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The role of the gut microbiota in infectious complications during immunochemotherapy for diffuse large B-cell lymphoma

Man Sun[1†], Duozhuang Tang[2,3†], Jie Jia[1], Yuanyuan Wu[1], Chenghui Yu[2,3], Rongrong Qiu[1], Hua Wang[1*] and Si Tao[1*]

Abstract

Background Infections are common complications and causes of death during immunochemotherapy in diffuse large B-cell lymphoma (DLBCL). The gut microbiota plays a significant role in bacterial infection, but its relationship and predictive capacity with infectious complications in DLBCL are unknown.

Methods We performed 16S rRNA gene sequencing of fecal samples collected from 41 patients with newly diagnosed DLBCL at baseline, after every two cycles of standard immunochemotherapy, during infection, and after infection recovery. Analysis of the diversity and species composition of these samples was used to evaluate the relationship between gut microbiota and bacterial infection.

Results Our findings demonstrate the dynamic changes of *Enterobacteriaceae* in patients with DLBCL during immunochemotherapy. The abundance of *Enterobacteriaceae* was markedly higher at baseline in patients who subsequently developed bacterial infection during immunochemotherapy than in those who did not ($P < 0.0001$), and showed a further increase during infection ($P < 0.01$), after recovery from the infection, the *Enterobacteriaceae* was significantly decreased ($P < 0.001$). While there was no significant change in patients who did not develop bacterial infection. The univariate and multivariate analysis showed that baseline abundance of *Enterobacteriaceae* > 4.5% was independently associated with post-immunochemotherapy bacterial infection.

Conclusions Our findings suggest that the gut microbiota signatures differ between patients with DLBCL who do and do not develop bacterial infection. The baseline abundance of *Enterobacteriaceae* is associated with the post-immunochemotherapy bacterial infection, and it has certain predictive value. Detecting the changes of gut microbiota can help predict the risk of bacterial infection after immunochemotherapy.

Keywords Diffuse large B-cell lymphoma, Gut microbiota, Bacterial infection, Immunochemotherapy

†Man Sun and Duozhuang Tang contributed equally to this work.

*Correspondence:
Hua Wang
ndefy19002@ncu.edu.cn
Si Tao
ndefy11188@ncu.edu.cn
Full list of author information is available at the end of the article

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Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of lymphoma, representing approximately 30%–40% of all non-Hodgkin's lymphoma [1]. Most patients are diagnosed at an advanced stage. With the emergence of new drugs, the survival of DLBCL patients has greatly improved. Combination immunochemotherapy with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (RCHOP) is the current standard treatment, over 60% patients being cured by this regimen [2]. However, high-dose combination immunochemotherapy can cause bone marrow suppression and immunosuppression [3, 4], making patients susceptible to infections. Randomized clinical trials have shown that the incidence of treatment-related infections with RCHOP immunochemotherapy is approximately 10%–20% [5], whereas real-world studies have reported incidences reaching 40%–60% [6, 7]. While mild infections prolong hospitalization and increase economic burden, severe infections can lead to treatment interruptions that impact treatment efficacy and even result in death. Currently, the process of diagnosing post-immunochemotherapy infections is time-consuming with low sensitivity, mainly relying on clinical symptoms, laboratory indicators, and pathogen test results. Furthermore, patients who develop and are then treated for infection may experience serious consequences, such as poor treatment effects and further disease progression. Therefore, it is necessary to strengthen the early identification and prediction of immunochemotherapy-related infections in patients with DLBCL.

A healthy gut microbiota is an important biological barrier that can prevent infection by inhibiting pathogenic species colonization [8–10]. Under normal circumstances, opportunistic pathogens do not infect hosts, but interventions such as chemotherapy, radiotherapy, and surgery can significantly disrupt the gut microecology and lead to dysbiosis of the intestinal microbiota. Such conditions allow opportunistic pathobionts to overgrow and expand, escape into the systemic circulation by translocation through damaged epithelial tissues, and induce infection [11–14]. Recent research has confirmed that the relative abundance of *Enterobacteriaceae* before treatment is associated with febrile neutropenia (FN) after RCHOP immunochemotherapy in patients with DLBCL [15]. Similar findings have been confirmed in patients with leukemia and those hematopoietic stem cell transplant(HSCT) patients [16–18]. For example, a low baseline Shannon diversity is associated with infection during neutropenia in patients with acute myeloid leukemia [16]. Therefore, pre-treatment characteristics of the gut microbiota could potentially serve as biomarkers to identify individuals at high risk of chemotherapy-related infections. However, most of these investigations have focused on infection during neutropenia. Considering that some infectious episodes occur in the absence of grade 4 neutropenia [19], we conducted this study to analyze the relationship between the gut microbiota and all bacterial infection during RCHOP immunochemotherapy in patients with DLBCL.

