

Role of gut microbiota in post-operative atrial fibrillation: a randomized trial of berberine

Huishan Wang (✉ huishanw@126.com)

General Hospital of Shenyang Military Area Command

Jian Zhang

Shenyang Northern Hospital

Yang Wang

General Hospital of Shenyang Military Area Command

Hui Jiang

General Hospital of Shenyang Military Area Command

Dengshun Tao

General Hospital of Shenyang Military Area Command

Keyan Zhao

General Hospital of Shenyang Military Area Command

Zongtao Yin

Shenyang Northern Hospital

Jinsong Han

Shenyang Northern Hospital

Fang Ran

General Hospital of Shenyang Military Area Command

Yan Jin

General Hospital of Shenyang Military Area Command

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Abstract

Post-operative atrial fibrillation (POAF) is one of the most common complications. However, the underlying factors governing POAF are not well understood. The aim of this study was to investigate the relationship between gut microbiota and POAF. Then, we conducted a randomized, double-blind, placebo-controlled trial with patients underwent isolated coronary artery bypass grafting (CABG) in China to measure gut microbiota altering and impact on the incidence of POAF by oral berberine. Compared with no-POAF patients, gut microbiota composition was significantly altered, including *Veillonella* increasing. The POAF incidence was reduced from 35–20% under the treatment of berberine. Oral berberine significantly decreased *Veillonella* abundances, and lipopolysaccharide (LPS), C-reactive protein (CRP) and Interleukin-6 (IL-6) level. Elevated LPS after surgery is associated with POAF. Our results showed that POAF patients show significantly gut microbiota shift. Altering gut microbiota like oral berberine reduced the POAF.

Introduction

Post-operative atrial fibrillation (POAF) is one of the most common complications of cardiac surgery, which is associated with prolonged hospital stay, hemodynamic instability, increased risk of stroke, and increased mortality.^{1,2} Some studies indicated inflammation, oxidative stress, and electrical remodeling are the main reasons for POAF.^{3,4} However, the detailed mechanisms underlying POAF are largely unknown. Uncovering the driving factors and identifying strategies to impede POAF have attracted considerable attention.

The collection of microbes living in the human intestinal tract (gut microbiota) have influence far beyond digestion.⁵ Increasing evidence showed that the gut microbiota plays an important regulatory role during the development of cardiovascular diseases, such as hypertension and atrial fibrillation (AF).^{6,7} Compared with no-AF patients, gut microbiota was disordered in AF patients. Along with the progression of AF, some distinctive and progressive alterations in the gut microbiota and metabolic structure has been identified.⁸ Further investigation found that AF patients had altered gut microbial composition in connection with dietary habits.⁹ However, extensive research is still needed to explore the clinical significance of the gut microbiota in AF initiation. To find the initiating gut microbiota which may promote new on-set AF, we investigated whether patterns of dysbiosis gut microbiota are associated with POAF in patients underwent isolated coronary artery bypass grafting (CABG).

Berberine (C₂₀H₁₈N₄O₄) is a quaternary ammonium salt derived from isoquinoline alkaloid, with a molar weight of 336.36 g/mol, a common traditional Chinese medicinal herb used for the therapy of diarrhea.^{10,11} Recently, it has been reported for other pharmacological effects, including anti-viral, antibacterial, anti-inflammatory, anti-cancer, hypoglycemic, and lipid regulation.¹² Previously, studies proposed that berberine has class III anti-arrhythmic effects for AF treatment.¹³ Therefore, we hypothesized that berberine might protect against POAF through modulating gut microbiota.

Results

Diversity of the fecal microbiota in POAF patients

Firstly, we included 45 POAF patients underwent isolated CABG and matched 90 no-POAF patients. The POAF was detected by 7-day Holter monitoring for any atrial tachyarrhythmia episode lasting ≥ 30 s. The clinical characteristics are shown in table supplementary 1. The baseline clinical characteristics among the POAF and no-POAF patients were similar.

The diversity index indicates the variety and richness of microbial entities in the gut and is known to be associated with AF recurrence and duration of persistent AF.^{8, 14} To assess gut microbial diversity in POAF patients, Shannon index and chao1 index were used to assess the α -diversity of the microbiota, Principal coordinate analysis (PCoA) was used for the β -diversity. The differences of Shannon indexes between the POAF and the no-POAF patients were analyzed and were not statistically different (Figure 1A, $P=0.067$). The difference in chao1 index between the POAF and no-POAF patients was statistically significant (Figure 1B, $P= 0.043$). For the β -diversity, the PCoA analysis showed significant difference between POAF and no-POAF patients (Figure 1C, $P<0.01$). These results show a distinctive bacterial composition in different groups.

The microbiota at genus level in POAF patients

To further explore discriminative features between POAF and no-POAF groups, Metastats was performed, and 21 distinct OTUs between the POAF and no-POAF groups were found. At the genus level, the composition of bacterial microbiota of the POAF patients and no-POAF was analyzed. *Brevundimonas*, *Caulobacter*, *Leuconostoc*, and *Veillonella* were the main up-regulated genus of the bacterial microbiota in POAF patients. *Klebsiella*, *Streptococcus* and *Escherichia-Shigella* are significantly down-regulated in POAF patients (Figure supplementary 1 and table supplementary 2).

