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Effect of perioperative probiotic intervention on postoperative cognitive dysfunction in elderly patients: a randomized double-blinded and placebo-controlled trial

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Abstract

Background Postoperative cognitive dysfunction (POCD) may last for days, months or even years, leading to prolonged hospitalization and increased costs, increased mortality, and poor quality of life. Although POCD is an important clinical problem, its prevention, treatment strategies and effects are still limited. Therefore, this study aims to investigate the preventive effect of perioperative probiotic intervention on POCD in elderly patients, and further explore the mechanism of probiotics in improving postoperative cognitive function.

Methods After obtaining ethical approval and written informed consent, 190 patients aged 65 years or older scheduled for elective lower-extremity orthopedic surgery were enrolled in this randomized, single-center, double-blind trial. Enrolled patients were randomized to probiotic or control groups receiving either probiotics or placebo (210 mgx4/dose orally, 2 times/day) from 1 day before surgery to 5 days after surgery. The primary outcome was the cognitive function assessed by Mini-mental State Examination (MMSE) on admission, the first day, the third day and the seventh day after surgery. The secondary outcomes included perioperative changes in plasma IL-1β, IL-6 and BDNF, postoperative pain intensity, perioperative activities of daily living (ADL), faecal microbiota composition and changes of intestinal metabolites

Results The incidence of POCD in the probiotic group was significantly lower than in the control group (6 of 90 patients [6.7%] vs. 16 of 93 patients [17.2%], $P = 0.028$). In addition, the plasma levels of proinflammatory cytokines IL-1β and IL-6 were significantly lower and BDNF levels were significantly higher in the probiotic group than in the control group 1–2 days after surgery ($U = 173.0$, $P < 0.01$; $U = 139.0$, $P < 0.01$; $U = 207.0$, $P < 0.01$).

Conclusion Perioperative probiotic intervention can reduce the incidence of POCD in elderly patients, which may improve cognitive function by inhibiting inflammatory response after anesthesia and surgery, and altering the composition of the postoperative gut microbiota and intestinal metabolites.

Keywords Probiotics, Postoperative cognitive dysfunction, Gut microbiota, Gut-brain axis

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Background

Postoperative cognitive dysfunction (POCD) is common in elderly patients after surgery, which is characterized by changes in learning and memory loss, reduced verbal thinking, and inattentiveness [1]. The incidence of POCD ranges from 4.1 to 41% at 7 days postoperatively, and 6.8–19.6% at 3 months postoperatively [2]. POCD leads to prolonged hospitalization, increased hospitalization costs, increased social burdens, and increased mortality rates, and imposes a greater burden on the family and the society. Currently, there are many strategies for the prevention and treatment of POCD, including cognitive and physical exercise [3, 4], pharmacological interventions (e.g., edaravone [5], methylthionine [6], dexmedetomidine [7], statins [8], and antibiotics for anti-inflammation [9]), control of fasting time and carbohydrate load [10], anesthesia management (e.g., multi-mode analgesia including preemptive analgesia, dexmedetomidine, and epidural analgesia [11]), and blood pressure management [12]. However, these strategies have not achieved the desired clinical outcomes. Therefore, new simple and effective prevention and treatment strategies are urgent tasks.

Gut microbiota refers to the microorganisms in the gastrointestinal tract of animals. An adult gastrointestinal tract hosts more than 10,000 species and 10^{14} microorganisms, and the main flora include *Mycobacterium phylum*, *Thick-walled Mycobacterium*, *Aspergillus*, and *Actinobacterium*. Gut microbiota and brain achieve bidirectional communication between brain and intestine through neural, endocrine and immune pathways to maintain normal physiological functions of brain and intestine, a pathway known as the gut microbiota-gut-brain axis [13]. Many factors in the perioperative period can affect the gut microbiota, including the surgery itself, antibiotics, opioids, or acid-inducing drugs [14]. Surgical anesthesia-induced abnormalities of the gut microbiota are age-dependent, as evidenced by a significant decrease in the abundance and diversity of the microbiota with age [15]. Decreases in beneficial bacteria (*lactobacilli*, *bifidobacteria*) increase the risk of postoperative cognitive impairment, and surgical anesthesia exacerbates gut microbiota dysbiosis and transforms the gut microbiota into a more toxic phenotype [16]. Abnormal gut microbiota has been linked not only to the development of autism, depression, schizophrenia, and Alzheimer's disease, but also to postoperative decline in learning and memory [17–20]. And previous study showed that increasing probiotics can reduce the production of inflammatory cytokines (such as IL-1β, TNF-a and IL-6) [21]. The above studies suggest that abnormal intestinal flora plays a key role in the pathogenesis of postoperative neurocognitive disorders. Therefore, regulating the composition of intestinal flora and improving its physiological function will help to prevent and treat postoperative cognitive decline.

This study was designed to investigate the effects of perioperative probiotic intervention on postoperative cognitive function in elderly patients and preliminarily investigate the effects of probiotics on intestinal flora and metabolites.

Methods

Design

This study was a prospective randomized controlled study, which was approved by the Ethics Committee of Jiaxing Second Hospital (JXEY-2021SW074). The study was conducted in accordance with the guidelines of Good Clinical Practice and the principles expressed in the Declaration of Helsinki. The study was registered at chictr.org (ChiCTR2000036157). All patients in this study were initiated after completion of clinical trial enrollment, and written informed consent was obtained from all enrolled patients or their guardians for this study.

Subjects

Inclusion criteria.

1. Age ≥ 65 years;
2. American Society of Anesthesiologists (ASA) Class I-III;
3. Elective lower extremity orthopedic surgery (unilateral femur or tibia fracture surgery and hip or knee arthroplasty);
4. A score of ≥ 20 on the Mini-mental State Examination (MMSE) scale;
5. Informed consent was obtained to voluntarily participate in this trial.

Exclusion criteria.

1. Have a communication disability (e.g., severe hearing, vision, speech impairment);
2. Comorbid or previous central nervous system disorders such as stroke, Parkinson's disease, Alzheimer's disease, epilepsy, and others;
3. Comorbid or previous mental disorders such as schizophrenia, depression, mania, anxiety, etc.;
4. Comorbid immune system disorders, or long-term treatment with anti-inflammatory drugs, painkillers, glucocorticoids, cytotoxic drugs, etc.;
5. History of alcoholism, drug abuse, or drug dependence;
6. Treatment with antibiotics, probiotics, or pro-gastrointestinal medications within 10 days prior to admission;
7. More than one surgery is required during hospitalization;

8. Complete parenteral nutrition was used postoperatively;
9. Postoperative hospital stay ≤ 7 days;
10. Participation in other clinical trials or refusal to participate in this trial or failure to cooperate with treatment.