Methods

Cohort study

This study participants comprised 41 treatment-naïve, newly diagnosed patients with DLBCL undergoing RCHOP in the Second Affiliated Hospital of Nanchang University from September 2021 to July 2023. The study protocol was approved by the Institutional Ethics Committee of Second Affiliated Hospital of Nanchang University and was conducted in compliance with the Declaration of Helsinki. The inclusion criteria were as follows: 1) pathologic diagnosis of DLBCL without previous chemotherapy; and 2) absence of chronic inflammatory gastrointestinal diseases or other tumors. The exclusion criteria were as follows: 1) history of acute inflammation or infectious disease within the past month; 2) history of antibiotic use within the past month; 3) presence of autoimmune disease and long-term use of steroids or immunosuppressants; 4) history of gastrointestinal surgery; and 5) use of probiotic preparations within the previous 3 months.

The following clinical information was recorded: sex, age, immunotype, serum lactate dehydrogenase (LDH) and β−2 microglobulin levels, Eastern Cooperative Oncology Group (ECOG) performance status, body mass index (BMI), Ann Arbor stage and International Prognostic Index (IPI), and absolute neutrophil and lymphocyte counts, infectious episodes, neutropenia. The response evaluation was based on the Lugano response criteria, which include complete remission (CR), partial remission (PR), stable disease (SD), and progressive disease (PD). The follow up of each patient started at the beginning of the immunochemotherapy and ended 3 weeks after the last dose or patients occurred bacterial infection.

Patients were treated with first-line standard R-CHOP regimen, which includes rituximab (375 mg/m^2, on day 0), cyclophosphamide (750 mg/m^2, on day 1), doxorubicin (50 mg/m^2, on day 1), vincristine (1.4 mg/m^2, with a maximum dose of 2 mg, on day 1), and prednisone (100 mg, administered from day 1 to 5) every 21 days, and pegylated G-CSF was administered prophylactically every cycle. None of the patients received prophylactic antibiotic therapy prior to the initiation of immunochemotherapy.

Neutropenia is defined as an absolute neutrophil count < 500 cells/mm^3 or that is expected to decrease to < 500 cells/mm^3 during the next 48 h [20].

Bacterial infections were determined when microbiological evidence was present or clinically diagnosed [21]. Microbiologically defined bacterial infection was diagnosed when there are signs and symptoms of infection with microbiologic culture confirmation. Clinically defined bacterial infection was diagnosed when symptoms (e.g. fever, cough, purulent sputum, acute diarrhea, abdominal pain, pyuria, dysuria), signs (e.g. pulmonary rales, abdominal tenderness, tenderness in lumbosacral region), clinical test (e.g. increased peripheral blood leukocytes and neutrophils, elevated C-reactive protein and procalcitonin, increased white blood cells in urine) and medical imaging (e.g. lobar consolidation, multiple patchy consolidations, pleural effusion, localized lesion exudation, intestinal edema) of infection were evident.

Stool sample collection and DNA extraction

A total of 41 patients were enrolled in the study. Stool specimens were obtained for microbiota analysis at baseline, after every two cycles of standard immunochemotherapy, during infection and after infection recovery. And all samples were frozen at − 80 °C until DNA extraction.

1. Fecal DNA isolation

Fecal samples were weighed and total DNA was extracted with the DP328 Fecal Genome Extraction Kit (Tiangen Biotech) according to the manufacturer's instructions. DNA concentration and purity were measured with Nanodrop 2000.

2. 16S rRNA gene sequencing

The 16S rRNA gene regions V3-V4 was amplified with universal primers (Invitrogen, Carlsbad, CA, USA) 341F and 806R using the PCR reactions. The libraries for sequencing were prepared using the NEBNext® Ultra™ DNA Library Prep Kit designed for Illumina® sequencing (New England Biolabs, USA). The prepared DNA libraries was sequenced on an Illumina Nova6000 platform, generating paired-end reads with a length of 250 bp paired-end reads [22].

3. Sequencing data processing

Paired-end raw reads quality control

Fastp (version 0.14.1, https://github.com/OpenGene/fastp) was used to control the quality of the Raw Data by sliding window (-W 4 -M 20). The primers were removed by using cutadapt software (https://github.com/marcelm/cutadapt/) according to the primer information at the beginning and end of the sequence to obtain the paired-end clean reads.

Paired-end clean reads assembly

Paired-end clean reads were merged using usearch-fastq_mergepairs (V10, http://www.drive5.com/usearch/) according to the relationship of the overlap between the paired-end reads, when at least 16 bp overlap the read generated from the opposite end of the same DNA fragment, the maximum mismatch allowed in overlap region was 5 bp, and the spliced sequences were called Raw Tags.

Raw tags quality control

Fastp (version 0.14.1, https://github.com/OpenGene/fastp) was used to control the quality of the raw Data by sliding window (-W 4 -M 20) to obtain the paired-end clean tags.

4. OTU cluster and Species annotation

The usearch software (Version 10, http://www.drive5.com/usearch/) was employed to perform sequence analysis. Sequences that demonstrated a similarity of 97% or above were categorized into the same OTU. The representative sequence of each OTU was the most recurrent sequence, and was screened for subsequent annotation.