Berberine prevented POAF: a randomized, double-blind, placebo-controlled trial.

As the shifts in gut microbiome composition in POAF patients, a bacteriostatic agent, berberine were used to altering the gut microbiota and conducted a randomized, double-blind, placebo-controlled trial. Two hundred patients underwent isolated off-pump CABG gave informed consent to participate in this trial and were randomized to the berberine group ($n = 100$) and the placebo group ($n = 100$) from January 1, 2020 to September 31 2020 (Figure 2). There is no difference at baseline characteristics between the two groups (Table supplementary 3). All the patients were randomized to receive either berberine or placebo for 5-7 days before surgery. Following cardiac surgery, patients received berberine or placebo via oral or gastric tube until 7 days after the surgery.

Primary Outcomes

All 200 patients were recorded by continuous Holter electrocardiographic and stayed in the hospital for more than 7 days. Thirty-five patients in the placebo group and 20 patients in the berberine group

developed POAF during the first 7 days after CABG, respectively (hazard ratio: 0.5; 95% confidence interval: 0.29 to 0.78; $p = 0.0143$) (Figure 3).

Secondary Outcomes: AF related measurements and clinical outcomes

AF burden, maximum times of AF and maximum ventricular rate were significantly lower in the berberine group (secondary outcomes in supplementary tables). However, length of hospitalization and postoperative ventilator support time did not differ between the 2 groups (secondary outcomes in supplementary tables).

Secondary Outcomes: a significant impact of berberine on the human gut microbiota.

To evaluate how does berberine prevented POAF through gut microbiota, 16S sequencing was performed in the patients allocated to berberine group. Seventy of 100 patients under the treatment of berberine were collected stool samples at both times, one is before berberine administration, the other time point is having berberine for 5-7 days before surgery. Analysis showed that the alterations in the gut microbiome after berberine treatment, regarding to the α -diversity and β -diversity (Figure 4).

Further Metastats analysis indicates that 50 distinct OTUs post berberine treatment. *Morganella*, *Pyramidobacter* and *Erysipelatoclostridium* were increased after berberine treatment. Also, *Faecalibacterium*, *Roseburia*, *Alistipes* and *Veillonella* were decreased significantly (Figure supplementary 2 and Table supplementary 4). Interestingly, patients with high *Veillonella* abundances were more likely to developed POAF, however, under the treatment of berberine, *Veillonella* was decreased more than two folds (Figure 5).

Berberine inhibited intestinal endotoxin levels and systemic inflammation.

As *Veillonella* was gram-negative diplococci and could produce lipopolysaccharide (LPS) and induce inflammation reactions. We next detected the biomarkers of intestinal endotoxin levels and systemic inflammation. The level of LPS, Interleukin-6 (IL-6), C-reactive protein (CRP) and neutrophil to lymphocyte ratio (NLR) were similar in both berberine and placebo groups at baseline (Table supplementary 5). After 48 hours of surgery, the LPS, IL-6 and CRP levels were significantly lower in berberine group (Table 1 and Figure 6).

Table 1
The biomarkers of intestinal endotoxin levels and systemic inflammation after 48 hour of surgery

	berberine	placebo	P
LPS	496.1(460.4-518.3)	560.0(528.2-613.5)	<0.001
IL-6	67.32(37.05-137.76)	179.18(107.59-283.70)	<0.001
CRP	161.90(157.00-172.30)	167.35(161.08-195.50)	0.001
NLR	8.97(6.80-12.46)	12.09(9.08-16.52)	<0.001
NLR: Neutrophil/Lymphocyte Ratio.			

Intestinal endotoxin levels and POAF

Then we investigated the role of LPS in peripheral blood after surgery 48 hours, identifying a significant increase in LPS in patients with POAF (Figure 7) ($p < 0.0001$). Further subgroup analysis found that LPS levels were increased in POAF patients but did not reach statistical significance in berberine groups (Figure 7) ($p = 0.0591$). However, in placebo group, we observed that the LPS levels were significantly higher in POAF patients (Figure 7) ($p = 0.0034$).

Discussion

In the present study, we acquired new evidence describing the characteristics of a dysbiotic gut microbiota in POAF patients. We used 16S rRNA sequencing to characterize POAF patients. Among the 21 distinct OTUs, *Veillonella* was significantly increased in POAF patients. Additionally, a randomized, double-blind, placebo-controlled clinical trial showed that berberine altered the gut microbiota and prevented POAF in isolated CABG patients. Further analysis indicates that berberine could decrease the abundances of *Veillonella* and alleviate levels of intestinal endotoxin and systemic inflammation. These findings are fundamental for further studies aiming to explore the precise contribution of the POAF.