Randomization and blinding

Using SPSS software (version 25.0, SPSS, Chicago, United States), random numbers were generated in a 1:1 ratio and patients were randomized into probiotic and control groups. During the study period, patients received either probiotic or placebo capsules from nurses according to the assignment of random numbers. Patients and clinicians (including surgeons and anesthesiologists), as well as all researchers who performed preoperative and postoperative assessments and data collection, were unaware of the grouping.

Intervention

The probiotic group received oral probiotic capsules (Bifico, Bifidobacterium trifidum capsule, 210 mg × 36, Shanghai SINE Pharmaceutical Co., Ltd., State Pharmaceutical License S10950032) from 1 day before to 5 days after surgery, containing *Bifidobacterium longum*, *Lactobacillus acidophilus*, and *Enterococcus faecalis* > 10^7 colony-forming unit[CFU]/210 mg, and 210 mg × 4/dose orally, 2 times/day. The medication was uniformly dispensed by the nurse after breakfast and dinner and taken half an hour after meals. On the day of surgery, it was taken on an empty stomach before surgery and half an hour after eating after surgery.

The control group received oral placebo capsules from 1 day before to 5 days after surgery, also provided by Shanghai SINE Pharmaceutical Co. Ltd, containing all ingredients except probiotics, which were identical in size, shape and smell to the probiotic capsules, and were given to the patients in the control group in the same manner during hospitalization.

Outcome measures

Primary outcome: in this trial, the incidence of POCD was set as the primary outcome. Cognitive function was assessed by MMSE scale at admission (baseline), postoperative day 1, day 3 and day 7, respectively. Significant cognitive decline was usually defined as a change of ≥ 3 points in MMSE score. Based on previously used criteria [22–24], POCD was defined as a decrease of 3 or more points in MMSE score from admission to postoperative day 7.

Secondary outcome: the secondary outcome measures included perioperative plasma IL-1β, IL-6, and brain-derived neurotrophic factor (BDNF) concentrations changes, perioperative fecal microbiota composition and metabolomics changes, perioperative activities of daily living (ADL) evaluates the individual in daily life, independent completion of the basic life activities. Postoperative rest pain scores, and the incidence of POD, postoperative nausea and vomiting (PONV).

Data collection

Cognitive function was assessed by the same researcher at the time of admission, on postoperative day 1, 3 and 7 by the MMSE scale and the MMSE scores were recorded. The patients were assessed for the occurrence of POD on postoperative days 1–3 by using the Confusion Assessment Method (CAM). The patients were asked about and recorded the postoperative 48 h PONV occurrence, the rest pain level was assessed using 0–10 points Visual Analogue Scale (VAS) (0 = no pain, 10 = worst pain) on days 1–3 postoperatively.

Information directly available to the researcher from the medical record included: age, gender, ASA classification, height, weight, Body Mass Index (BMI), type of surgery, preoperative comorbidities, use of intravenous analgesia (Patient controlled intravenous analgesia, PCIA), type and duration of perioperative antibiotic use, duration of surgery, duration of anesthesia, intraoperative bleeding and fluid replacement, ADL scores on admission and postoperative days 1–3, and length of hospital stay. Among them, preoperative comorbidities mainly included four types: hypertension, diabetes, cardiovascular disease, and respiratory disease. Cardiovascular diseases mainly include ischemic heart disease, arrhythmia, heart valve disease, cardiomyopathy, and having a pacemaker, etc. Respiratory diseases mainly include chronic obstructive pulmonary disease, asthma, bronchiectasis, and lung tumors.

Specimen collection

The researchers collected blood specimens from patients before induction of anesthesia, before leaving the recovery room after awakening, on postoperative days 1–2, and on postoperative days 5–7. Of these, postoperative specimens were collected between 8 a.m. and 10 a.m. After collection, the blood collection tubes were placed in a high-speed centrifuge at 4 ℃ and 3000 r/min for 10 min, and plasma was taken and injected into a sterile silicified plastic tube, which was sealed and placed in a -80 ℃ refrigerator for storage. The changes in the concentrations of plasma IL-1β, IL-6 and BDNF were detected by enzyme-linked immunosorbent assay (ELISA).

Fecal specimens were collected 1–2 days before and 5–7 days after surgery for 16 S rRNA and untargeted metabolomics analysis. The nurse educated the patients and their families about fecal specimen collection, discharged the feces into the bedpan when using the toilet, avoided feces stained with urine or other liquids as much as possible, and retained about 10 g of fresh feces in the mid-section of the feces using the scoop accompanying aseptic fecal sampler, which was transferred by the researcher using an ice box and placed in a refrigerator at -80 °C for preservation.

Anesthesia management

Before anesthesia, it was confirmed that the patient had signed the informed consent for anesthesia. The patients were fasted and abstained from food and drink, and 5-lead electrocardiogram, noninvasive blood pressure, pulse oximetry, partial pressure of end-tidal CO_2 (PetCO2), and temperature were monitored, an arterial cannula was placed in the left or right radial artery to monitor invasive arterial blood pressure and the bispectral index (BIS, Aspect, USA) was placed to measure the depth of sedation after admission to the operating room. Anesthesia was induced by intravenous administration of etomidate (Jiangsu Hengrui Pharmaceutical Co., Ltd) 0.2–0.3 mg/kg, cisatracurium (Jiangsu Hengrui Pharmaceutical Co., Ltd) 0.1–0.2 mg/kg, and sufentanil (Yichang Renfu Pharmaceutical Co., Ltd) 0.5–0.8 μg/kg. Patients underwent endotracheal intubation for mechanical ventilation, with a tidal volume of 8–10 ml/kg, respiratory rate of 12–14 times/min, inspiratory to expiratory ratio of 1:2, and PetCO2 was maintained at 35–45 mm Hg (1 mm Hg = 0.133 kPa). Anesthesia was maintained with remifentanil (Yichang Renfu Pharmaceutical Co., Ltd) 0.1–1.0 μg/(kg·min), propofol (Beijing Fresenius Kabi Pharmaceutical Co., Ltd) 50–150 μg/(kg·min), and oxygen with sevoflurane (Hangzhou Merck Sharp & Dohme Pharmaceutical Co., Ltd) 0.6-1.0 MAC. BIS values were maintained at 40–55. The heart rate and arterial blood pressure were maintained within 20% of baseline. Invasive arterial blood pressure is maintained by infusion of norepinephrine and fluid management. Nasopharyngeal temperature was maintained ≥ 36℃. Postoperative analgesics were used for patient-controlled intravenous analgesia (PCIA) within 48 h after surgery.