Statistical analysis

Differences between two groups were assessed using Student's t-test or the Mann–Whitney U test. One-way analysis of variance (ANOVA) was used for multi-group (more than two groups) datasets. A receiver operating characteristic (ROC) curve was generated and the corresponding area under the curve (AUC) value was calculated for baseline *Enterobacteriaceae* abundance as a predictor of infection during immunochemotherapy. Kaplan–Meier curves were used to visualize time to infection based on "high" and "low" abundance, and were compared using log-rank tests. Cox proportional hazard models were used to investigate the univariate and multivariate analyses of the association between infection and gut microbiota data. P values < 0.05 were considered to indicate statistical significance. SPSS 27.0 and GraphPad Prism 9.0 software were used for statistical analysis.

Results

Patient characteristics and outcomes

The clinical characteristics of the 41 study patients are presented in Table 1. There were 23 male patients

Table 1 Patients' characteristics

***N*=41**		
number (percent)		
Baseline characteristics		
Age, years	≤60	22 (54%)
	>60	19 (46%)
Sex	Male	23 (56%)
	Female	18 (44%)
ECOG PS	<2	18 (44%)
	≥2	23 (56%)
BMI, Kg/m^2	<18.5	3 (7%)
	18.5–24.9	24 (59%)
	≥25	14 (34%)
Serum LDH	Normal	28 (68%)
	Elevated	13 (32%)
β2-microglobulin	Normal	20 (49%)
	Elevated	21 (51%)
Cell of origin	GCB	17 (41%)
	Non-GCB	24 (59%)
Stage	I-II	16 (39%)
	III-IV	25 (61%)
IPI	Low	15 (37%)
	Low-Intermediate	10 (24%)
	High-Intermediate	6 (15%)
	High	10 (24%)
Baseline neutrophil count	Normal	38 (93%)
	Reduced	3 (7%)
Baseline lymphocyte count	Normal	13 (32%)
	Reduced	28 (68%)
Patients with infections	Totol	19 (46%)
	Grade 4 neutropenia	9 (47%)
	Without grade 4 neutropenia	10 (53%)
Treatment outcome after chemotherapy		
Complete response		26 (63%)
Partial response		8 (20%)
Progression		6 (15%)
Not evaluated		1 (2%)

Abbreviations: *BMI* body mass index, *ECOG PS* Eastern Cooperative Oncology Group Performance Status, *GCB* germinal center B cell, *IPI* International Prognostic Index

(56.0%) and 19 patients (46%) who were >60 years of age. According to the Hans classification, 24 patients (59%) had the non-germinal center B cell (non-GCB) subtype of DLBCL. At the time of initial treatment, 18 patients (44%) had an ECOG score <2, 16 patients (39%) were in Ann Arbor stages I–II, and 15 (37%) and 10 (24%) patients had low-risk and high-risk IPI scores, respectively. Thirteen patients (32%) had elevated LDH levels at initial treatment. There were 28 patients (68%) with a reduced absolute lymphocyte count, and three patients (7%) with a reduced absolute neutrophil count. Nineteen patients (46%) experienced at least one bacterial infection during the RCHOP immunochemotherapy period, 53% of which occurred in the absence of grade 4 neutropenia. The overall effectiveness of RCHOP immunochemotherapy was 83%: 26 patients (63%) achieved CR, eight patients (20%) had PR, six patients (15%) had PD, and one patient was not evaluated for treatment efficacy (Table 1).

Baseline abundance of *Enterobacteriaceae* increased in patients who developed bacterial infection

The 16S rRNA gene sequencing analysis showed a lower baseline level of alpha diversity of the gut microbiota in patients who developed bacterial infection than in those who did not; however, this difference did not reach statistical significance ($P=0.233$; Fig. 1A). By contrast, there was a significant difference in beta diversity between the two groups ($P<0.05$; Fig. 1B). In the taxonomic comparison of gut microbial composition at the phylum level, the baseline abundance of Proteobacteria was found to be significantly higher in the post-immunochemotherapy infection group than in the non-infection group ($P<0.0001$; Fig. 1C, E). At the family level, patients who developed bacterial infection had a greater abundance of *Enterobacteriaceae* ($P<0.0001$; Fig. 1D, F). Furthermore, linear discriminant analysis (LDA) effect size (LEfSe) analysis showed that, compared with those in the non-infection group, c_Gammaproteobacteria, p_Proteobacteria, o_Enterobacteriales, and *Enterobacteriaceae* with *g_Escherichia_Shigella* were higher in relative abundance in the infection group when the cutoff for the LDA score was set to 4.5 (Fig. 1G). These results suggested that the baseline abundance of *Enterobacteriaceae* differed between patients with DLBCL who did and did not develop bacterial infection.

