Effect of a Multistrain Probiotic on Cognitive Function and Risk of Falls in Patients With Cirrhosis: A Randomized Trial

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Probiotics can modulate gut microbiota, intestinal permeability, and immune response and could therefore improve cognitive dysfunction and help avoid potential consequences, such as falls, in patients with cirrhosis. The aim of this study was to evaluate the effect of a multistrain probiotic on cognitive function, risk of falls, and inflammatory response in patients with cirrhosis. Consecutive outpatients with cirrhosis and cognitive dysfunction (defined by a Psychometric Hepatic Encephalopathy Score [PHES] < –4) and/or falls in the previous year were randomized to receive either a sachet of a high-concentration multistrain probiotic containing 450 billion bacteria twice daily for 12 weeks or placebo. We evaluated the changes in cognitive function (PHES); risk of falls (Timed Up and Go [TUG] test, gait speed, and incidence of falls); systemic inflammatory response; neutrophil oxidative burst; intestinal barrier integrity (serum fatty acid–binding protein 6 [FABP-6] and 2 [FABP-2] and zonulin and urinary claudin-3); bacterial translocation (lipopolysaccharide-binding protein [LBP]); and fecal microbiota. Thirty-six patients were included. Patients treated with the probiotic (n = 18) showed an improvement in the PHES (P = 0.006), TUG time (P = 0.015) and gait speed (P = 0.02), and a trend toward a lower incidence of falls during follow-up (0% compared with 22.2% in the placebo group [n = 18]; P = 0.10). In the probiotic group, we observed a decrease in C-reactive protein (P = 0.01), tumor necrosis factor alpha (P = 0.01), FABP-6 (P = 0.009), and claudin-3 (P = 0.002), and an increase in poststimulation neutrophil oxidative burst (P = 0.002). *Conclusion:* The multistrain probiotic improved cognitive function, risk of falls, and inflammatory response in patients with cirrhosis and cognitive dysfunction and/or previous falls. (*Hepatology Communications* 2019;3:632-645).

Intestinal dysbiosis, gut barrier failure, bacterial translocation, and subsequent inflammatory response are key factors in the progression of liver diseases.[1] These factors have also been implicated in worsening of liver failure and portal hypertension and, consequently, in the development of complications,

including cognitive dysfunction, after advanced chronic liver disease or cirrhosis is established.[1-3]

Cognitive dysfunction, or minimal hepatic encephalopathy, is a risk factor for overt hepatic encephalopathy, worsening in health-related quality of life (HRQOL), traffic accidents, and mortality in patients with cirrhosis.[4-6] In addition, these patients are more predisposed to accidental falls.[7,8]

Falls are particularly important in patients with cirrhosis, because they have a greater risk of fracture than the general population.[9] Moreover, falls are a significant cause of complications, mortality, and HRQOL impairment.[6-9] In addition to their negative consequences for the patient, falls have implications for the patient's relatives and are an economic and social burden for the community.[10] Moreover, individuals with previous falls are frequently predisposed to recurrent falling,[7,8,11] thus supporting the growing concept of frailty in patients with cirrhosis[12] and the need for preventive measures.[7-9]

Probiotics can modulate gut microbiota, the intestinal barrier, and the immune response and could therefore decrease pathological bacterial translocation and ameliorate immune system alterations in cirrhosis.[1,13-15] These changes could not only improve liver function and help prevent bacterial infections and other complications, but also ameliorate cognitive dysfunction and avoid its consequences, such as overt hepatic encephalopathy, traffic accidents, deterioration of HRQOL, and falls.[1,13,14]

Studies have shown that certain probiotics improve the proinflammatory state,[1,13,15] liver function,[1] portal hemodynamics,[1] cognitive function,[1,16] incidence of overt hepatic encephalopathy,[1,16] and HRQOL[1] in patients with cirrhosis. However, the effect of probiotics on the risk of falling in these patients has not been previously evaluated.

The aim of this study was to evaluate the effect of a multistrain probiotic on cognitive function and the risk of falls in patients with cirrhosis who also have cognitive dysfunction and/or previous falls. We also studied potential mediators of these effects, particularly the systemic inflammatory response, the gut barrier, bacterial translocation, and intestinal microbiota.

Patients and Methods

Between February 2013 and March 2016, we performed a double-blind placebo-controlled randomized trial at Hospital de la Santa Creu i Sant Pau, a tertiary care hospital in Barcelona, Spain. The protocol conformed to the 1975 Declaration of Helsinki and Guidelines for Good Clinical Practice in Clinical Trials and was approved by the clinical research ethics committee (Comitè d'Ètica d'Investigació Clínica-CEIC) at our institution. Monitoring was performed by the Research Institute (IIB-Sant Pau) according to good clinical practice recommendations. All patients received information regarding their participation in the study and signed an informed consent form. The protocol was registered at ClinicalTrials.gov (NCT01686698).

PATIENT SELECTION

We included consecutive outpatients with cirrhosis who had cognitive dysfunction and/or falls during the previous year and were visited at the nursing outpatient office. Patients were selected by the nurses and hepatologists involved in the study. Cirrhosis was

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diagnosed by means of clinical, analytical, and ultrasonographic findings or by liver biopsy. The presence of cognitive dysfunction was defined as a Psychometric Hepatic Encephalopathy Score (PHES) of less than –4,[17,18] and previous falls were assessed using a described questionnaire.[7,8] Exclusion criteria were hospitalization during the previous month, hepatocellular carcinoma or any other neoplasia, acute or chronic overt hepatic encephalopathy, neurologic disease, active alcohol intake (in the previous 3 months), clinically significant cognitive impairment, inability to perform psychometric tests, severe comorbidities, life expectancy of less than 6 months, any treatment with nonabsorbable disaccharides, laxatives, antibiotics and/or antivirals in the previous 3 months, and refusal to participate in the study.