Sample size

The estimated sample size was calculated assuming a POCD rate of 40% in the control group and 20% in the probiotic group. The positive rate of the control group was determined by reference to previous literature [25, 26]. Given a significance set at the level of 0.5, with a 2-sided, power at 80%, 164 patients were needed. Considering the possibility of loss to follow-up or consent withdrawals, we recruited 190 patients to participate in this trial.

Statistical analysis

Data were analyzed using SPSS 25.0 statistical software and and GraphPad 7.0 software was used to construct all plots. Continuous data with a normal distribution were presented as mean ± standard deviation (SD) and compared using the unpaired, 2-tailed t test. Data that were not normally distributed were reported as median and interquartile range (IQR) and analyzed using the Mann-Whitney test. Categorical variables were reported as number (%) and were analyzed with Pearson's chi-square test or Fisher's exact test, as appropriate. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

From May 2021 to December 2022, a total of 190 patients were recruited into the study who agreed and met the inclusion criteria, and these 190 patients were randomly assigned to either the probiotic group ($n = 95$) or the control group ($n = 95$). During the actual operation of the study, 7 patients (5 in the probiotic group and 2 in the control group) were removed from the study due to cancellation of surgery, change in surgical approach, refusal of postoperative follow-up, multiple surgeries during hospitalization, and occurrence of postoperative hemolysis. Primary case information and data from 90 patients in the final probiotic group and 93 patients in the control group were included in the statistical analysis. The process of the participants participating in the experiment and the number of patients who dropped out are shown in Fig. 1.

Baseline characteristics such as age, gender, height, weight, BMI, ASA classification, type of surgery, preoperative comorbidities, and PCIA use were distributed evenly between the two groups without statistical differences (Table 1). It is worth mentioning that because the use of PCIA or not was an autonomous preoperative choice of the patients, it was also included in the baseline characteristics.

Primary outcome

There were no significant differences in MMSE scores between patients in the probiotic group and control group during perioperative period. POCD occurred in 6 out of 90 patients in the probiotics group and 16 out of 93 patients in the control group, and the incidence of POCD in the probiotic group was significantly lower than that in the control group (6.7% vs. 17.2%, $P = 0.028$). The distribution of MMSE scores is shown in Fig. 2.

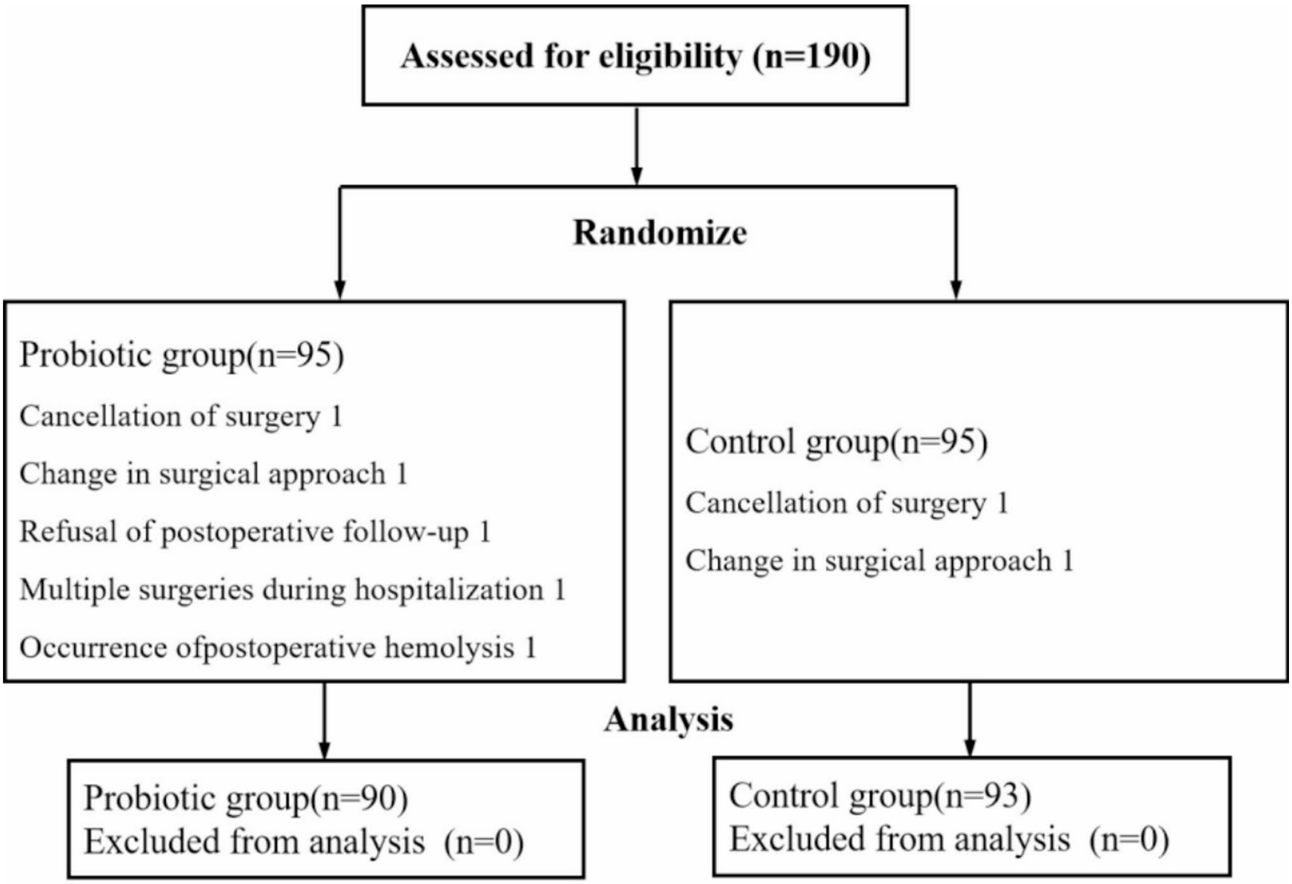

Fig. 1 Patient flowchart

Secondary outcomes

Perioperative-related variables

There was no statistical difference in the type of perioperative antibiotic use and duration of antibiotic use, surgery duration, anesthesia duration, intraoperative bleeding and fluid replacement, length of stay between the two groups ($P > 0.05$). There was no statistical difference between the two groups in the incidence of postoperative delirium (POD) on postoperative days 1–3 (4.4% vs. 3.2%, $P = 0.965$), and the incidence of postoperative nausea and vomiting (PONV) on postoperative day 48 h (28.9% vs. 20.4%, $P = 0.182$) (Table 2).

