Correlation of Gut Microbiota with Efficacy of Chemotherapy in Patients with Diffuse Large B-cell Lymphoma

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Abstract

The composition and diversity of gut microbiota (GMB) have been reported to be associated with the occurrence and progression as well as treatment outcome in many diseases. Our previous study demonstrated that the GMB was changed significantly and *Proteobacteria* phylum was the dominant microbiota in untreated diffuse large B-cell lymphoma (DLBCL) patients compared with healthy controls. This study aims to investigate the association of GMB with treatment outcomes in patients with DLBCL. 17 Patients with DLBCL and 18 healthy volunteers were recruited at Peking Union Medical College Hospital. The GMB of fecal samples was analyzed using 16S ribosomal RNA gene sequencing. We examined GMB compositions in 3 contexts: DLBCL patients (17) compared with healthy controls (18), DLBCL patients pretreatment (17) compared with posttreatment (17), and the association of GMB with chemotherapy treatment outcomes (10 complete remissions, 7 non-complete remisisons). We found that the GMB was changed considerably in posttreatment DLBCL patients. More specifically, the abundance of Proteobacteria phylum decreased significantly in patients following 4 courses of chemotherapy, although no significant difference from healthy comtrols(CG). In addition, the abundance of *Lactobacillaceae*, *Lactobacillus*, *Lactobacillus fermentum* were significantly higher and became dominant gut microbiota in DLBCL patients with complete remission, ,which may be associated with chemotherapy intervention and tumor extinction. *Lactobacillus fermentum* may have an inhibitory effect on DLBCL contributing to the disease remisison. The composition of gut microbiota in DLBCL patients changed from the healthy condition to the pretreatment condition, and then turned to the posttreatment condition after chemotherapy, which echoed the pathological succession of gut microbiota in DLBCL patients. Results from our current study together with previous research will provide a rational foundation for further investigation on the pathogenesis of gut microbiota in DLBCL to facilitate drug development and foster novel strategy for the better management of patients with DLBCL.

Background

Although the Diffuse large B-cell lymphoma (DLBCL) is the most common type of adult non-Hodgkin's lymphoma(NHL), the pathogenesis of DLBCL is not fully understood and the treatment is limited leading to huge challenges in clinical management of this disease[1]. Therefore, exploring the underlying pathogenesis of DLBCL is of great importance. Microecology especially the composition and diversity of gut microbiota (GMB) have been widely reported to be associated with the occurrence and progression of DLBCL[2]. All physiological changes in the human body may alter the composition of the microbiota, which is called physiological succession, while the changes in the composition of gut microbiota caused by diseases are pathological succession[3].

In the clinical treatment of hematological malignancies, it was found that patients suffered from the increased risk of infection due to their underlying malignant tumor background, frequent hospitalization, long-term antibiotic use, chemotherapy-induced immunosuppression, neutropenia and dysbiosis of the GMB [5]. Therefore, the succession of GMB may not only play a role in the occurrence of hematological

malignancies but also contribute to the treatment outcomes, which have become the main research directions in the correlation between GMB and hematological malignancies [6–10].

In our previous study we demonstrated the composition and diversity of GMB were altered and the *Proteobacteria* phylum was the dominant microbiota in the untreated DLBCL patients[4]. In this current study we are trying to identify whether there is an association between GMB and the treatment outcome of patients with DLBCL, and whether there are new dominant GMB, while explore the GMB which is beneficial to DLBCL treatment to provide more accurate treatment targets for DLBCL patients, and to facilitate drug development and foster novel strategy for the better management of patients with DLBCL.

Materials And Methods

The DLBCL patients(17) and matched healthy controls(18) were recruited in the Hematology Department of Peking Union Medical College Hospital from November 2019 to November 2020 and diagnosed as diffuse large B-cell lymphoma[11]. All 17 patients were Chinese living in mainland China, without chronic inflammatory diseases in the gastrointestinal tract and other tumors, and they did not receive treatment for DLBCL or antibiotic treatment within 4 weeks. The exclusion criteria included: history of indolent lymphoma, active viral infection at diagnosis, and history of autoimmune disease.

The 17 DLBCL patients were treated with chemotherapy regimen which was based on the disease risk stratification, IPI score, drug dose by their body surface area and the general condition of the patients (Eastern Cooperative Oncology Group, ECOG). The regimen and dose are optimized for each patient. Two regimens were administrated, R-CHOP[12](rituximab 375mg/m^2 iv d0, cyclophosphamide 750mg/m^2 iv d1, epirubicin 75mg/m^2 iv d1, vindesine 4mg iv d1, prednisone100mg po d1-5),R-DA-EPOCH[13]：rituximab 375mg/m^2 d0, dose adjustment VP-16 50mg/m^2/d civ d1-4, cyclophosphamide 750mg iv d5, epirubicin 15mg/m^2/d civ d1-4, vindesine 0.8mg/m^2/d civ d1-4, prednisone 100mg bid po d1-5. Positron emission tomography/computed tomography (PET/CT) was used to evaluate the efficacy to the Deauville five-point method after four-courses of chemotherapy[14]. Patients with score under 3 points is defined as complete remission (CR), while above 4 points is non-complete remission (NCR). In the course of 4 courses of chemotherapy, 13 cases had treated with antibiotics due to agranulocytosis with fever.

The microbiota composition of fecal samples from 17 DLBCL patients and 18 healthy volunteers matched for the place of birth, age, and sex were analyzed by16S ribosomal RNA gene sequencing. The inclusion criteria included: no history of tumor and chronic gastrointestinal inflammatory diseases, no use of antibiotics within 4 weeks, and no history of diarrhea within 2 weeks. 1 fecal sample from healthy control and 2 fecal samples from DLBCL patients before and after 4 courses chemotherapy were collected. The time point of fecal collection after chemotherapy was set when the routine test returned to normal without gastrointestinal and other clinical symptoms.

The above 35 participants gave signed informed consent for sample collection. When the samples were collected, each participant obtained a sterile plastic bag and a fecal collection tube with microbial culture

medium, and the fresh feces were collected aseptically in the fecal collection tube. Immediately after sampling, the specimens were stored in a − 20℃ freezer. Within 24 h, they were sent to the Hematology Laboratory of Beijing Union Hospital for storage at − 80℃ until DNA was extracted.

