Characteristics and prognostic value of gut microbiota in follicular lymphoma

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Abstract. The pathogenesis and progression of follicular lymphoma (FL) depends on immune evasion mechanisms. The gut microbiota has been reported to be associated with the development and outcome of several human diseases by modulating host immunity. Thus, the present study investigated the characteristics and prognostic value of the gut microbiota in FL. Fecal samples from treatment-naïve patients with FL (n=28) and healthy controls (n=18) were prospectively collected. The gut microbiota diversity and composition were examined by 16S ribosomal RNA sequencing. The results demonstrated that patients with FL had distinct microbiota compositions. The relative abundance of the *Ruminococcaceae* family was significantly increased in patients with FL (P=0.01). Furthermore, a high level of *Ruminococcus* was identified as a strong indicator of tumor burden (P=0.001), and was related to the FL International Prognostic Index score and serum lactate dehydrogenase levels. The present results indicated an association between the gut microbiota and FL prognosis. Findings from the present study may provide a rational foundation for further investigation of the role of gut microbiota in lymphoma management.

Introduction

Follicular lymphoma (FL) is a highly heterogeneous, indolent form of non-Hodgkin lymphoma (NHL). In China, FL represents 10-20% of all new NHL diagnoses (1). The majority of patients with FL have a favorable outcome; however, ~20% of patients still face the risk of disease progression and adverse outcomes despite intensive treatment (2-4). Given the heterogeneity of the disease, several models have been proposed to predict treatment outcomes (5,6). However, identifying high-risk patients at the time of FL diagnosis remains challenging. Thus, mechanistic studies into FL development and progression are vital for identifying more prognostic predictors.

It has become increasingly evident that the survival of indolent lymphoma cells is highly dependent upon their ability to escape host immunity (7); however, the immune evasion strategies remain unclear. Growing evidence has suggested that the gut microbiota is associated with immune cell dynamics and immune homeostasis in humans (8-10). Recent studies have provided strong evidence of the association between the gut microbiota and human diseases, including cancer (11-13). Studies have also demonstrated the prognostic roles of the gut microbiota in immunotherapy and allogeneic stem cell transplantation (14-16). These studies have garnered interest in other diseases where a connection to the gut microbiota is suspected. Recent studies that have assessed the gut microbiota in patients with NHL, especially diffuse large B-cell lymphoma, have revealed an association between gut microbiota composition and disease outcomes (17-19). However, research on the gut microbiota in FL remains limited. To the best of our knowledge, only one study has previously examined the gut microbiota composition in a small cohort of patients with primary gastrointestinal FL (20). The present study aimed to investigate the characteristics of the gut microbiota in patients with FL, and explored the prognostic value of the gut microbiota composition and the abundance of specific taxa.

Materials and methods

Patient enrollment and sample collection. Patients diagnosed with FL (n=28), and age- and sex-matched healthy controls (n=18) were enrolled from the Hematology Department, Peking Union Medical College Hospital (Beijing, China) between June 2021 and June 2022. All patients were enrolled

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Table I. Characteristics of patients with FL and healthy controls.

Characteristic	Patients with FL (n=28)	Healthy controls (n=26)	P-value
Median age, years (range)	51 (30-70)	56 (35-64)	0.57
Sex, n (%)			0.11
Male	9 (32.1)	14 (53.8)	
Female	19 (67.9)	12 (46.2)	
Ann Arbor stage, n (%)			
I	0 (0)		
II	3 (10.7)		
III	16 (57.1)		
IV	9 (32.2)		
WHO pathological grade, n (%)			
1	18 (64.3)		
2	7 (25.0)		
3a	3 (10.7)		
3b	0 (0)		
FLIPI score, n (%)			
Low risk	17 (60.7)		
Intermediate risk	5 (17.9)		
High risk	6 (21.4)		
Extranodal site, n (%)			
None	17 (60.7)		
Gastrointestinal tract	6 (21.4)		
Bone marrow	5 (17.9)[a]		
Skin	1 (3.6)		
Tumor burden, n (%)			
Low	17 (60.7)		
High	11 (39.3)		

[a]One patient had bone marrow and gastrointestinal involvement. FL, follicular lymphoma; FLIPI, FL International Prognostic Index; WHO, World Health Organization.