Changes of plasma IL-1β, IL-6 and BDNF during perioperative period

Plasma levels of pro-inflammatory cytokines IL-1β and IL-6 were elevated in patients within 1–2 days after surgery compared to those before induction of anesthesia (T0), with a mild elevation before discharge from the recovery room after awakening (T1), and a significant elevation on postoperative days 1–2 (T2), and this elevation was significantly greater in the control than in the probiotic group on postoperative days 1–2 (T2) (U = 173.0, $P < 0.01$; U = 139.0, $P < 0.01$). The levels of pro-inflammatory cytokines IL-1β and IL-6 in plasma decreased on postoperative days 5–7 (T3), essentially restoring preoperative (T0) levels (Figs. 3A, B). Similarly, compared with before the induction of anesthesia (T0), BDNF levels in plasma decreased during the 5–7 days after surgery, decreased significantly before leaving the recovery room after awakening (T1), and then showed a gradual increase, in which this increase was significantly greater in the probiotic group than in the control group on postoperative days 1–2 (T2) (U = 207.0, $P < 0.01$) (Fig. 3C).

Changes in perioperative activity of daily living and postoperative pain intensity

There were no significant differences in ADL scores on admission (U = 4137, $P = 0.889$), postoperative day 1 (U = 4109, $P = 0.821$), postoperative day 2 (U = 4055, $P = 0.710$) and postoperative day 3 (U = 3571, $P = 0.080$) between the two groups (Fig. 4A). Similarly, there were no significant differences in VAS scores for pain at rest between the two groups of patients on postoperative days 1–3 respectively (U = 4155, $P = 0.932$; U = 4102, $P = 0.723$; U = 4034, $P = 0.514$) (Fig. 4B).

Table 1 Patient demographic and baseline characteristics

	Probiotics (*n* = 90)	Control (*n* = 93)
Age(yr)	70 (66, 75)	72 (67, 75)
Gender		
Male	36 (40.0%)	35 (37.6%)
Female	54 (60.0%)	58 (62.4%)
Height(cm)	161.78 ± 6.94	160.85 ± 7.59
Weight (kg)	63.98 ± 9.00	64.22 ± 10.13
BMI (kg/ m^2)	24.44 ± 3.08	24.79 ± 3.27
ASA classification		
II	73 (81.1%)	84 (90.3%)
III	17 (18.9%)	9 (9.7%)
Surgery type		
Knee arthroplast	52 (57.8%)	59 (63.4%)
Hip arthroplasty	28 (31.1%)	22 (23.7%)
Femur fracture surgery	8 (8.9%)	8 (8.6%)
Tibia fracture surgery	2 (2.2%)	4 (4.3%)
Preoperative comorbidities		
Hypertension	57 (63.3%)	56 (60.2%)
Diabetes	21 (23.3%)	21 (22.6%)
Cardiovascular disease	6 (6.7%)	9 (9.7%)
Respiratory disease	1 (1.1%)	0
Usage rate of PCIA	73 (81.1%)	81 (87.1%)
Sufentanil consumption in PCIA (μg)	92.00 ± 8.48	93.55 ± 8.95

All data are expressed as mean ± SD, frequency (%), and median (first quartile, third quartile)

PCIA: patient controlled intravenous analgesia

Table 2 Perioperative-related indicators

	Probiotics (*n* = 90)	Control (*n* = 93)	*P*-value
Preoperative antibiotics			0.420
None	1 (1.1%)	0	
Cefuroxime sodium	44 (48.9%)	37 (39.8%)	
Clindamycin	1 (1.1%)	2 (2.2%)	
Cefuroxime sodium +vancomycin	44 (48.9%)	54 (58.1%)	
Intraoperative antibiotics			0.987
None	89 (98.9%)	93 (100%)	
Clindamycin	1 (1.1%)	0	
Postoperative antibiotics			0.419
None	2 (2.2%)	3 (3.2%)	
Cefuroxime sodium	86 (95.6%)	84 (90.3%)	
Clindamycin	1 (1.1%)	2 (2.2%)	
Cefoperazone sodium and sulbactam sodium	0	1 (1.1%)	
Piperacillin sodium and tazobactam sodium	0	3 (3.2%)	
Levofloxacin	1 (1.1%)	0	
Postoperative antibiotic use duration (d)	1 (1, 1)	1 (1, 1)	0.649
Surgery duration (min)	95 (80.75, 115)	100 (90, 120)	0.121
Anesthesia duration (min)	120 (100.75, 135)	125 (110, 146)	0.155
Intraoperative bleeding (ml)	50 (20, 200)	50 (20, 100)	0.129
Fluid replacement (ml)	1220 (1000, 1500)	1213 (1000, 1500)	0.913
POD	4 (4.4%)	3 (3.2%)	0.965
PONV	26 (28.9%)	19 (20.4%)	0.184
Length of stay (d)	12 (10, 14)	11 (9, 13)	0.111

All data are expressed as mean ± SD, frequency (%), and median (first quartile, third quartile)

Effects of probiotics on the α-diversity and β-diversity of gut microbiota

Fecal samples were collected on 5–7 days after surgery. We used 16SrRNA sequencing to detect gut microbiota composition in the C1 (control group sample 5–7 days