STUDY DESIGN

Patients were randomized by a hepatologist, other than those who selected the patients, to take either a probiotic (probiotic group) or a placebo (placebo group). Randomization was performed by means of a computer-generated sequence using blocks of four and consecutively numbered opaque sealed envelopes. All patients were treated for 12 weeks and assessed at baseline and at 12 weeks (end of treatment) for clinical and analytical data, complications of cirrhosis, side effects, adherence, cognitive function, risk of falls, systemic inflammatory response, and biomarkers of intestinal barrier and bacterial translocation. A subgroup of patients was evaluated for fecal microbiota. Additional visits were performed at week 6 and week 20 (8 weeks after the end of treatment) to check patients' clinical and analytical (routine standard analysis) status, side effects, adherence (only at week 6), and incidence of falls. Patients were instructed to return the boxes with the used and unused sachets at the week 6 and week 12 visits to assess the adherence. The blinding was maintained for participants and the study team until the end of the study and the analysis of the results.

STUDY PRODUCT

The probiotic formulation was a mixture of eight strains, namely, *Streptococcus thermophilus* DSM 24731, *Bifidobacterium breve* (*B. breve*) DSM 24732, *B. longum* DSM 24736, *B. infantis* DSM 24737, *Lactobacillus paracasei* (*L. paracasei*) DSM 24733,

L. acidophilus DSM 24735, *L. delbrueckii* subsp *bulgaricus* DSM 24734, and *L. plantarum* DSM 24730, at a total dose of 450 billion live bacteria per 4.4 g sachet with maltose and silicon dioxide as excipients. The probiotic mixture was manufactured by Danisco-DuPont (Madison, WI) and is currently marketed under the brand Vivomixx (MENDES, S.A., Lugano, Switzerland) in Europe and Visbiome (ExeGi Pharmaceuticals, Rockville, MD) in the United States. The placebo contained maltose and silicon dioxide as inactive agents and was formulated as identical in appearance to the active agent. Patients were instructed to keep the study product at 4ºC in the refrigerator at home and to take one sachet diluted in one glass of water, milk, or juice at room temperature twice daily (every 12 hours) over 12 weeks.

OUTCOMES AND ENDPOINTS

At baseline and at the end of treatment, we assessed cognitive function according to PHES and the risk of falls by means of the Timed Up and Go (TUG) test and gait speed. The main endpoints were cognitive function and risk of falls, and the secondary endpoints were fall incidence, HRQOL, systemic inflammatory response, gut barrier, bacterial translocation, and fecal microbiota.

CLINICAL AND ANALYTICAL DATA

At baseline, week 6, week 12 (end of treatment), and week 20 (end of study), we recorded demographic, clinical, and analytical data. We also recorded parameters that can influence the predisposition to fall, including comorbidity, muscular strength, visual acuity assessed by the Snellen test, and walking problems.[7,8]

COGNITIVE FUNCTION

Cognitive function was evaluated by the PHES, a neuropsychological battery widely used to assess cognitive function in patients with cirrhosis,[17] validated for the Spanish population,[18] using the computer program of the Spanish Network of Hepatic Encephalopathy (www.redeh.org). Patients were considered to show minimal hepatic encephalopathy or cognitive dysfunction when they showed a PHES score of less than –4.[17,18]

RISK OF FALLS

The risk of falling was assessed using the TUG test and gait speed.[8,19,20] The TUG test measures the time the patient needs to stand up from a chair, walk 3 m, turn around, walk back, and sit down in the chair without support. Gait speed was measured according to the time taken to walk 5 m. Gait speed is generally considered as abnormal and representative of increased risk of falling when it is 1 m/second or slower.[19]

INCIDENCE OF FALLS

Falls were prospectively assessed at each visit during the study using a described specific questionnaire[7,8] and a revision of clinical records. The number of falls, severity of injuries, and health care needed were also recorded. Injuries were categorized as contusion, wound or fracture, and health care needed was classified as primary care, emergency room care, or hospitalization.[7,8]

HAND GRIP MUSCULAR STRENGTH

Sarcopenia, a factor related to the predisposition to fall,[11] was evaluated by hand grip muscular strength using a dynamometer (KERN MAP-BA-s-0910) following the manufacturer's instructions.

HRQOL

To evaluate HRQOL, we used the Nottingham Health Profile (NHP), a general questionnaire that has been used in patients with cirrhosis.[21] The NHP has two parts. The first part includes 38 items that cover six domains, with scores ranging from 0 to 100. The second part consists of seven questions about limitations due to health problems in relation to seven functional activities of daily life: occupation, housework, social life, family life, sexual function, hobbies, and holidays. Higher scores or percentages indicate worse quality of life. We used the validated Spanish version of the NHP.[22]

SYSTEMIC INFLAMMATION

We determined serum C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), interleukin (IL) 10 (BD Pharmingen, San Diego, CA), and IL-6 (ImmunoTools, Friesoythe, Germany) in serum using specific enzyme-linked immunosorbent assays (ELISA). To determine neutrophil oxidative burst (reactive oxidants), we incubated 10^5 peripheral blood cells for 20 minutes either with 200 ng/mL phorbol 12-myristate 13-acetate (PMA) (Sigma-Aldrich, St. Louis, MO) or without stimulus at 37ºC. The formation of reactive oxidants was monitored by the oxidation of dihydrorhodamine 123 (Sigma-Aldrich) to rhodamine by flow cytometry.

INTESTINAL BARRIER AND BACTERIAL TRANSLOCATION

The integrity of the intestinal barrier was assessed indirectly by the determination of fatty acid–binding protein 6 (FABP-6; Cloud-Clone Corp., Houston, TX), FABP-2 (Cloud-Clone Corp.), and zonulin (Bioss Antibodies Inc., Woburn, MA) in serum, and claudin-3 in urine (Elabscience, Houston, TX)[23,24] using specific ELISA. We determined lipopolysaccharide-binding protein (LBP) concentration in serum using specific ELISA (Biometec GmbH, Greifswald, Germany) according to the manufacturer's instructions.

FECAL MICROBIOME ANALYSIS

We analyzed the fecal microbiome in the second half of patients included in the study. Patients provided fecal samples at baseline and at 12 weeks (end of treatment) to assess changes in their microbiomes. The methods for sample collection, genomic DNA extraction, and high-throughput DNA sequencing have been described[25] and are detailed in the Supporting Information.

