Effects of *Lactobacillus rhamnosus* GG on early postoperative outcome after pylorus-preserving pancreatoduodenectomy: a randomized trial

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Abstract. – OBJECTIVE: Pancreatoduodenectomy (PPPD) remains one of the most complex surgical procedures with high complication rates. Infectious complications, postoperative ileus and delayed gastric emptying in the perioperative period have a significant impact on the recovery from the treatment. Probiotics (PB) are known to have a beneficial effect as supportive therapy in major abdominal surgery but the evidence in pancreatic surgery is still limited. The aim of the study was to assess the influence of postoperative administration of PB on the early outcomes after PPPD.

PATIENTS AND METHODS: Forty patients undergoing pylorus-preserving PPPD were enrolled to prospective trial and randomized in two groups: A – control group (n=20) receiving standard nutrition and B – probiotic group (n=20) treated additionally with *Lactobacillus rahmnosus* GG (*L. rhamnosus* GG) in the postoperative period from the day of the surgery for 30 days. Gastrointestinal motility, infection complications, length of hospital stay, and mortality were compared in the perioperative period and during 2 follow-up (i.e., after 14 and 30 days).

RESULTS: There were no significant differences in mortality and infectious complications between groups. The length of hospital stay was shorter in the probiotic group compared to control (10 days *vs.* 8, respectively). The positive effect of *L. rhamnosus* GG on gastrointestinal tract's motility was observed, including earlier recurrence of postoperative bowel movements (group B: after 3.75 days vs. group A: 2.15 days), passing gasses (group B after 4 days vs. group A 2.9 days) and the first postoperative stool (group B after 5.84 days vs. group A 3.85 days). *L. rhamnosus* GG improved the appetite in postoperative day 1, 3, 5, 7 and 30 days after the surgery.

CONCLUSIONS: *L. rhamnosus* GG improves the function of the gastrointestinal tract after major pancreatic surgery and may reduce the length of hospital stay.

Key Words:
Lactobacillus rhamnosus GG, Pylorus-preserving pancreatoduodenectomy, Gastrointestinal motility, Delayed gastric emptying, Probiotics, Surgery.

Introduction

Despite the improvement of the surgical technique and perioperative management pylorus-preserving pancreatoduodenectomy (PPPD) remains a surgical procedure with high complication rates. The most common are postoperative ileus (POI) and infectious complications (IC). 20-50% of patients suffer from delayed gastric emptying[1-3] which may seriously influence the tolerance of postoperative nutrition and quality of life. Other major complications are pancreatic fistula (10 to 15 % of patients), gastrointestinal or intraabdominal bleeding (4-16%), as well as IC, such as wound infections (4.8-10%), abdominal abscess (1-12%), and postoperative pancreatitis (1-10%)[4].

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The growing interest towards the role of the intestinal microbiota in the pathogenesis of many diseases is currently observed, as well as the use of probiotic (PB) strains, in supporting the treatment of surgical patients. Perioperative bowel ischemia and reperfusion decreased bowel motility and inflammatory system dysregulation which may lead to dysfunction of the intestinal barrier function[5,6]. It is known that PB maintain the balance between non-pathogenic and harmful microorganisms and improve the function of the mucosal barrier through various mechanisms. They produce nutrients for enterocytes like short-chain fatty acids[7], lower local pH, and promote the production of the mucus layer on the epithelium[8-10]. Specific species, such as *Lactobacillus rhamnosus* GG (*L. rhamnosus* GG) or *Lactobacillus plantarum* 299v are able to block the receptor spot on the intestinal epithelium for pathogenic Escherichia coli[11]. Specific strains produce substances like bacteriocins or pyroglutamic acid, which eliminate competing bacteria from their habitat[8,12,13]. The immunomodulatory effect of PB is achieved *via* the activation of Natural Killer (NK) cells and macrophages, as well as the increased production of anti-inflammatory cytokines, interferon gamma, and immunoglobulin A[14-16].

