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Immune-microbiota dysregulation in maintenance hemodialysis: a 16S rRNA sequencing-based analysis of gut flora and T cell profiles

Yan Lv[a,b*], Xiuting Yang[c*], Xiaowu Sun[a,b] and Xiaohong Ren[d]

[a]Department of Nephrology, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Tongji Shanxi Hospital, Third Hospital of Shanxi Medical University, Taiyuan, China; [b]Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; [c]Department of Epidemiology, School of Public Health, Shanxi Medical University, Taiyuan, China; [d]Department of Intensive Care Unit, Lvliang People's Hospital, Lvliang City, China

ABSTRACT

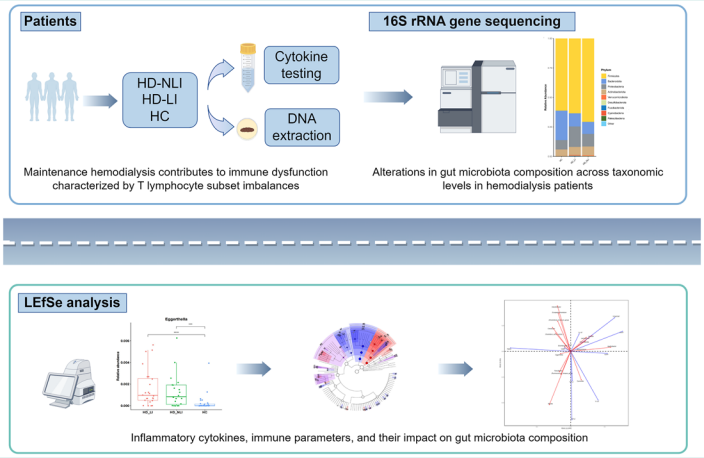
Background: Maintenance hemodialysis (MHD) patients frequently exhibit immune dysregulation and gut dysbiosis, both of which contribute to increased infection risk and adverse outcomes. However, the relationship between gut microbial composition and immune competence in this population remains underexplored.

Methods: This study assessed 45 MHD patients and 30 healthy controls, stratifying MHD patients into immunocompetent (HD-NLI, $CD4^+/CD8^+ \geq 1$) and immunodeficient (HD-LI, $CD4^+/CD8^+ < 1$) groups. Circulating cytokines (IL-6, IL-10, IL-12, TNF-α, IFN-γ) were quantified using ELISA. Gut microbiota profiles were derived *via* 16S rRNA gene sequencing (V3-V4 regions), followed by QIIME2 and LEfSe-based bioinformatics analyses.

Results: HD-LI patients displayed severe T cell dysregulation and elevated pro-inflammatory cytokines. Compared to controls, HD patients had reduced abundance of beneficial taxa (e.g., *Prevotella copri, Bacteroides vulgatus, Agathobacter*), and enrichment of pro-inflammatory taxa (e.g., *Escherichia-Shigella, Blautia, Citrobacter*). LEfSe identified 39 discriminatory taxa with distinct immune group signatures. Redundancy analysis revealed that $CD4^+$ levels, $CD4^+/CD8^+$ ratios, and TNF-α significantly shaped microbiota composition. Correlation analysis confirmed strong associations between immune parameters and microbial taxa involved in short-chain fatty acid (SCFA) metabolism.

Conclusion: This study provides novel evidence linking gut microbial dysbiosis to immune impairment in MHD patients. The findings suggest that SCFA-producing bacteria are depleted in immunodeficient states, offering a potential target for microbiota-directed immunomodulatory therapies in ESRD.

GRAPHICAL ABSTRACT



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CONTACT Xiaohong Ren renxiaohong72@126.com Department of Intensive Care Unit, Lvliang People's Hospital, No.17 Xueyuan Road, Lishi District, Lvliang City 033000, China

*These authors contributed equally to this work and should be considered co-first authors.