The underlying mechanism of POAF was still unknown. Inflammation and ROS are still the main reason for the occurrence of POAF.^{2,3,4,15} Recently, several studies have shown that intestinal bacterial microbiota dysbiosis was associated with different types of AF and duration of AF.^{7,8} In this study, we demonstrated the characteristics of gut microbiota in patients underwent CABG are prone to developing POAF. At the genus level, 12 OTUs were upregulated, and 9 OTUs were downregulated. The abundances of *Veillonella* were nearly twice fold higher in compared with no-POAF patients. *Veillonella* is part of the normal flora of the mouth and gastrointestinal tract. It is reported that *Veillonella* is highly associated with marathon running. The mechanism involves crossing of serum lactate from the epithelial barrier into the gut lumen.¹⁶ *Veillonella* were opportunistic pathogens and have been previously reported to be associated

with inflammatory diseases.¹⁷ Therefore, cardiac surgery may impair intestinal epithelial barrier, and high abundance of bacteria, such as *Veillonella*, may have a higher susceptibility to stimulate inflammations.

Berberine is an ancient anti-diarrheal medication. Its poor oral bioavailability has suggested a potential effect on the gut microbiome. It was widely used for diarrhea for low price and seldom side effects. Previous study found that berberine has class III anti-arrhythmic effects without a good mechanism. Here we suggested berberine inhibited the incidence of POAF through gut microbiota. Other study also showed that exposure to berberine alters the populations of intestinal bacteria by mediating the metabolism of bile acids, lipids, and glucose.¹⁸ Expectance of modulating lipid metabolic, berberine is able to regulate the expression of a range of related molecules, including the TLR4/MyD88/NF- κ B signaling pathway in the rat model of diabetes.¹⁹ Moreover, berberine has been shown to reduce inflammation by inhibiting the overexpression of TLR and phosphorylated c-Jun, and increasing the expression of PI3K.²⁰ Berberine has also been shown to inhibit the mRNA expression of inflammation factors, while also reducing low-grade inflammation.¹⁹ In several clinical trials, drugs with anti-inflammatory effects have shown to be effective in lowering AF incidence. Corticosteroids reduce the incidence of new-onset POAF by inhibition of cytokine release (IL-6).^{21, 22} Intestinal epithelial barrier dysfunction caused by cardiac surgery elevated the LPS levels. Recent study indicates that the circulating LPS released by aged-associated microbiota dysbiosis increased AF susceptibility.²³ In LPS induced pericarditis rabbits model, rapid atrial pacing-induced burst firing was increased.²⁴ In our study, oral berberine before surgery significantly changed gut microbiota. 24 OTUs were upregulated, and 27 OTUs were downregulated. The abundance of *Veillonella* was significantly decreased under the treatment of berberine. Interestingly, it was high level in POAF patients. In Consistent with *Veillonella* tendency, LPS was obviously decreased in berberine group and associated with incidence of POAF. What is interesting, under the treatment of berberine, LPS was not increased in POAF patients. That may indicate berberine prevented POAF through other mechanisms. *Veillonella* is a biofilm-forming commensal found in the gastro-intestinal tract of humans, yet it may develop into an opportunistic pathogen.²⁵ It was an important component of the human microbiome and possessed an outer membrane with LPS. A recent RCT suggested that fried meat intake increased *Veillonella* abundances and elevated intestinal endotoxin and systemic inflammation levels.²⁶ In our study, we found that patients with high *Veillonella* abundances were more likely to devolve POAF. Oral berberine before surgery significantly decreased *Veillonella* abundances and suppressed intestinal endotoxin and systemic inflammation levels.

Study limitation

Firstly, our report is a prospective randomized clinical trial study from one center. Further multicenter would allow confirmation of these initial findings with the use of a larger sample size. Secondly, this study was performed in a Chinese population. The cereals and cereal products are the main diet. Furthermore, gut microbiota is critically determined by diet. Therefore, simply diet may also limit our findings.

In conclusion, the present findings provide a comprehensive description of disordered gut microbiota profiles in patients underwent isolated CABG with a high risk of developing POAF. Intervention strategies targeting dysbiotic gut microbiota to prevent POAF may be clinically valuable. Berberine has multifunctional effects through modulating gut microbiota. Oral berberine before CABG inhibited gut microbiota derived LPS and inflammation and suppressed POAF.

Materials And Methods

Study cohort

Firstly, fecal samples were collected from the Department of Cardiovascular Surgery of the General Hospital of Northern Theater Command from January 1, 2020 to September 31 2020. All patients underwent off pump CABG. Fifty Forty-five POAF patients and 90 matched controls (1: 2) were enrolled. POAF was defined as any atrial tachyarrhythmia episode lasting ≥ 30 s after surgery. After completion of the surgery, continuous telemetry monitoring and 7-day Holter monitoring were started.¹ All samples in this study were collected after informed consent was obtained from the participants, and all procedures were conducted according to an established protocol that was approved by the Ethics Committee of the General Hospital of Northern Theater Command.