Scholars[17] show that preoperative bowel preparation, prophylactic antibiotic treatment and the surgical procedure, affects the microbiome. Preventing bacterial translocation may play an important role in the pathogenesis of surgical infectious IC, which are often initiated by bacteria derived from the intestinal microflora[11,18,19]. It was noted that zonulin concentration in the serum as a marker of mucosal barrier permeability and infection rates were lower for patients undergoing colorectal surgery after PB[20]. However, there are also studies[21,22] showing contradicting results with no effect of PB administration on IC for patients after major abdominal surgery or pancreatic resections[18]. Therefore, the role of bacterial translocation and the influence of specific PB strains remain unclear[23]. The efficiency of *L. rhamnosus* GG in prevention, as well as the treatment of gastrointestinal infections is well documented[24]; however, it has never been studied in the context of patients after PPPD.

Anastomosis leakage after pancreas resection is a serious complication leading to an increased mortality rate. There are many animal model studies confirming that specific bacterial strains promote damage to the healing anastomosis. It was shown that 95% of rats inoculated with *Pseudomonas aeruginosa* developed anastomosis dehiscence, whereas only 6% from the decontaminated group[25]. *Enterococcus faecalis* on the other hand was proven to activate intestinal tissue matrix metalloprotease 9 and collagen degradation leading to anastomotic leak[26,27].

POI and delayed gastric emptying cause prolonged hospital stay and increase complication rate after major abdominal surgery. As a consequence, it may lead to bacterial overgrowth and translocation causing septic complications[28]. Intestinal microbiota seems to have a regulatory role in the gastrointestinal tract's (GI) motility. Cross-talk between dendritic cells, macrophages and the microbiome was proven to be responsible for POI in experimental studies[29,30]. Microbiome modifications with PB to alternate bowel motility have solid scientific evidence in the treatment of diarrhea (for instance, post-antibiotic and travel diarrhea)[31-34] and reduce pain and symptom severity scores in Irritable Bowel Syndrome (IBS)[35]. On the other hand, specific PB strains decrease the intestinal transit time in constipated patients[36-38].

Considering the potential mechanism of PB action and insufficient data concerning patients after pancreatic surgery, we investigated the effects of single specimen PB therapy (*L. rhamnosus* GG) on GI motility, IC, and length of hospital stay in patients after PPPD. No previous study described beneficial effects of this intervention in the above-mentioned group of surgical patients.

Patients and Methods

Patients (n=40) undergoing pylorus-preserving Longmire-Traverso PPPD from April 2009 to August 2013 in Pomeranian Center Of Traumatology in Gdańsk were recruited to the study by a surgeon. Inclusion criteria were: age ≥18 yr., informed and written consent, planned PPPD. Exclusion criteria were: <18 yr., lack of informed consent, mental disorders, and a contradiction for postoperative enteral feeding.

There were no significant differences in groups regarding gender. Patients were significantly older in group A. The nutritional status was comparable in both groups with no differences in (Nutrition Risk Score) NRS 2002, Body Mass Index (BMI), Weight, preoperative albumin and transferrin level. Group characteristics and demographic data are presented in Table I. 20% Pa-

Table I. Basic demographic statistics

	Group A (control)	Group B (probiotic)	p
Age (medium; SD)	66.5; 10,36	57.3; 11,68	0.01
Men (%)/Female (%)	13.65%/7.35%	12.60%/8.40%	1
Cancer n (%)/Pancreatitis n (%)	n = 18 (90%)/n = 2 (10%)	n = 14 (70%)/n = 6 (30%)	0.2152
Nutritional status			
BMI (median; [95% Cl])	24.8; [22.96; 26.16]	23; [22.43; 25.57]	0.603
Albumin (g/l, medium; SD)	35.44; 8,16	36.79; 7,04	0.6074
Total protein (g/l, medium; SD)	57.6; 12,58	61.42; 11,84	0.3803
NRS2002 (median; [95%Cl])	3.45; [2.96; 3.94]	3; [2.54; 3.46]	0.1859
Surgery			
Operating time (min., medium; SD)	251.3; 45.08	289; 40.45	0.08
Blood transfusion (units)	0.85	0.5	0.2287

BMI – Body Mass Index (kg/m^2); NRS 2002 – Nutritional Risk Score 2002.

tient's histological examinations revealed benign disease (chronic pancreatitis), 80% - malignant diagnosis (mainly pancreatic adenocarcinoma, adenocarcinoma of bile duct or ampulla of Vater, one patient with a metastatic tumor in the pancreatic head from kidney cancer). The mean duration of the operating time was 270 minutes with no differences in study groups.