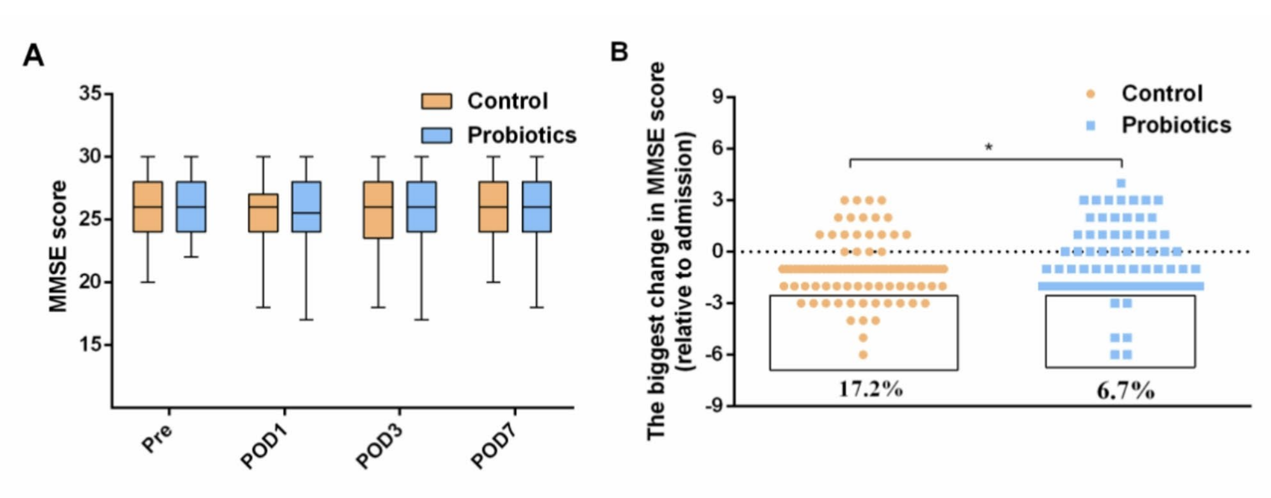

Fig. 2 (**A**) Box plots of the distribution of perioperative MMSE scores. (**B**) Scatter plot of maximum changes in MMSE scores before and after surgery, the incidence of POCD was significantly lower in the probiotic group than the control group. Pre standed for admission, POD1, POD3, and POD7 represented postoperative day 1, day 3, and day 7, respectively. The line represents the median, boxes represent the IQR, whiskers represent the range. IQR: interquartile range. **P* < 0.05 Probiotic VS control groups

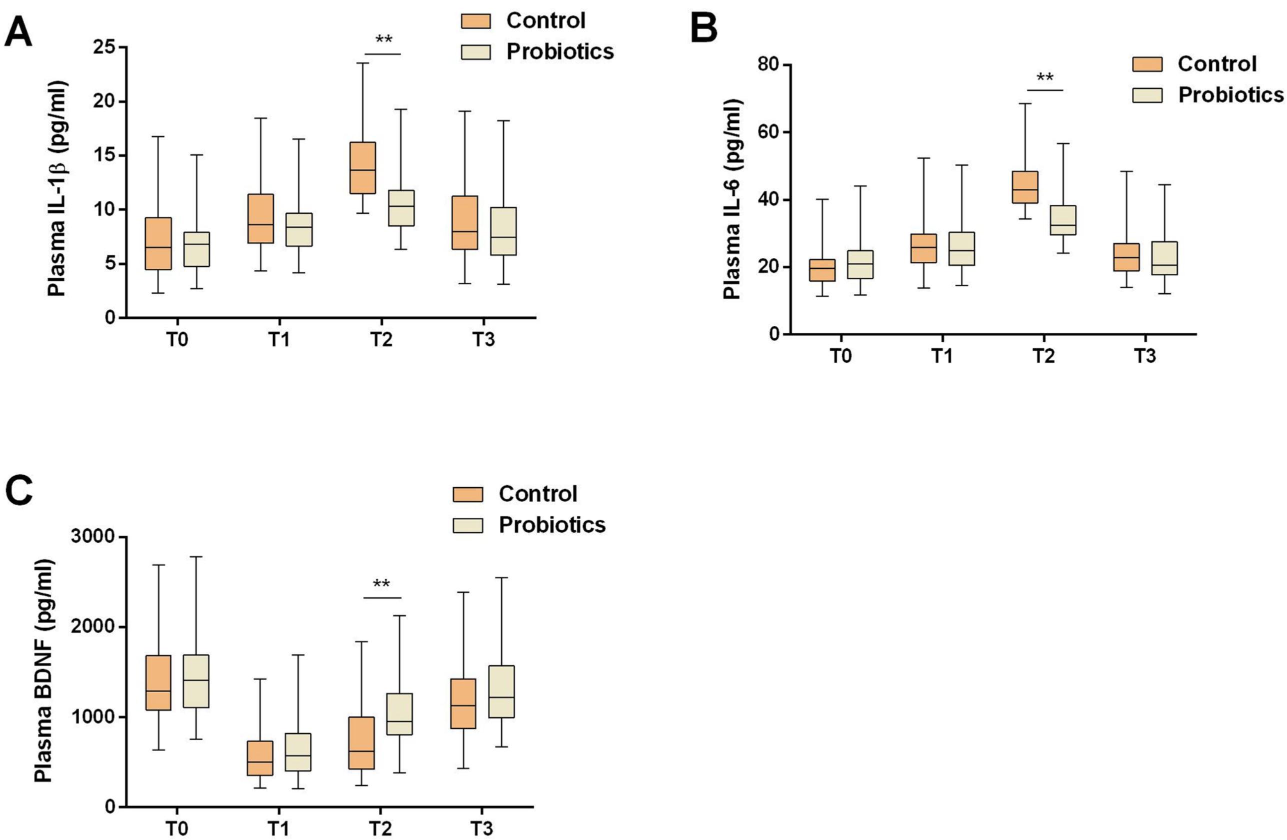

Fig. 3 Perioperative Changes in Plasma IL-1β, IL-6 and BDNF. T0, T1, T2, and T3 represented before anesthesia induction, before discharge from the recovery room after awakening, postoperative day 1–2, and postoperative day 5–7, respectively. The line represents the median, boxes represent the IQR, whiskers represent the range and $^{**}P < 0.01$ Probiotic VS control groups. IQR: interquartile range