This study population was divided into the pretreatment group (PRG) of DLBCL patients and control group (CG) of healthy volunteers, pretreatment group(PRG)and posttreatment group (POG) of DLBCL patients. The treated patients were further classified into the complete remission group (CRG) and non-complete remission group (NCRG) through the efficacy of chemotherapy. According to CRG and NCRG, PRG was divided complete remission before treated group (CR_PRE) and non-complete remission after treated group (NCR_PRE).

Fecal DNA was extracted using PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) following the revommended protocol. Purity and quantity of the genomic DNA were checked on 1% agarose gels and a NanoDrop spectrophotometer (Thermo Scientific), respectively. The V3-4 hypervariable regions of bacterial 16S rRNA gene were amplified with the primers 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT)[15]. The PCR products were purified using an Agencourt AMPure XP Kit. Deep sequencing was performed on Miseq platform at Allwegene Company (Beijing). After the run, image analysis, base calling and error estimation were performed using Illumina Analysis Pipeline Version 2.6. The original sequence was uploaded to SRA database of NCBI. The raw data were first screened and sequences were removed from consideration if they were shorter than 230 bp, had a low quality score (≤ 20), contained ambiguous bases or did not exactly match to primer sequences and barcode tags. Qualified reads were separated using the sample-specific barcode sequences and trimmed with Illumina Analysis Pipeline Version 2.6. And then the dataset were analyzed using QIIME. The sequences were clustered into operational taxonomic units (OTUs) at a similarity level of 97%[16] to generate rarefaction curves and to calculate the richness and diversity indices. The Ribosomal Database Project (RDP) Classifier tool was used to classify all sequences into different taxonomic groups[17]. QIIME1(v1.8.0) software was used to analyze α-diversity index (including Shannon, Simpson and Chao1 indexes). Partial least squares discrimination analysis (PLS-DA) was used to analyze β-diversity using R (v3.6.0). The differences between groups of metastats were analyzed by using the software of mothur (v.1.34.4[18], and LEfSe was analyzed by phython (V2.7) to analyze diversity between DLBCL patients and control group. The PICRUSt2 software was used to predict the function of gut microbiota of 16S sequence results, and the functional changes among groups were analyzed based on the prediction results[19].

Results

The study included 17 untreated DLBCL patients (PRG), with 8 (47.1%) men and 9 (52.9%) women. The median age was 55 years (range, 22–70 years). These 17 DLBCL patients were grouped into GCB (9 cases, 52.9%) or ABC (8 cases, 47.1%) types according to pathological subtypes. Six (35.3%) patients were GI and 11 (64.7%) were NGI. At the time of diagnosis, the prognosis of these 17 patients was assessed according to the IPI score (0–5) (Table 1). Sixteen (94.2%) cases were treated with R-CHOP

regimen and 1 case (5.8%) was treated with R-EPOCH regimen. In the course of 4 courses of chemotherapy, 13 cases (76.4%) had treated with antibiotics due to agranulocytosis with fever. After 4 courses of chemotherapy, according to the Deauville five - point method of PET/CT detection, 10 cases (58.5%) were CR and 7 cases (41.2%) were NCR; The median age of the CG was 53 years old (range, 24–74 years) with 10 (55.6%) men and 8 (44.4%) women. There was no significant difference in age and gender between the PRG and the CG (P = 0.50 and 0.81, respectively). Baseline characteristics of the cohort are listed in Table 1.

Table 1
Baseline characteristics of patients with DLBCL and healthy controls

	All patient(N=17)	Control group(N=18)
Median age,Y	55	53
Gender, n(%)		
Male	8(47.1)	10(55.6)
Female	9(52.1)	8(44.4)
Race/region,n		
East Asian/china	17	18
Pathological subtype, n(%)		
GCB	9(52.1)	
ABC	8(47.1)	
Organ involved,n(%)		
Gastrointestinal involvement	6(35.3)	
Non gastrointestinal involvement	11(64.7)	
IPI score,n(%)		
0	2(11.8)	
1	5(29.4)	
2	4(23.5)	
3	4(23.5)	
4	1(5.9)	
5	1(5.9)	
Treatment		
R-CHOP	16(94.2)	
R-EPOCH	1(5.8)	
Efficacy evaluation		
CR	10(58.5)	
NCR	7(41.2)	
Antibiotics due to agranulocytosis with fever after chemotherapy.		

	All patient(N=17)	Control group(N=18)
Received	13(76.4)	
Not received	4(23.6)	

1. GMB analysis in pretreatment DLBCL patients (PRG) and healthy controls (CG)

A total of 35 fecal specimens from the PRG and CG were analyzed by 16S rRNA gene sequencing. In total 3416551 reads were obtained and 60914 reads were achieved per sample. In 35 sequenced fecal samples, 852 OTUs were identified. Shannon-Wiener curves indicated that the amount of sequencing data is sufficient to reflect most microbial information in the sample. α-Diversity analysis indicates that there was no significant difference in species diversity and abundance between PRG and CG (chao1 index P = 0.24, Shannon index P = 0.34, observed_species index P = 0.23, PD_whole_tree index P = 0.43). However, β-diversity analysis showed significant difference between the two groups. The GMB showed a continuous evolutionary relationship at 6 levels: the abundance of *Proteobacteria* (phylum) (P = 0.019), *Gammaproteobacteria* (class) (P = 0.0078), *Enterobacteriales* (order) (P = 0.0052), *Enterobacteriaceae* (family) (P = 0.005), *Escherichia-Shigella* (genus) (P = 0.008), and *Escherichia coli* (species) (P = 0.008) was significantly higher in PRG that of CG. The abundance of *Lactobacillaceae* (family) (P = 0.79), *Lactobacillus* (genus) (P = 0.67), and *Lactobacillus fermentum* (species) (P = 0.24) was not significantly different between the two groups (Fig. 4B).