STATISTICAL ANALYSIS

To compare the two groups at baseline, we used the Fisher's exact test for categorical variables. The Student t test (if the data distribution was normal) or the Mann-Whitney U test (if the distribution was not normal) were used for quantitative variables. To compare the changes between baseline and the end of treatment in each group, we used the Student t test if the data distribution was normal and the Wilcoxon test if the distribution was not normal. Normality was

assessed by the Shapiro–Wilk test. Correlations were assessed by the Spearman test. Data are expressed as frequencies, percentages, and mean ± SEM. A two-sided P value of less than 0.05 was considered statistically significant. The methods used for statistical analysis of changes in the fecal microbiome are described in the Supporting Information.

Sample size was calculated according to previous data on the effect of the probiotic on cognitive function in patients with cirrhosis.[16] We used the computer program GRANMO version 7.12, released in 2012 and developed by the Institut Municipal d'Investigació Mèdica, Barcelona, Spain. We considered a resolution rate of minimal hepatic encephalopathy of 55% in patients receiving the probiotic versus 10% in patients receiving placebo. With a calculated 10% of patients lost during follow-up, an alpha error of 0.05, and a power of 0.80, 17 patients per group would be necessary to show statistically significant differences in the effect on cognitive function between the probiotic and the placebo. Statistical analysis was performed using the program released in 2017 (IBM SPSS Statistics for Windows, Version 25.0; IBM Corp, Armonk, NY).

Results

PATIENT CHARACTERISTICS

From February 2013 to March 2016, 279 patients with cirrhosis were evaluated for eligibility at the nursing outpatient office (Fig. 1). Of the patients, 158 had a PHES of at least –4 and no previous falls, and therefore did not fulfill the inclusion criteria. Of the remaining 121 patients, 85 met one or more exclusion criteria. Finally, 36 patients were randomized to one of two groups: 18 to the probiotic group and 18 to the placebo group. As indicated in Table 1, the two groups had similar clinical and analytical characteristics at baseline. In the probiotic group, 1 patient died during the treatment period and therefore was not evaluated at the 12-week visit (end of treatment), and we could not determine gait speed and TUG test at the end of treatment in another patient due to hip enthesitis. In the placebo group, 1 patient died after the end of treatment but before the 20-week follow-up visit.

COGNITIVE FUNCTION

Cognitive function evaluated by the PHES improved at the end of treatment (12-week visit) compared with baseline results in the probiotic group (–1.29 ± 0.72 to –0.35 ± 0.82; P = 0.006). No changes were observed in patients in the placebo group (Fig. 2).

RISK OF FALLING AND INCIDENCE OF FALLS

We observed a decrease in TUG (11.38 ± 0.57 to 10.00 ± 0.49 seconds; P = 0.015) and an increase in gait speed (0.90 ± 0.05 to 1.12 ± 0.10 m/second; P = 0.02) at the end of treatment (12-week visit) in comparison to the baseline results in the probiotic group, whereas we did not observe changes in the placebo group (Fig. 2).

The incidence of falls throughout the 20 weeks of the study was 0 of 18 (0%) in the probiotic group and 4 of 18 (22.2%) in the placebo group (P = 0.10). Regarding the severity of injuries due to falls, one fall caused a wound, whereas the others involved contusions only. The patients were treated at primary health care centers, and one was seen at the emergency room. No hospitalizations were required.

HAND GRIP MUSCULAR STRENGTH

No statistically significant differences were found between baseline and end-of-treatment results either in the probiotic group (20.76 ± 2.27 to 20.62 ± 2.05 kg; P = 0.78) or in the placebo group (20.98 ± 2.28 to 20.24 ± 2.05 kg; P = 0.12).

HRQOL

At baseline, patients in both groups showed a reasonably well-preserved HRQOL, as seen from the low values in each domain from the first part of the NHP and in the functional activities of daily life in the second part of the questionnaire (Supporting Fig. S1). We did not observe statistically significant changes at the end of the treatment in either group, but there was a trend to an improvement in the domain of social isolation in the probiotic group (P = 0.05).
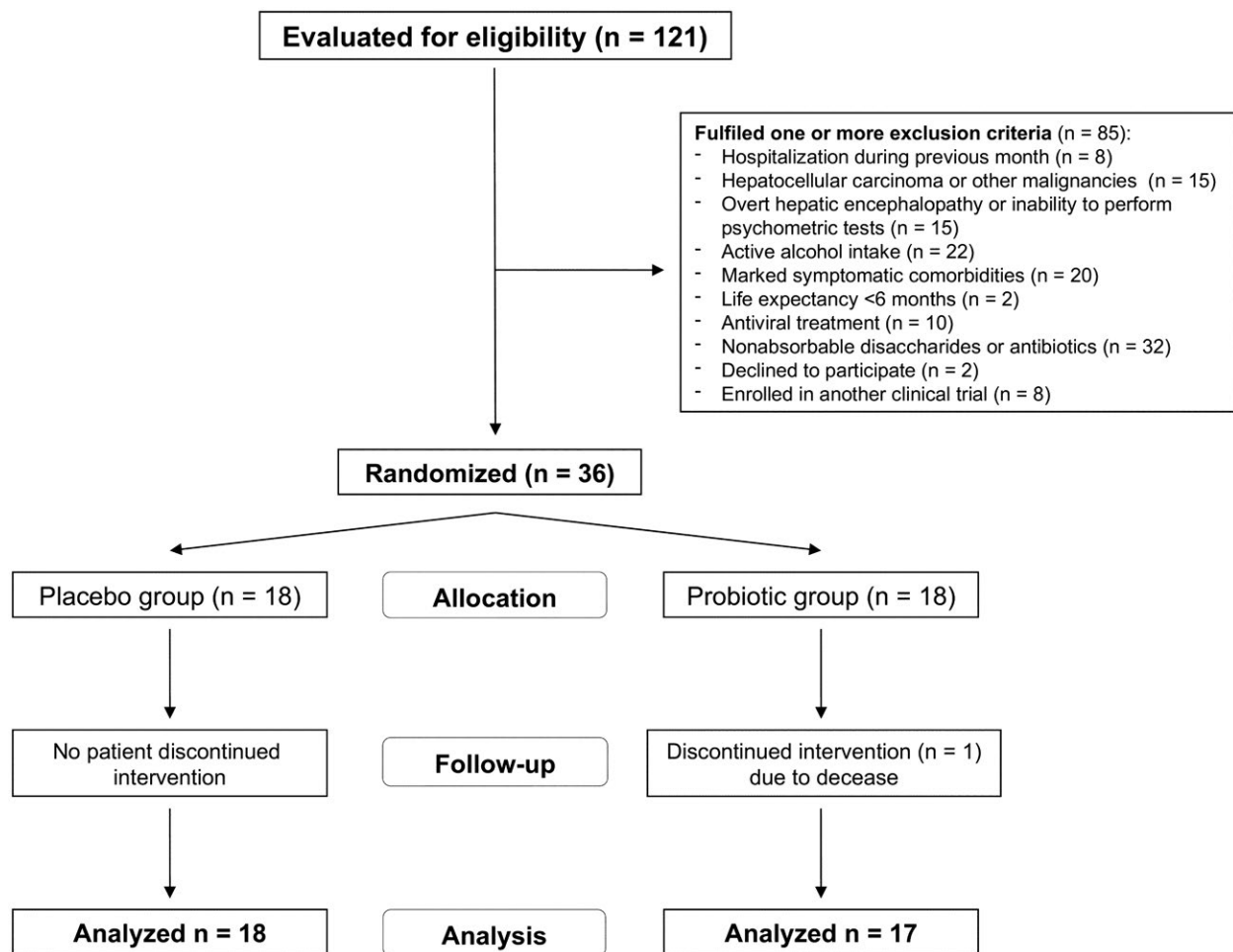
FIG. 1. Flowchart of the study. After the end of treatment (week 12), patients were followed up until week 20. One patient in the placebo group died during this period, at week 16.