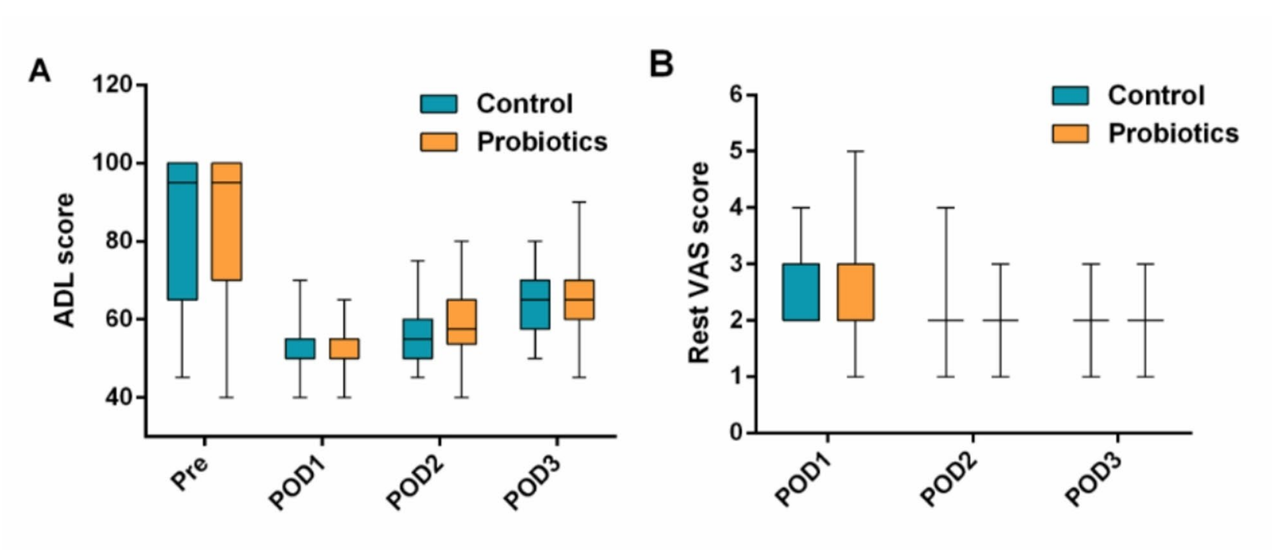

Fig. 4 Perioperative changes in ADL and rest VAS scores. The line represents the median, boxes represent the IQR, whiskers represent the range. ADL: activities of daily living, VAS: visual analogue scale. IQR: interquartile range

postoperatively) and P1 (probiotics group sample 5–7 days postoperatively) groups. a-diversity index reflects the richness and diversity of the community. Among them, the Chao1 index reflects community richness, and the Simpson index measures community diversity. There was no significant difference in the Chao1 index between the C1 and the P1 groups (Fig. 5A), but the Simpson index decreased in the P1 group compared with the C1 group (Fig. 5B). Principal coordinates analysis (PCoA) is a non-binding method of data dimension reduction, and performed to study similarities or differences in sample community composition. In two-dimensional data of PCoA, the dots of the C1 and P1 groups (Fig. 5C).

Changes of intestinal metabolites in the C1 and P1 groups

We collected fecal specimens from two groups of patients on 1–2 days before and 5–7 days after surgery, and performed fecal metabolomic analysis. The fecal metabolomic data of the two groups of patients were analyzed by orthogonal partial least squares discriminant analysis (OPLS-DA) model, and the scores of each group were plotted to further demonstrate the differences between the subgroups, as shown in Fig. 6A, there was a significant difference in the fecal metabolites of the C1 group and the P1 group on postoperative days 5–7. Overall, 587 intestinal metabolites were differentially expressed: 389 down-regulated and 198 up-regulated (Fig. 6B). Metabolic set enrichment analysis (MSEA) were performed for the C1 and P1 groups of differential metabolites, and the MSEA enrichment analysis diagram is shown in Fig. 6C.

Discussion

In this randomized, double-blind, placebo-controlled trial, perioperative probiotic intervention significantly reduced the incidence of POCD in elderly patients and altered the composition of the postoperative gut microbiota and intestinal metabolites. There were no significant differences in baseline characteristics, incidence of POD, incidence of PONV, type of perioperative antibiotic use and duration of antibiotic use, length of hospitalization, ADL scores in the perioperative period and change in postoperative pain intensity between the control and probiotic groups. Our study suggests that perioperative probiotic intervention may be a potential strategy to prevent the development of POCD in elderly patients.

The MMSE is the most widely used screening tool for cognitive decline and is characterized by ease of completion and less time for completion by patients [27, 28]. The MMSE is reliable in tracking for cognitive dysfunction [29]. The change in MMSE scores was consistent with the change in the 14-item cognitive subscale of the Alzheimer's Disease [30]. Previous studies have shown that Alzheimer's patients' cognitive function decreases by 3 points per year, as measured by the MMSE test, significant cognitive decline is generally defined as a change of ≥3 points in the MMSE score [31, 32]. Therefore, this experiment assessed cognitive function with reference to the criteria of previous studies.

Gut microbiota dysbiosis refers to an abnormal state in which the composition, quantity, distribution, or function of the gut microbial community (including bacteria, fungi, viruses, etc.) becomes imbalanced, disrupting the symbiotic relationship between microorganisms and the host, and potentially leading to health problems [33]. Increasing evidence suggests that gut microbiota dysbiosis can influence the development of neurodegenerative diseases, psychosomatic disorders, and perioperative neurocognitive dysfunction [13, 34, 35], and that gut microbiota communicate with the central nervous system and can influence brain function and behavior through neural, endocrine, and immune pathways [36].