Trial design

This trial was a single-center, double-blind, placebo-controlled, randomized clinical trial was conducted in accordance with the principles of the Declaration of Helsinki. The protocol was approved by the Ethics Committee of the General Hospital of Northern Theater Command and registered in the Chinese Clinical Trial Registry (registration number: ChiCTR2000028839). All study procedures were conducted after formal written consent. The outcome was to determine whether oral berberine could reduce the incidence of POAF after surgery. The inclusion criteria, exclusion criteria, surgical procedure, and monitoring of AF onset were performed as described previously.¹ Briefly, Adult patients undergoing isolated CABG were eligible for enrollment. Exclusion criteria included: 1) adult patients >80 years of age; 2) a history of arrhythmia, including atrial fibrillation (AF) and ventricular tachycardia; 3) significant valvular diseases 4) emergent surgery; 5) requiring mechanical or pharmacological therapy for hemodynamic support before surgery; 6) ejection fraction <40%; 7) prior cardiac surgery; 8) taking Class I or III antiarrhythmic agents before surgery; 9) concomitant surgery of any kind; 10) congenital heart disease; 11) abnormal liver or kidney function >3 times the upper normal limit; 12) diseases requiring radiotherapy, chemotherapy, or long-term hormone treatment; 13) poorly controlled hyperthyroidism; 14) participating in another clinical trial; and 15) refusal to enrollment.¹ The first patient enrolment is January 20, 2020 and the last is September 25, 2020.

Sample size calculation and randomization

The sample size was estimated referring to our previous study.¹ We determined that enrollment of 184 patients would provide a power 80% to detect a 50% reduction in the POAF incidence by berberine with a

two-sided type I error rate of 0.05. We assumed a 36% incidence of POAF in placebo group based on the historical POAF incidence in the study center. Assuming an 8% dropout rate, each group required 100 patients.

Eligible patients were randomly assigned to either the berberine group or the placebo group by means of a computer-generated randomization system with the group assignment concealed.

Intervention

Patients were randomized to receive berberine (0.4 g three times daily, Northeast Pharmaceutical Group, Shenyang, China) or placebo (generated by Shenyang Feilong pharmaceutical, Shenyang, China) before surgery for 7 days. Following cardiac surgery, patients continuously received medicine until hospital discharge or postoperative 7 days. The detection of POAF has been described previously.¹

DNA isolation, 16S rRNA gene amplification and bioinformatics

Bacterial gDNA was extracted from fecal samples using TIANamp DNA Stool Kit (TIANGEN, Beijing, China) according to the manufacturer's instructions. The V4, V5 regions of the bacteria 16S ribosomal RNA gene were amplified by PCR. After purification, the amplicon library was paired-end sequenced (2 × 250) on an Illumina HiSeq platform (Beijing Capitalbio Technology Co., Ltd) according to the standard protocols.²⁷

Intestinal endotoxin levels and systemic inflammation

Serum levels of lipopolysaccharide (LPS) was measured with ELISA kits (MEIMIAN. Jiangsu Meimian industrial Co., China). All serum inflammatory factors, including Interleukin-6 (IL-6) and C-reactive protein (CRP), were quantified with use of Luminex assay technology (R&D Systems, Minneapolis, MN).

Statistical analysis.

The basic data were statistically analyzed using SPSS software version 24.0 (IBM SPSS Statistics, IBM Corporation, Armonk, New York) and R software version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria). Continuous variables were compared using unpaired Student t tests. Categorical variables were compared using χ^2 tests.² Kaplan-Meier survival curves were used to describe the time-dependent incidence of POAF of both groups, the difference of which was compared by using the log-rank test.¹ $P < 0.05$ was considered significant in all comparisons.

Generation of α and β diversities and analysis and visualization of partial least squares-discriminant analysis (PLS-DA), principal coordinate analysis (PCoA), and principal component analysis (PCA) plots were performed using PAST.²⁸ Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 <http://drive5.com/uparse/>) and chimeric sequences were identified and removed using UCHIME.