LIVER AND RENAL FUNCTION

As indicated in Table 2, no variations in liver or renal function were found at the end of treatment in comparison to baseline values in either group.

SYSTEMIC INFLAMMATORY RESPONSE

There was a decrease in serum CRP (7.62 ± 2.64 to 3.45 ± 0.98 mg/L; $P = 0.01$) and TNF-α ($P = 0.01$) at the end of treatment in the probiotic group, without significant changes in IL-6 or IL-10. Neutrophil oxidative burst after stimulation with PMA increased significantly at the end of treatment in the probiotic

group ($P = 0.002$), without significant changes in resting oxidative burst. No changes were observed in the placebo group (Fig. 3).

INTESTINAL BARRIER AND BACTERIAL TRANSLOCATION

Regarding the intestinal barrier, there was a decrease in serum FABP-6 ($P = 0.009$) and urinary claudin-3 ($P = 0.002$) in the probiotic group at the end of treatment without changes in serum FABP-2 or zonulin. There were no variations in the placebo group. We observed a not statistically significant trend toward a decrease in LBP in the probiotic group ($P = 0.13$) (Fig. 4).

TABLE 1. BASELINE CLINICAL AND ANALYTICAL CHARACTERISTICS OF PATIENTS FROM THE PLACEBO AND PROBIOTIC GROUPS

	Placebo Group (n = 18)	Probiotic Group (n = 18)	P
Age (years)	64.0 ± 2.6	65.8 ± 3.1	0.65
Sex (male/female)	8 (44.4)/10 (55.6)	6 (33.3)/12 (66.7)	0.73
Etiology (%)			0.90
Alcohol	10 (55.6)	9 (50)	
Hepatitis C virus	3 (16.7)	3 (16.7)	
Hepatitis B virus	1 (5.6)	1 (5.6)	
Hepatitis C virus and alcohol	2 (11.1)	1 (5.6)	
Other	2 (11.1)	4 (22.2)	
Previous decompensations (%)	13 (72.2)	16 (88.9)	0.40
Previous hepatocellular carcinoma (%)	2 (11.1)	1 (5.6)	1.00
Child-Pugh score	6.0 ± 0.4	5.9 ± 0.2	0.42
MELD score	9.3 ± 0.8	9.0 ± 0.9	0.78
Minimal hepatic encephalopathy (%)	6 (33.3)	4 (22.2)	0.71
Previous falls (%)	15 (83.3)	15 (83.3)	1.00
Severe deficit visual acuity* (%)	0	0	1.00
Walking problems† (%)	4 (22.2)	2 (11.1)	0.66
Modified Charlson comorbidity score	2.8 ± 0.3	2.5 ± 0.4	0.46
Body mass index (kg/m^2)	28.9 ± 1.7	26.1 ± 0.9	0.27
Diabetes mellitus (%)	6 (33.3)	5 (27.8)	1.00
Diuretics (%)	10 (55.6)	11 (61.1)	1.00
Beta blockers (%)	7 (38.9)	8 (44.4)	1.00
Proton pump inhibitors (%)	10 (55.6)	9 (50)	1.00
Antidepressants (%)	2 (11.1)	2 (11.1)	1.00
Serum sodium (mmol/L)	138.7 ± 0.8	140.1 ± 0.8	0.35
Serum creatinine (μmol/L)	76.1 ± 6.2	80.5 ± 8.1	0.67
Serum bilirubin (μmol/L)	21.1 ± 4.6	19.2 ± 2.6	0.88
Serum albumin (g/L)	36.8 ± 1.4	37.7 ± 0.8	0.49
INR	1.18 ± 0.04	1.19 ± 0.06	0.44
Mean arterial pressure (mm Hg)‡	91.3 ± 3.4	94.7 ± 4.3	0.54

Note: Data are expressed as number (%) of patients and mean ± standard error of the mean.
*Less than 3 of 10 using Snellen number chart.
†Use of any walking-aid device.
‡Millimeters of mercury.
Abbreviations: INR, international normalized ratio; and MELD, Model for End-Stage Liver Disease.

FECAL MICROBIOTA

We analyzed the changes in the fecal microbiota in the second half of patients included in the study (9 patients from the probiotic group and 8 patients from the placebo group). We did not observe significant changes at the end of the treatment in comparison with baseline data in either group at a phylum, genus, or species level (false discovery rates > 0.21

for all phylogenetic levels and all bacterial groups). Sequence data were deposited in the National Center for Biotechnology Information database, with access number PRJNA499079.