Abundance of *Enterobacteriaceae* further increased above baseline during infection

Although the alpha diversity of gut microbes in the infection group did not differ significantly between the baseline and during-infection collection points ($P=0.061$; Fig. 2A), the beta diversity was lower during infection than at baseline ($P<0.05$; Fig. 2B). At the phylum level, the abundance of Proteobacteria exhibited a further significant increase between baseline and during infection ($P<0.05$; Fig. 2C, E). At the family level, patients had a greater abundance of *Enterobacteriaceae* during infection than at baseline ($P<0.01$; Fig. 2D, F). LEfSe analysis identified higher abundances of c_Gammaproteobacteria, p_Proteobacteria, o_Enterobacteriales, and *f_Enterobacteriaceae* with *g_Escherichia_Shigella* in patients during

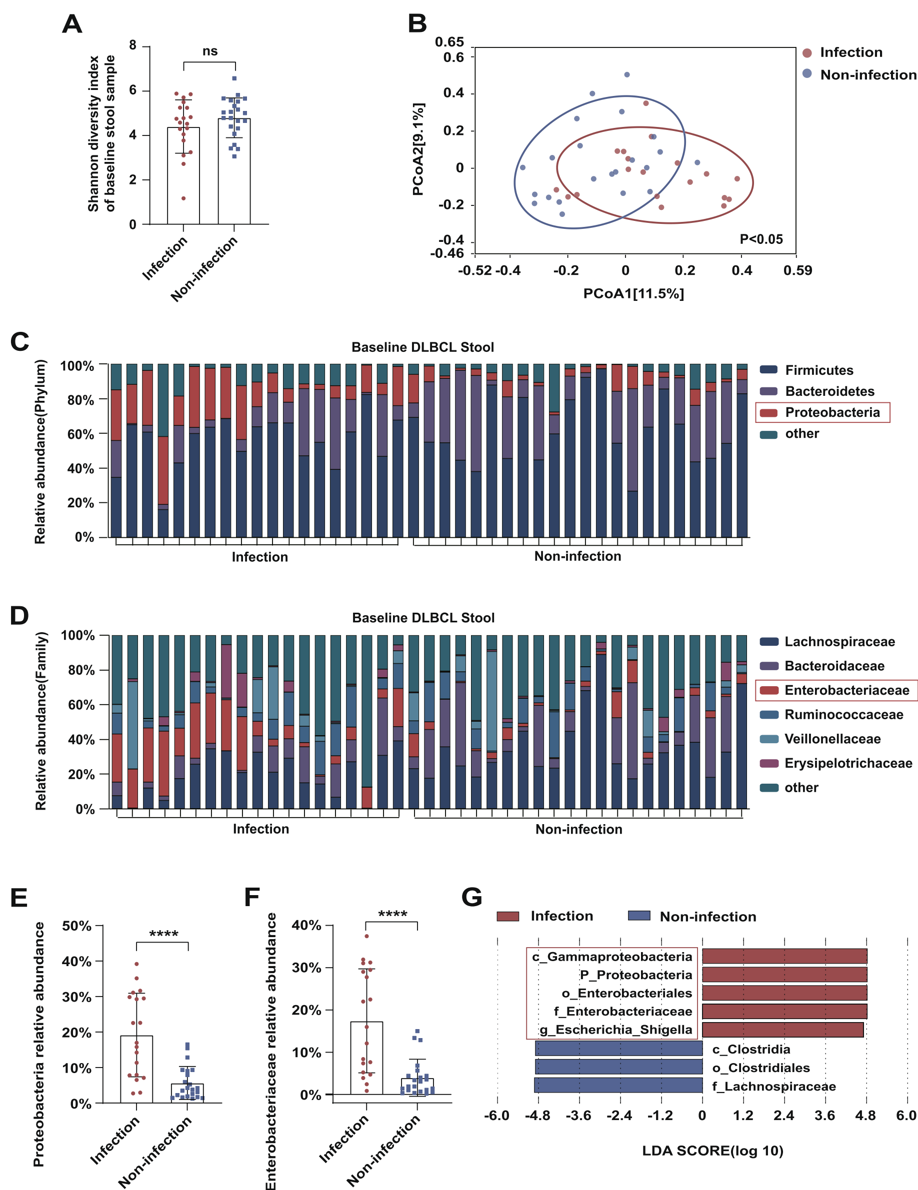

Fig. 1 **A**, **B** Shannon index of alpha diversity and Bray–Curtis index of beta diversity in baseline fecal samples of patients who did and did not develop bacterial infection during RCHOP. **C**, **D** Baseline relative abundance of bacteria at the phylum and family levels in stool samples of patients who did and did not develop bacterial infection during RCHOP. **E**, **F** Comparisons of Proteobacteria and *Enterobacteriaceae* abundance between patients who did and did not develop bacterial infection during RCHOP. **G** LEfSe analysis showing the higher baseline abundance of Proteobacteria and *Enterobacteriaceae* in patients who developed bacterial infection during RCHOP (LDA score cutoff: 4.5). Asterisks indicate significant differences identified using the two-tailed Mann–Whitney U test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. ns = not significant

infection compared with those at baseline when the LDA score cutoff was set to 4.5 (Fig. 2G).