Declarations

Acknowledgments and findings

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Figures

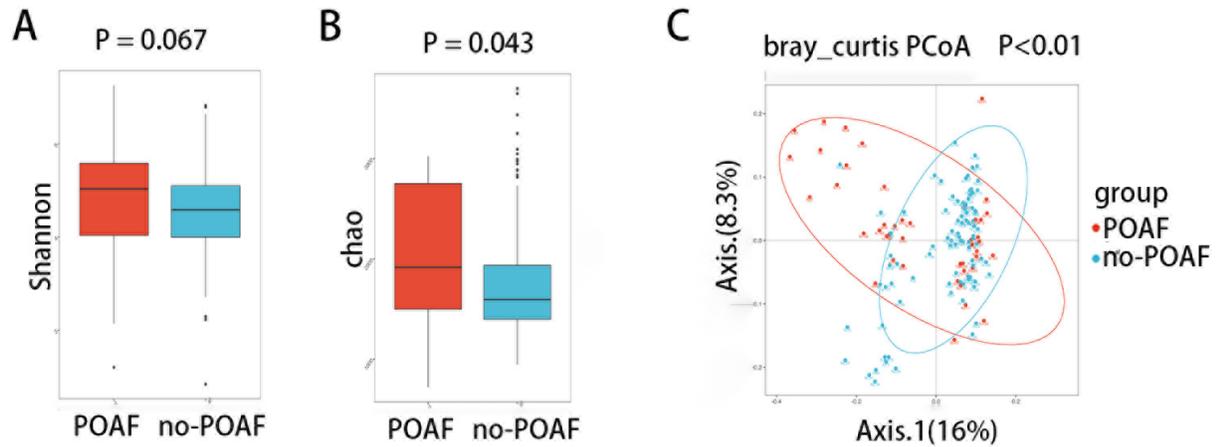


Figure 1

The diversity of the fecal microbiota. (A) The Shannon index in POAF and non-POAF patients. (B) The Chao1 index in POAF and non-POAF patients. (C) The β diversity of POAF and non-POAF patients based on the PCoA analysis. Samples were used in each analysis. Kruskal-Wallis H test was used in the comparison of Shannon index and Chao1 index. In the comparison of PCoA analysis, adonis test was used.

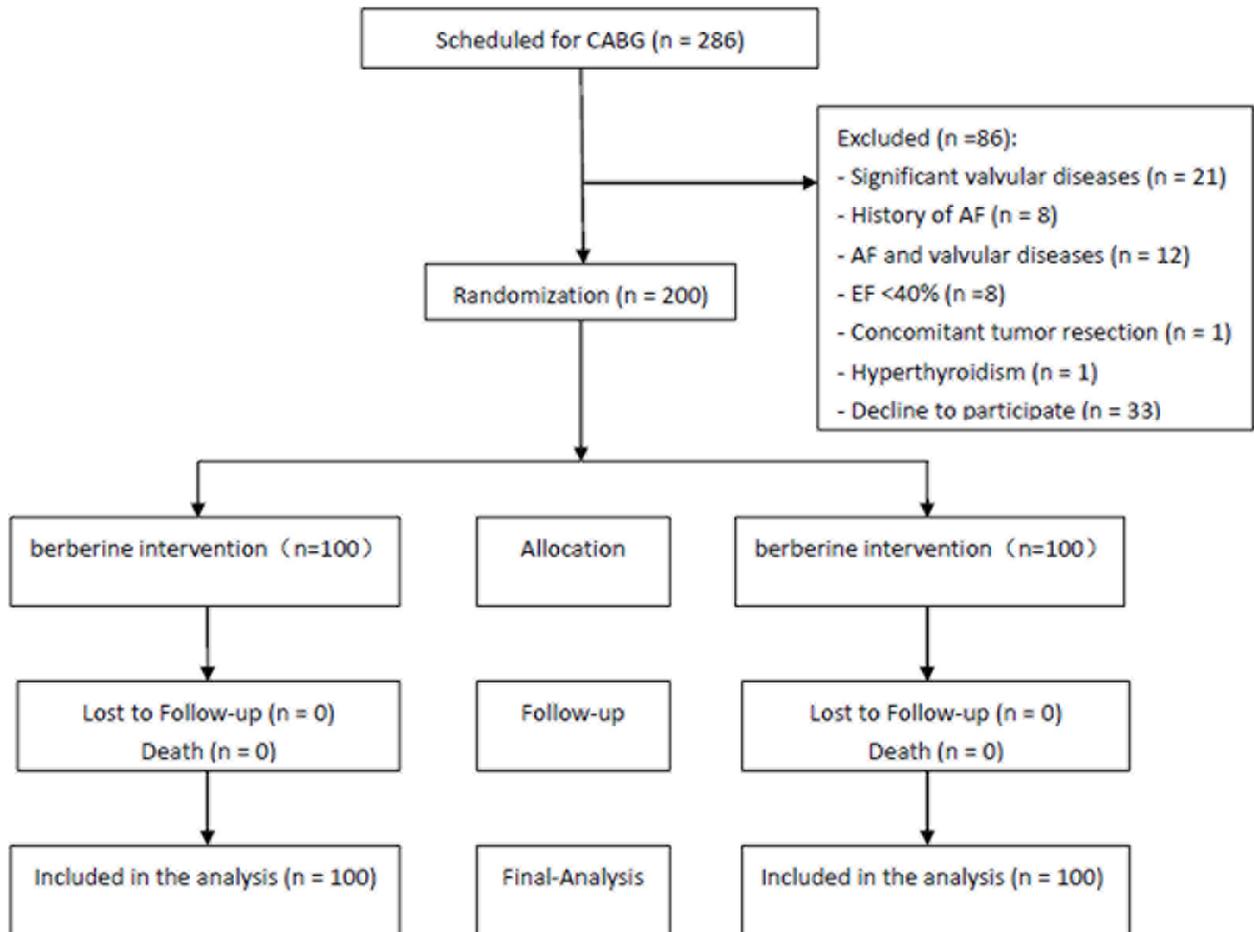


Figure 2

A total of 286 patients were screened. Two hundred patients consented to participate and were randomized 1:1 to receive berberine and placebo.