CORRELATIONS AMONG INTESTINAL BARRIER, INFLAMMATORY RESPONSE, COGNITIVE FUNCTION, AND RISK OF FALLING

Including all patients from the two groups at baseline, we observed mild but significant correlations between FABP-6 and LBP (r = 0.54; P = 0.002); FABP-6 and CRP (r = 0.40; P = 0.01); LBP and CRP (r = 0.42; P = 0.01); CRP and poststimulated neutrophil oxidative burst (r = –0.43; P = 0.01); poststimulated oxidative burst and TUG test (r = –0.37; P = 0.03); and TUG test and PHES score (r = –0.41; P = 0.01).

ADVERSE EVENTS, COMPLICATIONS OF CIRRHOSIS, AND MORTALITY

The incidence and number of adverse events and severe adverse events during treatment and follow-up were similar in the two groups (Supporting Table S1). Ten patients (55.5%) in the probiotic group and 12 patients (66.6%) in the placebo group (P = 0.73) presented 13 and 21 adverse events, respectively. Specifically, in the probiotic group, 2 patients presented complications of cirrhosis (1 patient died at week 6 due to respiratory infection and spontaneous bacterial peritonitis [SBP], and another presented grade 2 hepatic encephalopathy). In the placebo group, 3 patients presented complications of cirrhosis (1 patient was diagnosed with hepatocellular carcinoma, 1 patient developed ascites, and another died at week 16 due to SBP). Overall mortality during the study was therefore 2 of 36 (5.5%), 1 patient in each group. Regarding infections, 4 patients from the probiotic group presented five infections, and 5 patients from the placebo group presented five infections (Supporting Table S1). No adverse events attributable to the study product were observed. Overall adherence was considered satisfactory, as patients took 95.8% of the assigned sachets (97.2% in the probiotic group and 94.4% in the placebo group).
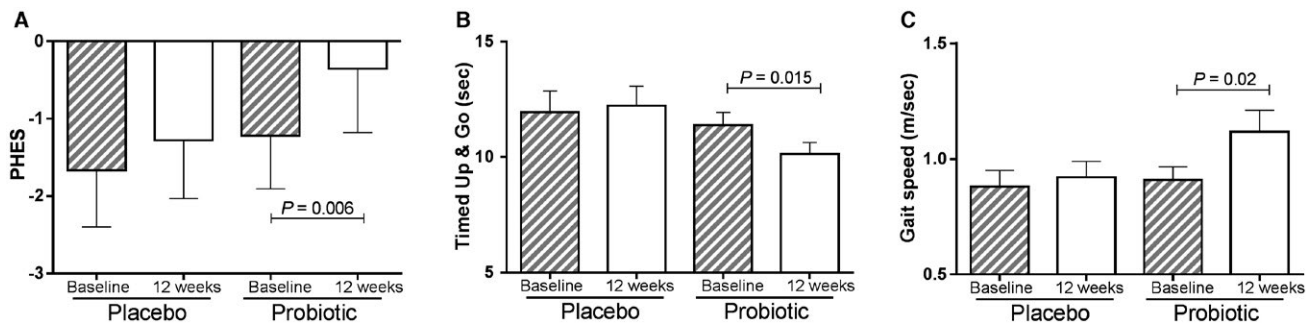
FIG. 2. Cognitive function assessed by the PHES (A) and risk of falls assessed by TUG test (B) and gait speed (C) at baseline and at the end of treatment (12 weeks) in the placebo group (n = 18) and in the probiotic group (n = 17 for PHES and n = 16 for TUG and gait speed). Data are expressed as mean ± SEM. *P* is not significant for any parameters in the placebo group.

TABLE 2. LIVER AND RENAL FUNCTION AT BASELINE AND AT THE END OF TREATMENT (WEEK 12) IN THE PLACEBO GROUP AND THE PROBIOTIC GROUP

	Placebo Group (n = 18)			Probiotic Group (n = 17)		
	Baseline	End of Treatment (week 12)	*P*	Baseline	End of Treatment (week 12)	*P*
Serum sodium (mmol/L)	138.7 ± 0.8	139.2 ± 0.7	0.47	139.7 ± 0.8	139.9 ± 0.8	0.84
Serum creatinine (μmol/L)	76.1 ± 6.2	75.3 ± 6.6	0.69	74.0 ± 5.2	75.0 ± 6.7	0.66
Serum bilirubin (μmol/L)	21.1 ± 4.6	23.2 ± 5.3	0.10	18.2 ± 2.6	18.6 ± 2.5	1.00
Serum albumin (g/L)	36.8 ± 1.4	37.3 ± 1.3	0.23	37.9 ± 0.8	38.1 ± 0.7	0.54
INR	1.18 ± 0.04	1.19 ± 0.04	0.58	1.20 ± 0.06	1.15 ± 0.05	0.69
AST (U/L)	43.5 ± 4.7	46.1 ± 4.9	0.43	38.6 ± 5.7	36.9 ± 5.4	0.80
ALT (U/L)	36.4 ± 5.1	41.3 ± 6.5	0.76	30.7 ± 8.1	36.3 ± 9.3	0.06
GGT (U/L)	92.7 ± 16.1	110.0 ± 22.2	0.34	68.4 ± 15.4	55.2 ± 10.4	0.05

Note: Data are expressed as mean ± SEM.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; and INR, international normalized ratio.

Discussion

The main finding of the present study is that treatment with a multistrain probiotic improved cognitive function and decreased the risk of falling in patients with cirrhosis who had cognitive dysfunction and/or previous falls.

Because of the strict exclusion criteria to avoid confounding factors, the patients included were highly selected and in a stable clinical situation, as can be observed by their low Child-Pugh and Model for End-Stage Liver Disease scores and the low prevalence of abnormal PHES results. Most of them were included in the study because of previous falls, and their TUG test and gait speed values were clearly impaired and indicative of risk of falling.[19,20] At the end of the probiotic treatment, however, the final results of the TUG test and gait speed indicated an improvement in the risk of falls in the probiotic group, but not in the placebo group.[19,20] Moreover, there was a not significant decrease in the incidence of accidental falls throughout the study in patients from the probiotic group in comparison with the placebo group (0% versus 22.2%), although the study was not designed with the statistical power to demonstrate differences in this clinical endpoint.