before initiating treatment or during monitoring of FL. The exclusion criteria were as follows: i) History of chronic gastrointestinal inflammatory diseases; ii) coexistence of other types of tumors; iii) history of diarrhea within 2 weeks; and iv) history of antibiotic use within 4 weeks (17,18,21,22). On their first visit to the clinic, fresh fecal samples were collected from the patients and stored at -20˚C. Patients were assessed by hematologists to determine their Ann Arbor stage (23), FL International Prognostic Index (FLIPI) score (24), pathological grade and tumor burden according to the modified Groupe d'Etude des Lymphomes Folliculaires (GELF) criteria (25,26). Patient data, including age, sex, Ann Arbor stage, World Health Organization pathological grade (25), FLIPI score, extranodal involvement, tumor burden and laboratory findings [IL-6, IL-8, IL-10 and lactate dehydrogenase (LDH)] were collected.

16S ribosomal RNA (rRNA) sequencing and statistical analysis. The microbiota diversity and composition of the samples were assessed using 16S rRNA gene sequencing. Briefly, fecal samples stored at -20˚C were processed within 24 h of collection. Sample DNA was extracted using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc.). The V3-4 hypervariable regions of the bacterial 16S rRNA gene were amplified with the forward primer 338F (5'-ACTCCTACGGGAGGCAGCAG) and reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT) using polymerase chain reaction (PCR) (27). The PCR products were purified using Agencourt AMPure XP Kit (Beckman Coulter, Inc.) and quantified by Nanodrop (Thermo Fisher Scientific, Inc.). The quality of the amplicons was assessed using the ABI StepOnePlus Real Time PCR system (Applied Biosystems, Inc.) and Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.). Deep sequencing was performed on the MiSeq platform using the MiSeq Reagent Kit v2 (cat. no. MS-102-2003; Illumina, Inc.) at Allwegene Technology (paired-end sequencing, raw read length, 300 bp; loading dose, ~1.6 pmol). After the run, image analysis, base calling and error estimation were performed using Illumina Analysis Pipeline Version 2.6 (Illumina, Inc.). The raw data were first screened and sequences were removed from consideration if they

