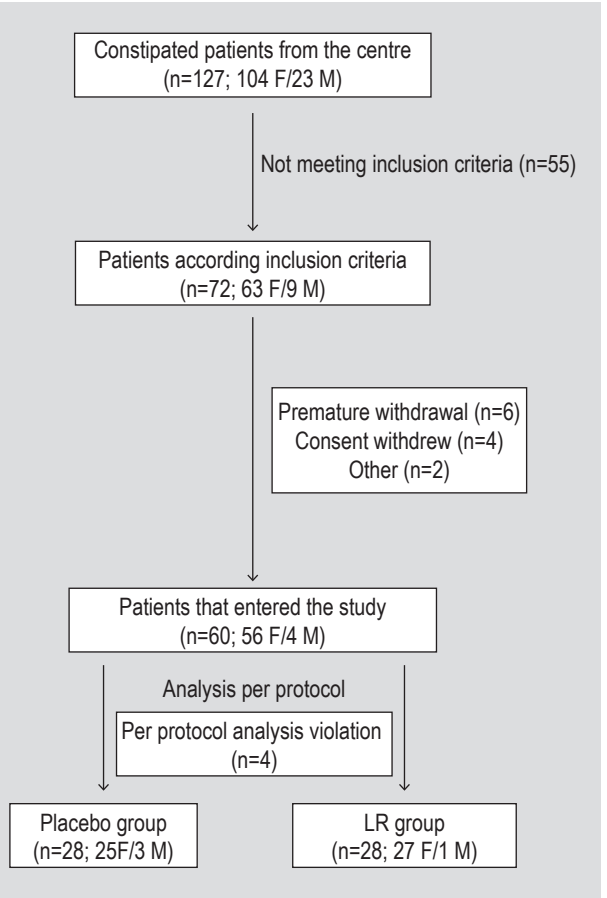

Figure 2. Flow chart of subjects throughout the study.

Table 2. Anthropometric data (expressed as mean ± standard deviation) at the start of the study (V0).[1]

Parameters	Whole group	LR group	Placebo group	T test
Sex	56/4 (total/male)	28/1(total/male)	28/3(total/male)	
Age (years)	43.8±11.5	42.1±11.6	45.5±11.3	ns
Weight (kg)	63.5±10.8	62.1±11.3	64.9±10.3	ns
Height (m)	1.61±0.1	1.61±0.1	1.61±0.1	ns
BMI	24.4±3.8	23.9±3.6	24.8±4.0	ns

[1] V0 = preliminary evaluation; LR = *Lactobacillus reuteri* DSM 17938; BMI = body mass index; ns = not significant.

Regression analysis showed that Constipaq score after 105 days (V4) could be significantly explained by Constipaq at V0 and treatment (F=15.05, df=2, $P<0.001$; adjusted $R^2=0.338$). Specifically, regression analysis demonstrated that the treatment account for a reduction in the Constipaq score at V4, corresponding to 17.25 score units (Table 3).

Table 4 shows each single CSS item and the cumulative items (CSS, PAC-QoL, Contipaq, Bristol score) at the basal value (V0) and after 105 days (V4) for both LR DSM 17938 or placebo group. Comparing LR DSM 17938 and placebo group at the end of the administration period, the CSS item 'N' (incomplete defecation), CSS item 'S' (abdominal discomfort, pain, bloating), and CSS item 'I-P' (helps for defecation: use of laxatives, suppositories and/or enema, digitation) were significantly lower in the LR DSM 17938 group than in the placebo group ($P=0.004$, $P=0.0177$, and $P=0.0067$, respectively). Furthermore, LR DSM 17938 administered for 105 days significantly reduced the CSS total score in treated patients compared to placebo ($P=0.0085$). More in detail, at the end of the supplementation period, significant improvements were observed in both Constipaq ($P<0.0001$) and the PAC-QoL score ($P=0.0001$) in the LR DSM 17938 group compared to the placebo group. No significant change in the stool consistency, calculated as Bristol score, was present due to the wide range of response observed (Table 4).