The cause of the predisposition of patients with cirrhosis to falling is probably multifactorial, but cognitive dysfunction (or minimal hepatic encephalopathy) appears to play an important role.[8,26] Cognitive function improved in patients from the probiotic group, as shown by the increase in the PHES results
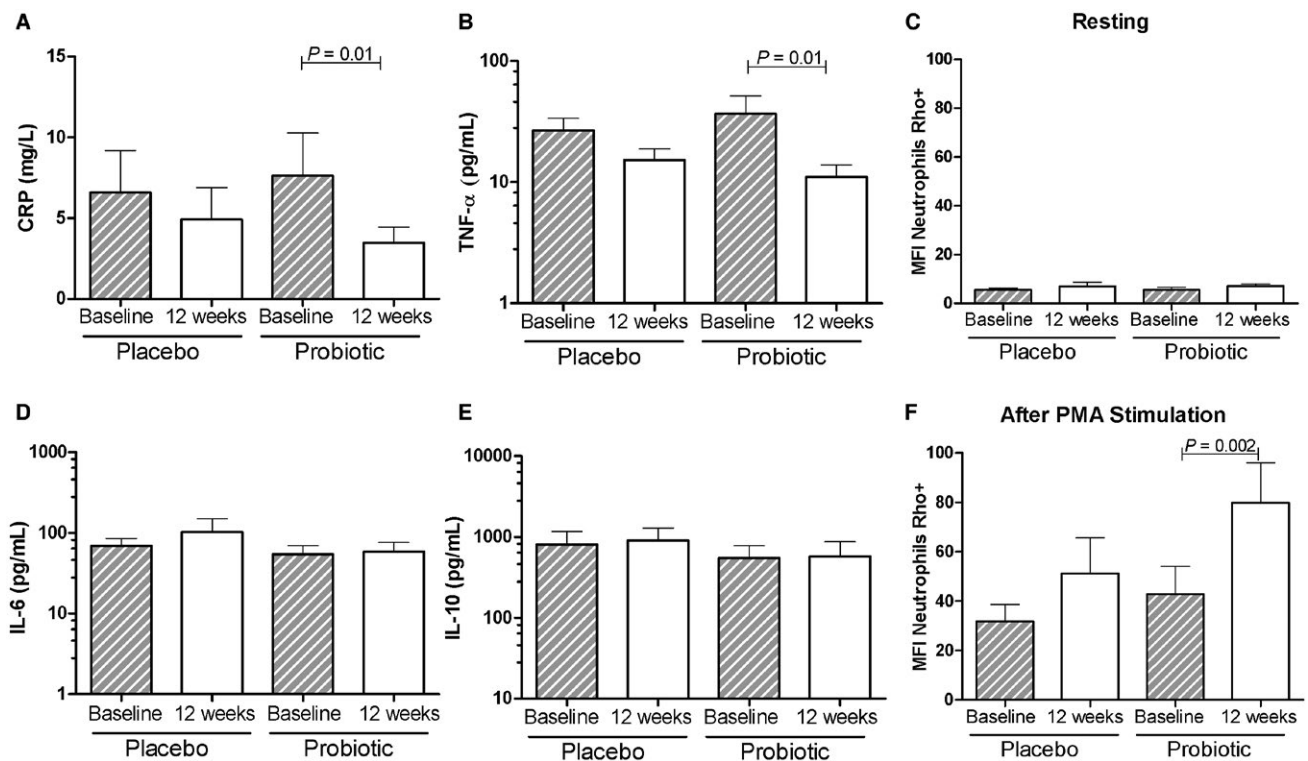
FIG. 3. Parameters of systemic inflammatory response at baseline and at the end of treatment (12 weeks) in the placebo group and in the probiotic group: CRP (A), TNF-α (B), neutrophil resting oxidative burst (C), IL-6 (D), IL-10 (E), and neutrophil oxidative burst after PMA stimulation (F). Data are expressed as mean ± SEM. *P* is not significant for any parameters in the placebo group and for IL-6, IL-10, and neutrophil resting oxidative burst in the probiotic group. Abbreviations: MFI, mean fluorescence intensity; Rho, rhodamine.

at the end of the treatment. This finding is in agreement with studies reporting an improvement in cognitive function in patients with cirrhosis treated with the probiotic used here[16,27] as well as in studies using other probiotics.[1,13,28]

Most patients in our study had a baseline PHES of at least –4, which is above the limit to diagnose minimal hepatic encephalopathy. Therefore, we could be skeptical about the clinical significance of the improvement in the PHES observed in the probiotic group. However, Giménez-Garzó et al.[29] recently demonstrated that patients with a normal PHES may exhibit different types of neuropsychological alterations that are mild but have clinical consequences. These data are in line with previous studies showing a lack of correlation between the incidence of falls and the degree of impairment in the PHES.[8] In addition, the alterations in brain white matter tracts observed by magnetic resonance–diffusion tensor imaging in patients with cirrhosis and previous falls were not

related to the PHES results, but to more accurate tests of executive function, such as the Wisconsin Card Sorting Test.[30] According to these data, impaired PHES could be a surrogate marker of the underlying brain alterations that predispose patients with cirrhosis to falling.[30] We consider that the improvement in the PHES observed in our study in patients treated with the probiotics may have clinical relevance, especially considering that it was associated with an improvement in the tests to assess the risk of falls. Recently, Pinto-Sanchez et al.[31] reported that treatment with the probiotic *B. longum* NCC3001 in patients with irritable bowel syndrome produced changes in the cerebral activation pattern evaluated by functional magnetic resonance imaging (MRI). It would be interesting to use this technique to study the effect of probiotics on brain function in patients with cirrhosis.