PICRUSt was used to predict the microbial function based on the 16S rRNA gene sequencing data, which showed that Thiamine metabolism, Phenylalanine, tyrosine and tryptophan biosynthesis were significantly lower in PRG than CG (P = 0.00085, P = 0.00037, respectively), while the function in Pentose phosphate pathway showed no significant difference between the two groups (P = 0.53).

2. GMB analysis in pretreatment (PRG) and posttreatment (POG) DLBCL patients

A total of 34 fecal specimens from the PRG and POG were analyzed by 16S rRNA gene sequencing. In total 3800257 optimized reads were obtained and 60830 reads were achieved per sample. From 34 sequenced fecal samples, 622 OTUs were identified and classified into 12 phyla, 25 classes, 32 orders, 55 families, 199 genera, and 41 species. There were 780 and 733 OTUs in the pretreatment (PRG) and posttreatment (POG), respectively and with 622 identical in both groups. Shannon-Wiener curves indicated that the amount of sequencing data is sufficient to reflect most microbial information in the sample. α-Diversity analysis indicates that there was no significant difference in species diversity and abundance between PRG and POG (chao1 index P = 0.70, Shannon index P = 0.76, observed_species index P = 0.68, PD_whole_tree index P = 0.44) (Fig. 1A). β-diversity analysis revealed significant difference

between the two groups (Fig. 1B). The abundance of the *Fusobacteria* (phylum) (P = 0.045), *Proteobacteria* (phylum) (P = 0.04); *Gammaproteobacteria* (class) (P = 0.019), *Fusobacteriia* (class) (P = 0.046); *Enterobacteriales* (order) (P = 0.0044), *Fusobacteriales* (order) (P = 0.045); *Enterobacteriaceae* (family) (P = 0.0044), *Fusobacteriaceae* (family) (P = 0.046); *Escherichia-Shigella* (genus) (P = 0.0015), *Fusobacterium* (genus) (P = 0.045); *Eubacterium infirmum* (species) (P = 0.0047), *Escherichia coli* (species) (P = 0.015) was significantly higher in PRG than that of POG (Fig. 2A) .

PICRUSt was used to predict the microbial function based on the 16S rRNA gene sequencing data, which showed that Pentose phosphate pathway function in POG was significantly higher than that of UEG (P = 0.0032).

3. GMB analysis in posttreatment(POG) DLBCL patients and healthy controls (CG)

A total of 35 fecal specimens from the POG and CG were analyzed by 16S rRNA gene sequencing. 3540774 optimized reads were obtained and 64438 reads were achieved per sample. In 35 sequenced fecal samples, 519 OTUs were identified and classified into 13 phyla, 23 classes, 32 orders, 54 families, 179 genera, and 36 species. There were 701 and 642 OTUs in the POG and CG, respectively with 519 identical in both groups. Shannon-Wiener curves indicated that the amount of sequencing data is sufficient to reflect most microbial information in the sample. α-Diversity analysis indicated that there was significant difference in species diversity and abundance between POG and CG (chao1 index P = 0.08, Shannon index P = 0.20, observed_species index P = 0.038, PD_whole_tree index P = 0.039) (Fig. 1C). However, β-diversity analysis revealed significant difference between the two groups (Fig. 1D). The abundance of Saccharibacteria (phylum) (P = 0.007) and Fusobacteria (phylum) (P = 0.045) in CG was significantly higher than that of POG; The abundance of *Deltaproteobacteria* (class) (P = 0.02), *Desulfovibrionales* (order) (P = 0.02), *Desulfovibrionaceae* (family) (P = 0.02) and *Fusobacteriia* (class) (P = 0.045), *Fusobacteriales* (order) (P = 0.045), *Fusobacteriaceae* (family) (P = 0.045) in CG was significantly higher than that of POG; The abundance of *Enterococcus* (genus) (P = 0.047), *Lactobacillus fermentum* (species) (P = 0.02) in POG was significantly higher than that of CG (Fig. 4B). There was no significant difference in *Proteobacteria* (phylum) (P = 0.94) between the two groups (Fig. 4A).

PICRUSt was used to predict the microbial function based on the 16S rRNA gene sequencing data, which showed that Thiamine metabolism function decreased significantly in CG (P = 0.00031) while the function of Pentose phosphate pathway was significantly higher in POG than that of CG (P = 0.0095).

4. GMB analysis in complete remission(CRG) and non-complete remisison (NCRG) DLBCL patients following chemotherapy

A total of 17 fecal specimens from the CRG and NCRG were analyzed by 16S rRNA gene sequencing. 1962239 optimized reads were obtained and 70164 reads were achieved per sample. In 17 sequenced stool samples, 649 OTUs were identified and classified into 12 phyla, 23 classes, 28 orders, 49 families,

166 genera, and 28 species. There were 469 and 537 OTUs in the CRG and NCRG, respectively with 357 identical in both groups. Shannon-Wiener curves indicated that the amount of sequencing data is sufficient to reflect most microbial information in the sample. α-Diversity analysis indicated that there was significant difference in species diversity and abundance between CRG and NCRG (chao1 index P = 0.16, Shannon index P = 0.25, observed_species index P = 0.19, PD_whole_tree index P = 0.17). β-diversity analysis showed significant difference between the two groups, which were *Lactobacillaceae* (family) (P = 0.018) (Fig. 3A), *Rikenellaceae* (family) (P = 0.0066), *Alistipes* (genus) (P = 0.006), *Lactobacillus* (genus) (P = 0.013), *Veillonella* (genus) (P = 0.043); *Lactobacillus fermentum* (species) (P = 0.024) (Fig. 3B). More specifically, the abundance of *Lactobacillaceae*, *Lactobacillus*, *Lactobacillus fermentum* and *Veillonella* in CRG was significantly higher than that of the NCRG (Fig. 2B).

PICRUSt was used to predict the microbial function based on the 16S rRNA gene sequencing data, which showed that glycine, serine and threonine metabolism functions from NCRG was significantly higher than that of CRG (p = 0.0065).