Study Design

This study was designed as a prospective single-center randomized trial to assess the efficiency of *L. rhamnosus* GG in patients undergoing PPPD. The study protocol has been registered and approved by the Local Ethics Committee (Identifier: 1904/11). Written informed consent was obtained from all patients. Standard pylorus-preserving Longmire-Traverso pancreatoduodenectomy with pancreatojejunostomy was performed in all patients. The naso-jejunal tube was intraoperatively placed 40 cm distal to the gastro-jejunal anastomosis. Data about operating time and perioperative blood transfusions were collected. Patients were treated with a single shot of perioperative antibiotic therapy (Cefazolin and Metronidazole) with no mechanical bowel preparation.

Randomization and Blinding

After the surgical procedure patients were randomized into two groups using charts sealed in envelopes generated with computer algorithm in MS Excel: control group (A) and probiotic (B). The study coordinator was blinded and had no information about the randomization outcome. Patients and the medical staff were not blinded. The treatment was initiated on the day of surgery and continued for 30 days with 2 follow up visits i.e., in 14 and 30 days after surgery. A total of 45 were assessed as eligible for this study. 5 Patients were excluded, due to lack of consent and 40 participants were randomized into 2 groups: A (n=20) and B (n=20). The study flow chart presented in Figure 1.

Study Product

The study product was a single specimen PB formula Dicoflor 60 (Vitis pharma, Warsaw, Poland) *containing L. rhamnosus* GG. Patients from the probiotic group (B) received standard postoperative nutritional treatment and one capsule containing 6 million colony forming units (CFU) of this PB strain every 12 hours from the day of the surgery for 30 days. The probiotic was administrated through the naso-jejunal tube (mixed

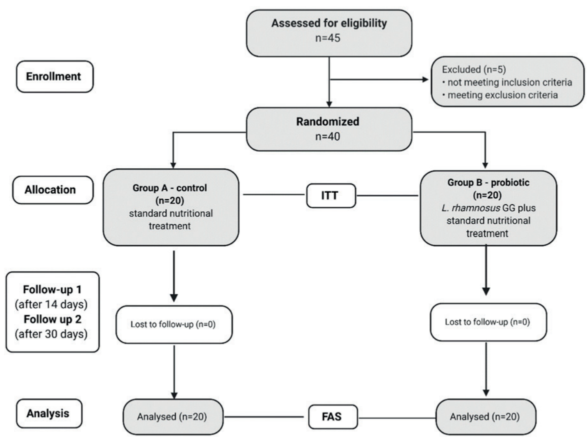

Figure 1. A flow chart of the study design. ITT – intention to treat; FAS – full set analysis; *L. rhamnosus* GG – *Lactobacillus rhamnosus* GG.

with saline water) and orally when a solid diet was introduced. Participants from the control group (A) were receiving only standard perioperative nutritional treatment. Enteral nutrition (EN) through the naso-jejunal tube was introduced 4 hours after the surgery with infusion rate 20 ml/h in the first 6 hours, 30 ml/h in the next 12 h, then 40 ml/h, and 60 ml/h in the 4th postoperative day. Elemental normocaloric enteral diet was used. EN was continued for at least 4 days until the patients met at least 70% of calorie needs with oral feeding.

Outcomes

Primary outcomes

The primary aim of this study was to investigate the influence of *L. rhamnosus* GG on GI motility and postoperative IC. The data were collected during the hospital stay and two follow-up visits (after 14 days and 30 days).