To further investigate the trend in *Enterobacteriaceae* abundance during immunochemotherapy, we analyzed fecal samples that had been collected at baseline, and after 2, 4, and 6 cycles of immunochemotherapy from 10 patients who did not develop bacterial infection. The results showed no significant change in the abundance of *Enterobacteriaceae* in these patients during RCHOP immunochemotherapy (Fig. 3A). Additionally, we analyzed fecal samples collected at baseline, during infection, and after infection recovery from 10 patients who developed bacterial infections. In these patients, the abundance of *Enterobacteriaceae* significantly increased between the baseline and infection periods,

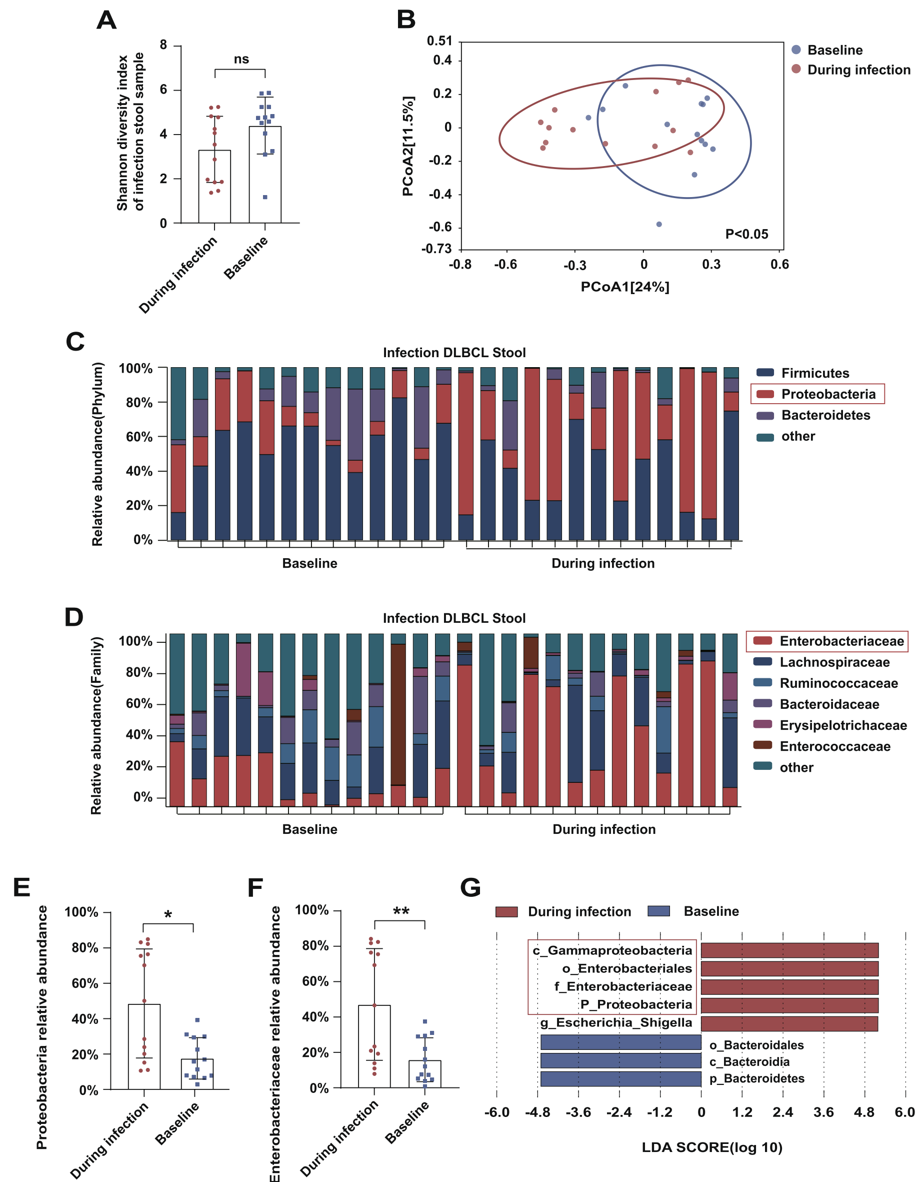

Fig. 2 **A, B** Shannon index of alpha diversity and Bray–Curtis index of beta diversity in fecal samples collected at baseline and during infection. **C**, **D** Relative abundance of bacteria at the phylum and family levels in stool samples collected at baseline and during infection. **E**, **F** Comparisons of Proteobacteria and *Enterobacteriaceae* abundance at baseline and during infection. **G** LEfSe analysis showing the higher abundance of Proteobacteria and *Enterobacteriaceae* during infection (LDA score cutoff: 4.5). Asterisks indicate significant differences identified using the two-tailed Mann–Whitney U test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. ns = not significant

then significantly decreased following anti-infection treatment and recovery from the infection (Fig. 3B). These findings demonstrated that the gut microbiota of patients with DLBCL had a greater abundance of *Enterobacteriaceae* during infection compared with that at baseline and compared with that in DLBCL patients who did not develop bacterial infection on RCHOP.