5. GMB analysis in the pretreatment fecal samples from complete remission (CR_PRE) and non-complete remission(NCR_PRE)

A total of 17 fecal specimens from the CRG_PRE and NCRG_PRE were analyzed by 16S rRNA gene sequencing. 1838020 optimized reads were obtained and 60884 reads were achieved per sample. In 17 sequenced stool samples, 670 OTUs were identified and classified into 11 phyla, 21 classes, 29 orders, 52 families, 167 genera, and 32 species. There were 519 and 579 OTUs in the CRG_PRE and NCRG_PRE, respectively with 428 identical in both groups. Shannon-Wiener curves indicated that the amount of sequencing data is sufficient to reflect most microbial information in the sample. α-Diversity analysis indicates that there was significant difference in species diversity and abundance between CRG_PRE and NCRG_PRE (chao1 index P = 0.10, Shannon index P = 0.11, observed_species index P = 0.19, PD_whole_tree index P = 0.20). β-diversity analysis showed that there was no significant difference in abundance between CR_PRE and NCR_PRE in terms of *Lactobacillaceae* (family) (P = 0.2), *Lactobacillus* (genus) (P = 0.6) and *Lactobacillus fermentum* (species) (P = 0.12) (Fig. 4B).

PICRUSt was used to predict the microbial function based on the 16S rRNA gene sequencing data, which showed that there was no significant difference in glycine, serine and threonine metabolism functions between the two groups (P = 0.84).

Discussion

The abundance and diversity of GMB has been reported to be associated with not only the occurrence but also the prognosis, especially the treatment outcome of many diseases. Our previous study indicated the GMB was significantly changed in patients with DLBCL, which suggested the GMB might play a role in the pathogenesis of DLBCL. However, whether the GMB is associated with the treatment outcome

remains to be defined. In this current study, we aimed to investigate the association of GMB with treatment outcome in patients with DLBCL.

We first analyzed the GMB in pretreatment DLBCL patients (PRG) compared with healthy controls (CG), which repeated our previous research with different cohort of patients[4]. In consistent with our previous research, β-diversity analysis showed that there were significant differences in the GMB between UEG and CG. At 6 levels, a continuous evolutionary relationship we observed. More specifically, the abundance of *Proteobacteria*, *Gammaproteobacteria*, *Enterobacteriales*, *Enterobacteriaceae*, *Escherichia-Shigella*, *Escherichia coli* was significantly higher in PRG than in CG, which was consistent with our previous research results to further validate that Proteobacteria phylum is the dominant gut microbiota in untreated DLBCL patients[4]

The prognosis of the 17 DLBCL patients after 4 courses chemotherapy intervention were assessed by PET/CT efficacy evaluation, which classified these patients into the CRG (n = 10) and NCRG (n = 7). β-diversity analysis show that the abundance of *Proteobacteria*, *Gammaproteobacteria*, *Enterobacteriales*, *Enterobacteriaceae*, *Escherichia-Shigella*, *Escherichia coli* was lower in posttreatment (POG) than that in pretreatment (PRG) DLBCL patients and *Proteobacteria* phylum is no longer the dominant gut microbiota in posttreatment DLBCL patients.These results indicated the GMB has been changed considerably following chemotherapy.

β-diversity analysis found that the abundance of *Lactobacillaceae* (family), *Lactobacillus* (genus), *Lactobacillus fermentum* (species) were significantly higher in CRG than NCRG, while there were no significant differences between CR_PRE and NCR_PRE. These results revealed that the abundance of *Lactobacillaceae*, *Lactobacillus*, *Lactobacillus fermentum* in CRG_PRE and NCRG_PRE were at the same baseline before the 4-courses chemotherapy intervention started, and there existed significant differences in CRG and NCRG after chemotherapy in DLBCL patients. The higher abundance of *Lactobacillaceae*, *Lactobacillus*, *Lactobacillus fermentum* in the posttreatment DLBCL patients suggested that these gut microbiota might be associated with the extinction of lymphomas and as dominant gut microbiota in CRG DLBCL patients, *Lactobacillus fermentum* was likely a succession of gut microbiota caused by chemotherapy intervention and tumor extinction.

DLBCL patients were treated with 4-courses chemotherapy interventions, such as anti-tumor chemotherapeutic drugs and other therapeutic drugs, which may have a direct impact on gut microbiota. *Fusobacteria* (phylum) was reported to be sensitive to antibiotics[20]. β-diversity analysis found the abundance of *Fusobacteria* (phylum), found *Fusobacteriia* (class), *Fusobacteriales* (order), *Fusobacteriaceae* (family), *Fusobacterium* (genus) were significantly lower in POG than in PRG, and were significantly lower than in CG; there were significant differences in α-diversity analysis between POG and CG, which was significantly lower in POG than in CG. Therefore, we may conclude that these changes of gut microbiota could be caused by the direct effect of chemotherapeutic intervention and were not associated with extinction in lymphomas in DLBCL patients. However, the abundance of *Proteobacteria* in POG decreased significantly and no longer the dominant gut microbiota, which showed no significant

difference between POG and CG at the same baseline, suggesting that the changes of *Proteobacteria* phylum were related to the extinction of lymphomas in DLBCL patients. The abundance of *Lactobacillaceae*, *Lactobacillus*, *Lactobacillus fermentum* CRG_PRE and NCRG_PRE at the same baseline before chemotherapy and were significantly higher in CRG than NCRG and CG after the the same chemotherapy intervention, indicating that they were obviously dominant GMB in CRG. Therefore, the abundance and diversity of *Proteobacteria* phylum and *Lactobacillaceae*, *Lactobacillus*, *Lactobacillus fermentum* could be completely excluded from the direct effects of drugs, but may directly related to the outcome of chemotherapy in DLBCL patients.

Studies have shown that *Escherichia coli* can produce colibactn and cytolethal distending toxin that can cause double-strand breaks in intestinal epithelial cells, leading to gene mutations that eventually lead to tumor formation[21]. Therefore, *Proteobacteria* phylum are likely to play an important role in the occurrence of DLBCL, which is the focus of the in-depth study of the microenvironment and pathogenesis of DLBCL. Therefore, regulating the abundance of gut *Proteobacteria* phyla may be a novel strategy for the prevention of DLBCL.