Gastrointestinal tract's Function

Patients were asked to measure their appetite (AT) using 0-10 points visual analog scale (VAS): the day before the surgery, every two days after the surgery and during follow-up visits. Clinical examination was performed every day during the hospital stay and the following data were collected: first day of bowel movement, first flatus and feces pass time, fluid retention in nasogastric tube and the day of its removal.

Infectious Complications Monitoring

Patients' clinical assessment included: lungs auscultation, temperature measurement (2 times a day), wound observation, and urine laboratory examination (when dysuria symptoms occurred). Laboratory tests, such as C-reactive protein, leukocytes and lymphocytes amount were performed. These parameters were measured on the day before the surgery and every 2 days starting from the day of the surgery.

Secondary Outcomes

The secondary outcomes were the impact of *L. rhamnosus* GG on: the length of hospital stay, perioperative mortality.

Definitions

Wound infection – detection of purulent discharge in the wound and a positive bacterial culture.

Pneumonia – fever, cough, dyspnea, characteristic pulmonary infiltrate on chest x-ray.

Intra-abdominal abscess – fluid collection requiring a drainage procedure with positive cultures.

Sepsis – fever, low arterial blood pressure, systemic inflammatory response, and positive bacterial blood cultures.

Urinary tract infection – dysuria, leukocyturia and a positive urine culture >105 CFU/mL with or without fever.

Venous catheter-related infection – it was defined according to catheter tip colonization, signs of systemic infection (fever, chills, and/or hypotension), with no apparent source of bacteremia except the catheter.

Sample size Calculation

One of the primary aims of this study was to investigate the influence of *L. rhamnosus* GG on postoperative infectious complications (IC). This parameter was used to calculate the sample size. Based on a study by Nomure et al[39] (2007) where the proportions of ICs in probiotic and placebo groups were 23 and 53% respectively, we calculated *a priori* that to obtain results at 80% power and with an α error probability of 0.05 we need a total of 94 persons allocated equally to both groups. Due to unplanned funding limitations, we had to close the recruitment for the study, and the planned sample size was not achieved (n=40). Therefore, we performed a post-hoc power calculation for critical evaluation of the results.

Statistical Analysis

Statistical computer analysis was calculated using StatSoft, Inc. (2011). STATISTICA (data analysis software system), version 10.0. www.statsoft.com. Quantitative variables were characterized using the arithmetic mean, standard deviation, median, minimum and maximum values (range) and 95% CI (confidence interval). Qualitative variables were presented using frequencies and percentages. To check whether the quantitative variable derived from a population of normal distribution the Shapiro-Wilk test was used. To test the hypothesis of equal variances Leven (Brown-Forsythe) test was used. The significance of differences between the two groups (model of unrelated variables) was controlled with tests: Student *t*-test (or in cases of the absence of homogeneity of variance – Welch test) or the Mann-Whitney test. The significance of differences among more than two groups was determined with test F (ANOVA) or Kruskal-Wallis test. For the groups with a significant differences post-hoc tests were used (Tukey's test for F and for Kruskal-Wallis the Dunn test). In the case of two variables related we used the Student-*t* or Wilcoxon couple sequence test. The significance of differences among more than two variables in the model was checked with an analysis of variance with repeated measurements or Friedman test. The Chi-square tests were used for categorical variables. In order to establish relationships, strength and direction between the variables we used the correlation analysis calculating the Pearson and/or Spearman correlation coefficients. In all the calculations the level of significance was p=0.05. *A priori* and post-hoc power analyses were done by means of GPower software Version 3.1.9.2[40] (Dusseldorf, Germany).

Results

Gastrointestinal Tract's Function

Bowel movement appeared statistically earlier in group B (probiotic) on a postoperative day 2.15 day vs. 3.74th day in group A. Patients in group B also passed their first flatus (after 2.9 days vs. 4 days in group A) and stool (medium day of first postoperative stool reported: group A-5.84, group B-3.85) earlier than in control group (Figure 2). The power for these analyses was as follows: 0.99, 0.98 and 0.85 respectively.