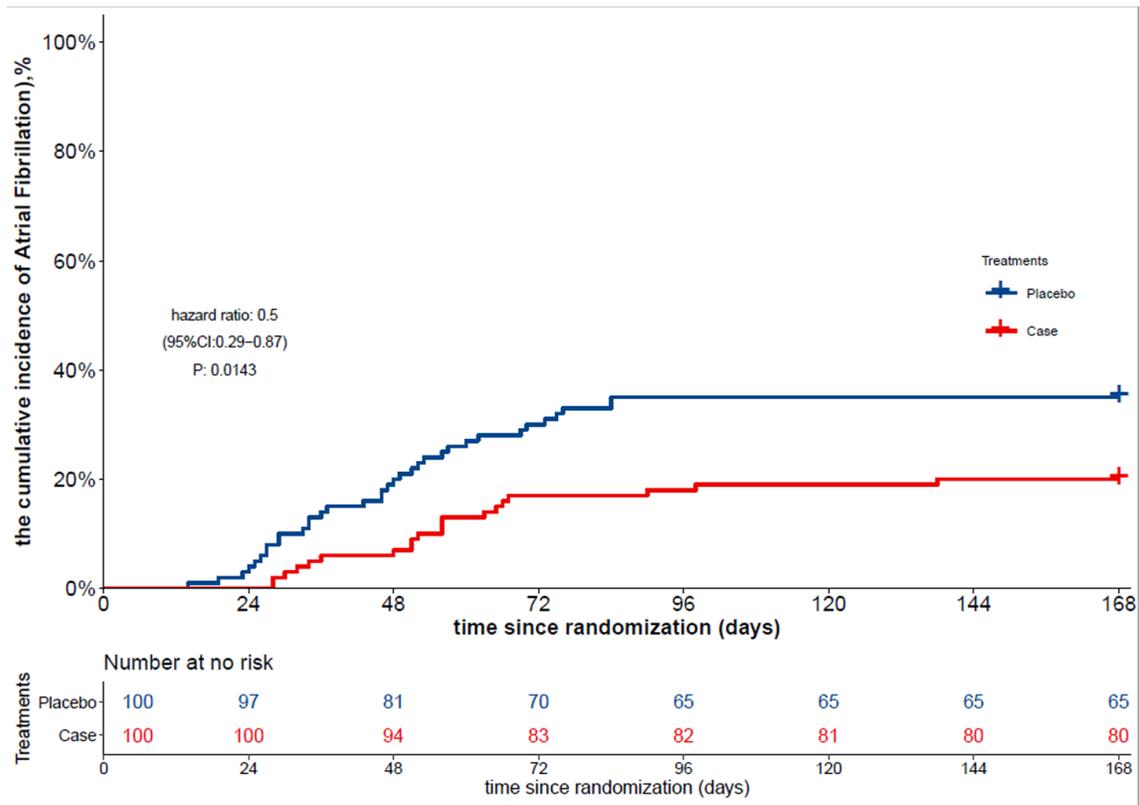


Figure 3

Kaplan-Meier Curve of the Primary Outcome. ***P= 0.00143. N = 100 per group.

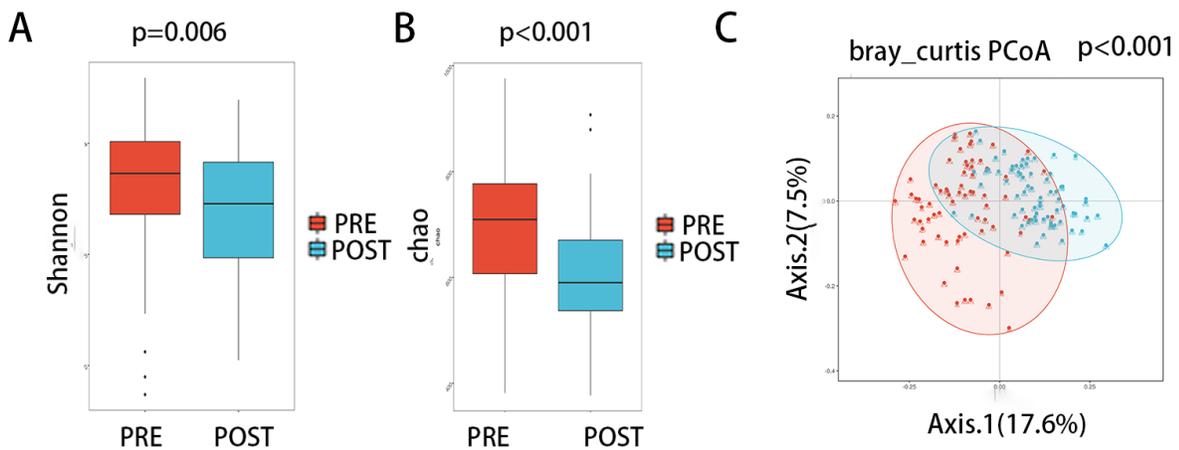


Figure 4

The diversity of the fecal microbiota. (A) The Shannon index before and post berberine. (B) The Chao1 index before and post berberine. (C) The β diversity before and post berberine based on the PCoA analysis. Kruskal-Wallis H test was used in the comparison of Shannon index and Chao1 index. In the comparison of PCoA analysis, adonis test was used.