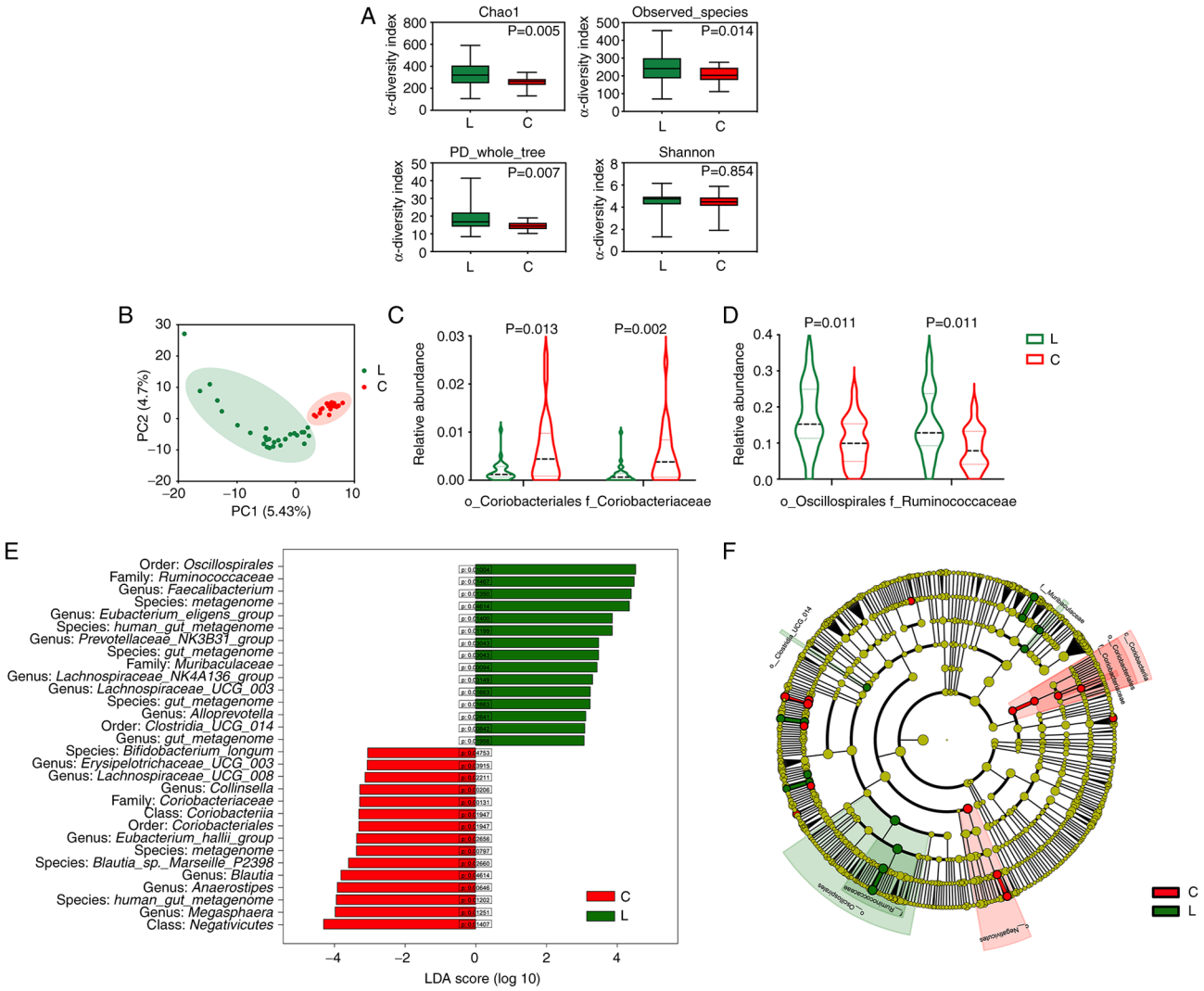

Figure 1. Patients with follicular lymphoma have an altered gut microbiota. (A) Bar plots of α-diversity indexes: Chao1, observed species, phylogenetic diversity whole tree and Shannon. (B) Principal coordinate analysis of β-diversity. (C) Violin plots of the relative abundance of the order Coriobacteriales and the family *Coriobacteriaceae* (Mann-Whitney U test) (D) Violin plots of the relative abundance of the order Oscillospirales and the family *Ruminococcaceae* (Wilcoxon rank-sum test). (E) Bar plots of the differentially abundant taxa defined by LDA score >3.0. (F) Relations of the enriched taxa by cladogram. C, control; L, lymphoma; LDA, linear discriminant analysis.

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. The dataset was then analyzed using QIIME1 (v1.8.0). The sequences were clustered into operational taxonomic units at a similarity level of 97% to generate rarefaction curves, and to calculate the richness and diversity indices (data not shown). The Ribosomal Database Project Classifier tool was used to classify all sequences into different taxonomic groups (28). QIIME1 (v1.8.0) software was used to calculate the chao1 index, observed species and phylogenetic diversity whole tree (29). Principal coordinate analysis was used to analyze β-diversity using R (v3.6.0) (30). The differences between groups were analyzed using Mothur software (v.1.34.4) (31), and linear discriminant analysis effect size (LEfSe) was analyzed by Python (V2.7) (32). The PICRUSt2 was used to predict the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment from 16S sequence results (33).

The χ^2 test, Fisher's exact test, and Mann-Whitney U test were applied, where appropriate, to compare the baseline demographics. Receiver operating characteristic (ROC) curve analysis, logistic regression, and simple linear regression analyses were conducted to analyze the association between microbiota and tumor burden. Statistical analysis was conducted using R (v3.6.0) and data were visualized using GraphPad Prism 9 (Dotmatics). P<0.05 was considered to indicate a statistically significant difference.

Results

Baseline characteristics. Among the 28 patients, the median age was 51 years (range, 30-70 years). The majority of patients had low-grade advanced-stage disease (Ann Arbor stage III-IV, pathological grade 1-2). Most patients were at low risk as defined by the FLIPI score (24). The