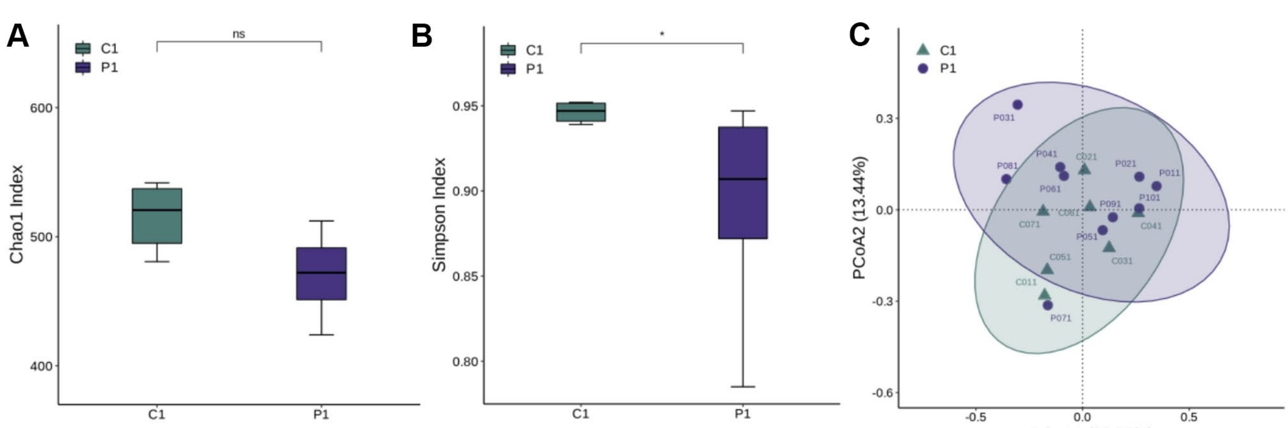

Fig. 5 Differential gut microbiota profiles between the C1 and the P1 groups. (**A**) Chao1 index between the groups. (**B**) Simpson index between the groups. (**C**) a β-diversity indicator PCoA analysis of gut bacteria data (Bray-Curtis dissimilarity) between the groups. C1: control group, P1: probiotic group. * $P < 0.05$ Probiotic VS control groups

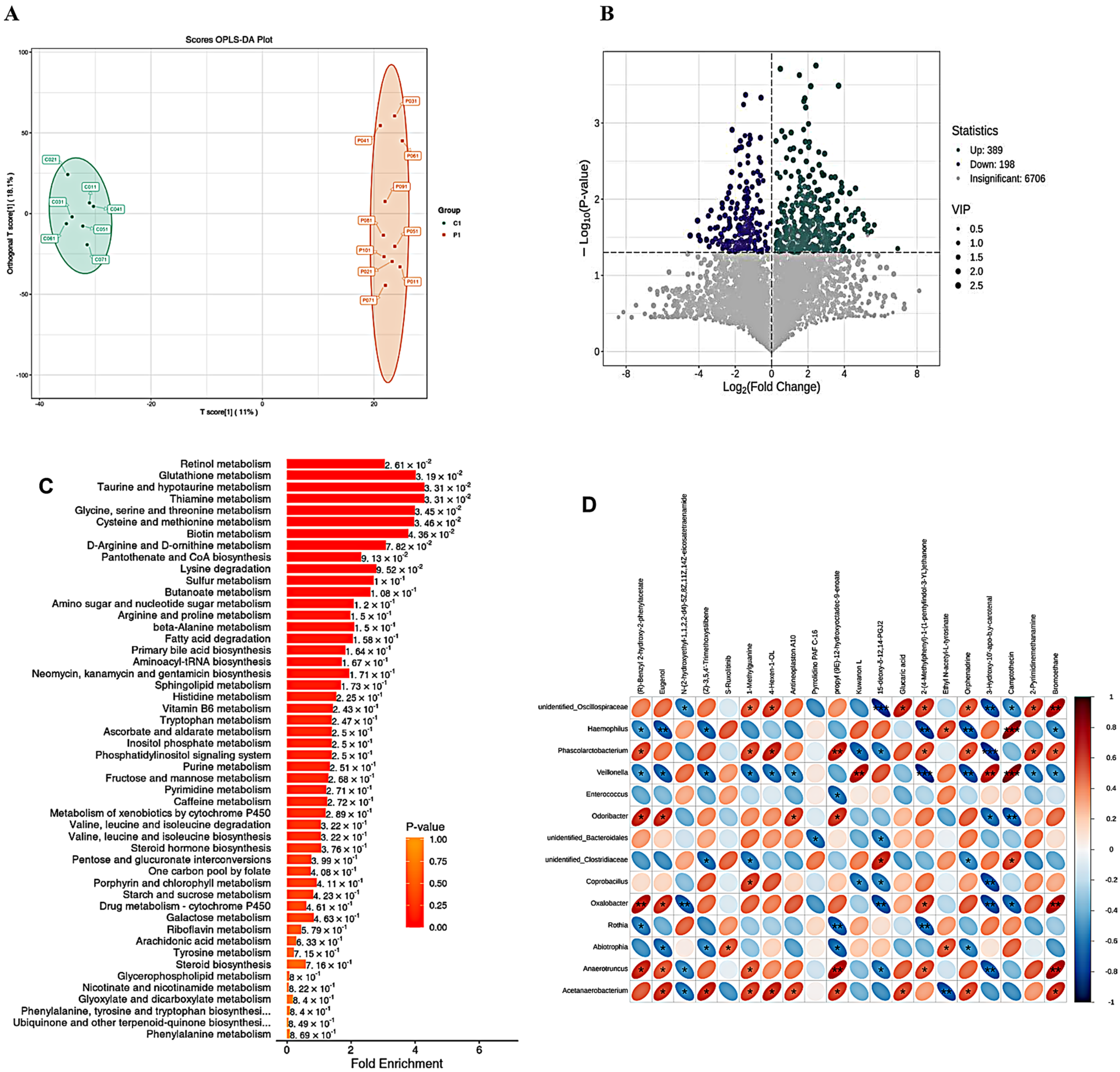

Fig. 6 (**A**) OPLS-DA Plot Horizontal coordinates indicate predicted component score values, with the horizontal direction showing the gap between groups; vertical coordinates indicate orthogonal component score values, with the vertical direction showing the gap within groups; and percentages indicate the rate of explanation of the component to the data set. (**B**) Volcanic maps showing the overall distribution of differential metabolites (389 down-regulated, 198 up-regulated). (**C**) MSEA enrichment analysis plot. The vertical coordinate indicates the name of the metabolic set (sorted by *P*-value), which corresponds to the *P*-value of the labeled metabolic set; the horizontal coordinate indicates Fold Enrichment, the degree of enrichment; the color indicates the *P*-value, the closer the *P*-value is to 0, and the redder the color is, the more significant the enrichment is. (**D**) Heat map correlation analysis. The horizontal direction shows different bacteria, the vertical direction shows different metabolites, and the legend on the right shows the correlation coefficient. Red is positive correlation, blue is negative correlation. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. C1: Control group sample 5–7 days postoperatively; P1: Probiotics group sample 5–7 days postoperatively

Probiotic treatment can significantly improve intestinal dysbiosis and its associated pathological effects. We also pay attention to whether the therapeutic effect of probiotics during the perioperative period is affected by antibiotics. Antibiotics are commonly used for preventing or treating surgical infections. In our study, in order not to interfere with the clinical work of surgeons, we did not restrict the use of antibiotics. To reduce the effect of antibiotics on the composition of the gut microbiota, patients who had been treated with antibiotics within the first 10 days of admission were excluded from the study, and there was no statistical difference in the type and duration of perioperative antibiotic use between the two groups. Despite the use of antibiotics, we still observed that perioperative probiotics protected postoperative cognitive impairment.