As regards the LR DSM 17938 group, the comparison of each single CSS item and the cumulative items (CSS, PAC-QoL, Contipaq, Bristol score) between V0 and the end of administration period (V4) (Table 3) showed an evident effect of probiotic on constipation. In fact, LR DSM 17938 treatment not only induced an increase in stool frequency, but also a reduction of the sensation of incomplete defecation as well as the time spent in toilet (CSS 'C' $P=0.0353$, 'N' $P=0.0124$, and 'T' $P<0001$). Besides, LR DSM 17938 significantly reduced GI symptoms (item 'O' obstruction, pain straining $P<0001$; CSS item 'S' (abdominal discomfort, pain, bloating, $P<0001$) as well the use of laxatives, suppositories and/or enema, digitation (CSS item 'I-P' helps for defecation, $P=0.0186$). Lastly, LR DSM 17938 reduced dramatically and significantly the total score of CSS, PAC-QoL, and Constipaq ($P<0001$, $P=0.0018$, $P<0001$, respectively). By opposite, the placebo administration did not modify the item scores at V4, apart from the unfruitful attempt (CSS 'A) and, as a consequence, the total score of CSS (Table 4). As regards the laxative use, only 5 patient in the LR DSM 17938 group took them (17.86%) compared to 17 patients in the placebo group (60.71%) with a difference of 42.85% in the number of patients taking laxatives.

Finally, Figure 3 shows the graph of the profile of the Constipaq score (i.e. CSS + PAC-QoL) throughout the duration of the clinical trial in LR DSM 17938 and placebo groups. The figure shows a clear long-term efficacy of the probiotic compared with the placebo that becomes gradually more evident after 60 days up to 105 days of treatment.

Table 3. Regression analysis of Constipaq score at the end of the treatment (V4).[1]

Parameters	β	Standard error (β)	*P*-value	95% CI
Constipaq (V0)	0.40	0.12	0.003	0.16 to 0.63
Treatment LR/placebo	-17.25	4.41	<0.001	-25.89 to -8.60

[1] V0 = preliminary evaluation; V4 = 105 days of treatment; LR= *Lactobacillus reuteri* DSM 17938; CI = confidence interval

Table 4. Cumulative and single item scores (median and range) of CSS, Constipaq, QoL, and Bristol at V0 and V4.[1]

Parameters	V0		*P*-value	V4 (105 days)		*P*-value	A vs C	B vs D
	LR (A)	Placebo (B)		LR (C)	Placebo (D)			
C stool frequency (n of defecation)	1.0 [0.0-3.0]	0.0 [0.0-3.0]	ns	0.0 [0.0-3.0]	1.0 [1.0-3.0]	ns	0.0353	ns
N incomplete defecation	3.0 [0.0-4.0]	4.0 [0.0-4.0]	ns	2.0 [0.0-4.0]	3.0 [1.0-4.0]	0.0040	0.0124	ns
T time spent in toilet (min)	2.0 [0.0-4.0]	1.0 [0.0-4.0]	ns	1.0 [0.0-4.0]	1.0 [0.0-4.0]	ns	<0.0001	ns
A unfruitful attempt (n)	1.0 [0.0-1.0]	1.0 [0.0-2.0]	ns	0.0 [0.0-3.0]	1.0 [0.0-1.0]	ns	ns	0.0170
O obstruction, pain, straining	3.0 [2.0-4.0]	3.0 [0.0-4.0]	ns	1.5 [0.0-4.0]	2.0 [0.0-4.0]	ns	<0.0001	ns
S abdominal discomfort, pain, bloating	3.0 [1.0-4.0]	3.0 [0.0-4.0]	ns	2.0 [0.0-4.0]	3.0 [0.0-4.0]	0.0177	<0.0001	ns
I+P helps for defecation	1.0 [0.0-2.0]	1.0 [0.0-2.0]	ns	0.0 [0.0-2.0]	1.0[0.0-3.0]	0.0067	0.0186	ns
Q duration of constipation (years)	3.0 [0.0-4.0]	4.0 [1.0-4.0]	ns	3.0 [0.0-4.0]	4.0 [1.0-4.0]	ns	ns	ns
CSS total score	23.0 [15.0-29.0]	24.0 [11-28]	ns	14.0[4.0-29.0]	20.0 [11.0-28.0]	0.0085	<0.0001	0.0274
PAC-QoL score	34.5 [7.0-72.0]	40.0 [12.0-67.0]	ns	19.0[2.0-63.0]	38.0 [9.0-65.0]	0.0001	0.0018	ns
Constipaq score	53.0 [25.0-99.0]	65.0 [31.0-95.0]	ns	31.0[6.0-86.0]	59.0 [30.0-89.0]	<0.0001	<0.0001	ns
Bristol score	2.0 [2.0-4.0]	2.0 [2.0-4.0]	ns	4.0[1.0-5.0]	2.0 [1.0-5.0]	ns	ns	ns

[1] LR= *Lactobacillus reuteri* DSM 17938; CSS = constipation scoring system; QoL = quality of life; V0 = preliminary evaluation; V4 = 105 days of treatment.