1. Introduction

End-Stage Renal Disease (ESRD) poses a major challenge to global public health [1]. The growing number of ESRD cases, influenced by an aging population and conditions like hypertension and diabetes, has resulted in a significant rise in affected individuals [2]. Maintenance hemodialysis (MHD), a critical and widely adopted renal replacement therapy, plays a central role in managing ESRD patients [3]. Approximately 90% of these patients depend on MHD to sustain essential life functions. However, infection remains a prevalent complication during hemodialysis. According to data from China's Dialysis and Transplantation Registry System, ESRD accounts for over one million deaths annually in China, with infection ranking as the second leading cause of mortality after cardiovascular disease. Immune dysfunction has been identified as a key contributor to infection in MHD patients [4,5], characterized by a pronounced suppression of T lymphocyte-mediated cell immunity. Additionally, gut microbiota imbalance, often found in MHD patients, is considered a significant cause of their compromised immune response [6,7].

The gut microbiota is a complex ecosystem comprising 500–1000 species and approximately 10^{14} bacterial cells [8], exceeding the total number of human cells by over tenfold [9]. Its collective genome, termed the microbiome, is more than 100 times larger than the human nuclear genome [10]. The gut microbiota, now seen as a crucial factor in human health, shows notable differences in composition and function between dialysis ESRD patients and healthy individuals [11]. Luo et al. further observed that hemodialysis patients exhibit more pronounced alterations in gut microbiota than pre-dialysis or peritoneal dialysis patients, suggesting an interactive relationship between microbial composition and clinical outcomes in ESRD patients [7]. Despite these findings, comparative analyses of immune function among dialysis ESRD patients remain limited. Addressing this gap necessitates elucidating the interplay between immune function and gut microbiota in post-dialysis ESRD patients.

To comprehensively investigate the mechanisms linking gut microbiota to immune function in maintenance hemodialysis patients, this study selected participants with varying immune function levels alongside healthy controls. Employing rigorous experimental design and advanced methodologies, the study conducted an in-depth comparison of gut microbiota alterations across these groups. Additionally, differential effects on T lymphocyte subsets and cytokine profiles were explored. These findings aim to provide novel, practical insights into improving immune function in hemodialysis patients, establishing a robust theoretical foundation for effective microbiota-targeted interventions to improve quality of life and overall health outcomes in this unique patient population.

2. Materials and methods

2.1. Patients

This study enrolled 45 patients undergoing maintenance hemodialysis at the Department of Nephrology, Shanxi Bethune Hospital, between June 2022 and December 2023. Patients were categorized into an immunocompetent group (HD-NLI, $n = 21$, CD4+/CD8+ ≥ 1) and an immunodeficient group (HD-LI, $n = 24$, CD4+/CD8+ < 1) based on CD4+/CD8+ ratios [12]. Furthermore, 30 healthy people who had physical exams at the same time were included as the control group (HC). The HD-LI group had an average age of 44.42 ± 13.48 years, including 10 males (41.6%) and 14 females (58.3%), whereas the HD-NLI group had an average age of 44.75 ± 13.68 years, with 12 males (57.1%) and 9 females (42.9%). The HC group had an average age of 41.29 ± 12.64 years, including 15 males (50%) and 15 females (50%). Inclusion criteria required patients to have undergone regular hemodialysis for ≥3 months, three times per week, with each session lasting 3–4 h, and to maintain a stable condition with normal dietary intake and independent living. Participants were excluded if they had acute or chronic infections, required enteral or parenteral nutritional support, or had coexisting conditions such as diabetes mellitus, immune system disorders, malignancies, or chronic gastrointestinal diseases. Additional exclusion criteria included a history of gallbladder or colon resection and recent use of antibiotics, steroids, immunosuppressants (within 1 month), or prebiotic-/probiotic-containing substances (within 6 months). Ethical approval for the study was obtained from the Medical Ethics Committee of Shanxi Bethune Hospital (Approval No.: YXLL-2022-105). All individuals consented to participate after being given comprehensive details about the study.