Previous studies have shown that perioperative peripheral inflammatory response is the main mechanism for the pathogenesis of POCD, which triggers cognitive decline by inducing neuroinflammation and disrupting synaptic connectivity [37–39], and inhibition of the perioperative peripheral inflammatory response can significantly alleviate POCD [40]. It has been demonstrated that pro-inflammatory cytokines IL-1β and IL-6 intervene in neuronal regeneration due to inflammatory inhibition [41], and that the effect of inflammatory response on POCD is more pronounced in the elderly because of the decreased regenerative capacity of the cells. Li et al. studied 37 elderly patients who underwent total hip arthroplasty, and the serum levels of inflammatory markers IL-1β and IL-6 were significantly higher in patients who developed POCD [42]. Brain derived neutrophic factor (BDNF) is a member of the neurotrophic factor family. BDNF is important in the development, survival and maintenance of neurons in the central nervous system, and is mainly expressed in the hippocampus and cortex, which is related to cognitive function. Liu et al. found that surgical incision induced POCD by inhibiting the BDNF signaling pathway in the hippocampus and amygdala [43], and Vignoli et al. found that learning consolidation in mice declined with decreasing BDNF levels [44]. A clinical study found that about 75% of stroke patients were associated with cognitive dysfunction and had significantly lower BDNF levels than patients without cognitive impairment [45]. This suggests a significant correlation between BDNF and cognitive dysfunction. Since BDNF produced by the CNS can rapidly cross the blood-brain barrier, plasma BDNF levels can reflect brain tissue BDNF levels [46]. The above suggests that changes in the levels of IL-1β, IL-6, and BDNF can be used as predictive parameters for the occurrence of POCD. Our further mechanistic study found that perioperative probiotic supplementation significantly reduced the levels of proinflammatory cytokines IL-1β and IL-6 and elevated the levels of BDNF in plasma 1–2 days after surgery. Other studies have also demonstrated that probiotic interventions reduce inflammatory responses [47–50]. Therefore, perioperative probiotic intervention may improve patients' cognitive function by suppressing the inflammatory response after anesthesia and surgery.

Gut microbiota has been reported to influence brain function and behavior through the microbiota-gut-brain axis [51–53]. This study demonstrated that after 7 consecutive days of perioperative probiotic therapy, 16 S rRNA analysis revealed an abnormality in the composition of the fecal microbiota in the probiotic and control groups. Given the critical role of microbial metabolites in regulating the gut microbiota and host pathophysiology, we investigated the effect of perioperative probiotic intervention on fecal metabolites in patients undergoing anesthesia and surgery. Fecal LC-MS metabolomics analysis revealed significant abnormalities in the composition of fecal metabolites in the probiotic and control groups. The metabolism of these substances is obviously disordered, including Retinol metabolism, Glutathione metabolism, Taurine and hypotaurine metabolism, Thiamine metabolism, Glycine, serine and threonine metabolism, Cysteine and methionine metabolism, Biotin metabolism. In addition, significant changes in fatty acid and tryptophan metabolism were also found in this study. Recent studies have shown that the gut microbiota can shape the tryptophan metabolic pathway in a variety of ways through direct or indirect effects to regulate host immune function, anxiety and depression behavior, and cognitive function [54]. Gut microbiota can affect fatty acid metabolism through the vagus nerve, humoral, immune and endocrine pathways of the brain-gut axis, thereby affecting learning and memory [55, 56]. These results provide theoretical basis for further exploring the mechanism of intestinal flora affecting postoperative cognitive function. The present study also revealed strong relationships between various metabolites and key gut microbes at the genus level. Among the genera with significant differences, *Haemophilus* and *Veillonella* were mainly positively correlated with several metabolites ((R)-Benzyl 2-hydroxy-2-phenylacetate, eugenol, 2-(4-Methylphenyl)-1-(1-pentylindol-3-YL) ethenone and bromoethane), while Oxalobacter and Anaerotruncus were negatively correlated.

Our study has several limitations. First, as a single-center study with a small sample size and a simple surgical population, the enrolled patients may not be fully representative of the patient population. Second, long-term follow-up of the patients was not performed, so whether probiotic intervention improves the long-term outcome of POCD remains unknown, and the long-term effects of perioperative probiotic intervention on postoperative cognitive function in elderly patients remain to be further explored. More importantly, it remains to be investigated which microbiota and metabolites play the most important roles in improving postoperative cognitive impairment. We plan to focus on this in future studies.

Conclusion

Our study indicated that perioperative probiotic interventions can attenuate postoperative cognitive impairment and reduce the levels of pro-inflammatory cytokines IL-1β and IL-6, as well as elevate the levels of BDNF in postoperative plasma of elderly patients.

Moreover, perioperative probiotic interventions altered gut microbial diversity and metabolite profiles.

Abbreviations

POCD	Postoperative cognitive dysfunction
MMSE	Mini-mental State Examination
ADL	Activities of daily living
PONV	Postoperative nausea and vomiting
CAM	Confusion assessment method
VAS	Visual analogue scale
PCoA	Principal coordinates analysis
OPLS-DA	Orthogonal partial least squares discriminant analysis
MSEA	Metabolic set enrichment analysis
BDNF	Brain derived neutrophic factor
SD	Standard deviation
IQR	Interquartile range
ASA	American society of snesthesiologists
BMI	Body mass index
PCIA	Patient controlled intravenous analgesia

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Author contributions
SG, GC and JL designed the experiments. SG, HD, QH, KJ and HX performed the experiments. SG, GC and JL contributed to data analyses. SG and JS drafted this manuscript. JL reviewed and edited the manuscript. All authors interpreted the data, revised the manuscript, approved the final content, and read and approved the final manuscript. All authors contributed to the article and approved the submitted version.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The Study was approved by the Second Hospital of Jiaxing Ethics Committee (approval number: JXEY-2021SW074). Besides, all the participants have written informed consent.

Consent for publication
Not applicable.

Conflict of interest
The authors have declared that no competing interests exist regarding the publication of this paper.

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