The average retention rate in the naso-gastric tube in similar postoperative days was the same in both groups. The naso-gastric tube was removed earlier in group B (medium day of the removal in group A-5.75 *vs.* 4 in group B, p = 0.0093; power = 0.63). AT score was significantly higher in group B on postoperative day 1, 3, 5, 7 (power 0.96, 0.91, 0.99, 0.43 respectively) and during second follow-up visit after 30 days (power 0.75). The difference between the vas scores during the first follow-up after 14 days was not statistically significant (power 0.12) (Figure 3).

Perioperative Complications

In 31 patients (77%) no IC were observed, 14 in group A (70%) and 17 (85%) in group B (probiotic) and the difference was not statistically significant (p=0.2253). There were also no significant differences in groups considering: wound infections (A-15% *vs.* B-10%), pneumonia (A-5% *vs.* B-0%), abdominal abscess (A-5% *vs.* B-5%) and urinary tract infections (A-5% *vs.* B-5%). Anastomosis leakage was observed in 6 (15%) patients (A-20% *vs.* B-10%, p=0.6614) and all required relaparotomy. 2 Patients (5%) died due to septic shock and multiorgan dysfunction in course of anastomosis leakage, 4 (10%) had a pancreatic fistula (type C) successfully treated

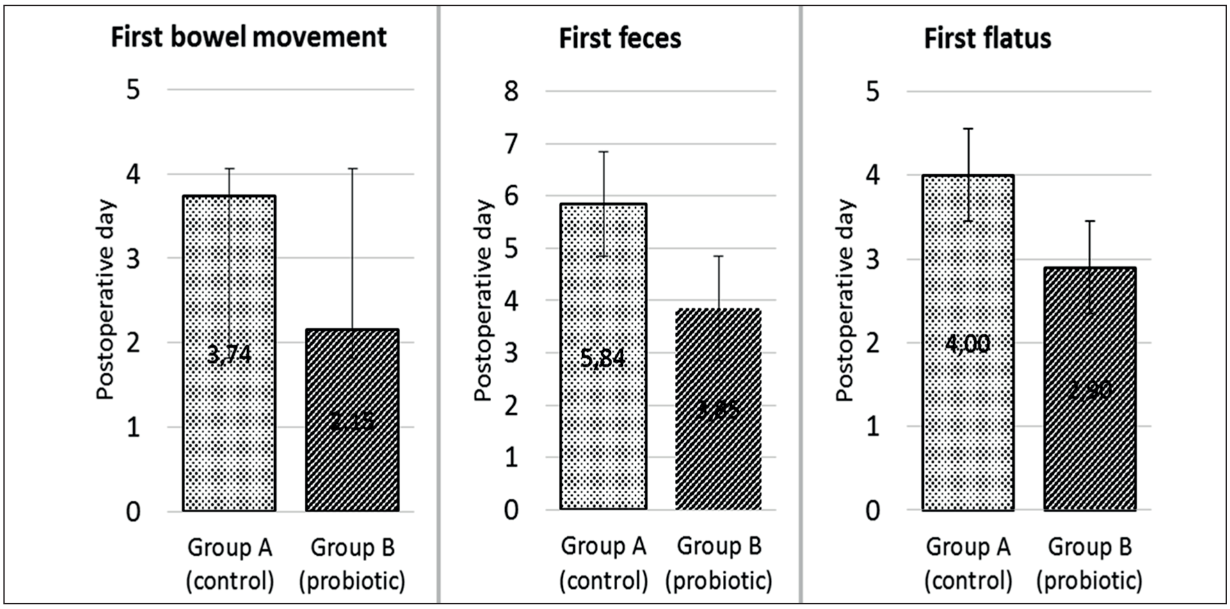

Figure 2. Restoration of postoperative bowel movement.