2.2. Blood sample collection and cytokine testing

Peripheral blood (3–5 mL) was collected from participants in the HD-NLI, HD-LI, and HC groups into EDTA anticoagulant tubes. After centrifugation at 3000 rpm for 10 min, plasma was isolated and stored at −80 °C. The concentrations of IL-6, IL-10, IL-12, TNF-α, and IFN-γ were measured using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol.

2.3. Fecal collection and DNA extraction

All participants independently collected fecal samples following detailed instructions to ensure sample integrity. Participants were advised to empty their bladder and prevent contamination with urine, water, or blood. Approximately 0.6 g of fecal matter was placed into sterile collection tubes containing DNA stabilization solution. Samples were flash-frozen in liquid nitrogen for 5–10 min and stored at −80 °C. The entire collection process was completed within 30 min. Quality control was performed on all samples to confirm compliance with the 'Quality Standards for DNA Sequencing Samples', and only those passing these criteria were processed further. Genomic DNA was extracted using a kit appropriate for the sample type. DNA concentration was quantified with a Qubit fluorometer, and integrity was verified *via* 1% agarose gel electrophoresis to ensure all samples met experimental standards.

2.4. 16S rRNA gene sequencing and data preprocessing

PCR was used to amplify the V3–V4 region of the 16S rRNA gene with the primer 341 F (5′-CCTACGGGNGGCWGCAG-3′). PCR conditions were as follows: initial denaturation at 95 °C for 3 min; 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 15 s; followed by a final extension at 72 °C for 5 min and storage at 4 °C. The resulting PCR products were pooled to construct sequencing libraries. Sequencing adapters were then added, followed by a second round of PCR with conditions as follows: initial denaturation at 98 °C for 45 s; 8 cycles of denaturation at 98 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s; with a final extension at 72 °C for 10 min and storage at 4 °C. Magnetic bead selection was used to purify libraries by eliminating primer dimers and small fragments, and the library index details were documented. The Illumina MiSeq platform was used to perform sequencing with the PE250 strategy. Preliminary sequencing data were retrieved, and paired-end reads were merged using an overlapping strategy. High-quality sequences were obtained through rigorous quality control and chimera removal. The DADA2 algorithm [13] was employed for data denoising, producing amplicon sequence variants (ASVs). Based on the ASVs, an operational taxonomic unit (OTU) table was constructed, resulting in an ASV feature table. The feature table was normalized using the Rarefy method [14].

2.5. Bioinformatics analysis of 16S rRNA gene sequencing

The QIIME2 plugin [15] feature-classifier fit-classifier-naive-bayes was employed to construct a classifier based on the specified database, and feature sequences were annotated using the feature-classifier classify-sklearn plugin. The gut microbiota α-diversity across the three groups was analyzed, including indices such as the observed Simpson index, Chao index, and Shannon index. β-diversity, reflecting overall differences between sample groups, was also assessed. Euclidean distance matrices were calculated based on bacterial composition, and principal coordinate analysis (PCoA) was performed using QIIME (v.1.9.1)[16] to visualize β-diversity among groups. To identify species with notable abundance differences between groups, the Kruskal-Wallis rank-sum test, a non-parametric method, was used for initial screening. This was followed by Wilcoxon rank-sum tests to confirm species-level variations across subgroups. Linear discriminant analysis (LDA) was then applied to quantify the contribution of species abundance to observed differential patterns. The composition of gut microbiota among the three groups was further examined using LEfSe [17], identifying microbial taxa with significant intergroup differences based on an LDA score >2 and $p < 0.05$. The connections between gut microbiota communities and environmental factors were analyzed using redundancy analysis (RDA).