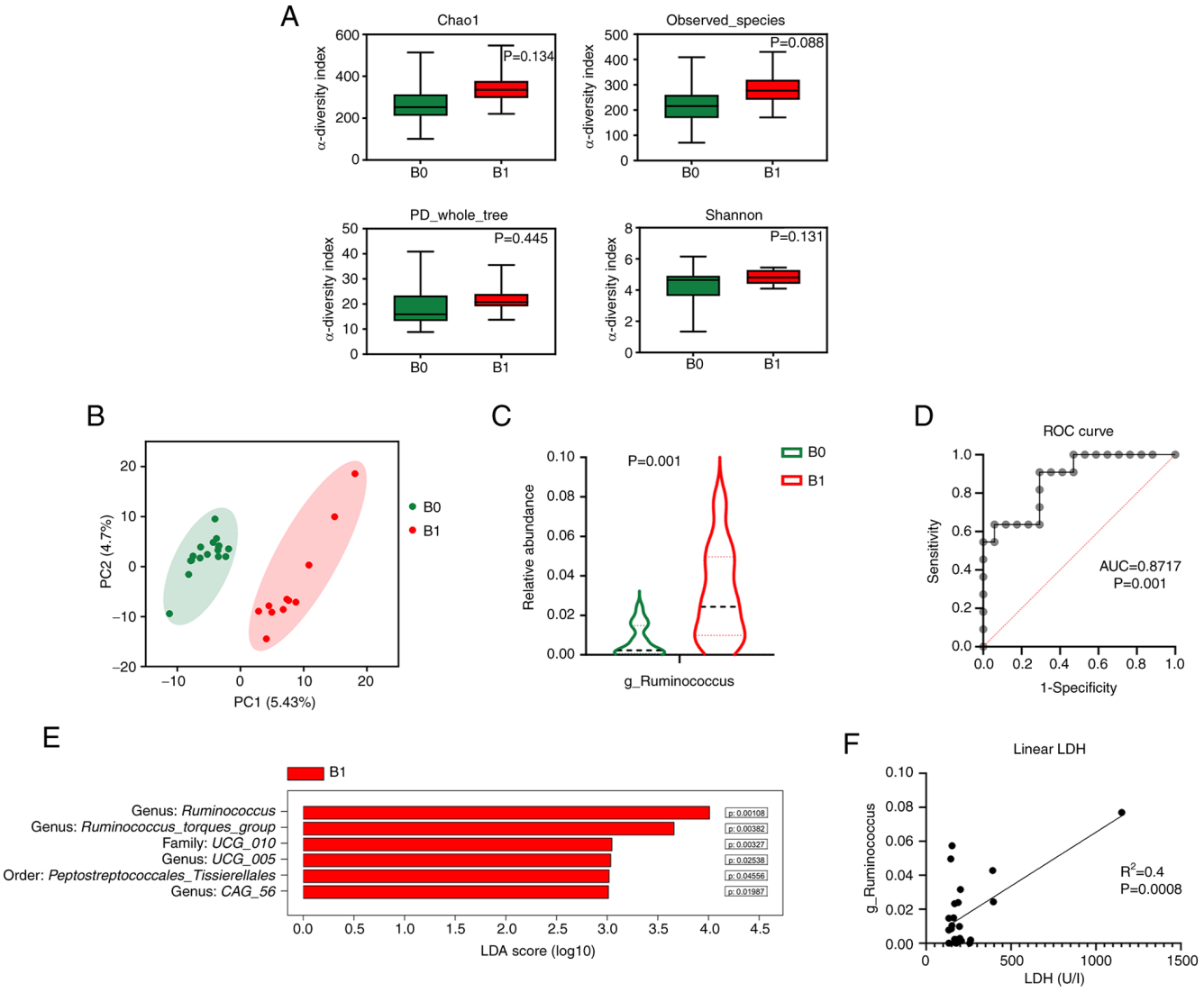

Figure 2. Gut microbiota is associated with tumor burden. (A) Bar plots of α-diversity indexes: Chao1, observed species, phylogenetic diversity whole tree and Shannon. (B) Principal coordinate analysis of β-diversity. (C) Violin plots of the relative abundance of the genus *Ruminococcus* (Wilcoxon rank-sum test). (D) ROC curve for predicting high tumor burden based on the relative abundance of *Ruminococcus*. (E) Bar plots of the differentially abundant taxa defined by LDA score >3.0. (F) Estimated regression line obtained by linear regression model linking LDH levels to the relative abundance of *Ruminococcus*. AUC, area under the curve; B0, low tumor-burden group; B1, high tumor-burden group; C, control; FLIPI, Follicular Lymphoma International Prognostic Index; L, lymphoma; LDA, linear discriminant analysis; LDH, lactate dehydrogenase; ROC, receiver operating characteristic.

extranodal involvement sites included the gastrointestinal tract, bone marrow and skin. According to the modified GELF criteria (26), 11 (39.3%) patients were classified as having a high tumor burden. There was no significant difference in age or sex between the patient and control groups (age: P=0.57, sex: P=0.11). Baseline characteristics are listed in Table I.