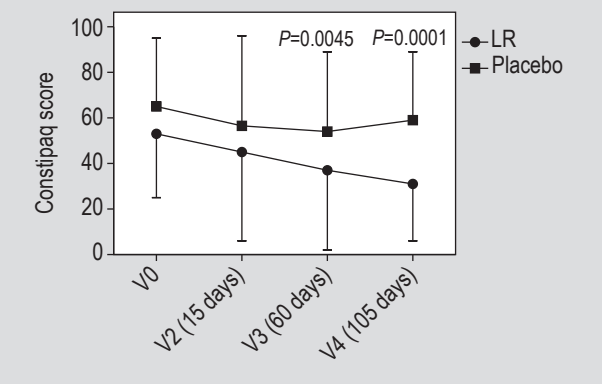

Figure 3. Constipaq profile throughout the duration of the clinical trial in *Lactobacillus reuteri* DSM 17938 (LR) group and placebo group. A significant lower Constipaq score in LR group than placebo group was evident at V3 (60 days of treatment) and V4 (105 days of treatment). Data are expressed as Median and range.

4. Discussion

Our study demonstrated that LR DSM 17938 seems to control symptoms other than stool consistency, more related to gas production and dysbiosis (namely, incomplete defecation, abdominal discomfort, pain, and bloating). Additionally, data highlighted the importance of considering both the symptom profile and the QoL in FC patients.

NCT patients suffer from symptoms largely overlapping with those in STC patients (e.g. difficulty with evacuation and/or the presence of hard stools). However, these patients should be regarded as a distinct class, not showing abnormal colonic motility patterns, nor putative abnormal functioning of the enteric nervous system. They frequently do not respond to medications (i.e. laxatives) and also may experience bloating, abdominal discomfort, or psychosocial distress. Due to these reasons, all those patients suffering from anorectal disorders or pelvic floor dysfunction, along with STC patients were also excluded from the present study.

Constipation may have a significant impact on QoL indicators irrespective of culture and nationalities (Enck *et al.*, 2016). A recent systematic review showed that impairment caused by constipation, as measured by QoL scores, predominates in the mental health domain and is similar to that caused by serious organic conditions (Belsey *et al.*, 2010). This is especially true in women with constipation who may suffer also from anxiety, depression, somatization, all conditions that contribute to lessening QoL (Koloski *et al.*, 2013).

The value of assessing QoL has become increasingly evident in clinical research (Bergner, 1989; Testa and Simonson, 1996; Wilson and Cleary, 1995). Socioeconomic outcomes, such as QoL status and resource utilisation, are more closely related to the patients' well-being perception and impact of disease than results from laboratory tests and measures of disease severity (Bergner, 1989; O'Keefe *et al.*, 1992; Testa and Simonson, 1996; Wilson and Cleary, 1995). Several studies have suggested that FC, but in a more general sense functional diseases, are responsible for substantial health care seeking in both community and referral settings (Koloski *et al.*, 2000), since the patients are more concerned about their QoL and disability than on how long they might live (McNeil *et al.*, 1981).

On these bases, if clinicians really want to obtain a useful tool for measuring the efficacy of treatment, investigation of constipation should contemporary include the symptom profile along with QoL-constipation related items. There are different scales for clinical evaluation of constipations and, among them, Constipaq is a validated questionnaire consisting of CSS that takes into account the constipated patient's QoL. CSS, PAC-Qol and Constipaq have already been proven to be reliable for evaluating the results in constipated patients (Agachan *et al.*, 1996; McMillan and Williams, 1989; O'Keefe *et al.*, 1992, 1995; Osterberg *et al.*, 1996).

Published data globally suggests a superior efficacy of probiotics when compared with laxatives and fibres in relieving constipations via the correction of dysbiosis (Shukla *et al.*, 2015). Probiotics induce the production of short chain fatty acids (butyric acid, propionic acid, and acetic acid) and lower the pH and, in such way, they improve the colonic peristalsis and reduce the intestinal transit time (Rios-Covian *et al.*, 2016). However, there are few well-designed controlled studies on the effect of the administration of probiotics on FC. This is partly due to the lack of standardisation in the definition of constipation and in the different adopted study designs (i.e. collection symptoms, probiotic strain, the concentration of probiotic, duration of treatment, etc.).

As concerns the probiotic administered in the present study, LR DSM 17938 has the ability to colonise the entire human GI tract with a blood safety profile similar to *L. reuteri* ATCC 55730 (LR DSM 17938 is daughter strain of *L. reuteri* ATCC 55730). Its colonisation is only temporary and genome annotation did not reveal any further gene or gene cluster known to be involved in virulence or antibiotic resistance (Rosander *et al.*, 2008). Besides, in accordance with the literature regarding the safety of LR DSM 17938 and of other probiotics, no side effects in the group treated with this strain were recorded during our study.