The lack of statistically significant improvements in HRQOL measured by the NHP could be due to
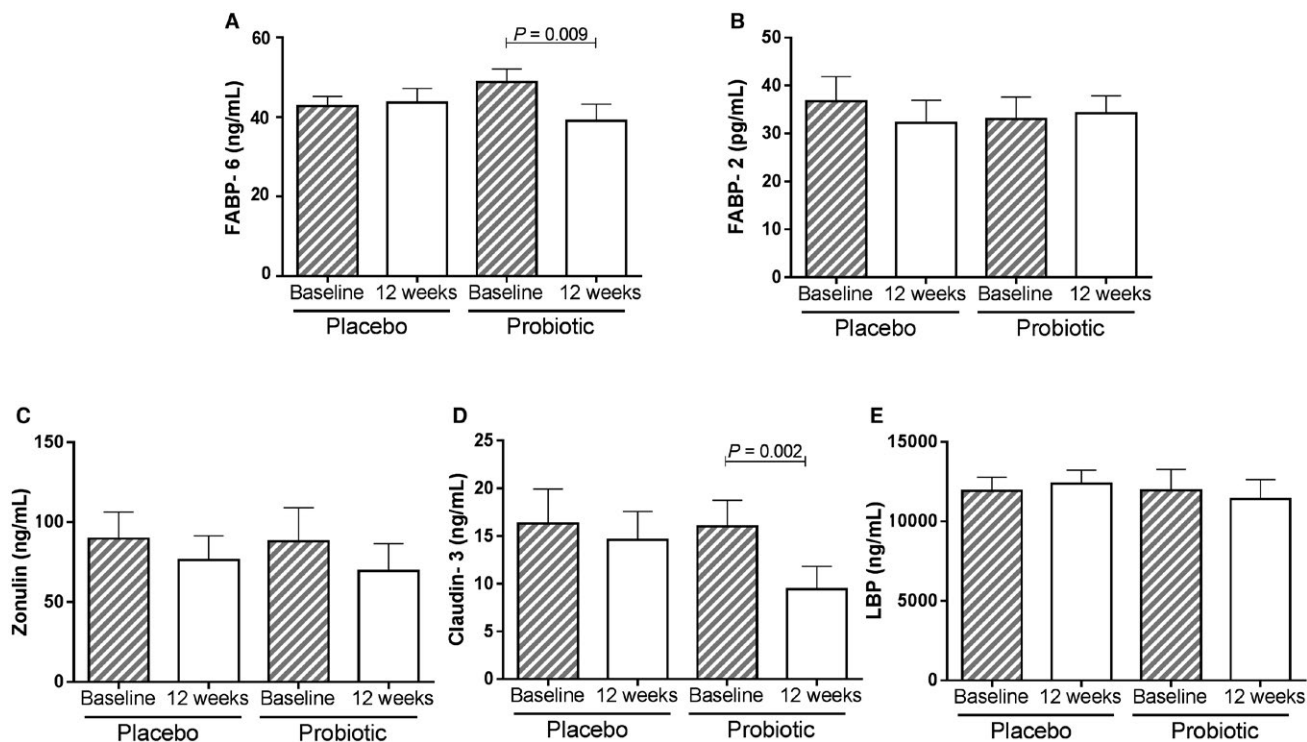
FIG. 4. Parameters of intestinal barrier and bacterial translocation at baseline and at the end of treatment (12 weeks) in the placebo group and in the probiotic group: FABP-6 (A), FABP-2 (B), zonulin (C), urinary claudin-3 (D), and LBP (E). Data expressed as mean ± SEM. Higher values indicate more impaired intestinal barrier. *P* is not significant for any parameters in the placebo group and for FABP-2, zonulin, and LBP (*P* = 0.13) in the probiotic group.

the fact that patients presented a relatively well-preserved baseline HRQOL that was therefore difficult to ameliorate. Another possible explanation is that the improvements in physical and cognitive functions were insufficient to improve the multifactorial HRQOL in patients with cirrhosis. Nevertheless, we observed a not significant trend to improvement in the domain of social isolation in the probiotic group.

We aimed to explore the potential mechanisms by which the probiotic may have improved cognitive function and decreased the risk of falling, such as modulation of systemic inflammation, improvement of the intestinal barrier, and changes in bacterial translocation and gut microbiota.[1,3,13,14]

Regarding systemic inflammation, baseline serum CRP levels were slightly increased in the two groups, suggesting a mild proinflammatory state, as may have been expected considering the characteristics of the patients included in the study. However, only patients in the probiotic group presented a decrease in CRP levels to almost normal values at the end of

the treatment, and this was associated with a significant decrease in serum TNF-α. Taking into consideration the importance of a proinflammatory state, and CRP in particular, in the prognosis of patients with cirrhosis,[2,32] we consider this one of the most relevant findings in our study. Specifically, systemic inflammation is related to neuroinflammation, which in turn is a main factor involved in the pathophysiology of covert and overt hepatic encephalopathy.[1-3] In common bile duct ligated mice, D'Mello et al.[33] showed that treatment with the same probiotic mixture as that used here reduced systemic inflammation, improved activation of brain microglia and cerebral monocyte infiltration, and ameliorated sickness behavior. In patients with cirrhosis treated with the same probiotic, a decrease in systemic inflammation, an improvement in cognitive function, and a decrease in the recurrence rate of hepatic encephalopathy have been reported.[1,16]

In the setting of the proinflammatory state, patients with cirrhosis present alterations in the neutrophil

oxidative burst that can contribute to worsening prognosis and increase the risk of bacterial infections.[34,35] Among these alterations, the impaired production of reactive oxygen species after stimulation is especially relevant.[35] This exhaustion of neutrophils is considered to be mainly due to the maintained proinflammatory state in the setting of repeated translocation of intestinal bacteria or their products (i.e,. endotoxin) as a consequence of the impaired intestinal barrier.[35] In our study, patients receiving the probiotic showed an improvement in neutrophil oxidative burst after stimulation. This finding is probably related to the decrease in the proinflammatory state and may help prevent infections and improve prognosis in these patients.[34,35] Interestingly, Stadlbauer et al.[36] observed that treatment with a single species probiotic (*Lactobacillus casei* Shirota) restored neutrophil phagocytic capacity in patients with compensated alcoholic cirrhosis. This effect was probably a consequence of an improvement in pathological bacterial translocation, as suggested by the decrease in the neutrophil toll-like receptor 4 expression after stimulation with endotoxin in patients treated with the probiotic.[36] In a more recent study, Horvath et al.[37] reported that treatment with a different multispecies probiotic in outpatients with cirrhosis produced an immune modulation consisting of an increase in neutrophil resting oxidative burst and serum neopterin, a marker of macrophage activation.