Our results from this study showed that the composition of gut microbiota in DLBCL patients had a succession after 4 courses chemotherapy intervention and *Proteobacteria* phylum was no longer the dominant gut micorbiota which was replaced by *Lactobacillus fermentum*. The change and succession of these gut microbiota composition can be excluded from the direct effect of therapeutic drugs on microbiota. *Lactobacillus fermentum* became the dominant microbiota in complete remission patient, which was related to the changes in lymphoid tumor and resulted from the gut microbiota succession caused by chemotherapy intervention and tumor extinction. Recent studies have indicated that signifificant microbiome alterations represented an increase in *Lactobacillus* during nCRT (neoadjuvant chemoradiotherapy)[22]. Therefore, the product of *Lactobacillus fermentum* may directly involve in anti-tumor,synergistically with chemoradiotherapy, and enhance the therapeutic effect of chemotherapeutic drugs.

Recent studies have found that some microbial bacteria have shown potential anti-tumor activity, such as Lactobacilli in vitro experiments. *Lactobacillus rhamnosus GG* can not only suppress the growth of tumor cells but also has anti-metastasis function[23–26]. Bladder cancer patients have been shown to have reduced recurrence of superficial bladder cancer by oral Lactobacillus casei[27] and the underlying mechanism might be related to the direct effect of anti-tumor immune responses induced by the activated host NK cells and macrophages [28]. *Lactobacillus casei* inhibited colon cancer in two ways: direct stimulation of immune cells and secreting metabolites with anti-tumor effect. *Lactobacillus fermentum* has the same effect, but its inhibition of colon cancer cells was less intense than *Lactobacillus casei*[29]。

Studies have shown that the anti-tumor effect of *Lactobacilli* is achieved by mTOR (molecular target of rapamycin) and Wnt/β-cantenin signaling pathway, which can significantly up-regulate secretory crimp-related protein 2 (SFRP2) and reduce CCND1、mTOR、S6K1、EIF4E expression[30]. Wnt/β-catenin signaling

pathway consists of Wnt proteins, transmembrane receptors, cytoplasmic proteins, nuclear transcription factors, and downstream target genes[31]. The phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of mTOR signaling pathway is an important pathway leading to cell growth and tumor proliferation[32]. By regulating the expression of relevant signal transduction genes in mTOR and Wnt/β-cantenin signaling pathways in tumor cells, *Lactobacilli* plays an important role in tumor cell growth. And studies have shown that, activation of mTOR complexes can lead to the phosphorylation of various signaling molecules, and thus over-expression of cyclinD1、Bcl2 proteins leads to tumor formation. Therefore, the results from our study showed that the abundance of *Lactobacillus fermentum* was significantly higher in CRG than that of the NCRG, suggesting that *Lactobacillus fermentum* may inhibit DLBCL [29]。

PICRUSt was used to predict the microbial function based on the 16S rRNA gene sequencing data, which showed that the Thiamine metabolism function of gut microbiota in PRG and POG was significantly lower than that of CG. High dose Thiamine can reduce pyruvate dehydrogenase kinase activity and play anti-tumor role in xenograft mice[33]. Therefore, it is speculated that the gut microbiota causes the DLBCL micro-environment to remain in the state of low thiamine, which leads to the over-expression of pyruvate dehydrogenase kinase, increasing the glycolytic process of cancer cells leading to tumor growth. Pentose phosphate pathway function was significantly higher in PRG than POG, but there was no significant difference in PRG and CG. Studies have shown that over-expression of enzymes with different oxidation and non-oxidation branches in the pentose phosphate pathway increases cisplatin resistance, and inhibition of pentose phosphate pathway restores cisplatin sensitivity[34]. Therefore, gut microbiota may increase the function of pentose pathway to promote the resistance of tumor cells to chemotherapeutic drugs. Glycine,serine and threonine metabolism function was significantly higher in NCRG than in CRG, and the two groups were at the same baseline before chemotherapy intervention. Studies have shown that glycine and serine are involved in mitochondrial carbon synthesis during cancer development, and serine can also act as a bridge between mTOR signaling pathways and DNA methylation[35]. In melanoma cells with low serine, p53 protein-mediated modulation of glycerate phosphate dehydrogenase (PHGDH) enhances apoptosis[36].

Conclusions

Our study indicated that the composition of gut microbiota in DLBCL patients was altered significantly after 4-courses chemotherapy. *Proteobacteria* phylum is no longer the dominant gut microbiota in DLBCL patients following chemotherapy, instead *Lactobacillaceae*, *Lactobacillus*, *Lactobacillus fermentum* became the dominant GMB, which were a succession of gut microbiota associated with extinction of lymphomas. The abundance of *Fusobacteria*, *Fusobacteriia*, *Fusobacteriales*, *Fusobacteriaceae*, *Fusobacterium* was decreased in POG, which could be the direct effect of chemotherapeutic drugs, and could not be associated with the extinction of lymphomas. *Proteobacteria* phylum, *Lactobacillaceae*, *Lactobacillus*, *Lactobacillus fermentum* were the dominant gut microbiota in different states of DLBCL patents and their changes and succession can be completely excluded as a direct result of therapeutic drugs. *Lactobacillus fermentum* is the dominant gut micorbiota in CR DLBCL patients, which is a

highlight of this study, indicating that *Lactobacillus fermentum* could be a valuable gut microbiota to be further explored in their inhibitory effect on DLBCL, and the underlying mechanisms, in order to establish a novel strategy for the better management of DLBCL patients.

Abbreviations

GMB：gut microbiota

DLBCL：diffuse large B-cell lymphoma

NHL：non-Hodgkin's lymphoma

ECOG：Eastern Cooperative Oncology Group

PET/CT：Positron emission tomography/computed tomography

CR：complete remission

NCR：non-complete remission

CRG：complete remission group

NCRG：non-complete remission group

PRG：pretreatment group

POG：posttreatment group

CR_PRE：complete remission before treated group

NCR_PRE：non-complete remission after treated group

CG：control group, healthy comtrols

PLS-DA：Partial least squares discrimination analysis

Declarations

Ethics approval and consent to participate

Ethics committee of Peking Union Hospital

Consent for publication

all the authors agree to publish this manuscript.