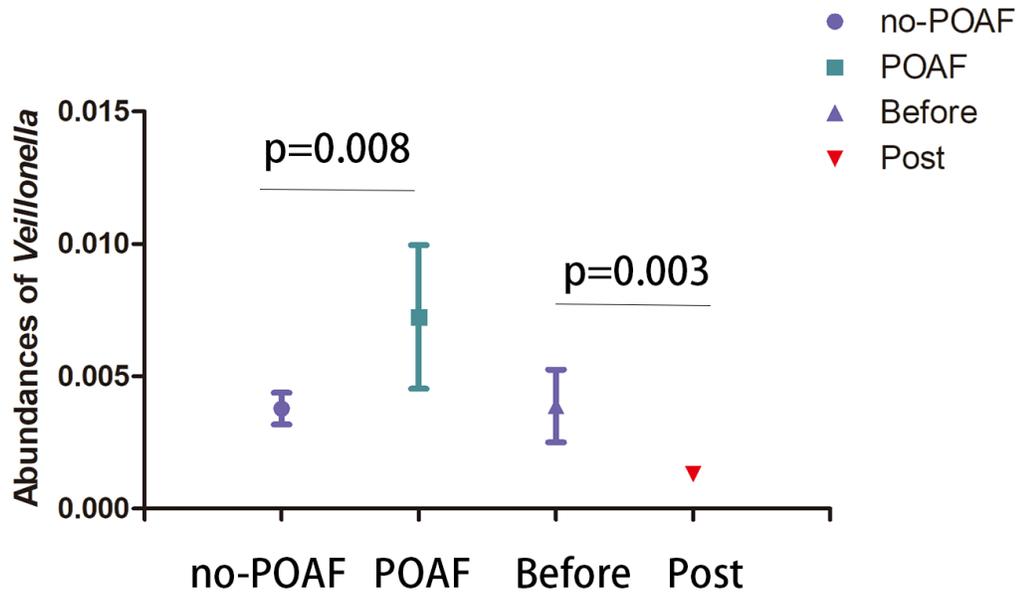


Figure 5

Relative abundance of *Veillonella*. Wilcoxon-Mann-Whitney test. T was used.