Patients with FL have an altered gut microbiota. To investigate whether the gut microbiota was altered in treatment-naïve patients with FL, patients with FL were compared to healthy controls. The α-diversity analysis showed that patients with FL had higher taxonomic diversity of gut microbiota (Fig. 1A). The β-diversity analysis showed a significant difference between patients with FL and healthy controls regarding microbiota composition (Fig. 1B). The LEfSe analysis revealed that the main difference in β-diversity was related to the overabundance of *Ruminococcaceae* (family of the Oscillospirales order) in patients with FL (Fig. 1D and E). A decrease in *Coriobacteriaceae* abundance at the family level were also observed in patients with FL (Fig. 1C and E). The cladogram shows the relationships of the enriched taxa (Fig. 1F). To further investigate the functional alteration of gut microbiota, PICRUSt2 was used to predict the KEGG pathway enrichment based on the 16S rRNA sequencing data. The activity of the 'bacterial secretion system' was significantly enriched in patients with FL (P=0.005), with a concomitant decrease in nutrient metabolism function, including 'carbohydrate metabolism' (P=0.003), 'fructose and mannose metabolism', and 'thiamine metabolism' (P=0.01) (Table SI). These results suggested that the composition and function of the gut microbiota were altered in patients with FL prior to treatment.

Gut microbiota is associated with tumor burden. To explore the prognostic value of the gut microbiota in FL,

Table II. Characteristics of patients with FL and high or low *Ruminococcus* abundance.

Characteristic	High *Ruminococcus* (n=15)	Low *Ruminococcus* (n=13)	P-value
Median age, years (range)	43 (30-70)	51 (41-64)	0.32
Sex, n (%)			>0.99
Male	5 (33.3)	4 (30.8)	
Female	10 (66.7)	9 (69.2)	
Ann Arbor stage, n (%)			0.87
I	0 (0)	0 (0)	
II	1 (6.7)	2 (15.4)	
III	9 (60.0)	7 (53.8)	
IV	5 (33.3)	4 (30.8)	
WHO pathological grade, n (%)			0.27
1	8 (53.3)	10 (76.9)	
2	4 (26.7)	3 (23.1)	
3a	3 (20.0)	0 (0)	
3b	0 (0)	0 (0)	
FLIPI score, n (%)			0.18
Low risk	7 (46.7)	10 (76.9)	
Intermediate risk	3 (20.0)	2 (15.4)	
High risk	5 (33.3)	1 (7.7)	
Extranodal site, n (%)			0.64
None	9 (60.0)	8 (61.5)	
Gastrointestinal tract	2 (13.3)	4 (30.8)	
Bone marrow	3 (20.0)	2 (15.4)[a]	
Skin	1 (6.7)	0 (0)	
Tumor burden, n (%)			0.0003
High	10 (66.7)	1 (7.7)	
Low	5 (33.3)	12 (92.3)	

[a]One patient had bone marrow and gastrointestinal involvement. FL, follicular lymphoma; FLIPI, FL International Prognostic Index; WHO, World Health Organization.

patients were grouped into a low tumor burden group (B0, n=17) and a high tumor burden group (B1, n=11). Although the α-diversity did not differ significantly (Fig. 2A), the β-diversity differed between patients with low and high tumor burden (Fig. 2B). In the taxonomic comparison of gut microbial composition, an overabundance of *Ruminococcus* (genus of the *Ruminococcaceae* family) was observed in patients with high tumor burden (Fig. 2C and E). The present study then specifically investigated the predictive value of the abundance of *Ruminococcus* on tumor burden. The ROC showed that the abundance of *Ruminococcus* could be used to distinguish high and low tumor burdens in patients (area under the curve=0.87, P=0.001; Fig. 2D). As determined by logistic regression analysis, it was revealed that the high abundance of *Ruminococcus* (defined by a relative abundance ≥0.8%; the cut-off value defined by ROC curve analysis) was associated with an increased risk of high tumor burden (OR 3.2, 95% CI 1.5-10.5, P=0.0003) (Table SII). Additionally, as determined by linear regression analysis, the abundance of *Ruminococcus* was associated with LDH levels of the patients (Fig. 2F). Baseline cytokine concentrations (IL-6, IL-8 and IL-10) were also assessed; however, no significant association was found between ILs and *Ruminococcus* (data not shown). In addition, the clinical characteristics of the patients with high or low *Ruminococcus* were compared. Although not statistically significant, patients with a high abundance of *Ruminococcus* tended to have a higher pathological grade (P=0.16) and FLIPI score (P=0.09) (Table II).