Availability of data and material

Data and material are available from corresponding author upon reasonable request.

Competing interests

All the authors declare that they have no competing interests.

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Author contributions

Daobin Zhou and Wei ZHANG obtained funding for the study. Daobin ZHOU, Jingnan Li and Li Yuan designed this study. Li Yuan, Wei Wang, and Yan Zhang participated in literature searches and study selection. Li Yuan，Danqing ZHAO and Chong Wei extracted data, evaluated study quality and assessed bias risk of eligible trials. Li Yuan，Danqing ZHAO and Chong Wei carried out all statistical analyses. Li Yuan and Daobin Zhou interpreted the data and Li Yuan drafted the report. Daobin Zhou and Wei Zhang revised the manuscript critically. All the authors contributed to resolve divergence and approved the final submitted version. The corresponding author has full access to all data in the study and takes final responsibility for the decision to submit for publication.

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Figures

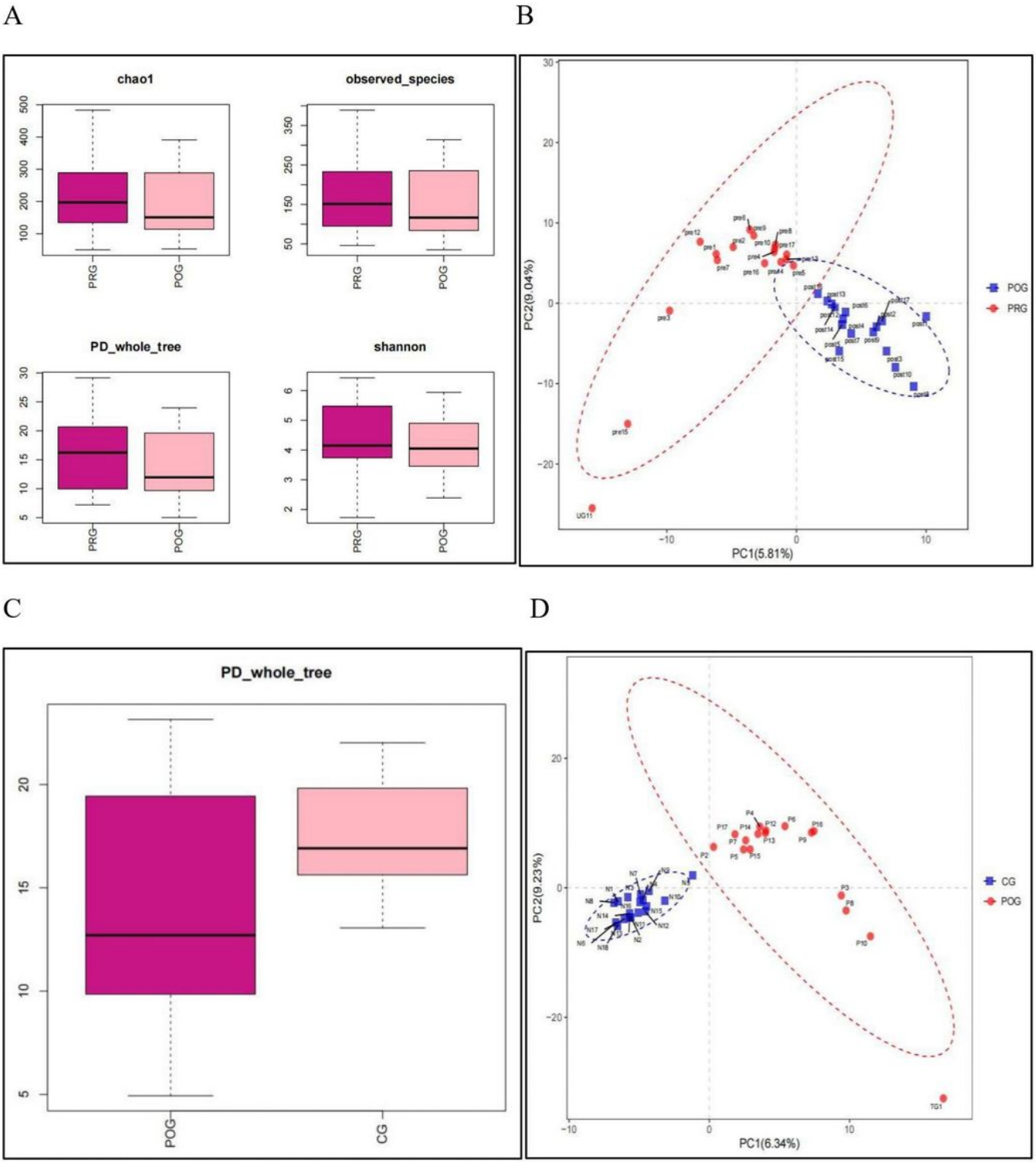

Figure 1

α-diversity and β-diversity analysis between PRG and POG, POG and CG (A) There was no significant difference in α-diversity between PRG and POG. (B) There are significant differences in β-diversity between PRG and POG, with blue being PRG, red being POG. (C) There are obvious differences in α-diversity between POG and CG, PD_whole_tree the diversity index of lineage diversity, species abundance

and evolutionary distance. The larger the value, the higher the community diversity. (B) There are significant differences in β- diversity between POG and CG, with red being POG, blue being CG.