This bacterial strain has already been used in a paediatric population (Urbanska and Szajewska, 2014) and a recent paper (Wu *et al.*, 2013) showed that LR DSM 17938 increased both colonic migrating motor complex frequency and velocity in an animal model. The authors, based upon the effects of LR DSM 17938 on the adult mouse colon, speculated that this approach may help to screen and identify the therapeutic effect of LR DSM 17938 on constipation and, generally, to correlate the given effect of the probiotic on the enteric nervous system with the action on GI motility.

Data from the present study demonstrate that LR DSM 17938 is better than placebo in improving the Constipaq score, as reported by the linear regression parameters. Besides, LR DSM 17938 ameliorated several specific symptoms related to constipation. In fact, LR DSM 17938 improved the sensation of incomplete defecation (CSS item 'N'), abdominal bloating, discomfort and pain (CSS item 'S'), as well as it reduced the use of enema or laxatives (CSS item 'I-P'). Of note, there was about a 40% reduction in the percentage of patients which assumed laxatives. Last, LR DSM 17938 group improved, although non-significantly, their Bristol score in comparison to the placebo group. The comparison of the questionnaire scores within the LR DSM 17938 group (V0 vs V4) confirmed the beneficial effects, with a significant improvement on the single CSS items and the cumulative items (CSS, PAC-QoL, Constipaq score) apart from unfruitful attempt (CSS 'A') and the Bristol score. The same comparison within the placebo group put in evidence only a reduction in CSS'A' (unfruitful attempts), actually linked to the use of laxatives during the administration period. Therefore, the clinical investigation should not be focused on classical symptoms only (e.g. bowel movements, consistency, and obstruction and straining as well), but, preferably, it should take into account also other symptoms such as discomfort, abdominal pain and bloating. This is quite conceivable in view of the fact that abdominal bloating not only depends on the gas content inside the gut lumen, but also on gas preferential retention within the small bowel, as well as methane effects on gut motility, visceral sensation and regularity, and completeness of defecation (Triantafyllou *et al.*, 2014).

Considering that the PAC-QoL is significantly associated with abdominal pain and constipation severity (Marquis *et al.*, 2005), it might be speculated that LR DSM 17938 could improve constipation via a correction of gas content and dysbiosis, thus reducing methane production and further studies will be aimed at investigating this particular issue.

But, what is the optimal duration of probiotic treatment for an adequate therapeutic response? This is a difficult question to answer. Short term use of probiotics has been found to either reduce the severity of diarrhoea induced by rotavirus (Das *et al.*, 2016) or the incidence of diarrhoea induced by antibiotics administration (Hayes and Vargas, 2016). Another probiotic, *Lactobacillus casei* Shirota, administered for four weeks, proved to be ineffective in ameliorating the stool frequency, consistency, and quantity in constipated patients when compared with controls and the authors suggested that further studies with longer intervention were needed to obtain conclusive results (Mazlyn *et al.*, 2013). A study on short-term *L. reuteri* administration in patients with FC (the same strain administered in our study) proved that this probiotic was able to improve stool frequency, but not consistency after four weeks (Ojetti *et al.*, 2014). The same authors suggested that the lack of a statistically significant difference in stool consistency could be due to the limited time of treatment. However, in our study stool frequency changed, considering the within comparison, but stool consistency did not change, even

after 105 days of treatment. In adults, a long-term treatment modality of probiotics and anti-inflammatory drugs has been demonstrated to be sustainable and it has already been considered as an alternative to corticosteroids in mild-to-moderate ulcerative colitis (Palumbo *et al.*, 2016). A recent randomised, double-blind, placebo-controlled study examined for the first time the long-term effects of probiotic bacteria on infections in normally healthy children. The intervention lasted seven months during the season in which the infection rate is usually highest and it was safe and able to reduce the incidence of infection (Hatakka *et al.*, 2001). Overall, the duration of administration is an important variable to consider for obtaining an effective response in the treatment of constipation. The comparison of the Constipaq profiles from LR DSM 17938 and placebo groups highlighted a significant difference only after a prolonged time of probiotic administration starting from 60th day and persisting up to the end of treatment. In agreement with this notion, present results confirmed that a length longer than 4 weeks of treatment is a necessary condition to obtain significant results.

In conclusion, our study demonstrated the beneficial effect of LR DSM 17938 on several constipation-related symptoms, other than stool consistency. These effects seem to be related to those symptoms (incomplete defecation, abdominal discomfort, pain, bloating) prevalently due to gas and dysbiosis. Finally, according to our opinion, probiotics may represent an effective association with standard protocols even if they are not yet recommended in the pyramid of FC management.

Disclosure of potential conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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