Discussion

In the present study, it was revealed that patients with FL at diagnosis had an altered gut microbiota composition compared with that in healthy individuals, characterized by an overabundance of *Ruminococcaceae* and increased bacterial secretion function. Furthermore, it was demonstrated that the relative abundance of *Ruminococcus* was a significant predictor of tumor burden.

Based on these findings, it was hypothesized that microbial dysbiosis may be involved in the development and progression of FL. It is generally believed that lymphoma cells must evolve some immune escape strategy to develop from lymphoid organs and survive in the periphery; however, the immune

evasion mechanisms remain poorly characterized (7). There is mounting evidence to support the role of the microbiome in altering the immune system of the host. The crosstalk between the gut microbiota and the immune system influences localized mucosal immunity and has broader effects, contributing to innate and adaptive immunity at multiple levels (9,34). Mechanistic studies have shown that gut microbial dysbiosis can coordinate helper T-cell responses via the secretion of inflammatory molecules and induction of local oxidative stress (35-37). As determined by PICRUSt2 prediction, it was demonstrated in the present study that the bacterial secretion system pathway was significantly enriched in patients with FL, whereas the nutrient metabolism pathway was not. This result indicated a functional shift in the gut microbiota toward a pathological state. To determine the inflammatory status of the patients, baseline cytokine concentrations (IL-6, IL-8, IL-10) and LDH levels were measured in serum samples obtained along with the fecal samples. Although no significant difference was determined in cytokines due to the limited sample size, an association was identified between *Ruminococcus* abundance and LDH levels. However, increased LDH could be caused by various factors other than the gut microbiome, and additional functional studies, especially metagenomic studies, are warranted to elucidate the mechanism underlying the interaction between the gut microbiota and inflammation in patients with FL.

As determined by taxonomic analysis, it was revealed that members of the *Ruminococcaceae* family, especially the genus *Ruminococcus,* were associated with FL and a high tumor burden. In the literature, *Ruminococcaceae* has been reported to produce short-chain fatty acids that can induce the *Wnt* pathway in intestinal stem cells to promote their proliferation (38). Furthermore, high abundance of *Ruminococcus* has been associated with several autoimmune diseases, including inflammatory bowel disease and spondylarthritis (39,40). This evidence, together with the present findings, suggested a novel role for *Ruminococcus* in the pathogenesis of several diseases.

To the best of our knowledge, the present study is the first comprehensive microbiota analysis of treatment-naïve patients with FL. The results suggested an association between gut microbiota composition and the disease progression of FL. However, the impact of these initial findings is limited by the small sample size. Validation of these findings in larger clinical studies is required. Moreover, long-term survival data will help to further consolidate the prognostic value of gut microbiota. Future studies should also investigate the regulatory mechanisms of the gut microbiota in preclinical models to understand the interplay of bacterial taxa and bacterial metabolites on the immune system.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the figshare repository (http://doi.org/10.6084/m9.figshare.22559515).

Authors' contributions

ZFX, CW and WW participated in the patient data acquisition. DQZ and YZ confirm the authenticity of all the raw data. ZFX, DQZ and YZ performed statistical analyses. DBZ, DQZ, and WZ designed the study. DBZ, WZ, and DQZ obtained funding for the study. DBZ and WZ revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Peking Union Hospital (protocol code 202104293005224, 13rd May 2021; Beijing, China). All patients and healthy volunteers provided informed written consent for the collection of samples.

Patient consent for publication

All patients and healthy volunteers provided informed written consent for the publication of this article.

Competing interests

The authors declare that they have no competing interests.