Predictive value of baseline *Enterobacteriaceae* abundance for bacterial infection

The finding of higher baseline *Enterobacteriaceae* abundance in stool samples from patients that developed bacterial infection during immuno chemotherapy led us to further examine the relationship between baseline *Enterobacteriaceae* abundance and post-immunochemotherapy bacterial infection. An ROC curve showed the

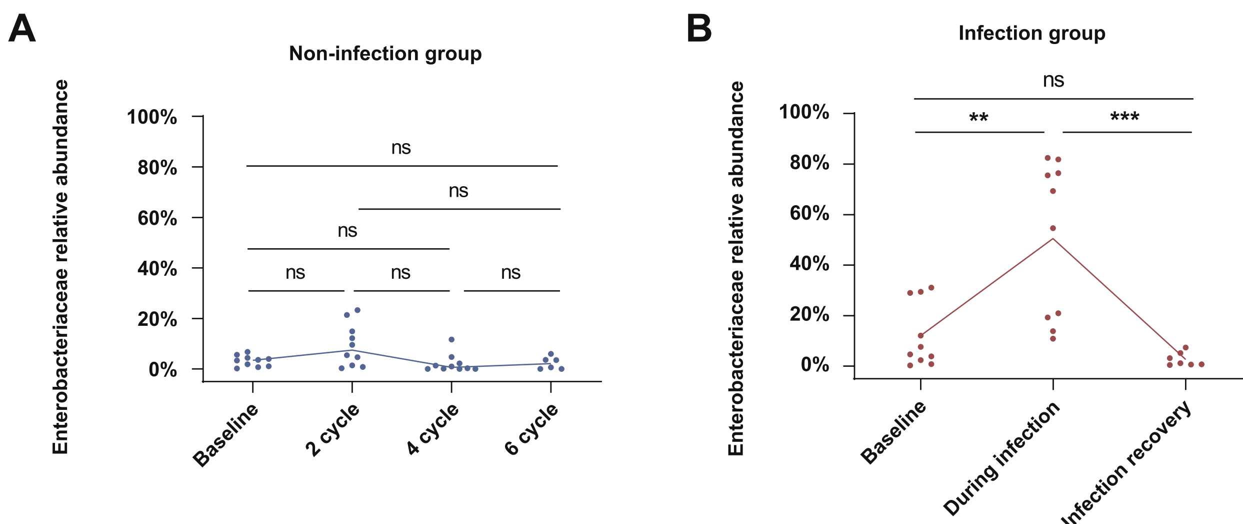

Fig. 3 **A** Change in *Enterobacteriaceae* abundance in fecal samples collected from 10 patients without bacterial infection during RCHOP for DLBCL at four time points: baseline, and after two cycles, four cycles, and six cycles. **B** Change in *Enterobacteriaceae* abundance in fecal samples collected from 10 patients who contracted a bacterial infection during RCHOP for DLBCL at three time points: baseline, during infection, and after infection recovery. Significant differences were identified using the two-tailed Mann–Whitney U test, Student's t-test, or one-way ANOVA; *$P<0.05$, **$P<0.01$, ***$P<0.001$. ns = not significant

strong predictive ability of baseline *Enterobacteriaceae* abundance for post-immunochemotherapy bacterial infection in DLBCL patients (AUC = 0.861; 95% confidence interval [CI]: 0.747–0.975; $P<0.0001$). An *Enterobacteriaceae* abundance threshold of 4.5% exhibited a sensitivity of 84% and a specificity of 77% (Fig. 4A). The patient cohort was divided into high and low *Enterobacteriaceae* abundance groups based on the threshold value derived from ROC curve analysis. The Kaplan–Meier curve showed that the group with high baseline *Enterobacteriaceae* abundance had a significantly higher rate of bacterial infection compared with the low-abundance group ($P<0.0001$; Fig. 4B).

To identify other baseline clinical indicators that might be predictive of the occurrence of bacterial infection, we used univariate and multivariate Cox proportional