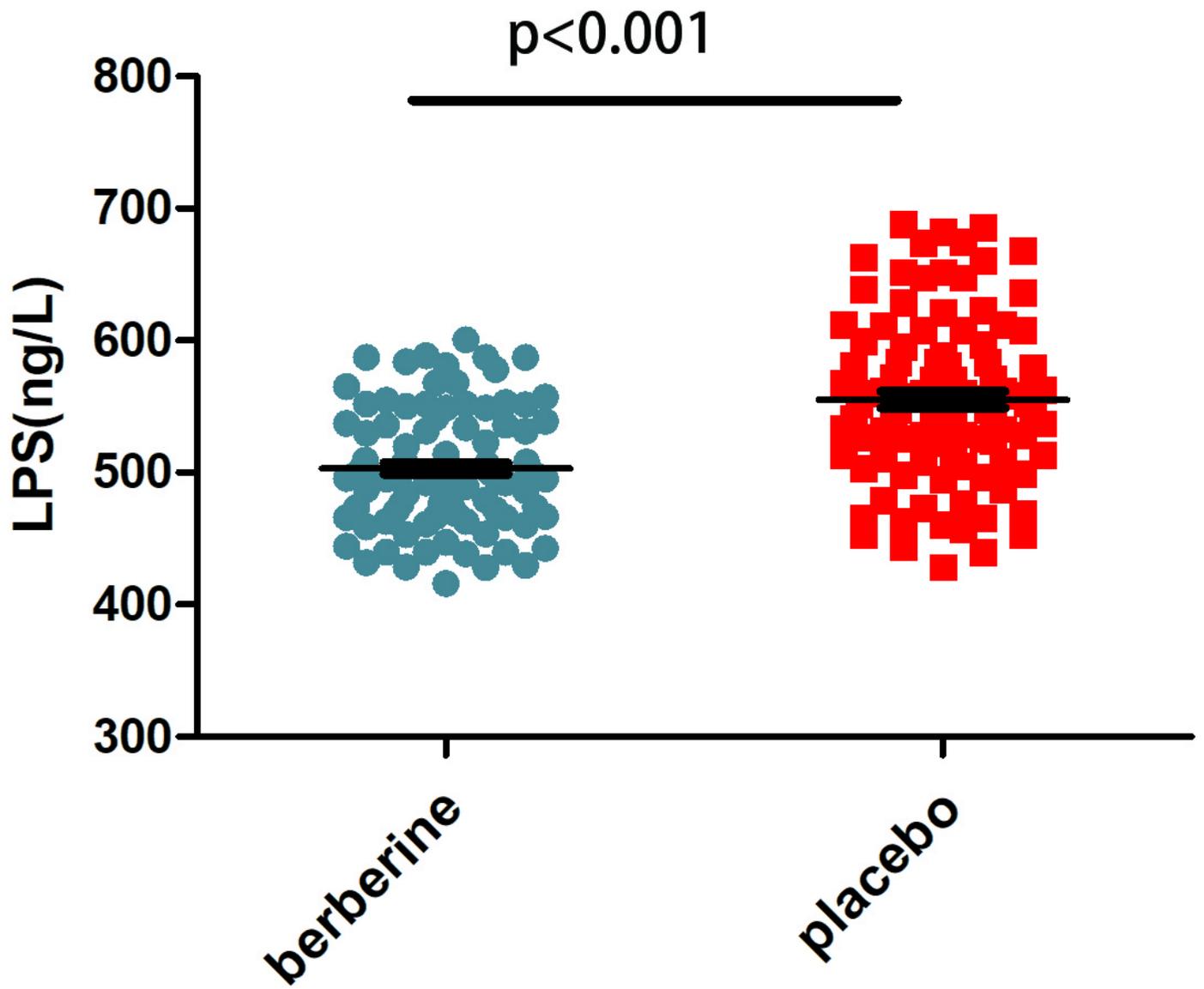


Figure 6

Relative expression of LPS. Unpaired Student's t-test was used.

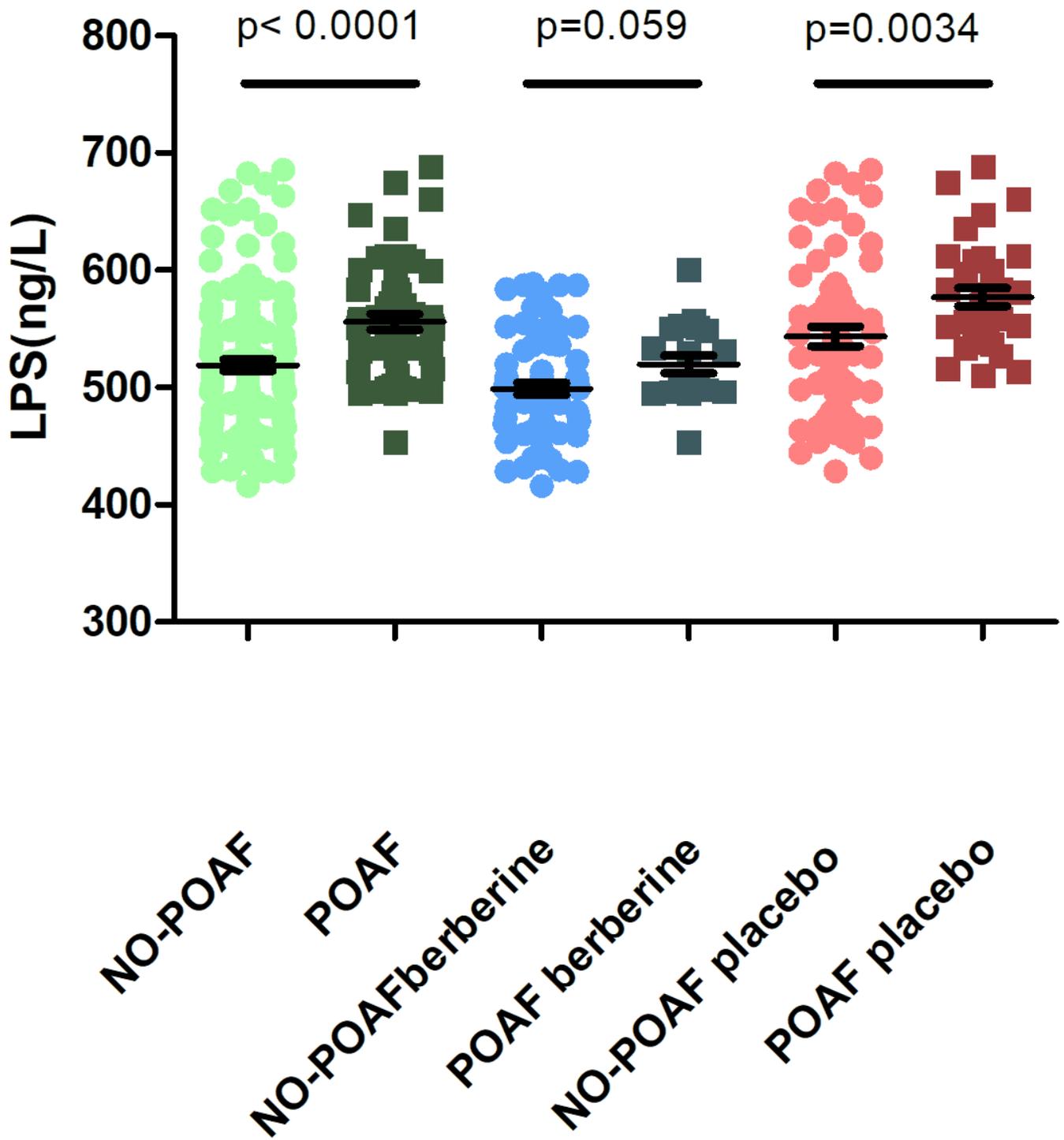


Figure 7

Relative expression of LPS. Unpaired Student's t-test was used.

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