References

1. Chinese Society of Lymphoma, Chinese Anti-cancer Association; Chinese Society of Hematology, Chinese Medical Association: Chinese guidelines for diagnosis and treatment of follicular lymphoma (2020). Zhonghua Xue Ye Xue Za Zhi 41: 537-44, 2020 (In Chinese).
2. Bachy E, Seymour JF, Feugier P, Offner F, López-Guillermo A, Belada D, Xerri L, Catalano JV, Brice P, Lemonnier F, *et al*: Sustained progression-free survival benefit of rituximab maintenance in patients with follicular lymphoma: Long-term results of the PRIMA study. J Clin Oncol 37: 2815-2824, 2019.
3. Casulo C, Byrtek M, Dawson KL, Zhou X, Farber CM, Flowers CR, Hainsworth JD, Maurer MJ, Cerhan JR, Link BK, *et al*: Early relapse of follicular lymphoma after rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone defines patients at high risk for death: An analysis from the national lymphocare study. J Clin Oncol 33: 2516-2522, 2015.
4. Zha J, Fan L, Yi S, Yu H, Zheng Z, Xu W, Deng M, Lin Z, Li Z, Ping L, *et al*: Clinical features and outcomes of 1845 patients with follicular lymphoma: A real-world multicenter experience in China. J Hematol Oncol 14: 131, 2021.
5. Silva A, Bassim S, Sarkozy C, Mottok A, Lackraj T, Jurinovic V, Brodtkorb M, Lingjaerde OC, Sehn LH, Gascoyne RD, *et al*: Convergence of risk prediction models in follicular lymphoma. Haematologica 104: e252-e255, 2019.
6. Roschewski M, Staudt LM and Wilson WH: Dynamic monitoring of circulating tumor DNA in non-Hodgkin lymphoma. Blood 127: 3127-3132, 2016.
7. Laurent C, Charmpi K, Gravelle P, Tosolini M, Franchet C, Ysebaert L, Brousset P, Bidaut A, Ycart B and Fournié JJ: Several immune escape patterns in non-Hodgkin's lymphomas. Oncoimmunology 4: e1026530, 2015.