Because probiotics can improve the intestinal barrier, we assessed several biomarkers of gut barrier integrity. We observed a decrease in serum FABP-6 and urinary claudin-3 in the probiotic group at the end of treatment, suggesting a possible improvement in the intestinal barrier,[23] without significant changes in serum FABP-2 and zonulin. Interestingly, FABP-2 is considered to reflect the intestinal barrier in the whole gut, whereas FABP-6 is more specific of the ileum.[23] Therefore, our findings suggest a more specific effect of the probiotic in this area. Previous studies have demonstrated a beneficial effect of certain probiotics on the intestinal barrier in several experimental and clinical settings.[38,39] This finding has also been reported in models of experimental cirrhosis, in which several probiotics improved the intestinal barrier, decreased bacterial translocation, and ameliorated the proinflammatory state.[40,41] However, in the study by Horvath et al.[37] in patients with cirrhosis treated with a different probiotic mixture, the authors did not

observe any significant effect on gut permeability, as assessed by a panel of markers including lactulose/mannitol ratio and fecal zonulin, or inflammatory biomarkers. Liu et al.[42] evaluated patients undergoing colorectal surgery treated with a three-strain probiotic and reported a decrease in serum zonulin, a parameter that was unchanged in our study. These discrepancies between studies may be explained by the differences in study populations, the probiotic product used,[13,14,39] and the assessment parameters. It should be pointed out that the biomarkers we used to evaluate the intestinal barrier have not been validated in cirrhosis. Although previous data in other settings, such as intestinal ischemia, intense exercise, human immunodeficiency virus infection or chronic hepatitis, suggest they could be surrogate parameters to assess the integrity of the intestinal barrier,[23,43-45] studies in patients with cirrhosis are largely lacking.

Regarding bacterial translocation, we quantified LBP in blood and failed to demonstrate significant changes after probiotic treatment. However, we should again emphasize that the patients were in a stable situation and showed relatively well-preserved liver function. Therefore, patients presented low levels of LBP at baseline, suggesting a low degree of bacterial translocation that would be difficult to improve with any treatment. We cannot rule out the possibility that the probiotic treatment produced a decrease in bacterial translocation of enterobacteria, suggested by the slight decrease in LBP. This hypothesis should be confirmed by sequencing analysis of the composition of blood bacterial DNA.[46] Such analysis was not conceptualized and therefore not performed in the present study. Using a different probiotic, *Lactobacillus* GG, Bajaj et al.[15] found that endotoxemia and serum TNF-α decreased and fecal microbiota dysbiosis improved in patients with cirrhosis with minimal hepatic encephalopathy.

We studied the fecal microbiota by 16S ribosomal DNA sequencing in a subgroup of patients and failed to demonstrate significant changes at the end of the treatment in either group. This finding must be considered with caution due to the small number of patients studied (9 in the probiotic group and 8 in the placebo group) and the potential confounding factors, such as inadvertent changes in life style. Moreover, the analysis of the fecal microbiota does not necessarily reflect changes in upper areas of the gut, where these changes could have more physiopathological

consequences in the setting of cirrhosis.[47] Another possible explanation is that the probiotic treatment did not produce a change in the microbiota composition but in its functions, as has been reported with rifaximin[48] and with the same probiotic as that used here in other settings.[49]

Adverse events were similar in the two groups, and no side effects attributable to the probiotic were observed, confirming previous studies and suggesting that this probiotic is safe in this setting.[16,27]

We hypothesize that the probiotic ameliorated the intestinal barrier and modulated the crosstalk between intestinal bacteria and the immune system. As a consequence, there was a decrease in systemic inflammation, and probably in neuroinflammation (not evaluated in the present study), and therefore an improvement in cognition and neural circuits involved in gait, which finally would decrease the risk of falls. The correlations between the parameters of the intestinal barrier, the inflammatory response, cognitive function, and the risk of falling observed in the present study further support this hypothesis.

Our study has several limitations. The first of these is related to the fact that to obtain more robust conclusions about the effects of the probiotic, we applied strict exclusion criteria to control for potential confounding factors. However, this resulted in a study population with a relatively well-preserved liver function and a small sample size, as we were able to include only 36 patients over a 3-year period. Consequently, it was difficult to show an improvement in several mildly impaired parameters, and the high number of patients excluded limited the potential applicability of the study results to a broader group of patients with cirrhosis in clinical practice.

A second limitation is that the biomarkers used to evaluate the gut barrier are not validated in cirrhosis. We preferred not to use other more standardized methods, such as the administration of lactulose/mannitol or polyethylene glycol,[23] because they included the administration of medications that were not permitted in accordance with the study design. Lactulose and polyethylene glycol are used to treat hepatic encephalopathy and could interfere with the parameters we aimed to evaluate, such as intestinal microbiota or cognitive function,[27,50] and therefore many of the other related parameters, such as inflammatory response or risk of falls. Nevertheless, we would like to point out that the results from the biomarkers used in the present study that could suggest an improvement in the intestinal barrier after probiotic treatment are consistent with previous data.[23,38-45]

As future research, a study with a larger number of patients and with a longer treatment protocol is warranted to assess the usefulness of the probiotic in decreasing the incidence of falls. Moreover, it would be interesting to analyze the intimate mechanisms determining the improvement in the risk for falls through accurate neurological tests and MRI-based techniques[30,31] as well as the changes in the microbiota function after probiotic treatment.[48,49]

We conclude that this multistrain probiotic improved cognitive function, decreased the risk of falls, and modulated the systemic inflammatory response in patients with cirrhosis and cognitive dysfunction and/or previous falls. This probiotic mixture could be useful in the prevention of falls and complications, especially hepatic encephalopathy and bacterial infections, in patients with cirrhosis.

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