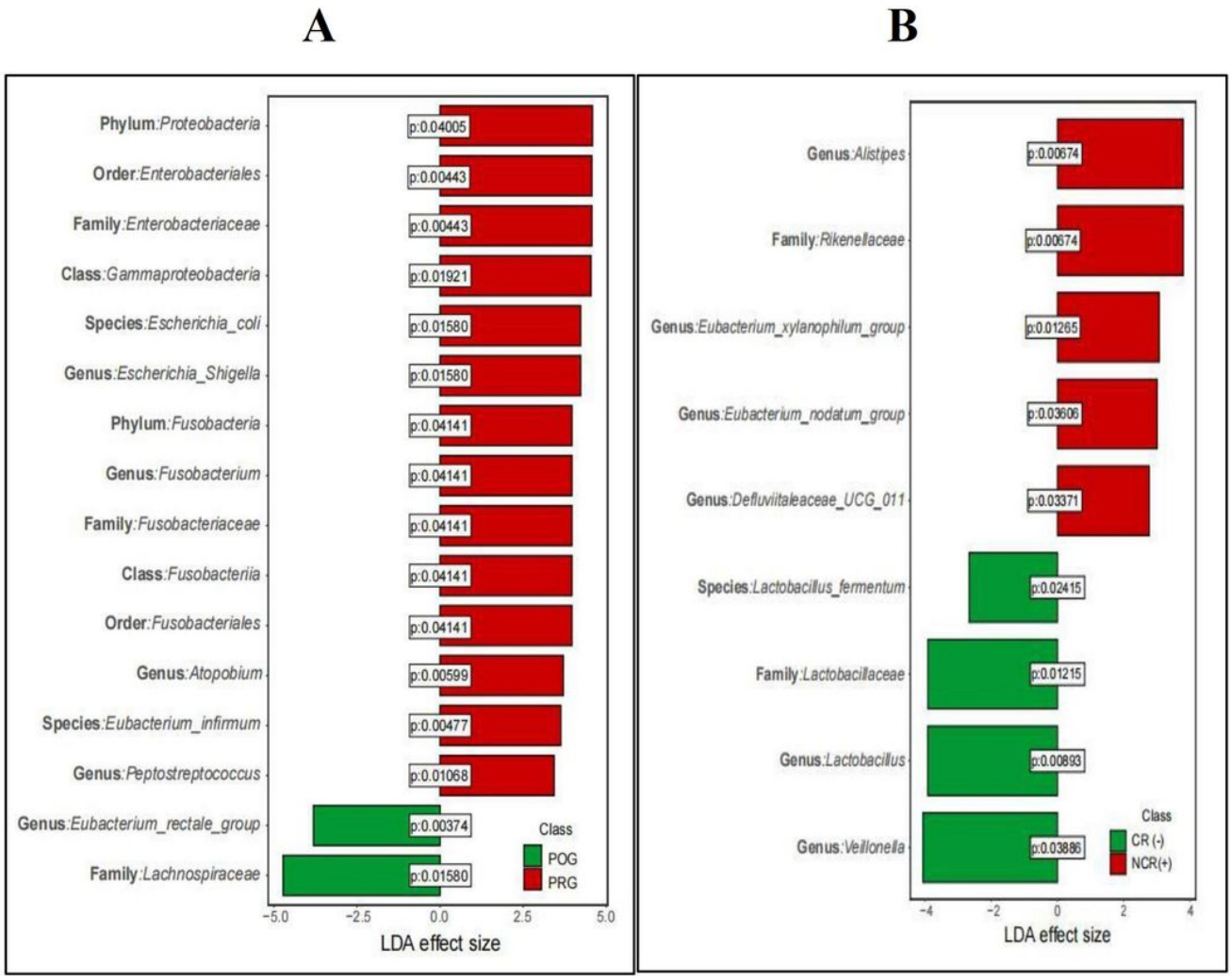

Figure 2

LEfSe analysis between PRG and POG, CRG and NCRG (A) There were dominant gut microbiota in PRG and POG; (B) There were dominant gut microbiota CRG and NCRG. Green is TEG and CRG, Red is UEG and NCRG.