8. Gopalakrishnan V, Helmink BA, Spencer CN, Reuben A and Wargo JA: The influence of the gut microbiome on cancer, immunity, and cancer immunotherapy. Cancer Cell 33: 570-580, 2018.
9. Honda K and Littman DR: The microbiota in adaptive immune homeostasis and disease. Nature 535: 75-84, 2016.
10. Schluter J, Peled JU, Taylor BP, Markey KA, Smith M, Taur Y, Niehus R, Staffas A, Dai A, Fontana E, *et al*: The gut microbiota is associated with immune cell dynamics in humans. Nature 588: 303-307, 2020.
11. Okumura S, Konishi Y, Narukawa M, Sugiura Y, Yoshimoto S, Arai Y, Sato S, Yoshida Y, Tsuji S, Uemura K, *et al*: Gut bacteria identified in colorectal cancer patients promote tumourigenesis via butyrate secretion. Nat Commun 12: 5674, 2021.
12. Davar D, Dzutsev AK, McCulloch JA, Rodrigues RR, Chauvin JM, Morrison RM, Deblasio RN, Menna C, Ding Q, Pagliano O, *et al*: Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. Science 371: 595-602, 2021.
13. Zhang Q, Ma C, Duan Y, Heinrich B, Rosato U, Diggs LP, Ma L, Roy S, Fu Q, Brown ZJ, *et al*: Gut microbiome directs hepatocytes to recruit MDSCs and promote cholangiocarcinoma. Cancer Discov 11: 1248-1267, 2021.
14. Matson V, Fessler J, Bao R, Chongsuwat T, Zha Y, Alegre ML, Luke JJ and Gajewski TF: The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. Science 359: 104-108, 2018.
15. Viaud S, Saccheri F, Mignot G, Yamazaki T, Daillère R, Hannani D, Enot DP, Pfirschke C, Engblom C, Pittet MJ, *et al*: The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science 342: 971-976, 2013.
16. Peled JU, Gomes ALC, Devlin SM, Littmann ER, Taur Y, Sung AD, Weber D, Hashimoto D, Slingerland AE, Slingerland JB, *et al*: Microbiota as predictor of mortality in allogeneic hematopoietic-cell transplantation. N Engl J Med 382: 822-834, 2020.
17. Yuan L, Wang W, Zhang W, Zhang Y, Wei C, Li J and Zhou D: Gut microbiota in untreated diffuse large B cell lymphoma patients. Front Microbiol 12: 646361, 2021.
18. Diefenbach CS, Peters BA, Li H, Raphael B, Moskovits T, Hymes K, Schluter J, Chen J, Bennani NN, Witzig TE and Ahn J: Microbial dysbiosis is associated with aggressive histology and adverse clinical outcome in B-cell non-Hodgkin lymphoma. Blood Adv 5: 1194-1198, 2021.
19. Yoon SE, Kang W, Choi S, Park Y, Chalita M, Kim H, Lee JH, Hyun DW, Ryu KJ, Sung H, *et al*: The influence of microbial dysbiosis on immunochemotherapy-related efficacy and safety in diffuse large B-cell lymphoma. Blood 141: 2224-2238, 2023.
20. Zeze K, Hirano A, Torisu T, Esaki M, Shibata H, Moriyama T, Umeno J, Fujioka S, Okamoto Y, Fuyuno Y, *et al*: Mucosal dysbiosis in patients with gastrointestinal follicular lymphoma. Hematol Oncol 38: 181-188, 2020.
21. Jakobsson HE, Jernberg C, Andersson AF, Sjölund-Karlsson M, Jansson JK and Engstrand L: Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PLoS One 5: e9836, 2010.
22. Rashidi A, Ebadi M, Rehman TU, Elhusseini H, Nalluri H, Kaiser T, Holtan SG, Khoruts A, Weisdorf DJ and Staley C: Gut microbiota response to antibiotics is personalized and depends on baseline microbiota. Microbiome 9: 211, 2021.
23. Carbone PP, Kaplan HS, Musshoff K, Smithers DW and Tubiana M: Report of the committee on Hodgkin's disease staging classification. Cancer Res 31: 1860-1861, 1971.
24. Solal-Céligny P, Roy P, Colombat P, White J, Armitage JO, Arranz-Saez R, Au WY, Bellei M, Brice P, Caballero D, *et al*: Follicular lymphoma international prognostic index. Blood 104: 1258-1265, 2004.
25. Alaggio R, Amador C, Anagnostopoulos I, Attygalle AD, Araujo IBO, Berti E, Bhagat G, Borges AM, Boyer D, Calaminici M, *et al*: The 5th edition of the World Health Organization classification of haematolymphoid tumours: Lymphoid neoplasms. Leukemia 36: 1720-1748, 2022.
26. Brice P, Bastion Y, Lepage E, Brousse N, Haïoun C, Moreau P, Straetmans N, Tilly H, Tabah I and Solal-Céligny P: Comparison in low-tumor-burden follicular lymphomas between an initial no-treatment policy, prednimustine, or interferon alfa: A randomized study from the groupe d'etude des lymphomes folliculaires. groupe d'etude des lymphomes de l'adulte. J Clin Oncol 15: 1110-1117, 1997.
27. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N and Knight R: Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci USA 108 (Suppl 1): S4516-S4522, 2011.
28. Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, Brown CT, Porras-Alfaro A, Kuske CR and Tiedje JM: Ribosomal database project: Data and tools for high throughput rRNA analysis. Nucleic Acids Res 42 (Database Issue): D633-D642, 2014.
29. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, *et al*: QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7: 335-336, 2010.
30. R Core Team: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2022. URL https://www.R-project.org/
31. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, *et al*: Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75: 7537-7541, 2009.
32. Pilgrim M and Willison S: Dive into python 3. Vol. 2. Springer, 2009.
33. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C and Langille MGI: PICRUSt2 for prediction of metagenome functions. Nat Biotechnol 38: 685-688, 2020.
34. Johansson ME, Jakobsson HE, Holmén-Larsson J, Schütte A, Ermund A, Rodríguez-Piñeiro AM, Arike L, Wising C, Svensson F, Bäckhed F and Hansson GC: Normalization of host intestinal mucus layers requires long-term microbial colonization. Cell Host Microbe 18: 582-592, 2015.
35. Gaboriau-Routhiau V, Rakotobe S, Lécuyer E, Mulder I, Lan A, Bridonneau C, Rochet V, Pisi A, De Paepe M, Brandi G, *et al*: The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. Immunity 31: 677-689, 2009.
36. Fattizzo B, Cavallaro F, Folino F and Barcellini W: Recent insights into the role of the microbiome in malignant and benign hematologic diseases. Crit Rev Oncol Hematol 160: 103289, 2021.
37. Yamamoto ML and Schiestl RH: Intestinal microbiome and lymphoma development. Cancer J 20: 190-194, 2014.
38. Xie J, Li LF, Dai TY, Qi X, Wang Y, Zheng TZ, Gao XY, Zhang YJ, Ai Y, Ma L, *et al*: Short-chain fatty acids produced by *Ruminococcaceae* mediate α-linolenic acid promote intestinal stem cells proliferation. Mol Nutr Food Res 66: e2100408, 2022.
39. Hall AB, Yassour M, Sauk J, Garner A, Jiang X, Arthur T, Lagoudas GK, Vatanen T, Fornelos N, Wilson R, *et al*: A novel *Ruminococcus* gnavus clade enriched in inflammatory bowel disease patients. Genome Med 9: 103, 2017.
40. Breban M, Tap J, Leboime A, Said-Nahal R, Langella P, Chiocchia G, Furet JP and Sokol H: Faecal microbiota study reveals specific dysbiosis in spondyloarthritis. Ann Rheum Dis 76: 1614-1622, 2017.