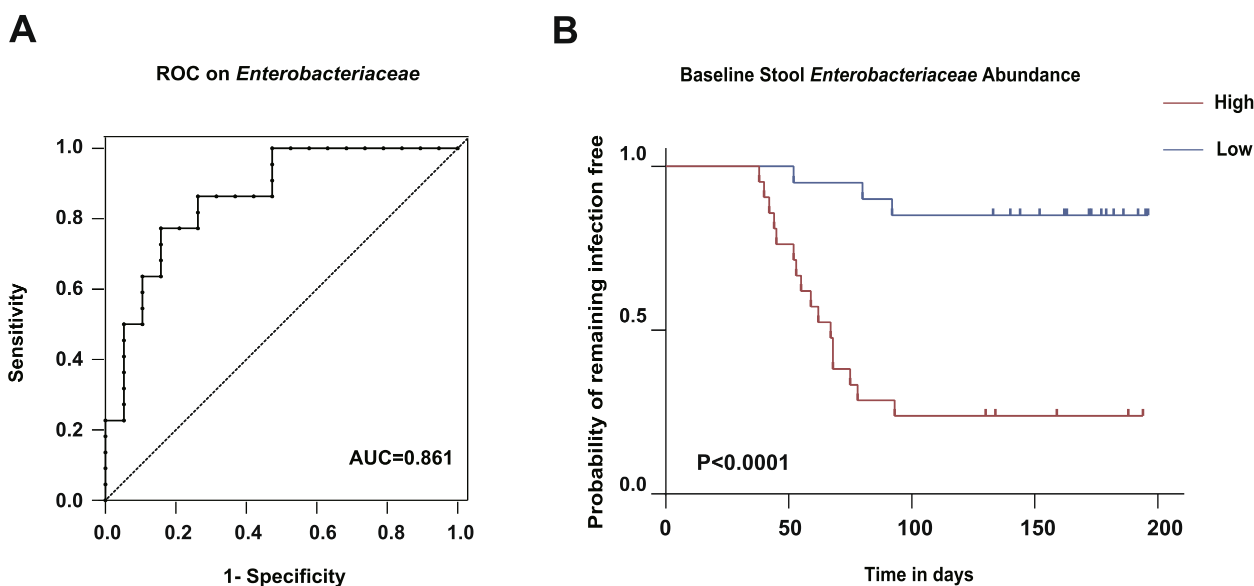

Fig. 4 **A** ROC curve of baseline *Enterobacteriaceae* abundance. **B** Kaplan–Meier (KM) plot illustrating the difference in infection outcome between two groups stratified by low abundance (blue) and high abundance (red) of *Enterobacteriaceae* (abundance threshold: 4.5%)

hazards models. In addition to the baseline abundance of *Enterobacteriaceae*, we incorporated age, sex, ECOG score, BMI, IPI and lymphocyte count. The univariate and multivariate Cox analyses showed that baseline *Enterobacteriaceae* abundance (hazard ratio: 11.307; 95% CI: 2.983–42.861; $P < 0.001$) was independently associated with the risk of post-immunochemotherapy bacterial infection (Table 2).

Discussion

The current RCHOP regimen has become the standard first-line treatment for DLBCL. However, while approximately 60% of patients are cured [2], infection remains a non-negligible complication and cause of death during immunochemotherapy [6, 7]. In this study, we used 16S rRNA gene sequence analysis of fecal samples to investigate the relationship between baseline characteristics of the gut microbiota and bacterial infection after RCHOP in 41 patients with DLBCL. We found that patients who developed bacterial infection after immunochemotherapy had a higher baseline abundance of *Enterobacteriaceae* compared with those who did not, and showed a further increase during infection. The baseline *Enterobacteriaceae* abundance > 4.5% was independently associated with bacterial infection and, after including potential clinical factors [3, 19], it remained an independent risk factor. Previous studies on DLBCL and gut microbiota have focused on disease characteristics and treatment outcomes, there are fewer studies on infectious complications. Currently, the means to predict infectious complications during immunochemotherapy before treatment are extremely limited. Our study for the first time demonstrates the trend of changes in *Enterobacteriaceae* in patients with DLBCL during immunochemotherapy by continuously collecting fecal samples, reveals its difference between infection and non-infection group. These findings show that baseline *Enterobacteriaceae* may have important predictive value for subsequent bacterial infection. Physicians treating DLBCL cases with high baseline abundance of *Enterobacteriaceae* may need to pay more attention to the risk of bacterial infection and take corresponding measures, as necessary.

The typical causative pathogens of infections in patients with malignant tumors mainly originate from the gut, such as *Escherichia coli*, *Klebsiella*, *Enterococcus*, *Pseudomonas aeruginosa* [23–25], most of these opportunistic pathogens belong to the *Enterobacteriaceae*. Mancini et al. found that the abundance of *Enterobacteriaceae* before transplantation is an independent risk factor for sepsis after allogeneic hematopoietic stem cell transplantation (allo-HSCT) [26], which is consistent with our findings. Another study discovered that the baseline abundance of Proteobacteria in the gut could predict FN after chemotherapy in children with acute lymphoblastic leukemia [27]. Recently, studies have also found that the baseline abundance of *Enterobacteriaceae* was related to FN after RCHOP in patients with DLBCL [15]. In line with the findings of previous report, our study found that the baseline abundances of Proteobacteria and *Enterobacteriaceae* were significantly increased in patients who later developed bacterial infection during immunochemotherapy. What's more, our results have shown the dynamic changes of *Enterobacteriaceae* in patients who do or do not develop bacterial infection. And these studies mainly focused on infection during neutropenia or sepsis. However, more than half of the infectious episodes in our cohort occurred without grade 4 neutropenia, which has also been reported in the literature [19]. This implies that patients who do not develop innate immunity deficiencies through chemotherapy may still experience severe bacterial infection, and these patients may have been overlooked in scientific literature. Our findings show that the *Enterobacteriaceae* still has predictive value for those patients.