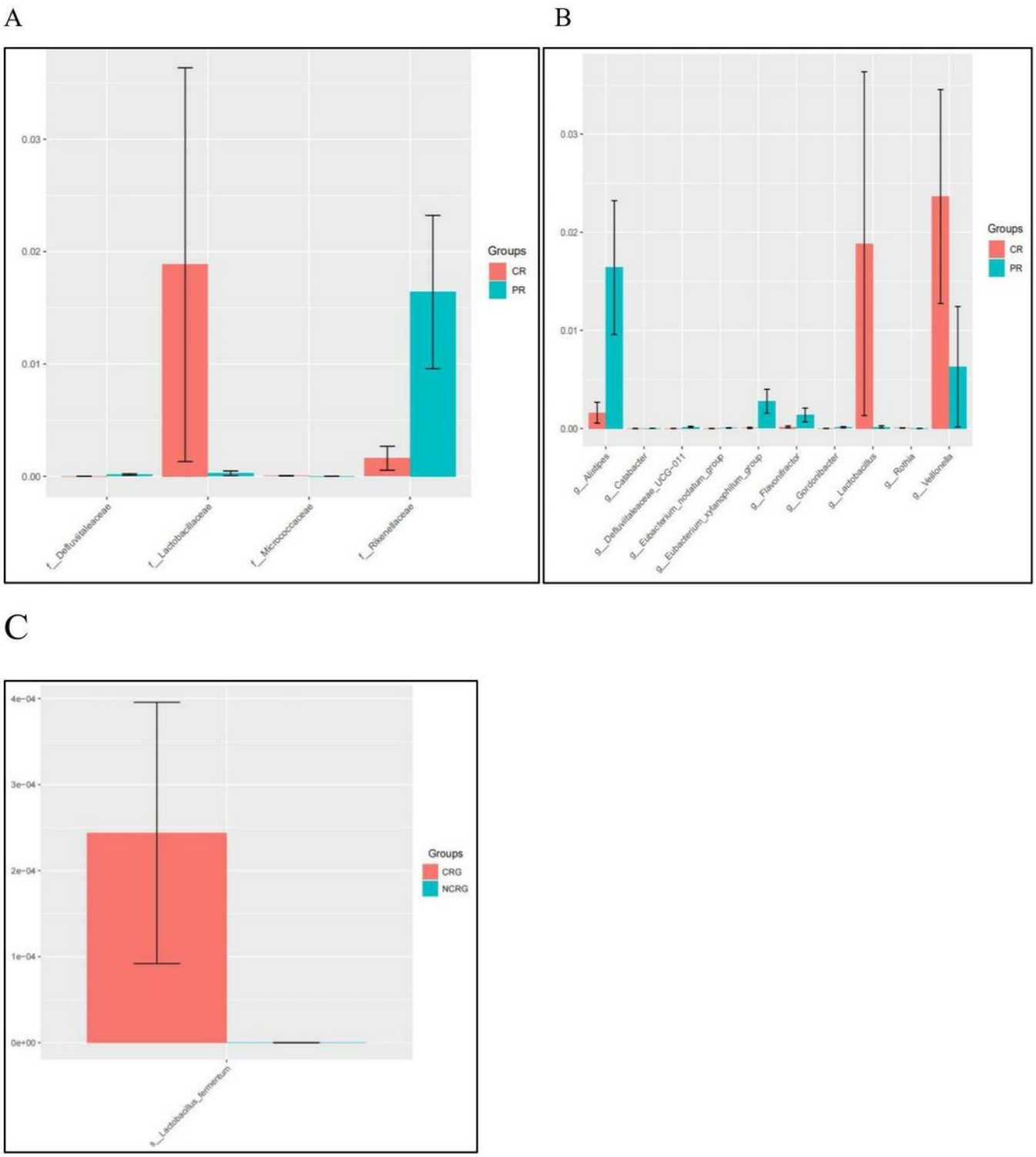

Figure 3

The difference of gut microbiota between CRG and NCRG (A) At the family level, The abundance of Lactobacillaceae was significantly higher in CRG than in NCRG; (B) at the genus level, The abundance of Lactobacillus was significantly higher in CRG than in NCRG; (C) At the species level, the abundance CRG of Lactobacillus fermentum was also significantly higher than that of NCRG.

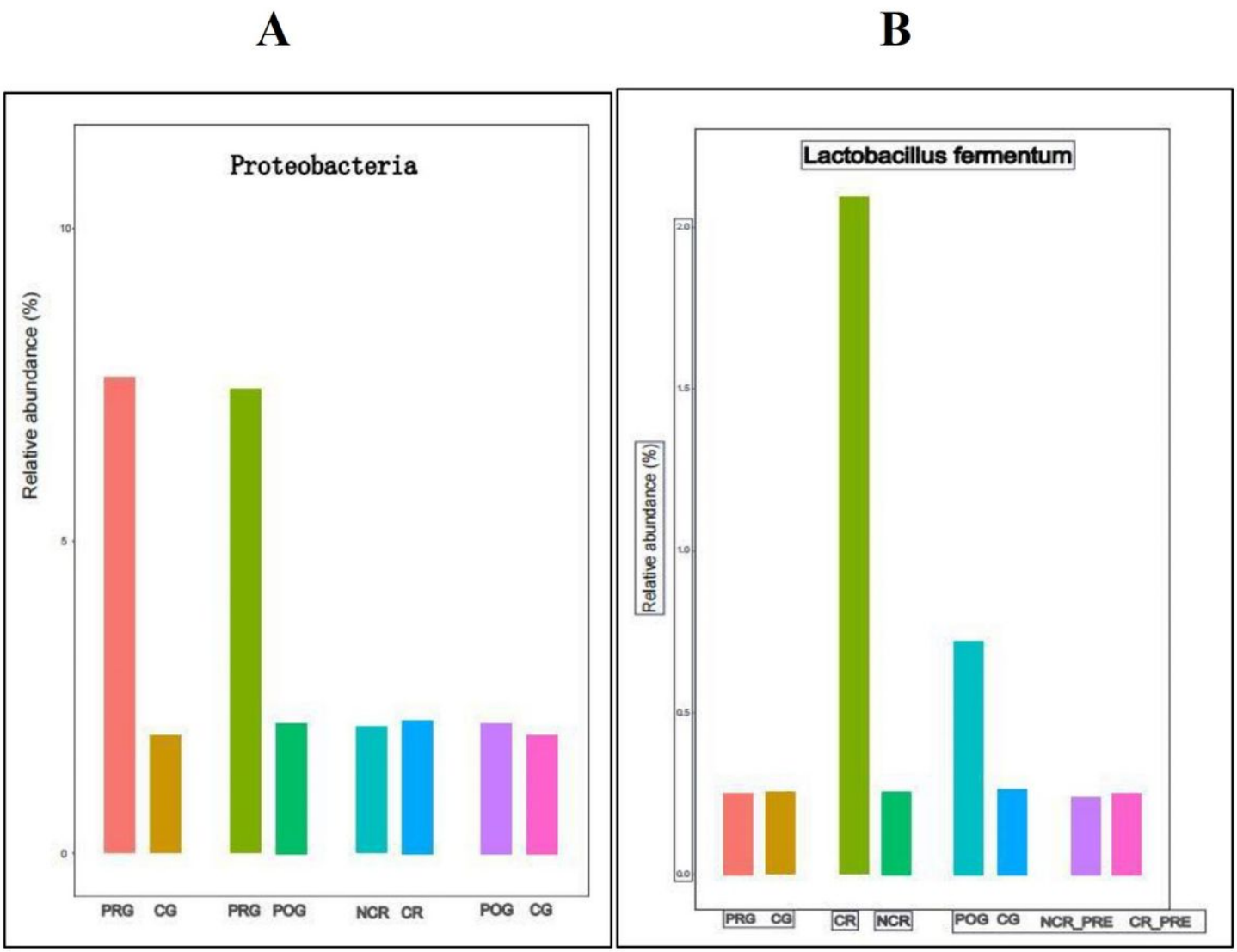

Figure 4

The succession of gut microbiota in DLBCL patients. (A) The comparison of abundance of Proteobacteria were between PRG and CG, PRG and POG, CRG and NCRG, POG and CG; The abundance of Proteobacteria phylum in PRG was higher than that of CG and POG. There was no significant difference between POG and CG, CRG and NCRG. (B) The comparison of abundance of Lactobacillus fermentum were between PRG and CG, CRG and NCRG, POG and CG, CR_PRE and NCR_PRE; The abundance of Lactobacillus fermentum in CRG was higher than that of NCRG and CG. There was no significant difference between PRG and CG, CR_PRE and NCR_PRE

Supplementary Files

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- KruskalresultgenusCRANDNCR1.xlsx

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- RawOTUstaxonomyCRANDNCR.xlsx
- RawOTUstaxonomyPRGANDPOG.xlsx
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