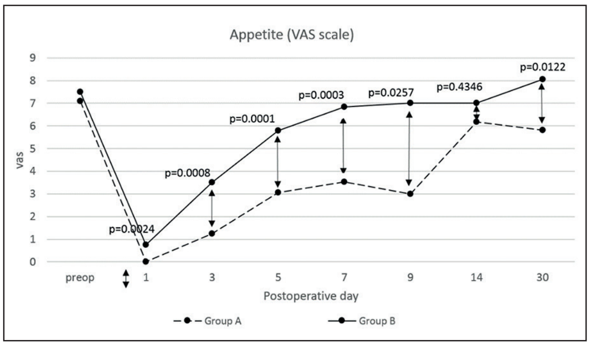

Figure 3. Postoperative appetite. VAS – Visual Analogue Scale.

with drainage. All of these analyses were underpowered (<0.8). Analysis of laboratory infection markers showed no statistical differences in groups. There were no differences in medium lab test results of C-reactive protein, leukocytes level or lymphocytes level during the following days of hospital stay and follow up visits. The analyses of statistical power demonstrated that in 1, 3, 5 and 7 days post-surgery and 14 and 30 days follow-ups the power of statistical analyses were under the recommended level of 0.8 (Figure 4).

Mortality and Length of Hospital Stay

General perioperative mortality rate was 5% (n=2) all patients who died were from the control group (power <0.8). No statistical differences in mortality were observed between study groups. The median of postoperative hospital stay was 9 days and was significantly shorter in group B (probiotic) (Group A: 10 days *vs.* Group B: 8, p=0.0303, power <0.8).

Discussion

To our knowledge, this is the first study investigating the influence of single specimen PB containing *L. rhamnosus* GG strain on early postoperative outcomes after PPPD. Several studies described the use of other PB strains, symbiotics or prebiotics. In Rayes et al[41] a prospective randomized double-blind trial including 80 patients after PPPD the effect of symbiotics on bacterial infection rate was assessed. All participants were receiving EN and were divided into two groups: first receiving symbiotics (i.e., 4 strains of *Lactobacillus* and 4 types of prebiotic fibers) and control group consuming only fibers. It was observed that the incidence of bacterial infections was lower in the symbiotics group in comparison to control (12.5% *vs.* 40%, respectively). In Nomura et al[39] study the participants (n=64) were divided into 2 groups: receiving PB (n=34) and control (n=30). PB (i.e., *Enterococcus faecalis* T-110, *Clostridium butyricum* TO-A, and *Bacillus mesentericus* TO-A) were given after admission for a scheduled PPPD, 3 to 15 days before the operation, and continued at second day in postoperative period until discharge. It was confirmed that the incidence of IC was significantly lower in the PB group compared to control (23% *vs.* 53%, p=0.02, respectively).

A recent meta-analysis has proven that PB administration after major surgery reduces the occurrence of surgical site infections and other complications like abdominal distention, diarrhea and time of fluid and solid diet introduction leading to decreased duration of hospital stay[42]. Nevertheless, in our study, though we observed a trend towards a beneficial effect of PB inter-