Table 2 Univariate and multivariate analyses of infection complication

	Univariate			Multivariate		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Female	1.327	0.538–3.271	0.539	0.748	0.271–2.064	0.575
Age > 60	0.928	0.334–2.579	0.886	0.816	0.240–2.776	0.745
ECOG ≥ 2	0.822	0.334–2.027	0.671	1.320	0.435–4.006	0.624
BMI	0.983	0.867–1.113	0.784	1.016	0.880–1.172	0.831
IPI > 2	0.985	0.388–2.505	0.975	1.134	0.373–3.444	0.825
Lymphocyte	1.167	0.519–2.622	0.709	1.510	0.576–3.961	0.402
Enterobacteriaceae > 4.5%	9.015	2.591–31.359	**< .001**	11.307	2.983–42.861	**< .001**

Abbreviations: *ECOG* Eastern Cooperative Oncology Group Performance Status, *BMI* body mass index, *IPI* International Prognostic Index, *HR* hazard ratio, *CI* confidence interval

Previous studies have shown before the occurrence of an infection, a corresponding microbial community already dominates in the gut. Taur et al. studied the relationship between the gut microbiota and bacteremia during allo-HSCT and found that patients with a predominance of *Enterococcus* in the gut had a nine-fold increased risk of vancomycin-resistant *Enterococcus* bacteremia, and those with a predominance of *Enterobacteriaceae* had a five-fold increased risk of Gram-negative bacilli bacteremia, compared with other patients [28]. And there is another research discovered that colonization by Bacteroidetes, *Lachnospiraceae*, and *Ruminococcaceae* bacteria before transplantation has a protective effect against *Clostridium difficile* infection after allo-HSCT [29]. And it was also found that patients with a higher abundance of fecal butyrate-producing bacteria after allo-HSCT had a five-fold lower risk of respiratory viral infections compared with other patients [30]. In recent years, increasingly more evidence suggests that supplementation with probiotics may help prevent infections [31–34]. For example, both in vivo and in vitro experimental studies have shown that certain probiotics have a protective effect against *C. difficile* infections, such as yeasts, Bifidobacteria, and *Lactobacillus* [31]. Research by Piewngam et al. showed that *Bacillus* species can inhibit colonization by *S. aureus* in the human gut and nasal passages, and these results were validated in mouse models [32]. These studies reveal the efficacy of using probiotics for pathogen decolonization and suggest the potential for probiotics to prevent pathogen colonization. Whether probiotics can be safely used to prevent post-immunochemotherapy infections requires further in-depth research.

However, our study has some limitations. First, not all patients provided stool samples at all points, and it did not adjust for additional factors such as diet, BMI, age that might affect changes in gut microbiota. Secondly, its single-center design and a larger sample size is needed to confirm the current results. Thirdly, dynamic changes of the gut microbiota during treatment may impact the risk of acquiring infection. Previously, we have shown in mouse models that fluorouracil induces differential regulatory effects on the gut microbiota across individual mouse. Fluorouracil induced an increase and translocation of pathogenic bacteria in the intestines of in certain mice, leading to infection and mortality. Conversely, other mice did not exhibit a rise in intestinal pathogenic bacteria nor significant infection-related mortality [35]. In line with our mouse experiment findings, in the current study, we observed that in patients who did not develop bacterial infection, the abundance of *Enterobacteriaceae* did not significantly change post-immunochemotherapy. However, insufficient number of samples were available for an investigation on whether immunochemotherapy induced change in gut microbiota would impact on the risk of bacterial infection in the current study. In future, larger investigation into the differential responses and dynamic changes of individual gut microbiota to immunochemotherapy and its correlation with infection incidence will be of significant interest.

In summary, our study has revealed the predictive capability of the baseline abundance of *Enterobacteriaceae* for post-immunochemotherapy bacterial infections in DLBCL, and it may be a biomarker. Although we did not address the effect of chemotherapy on the change of the gut microbiota. we think further investigation into the dynamic alterations of the gut microbiota during chemotherapy is imperative, as it facilitates a deeper understanding of the processes and mechanisms underlying bacterial infection development. The influence of chemotherapy on the microbiome and its subsequent impact on bacterial infections represents a promising and valuable avenue for future research. We are engaged in further studies, with the aim of reducing the rate of post-immunochemotherapy infectious complications and thereby improving the prognosis for patients with DLBCL.

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Authors' contributions
MS and DT collected samples and analyzed the majority of data. JJ, YW, CY, and RQ helped with sample collection and data recording. HW and ST conceived and designed the study. The manuscript was written by MS and ST and commented on by all other authors.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Declaration of Helsinki. The study protocol was approved by the Institutional Ethics Committee of Second Affiliated Hospital of Nanchang University (Review [2023] NO.098), Nanchang, China. All methods were carried out in accordance with approved rules and regulations. Informed consent was waived because of the retrospective nature of this study. No personal patient information was disclosed.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Oncology, The Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, 1 Min-De Road, Nanchang, Jiangxi 330006, China. [2]Department of Hematology, The Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, 1 Min-De Road, Nanchang, Jiangxi 330006,

China. [3]Jiangxi Provincial Key Laboratory of Hematological Diseases(2024SSY06052),Department of Hematology , The Second Affiliated Hospital of Nanchang University, 1 Min-De Road, Nanchang, Jiangxi 330006, China.

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