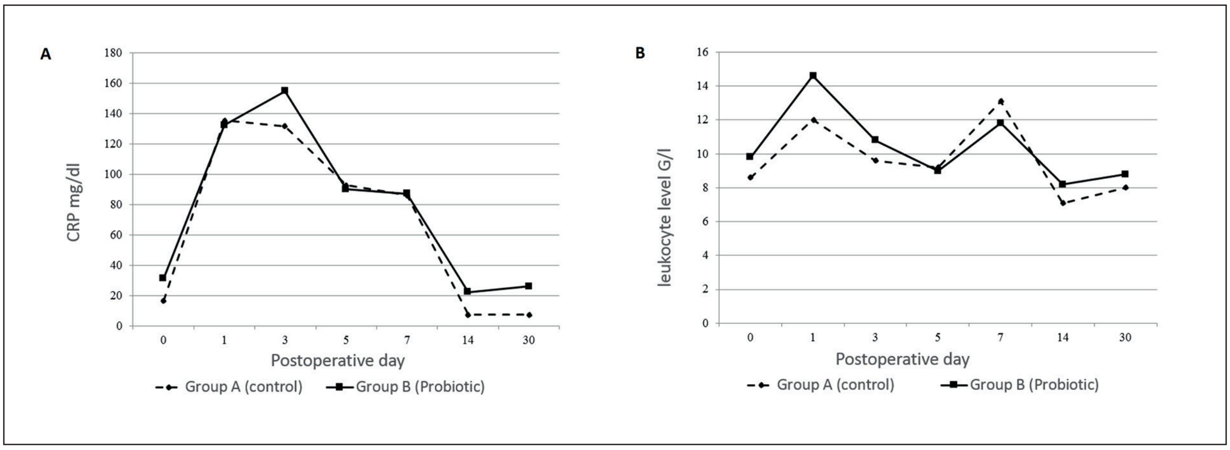

Figure 4. Laboratory inflammatory markers. A – C-reactive protein (CRP), B – Leukocytes level.

vention in the reduction of IC between PB (*L. rhamnosus* GG) and control group, it was not statistically significant. This might have been the effect of a small sample size which was confirmed by the post-hoc power calculation (under 0,8). Moreover, strain-dependent properties of PB or the duration of preoperative PB treatment may explain the disparity and provide directions for future studies.

Delayed gastric emptying is one of the most frequent complications in the early period after PPPD prolonging hospital stay, as well as impairing patients' quality of life (QOL)[43,44]. Numerous interventions like coffee drinking, chewing-gum together with enhanced recovery after surgery (ERAS) protocol are introduced in major abdominal surgery to reduce the postoperative incidence of POI[45,46]. There are only a few studies concerning the influence of PB on the postoperative recurrence of bowel motility reporting: earlier day of first flatus and first defecation or reduced rate of postoperative diarrhea[47-49]. None of those studies concerned patients after PPPD. In our study, we observed that *L. rhamnosus* GG has beneficial effects on GI motility causing earlier recurrence of bowel movements, passing gases and first postoperative stool.

Appetite level as an additional clinical manifestation of postsurgical bowel movement recurrence is an indirect sign of recovery after major abdominal surgery. As a result of standardized assessment using a visual analogue scale, we were able to confirm the improvement of AT after PB treatment. All the above-mentioned effects might have also contributed to the shorter hospitalization time in the reported PB group in analyzed material.

The present study has some limitations. Firstly, due to funding limitations, the intervention was not compared to placebo and the sample size was reduced from the planned study design. This might have caused the differences in age between groups. However, post-hoc analysis showed a high power of calculations concerning GI motility and AT as the main study outcomes. Microbiome alterations after surgery and PB treatment were not examined and results were based only on clinical observations of the intervention effect. Secondly, the PB intervention was not started in the preoperative period only on the day of the surgery. We were aware that most surgical patients in our unit are admitted to the hospital on the day before the planned surgery. Prehabilitation and preoperative nutritional assessment and preparation for the procedure are based on the ambulatory process guided by the outpatient clinic. Chowdhury et al[50] show that most effects of PB in surgery are not strongly correlated with the duration of treatment. Postoperative administration of PB with naso-jejunal tube in the early postoperative period enabled the tolerance and adequate dosage for patients with delayed gastric emptying. However, despite the lack of preoperative preparation with PB the effect on GI tract motility was statistically significant.

Conclusions

In brief, postoperative administration of PB containing *L. rhamnosus* GG strain improves the recurrence of GI motility and appetite and may reduce the length of hospital stay after PPPD. A larger sample size is required to confirm the effect on IC. More studies with similar bacterial strains and comparable administration protocols are needed to confirm positive recommendations for perioperative use of PB after PPPD.

Conflict of Interest

K.S.-Z. receives remuneration from probiotic company. The rest of the authors declare no conflict of interest.

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Authors' Contribution

Conceptualization, M.F, D.M; Data curation, M.F; Formal analysis, M.F, D.M; Investigation, M.F; Methodology, M.F, D.M, K.S-Z; Resources, M.F, D.M, S.M; Supervision M.F, M.D; Writing-original draft, M.F and D.M.; Writing-review & editing, M.F, D.M, S.M, K.K-S, K.S-Z.

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