2.6. Statistical analysis

SPSS 26.0 statistical software was used to perform data analysis. Continuous variables underwent tests for normality and variance homogeneity. Normally distributed data were expressed as mean ± standard deviation ($x \pm s$), while non-normally distributed data were expressed as the median (interquartile range). Independent sample t-tests were used for comparisons of normally distributed data, while non-parametric rank-sum tests were applied for non-normal distributions. Categorical variables, presented as frequencies and percentages, were analyzed using the χ^2 test or Fisher's exact test. Statistical significance was defined as $p < 0.05$ ($\alpha = 0.05$). Correlation analyses were also conducted using Spearman's method.

3. Results

3.1. Patient clinical characteristics

This study contained 45 patients undergoing maintenance hemodialysis and 30 healthy individuals as controls (Table 1). Baseline characteristics comparison revealed significant differences in monocytes, hemoglobin, platelets, albumin, serum creatinine, parathyroid hormone, urea, and uric acid levels across the three groups ($p < 0.001$), while no significant differences were observed for other clinical parameters. Additionally, T lymphocyte subsets were analyzed among hemodialysis patients with varying immune statuses and healthy controls (Table 2). Compared to the HC group, both the HD-NLI and HD-LI groups exhibited significant reductions in peripheral blood CD3+ cells [76.90 ± 4.98%, 71.11 ± 7.27%, and 67.61 ± 5.51%], CD4+ cells [49.67 ± 5.43%, 40.70 ± 5.29%, and 30.52 ± 3.28%], and CD4+/CD8+ ratios [1.69 (1.53, 1.82), 1.43 (1.22, 1.64), and 0.83 (0.77, 0.92)]. Moreover, the HD-LI group demonstrated significantly lower CD4+ cells [40.70 ± 5.29% vs. 30.52 ± 3.28%] and CD4+/CD8+ ratios [1.43 (1.22, 1.64) vs. 0.83 (0.77, 0.92)] compared to the HD-NLI group. Conversely, CD8+ cells were significantly elevated in the HD-LI group compared to both the HC and HD-NLI groups [29.83 (27.42, 32.10), 28.13 (24.95, 30.99), and 36.68 (30.70, 39.33), respectively] ($p < 0.05$). These results suggest that maintenance hemodialysis contributes to immune dysfunction characterized by T lymphocyte subset imbalances, particularly pronounced in HD-LI patients.

3.2. Alterations in gut microbiota composition across taxonomic levels in hemodialysis patients

Annotation analysis identified six taxonomic levels: phylum, class, order, family, genus, and species (Figure 1A–F). At the phylum level, *Firmicutes* and *Proteobacteria* showed increased abundance in both the HD-NLI and HD-LI groups compared to the HC group. At the class level, *Gammaproteobacteria* abundance was significantly elevated in both hemodialysis groups. At the order and family levels, *Enterobacterales* and *Enterobacteriaceae* showed significant increases in both the HD-NLI and HD-LI groups. At the genus level, *Escherichia-Shigella* and *Blautia* were markedly more abundant in hemodialysis patients, while at the species level, *Prevotella_copri* and *Bacteroides_vulgatus* were significantly reduced in both groups. These results highlight distinct

Table 1. Comparison of basic clinical data of the study subjects.

	HC group ($n=30$)	HD-NLI group ($n=21$)	HD-LI group ($n=24$)	F/x2	p
Age (years)	41.29 ± 12.64	44.75 ± 13.68	44.42 ± 13.48	0.51	0.608
Sex (male, %)	15 (50%)	12 (57.1%)	10 (41.6%)	–	0.517*
BMI (kg/m^2)	22.35 ± 3.01	22.31 ± 2.83	22.05 ± 3.43	0.06	0.937
Dialysis vintage (months)	–	59.38 ± 43.56	68.25 ± 53.89	0.19	0.665
Lymphocytes (10^9/L)	2.01 ± 0.54	1.50 ± 0.53	1.16 ± 0.52	1.97	0.146
Neutrophils (10^9/L)	5.32 ± 1.43	4.38 ± 2.01	3.65 ± 0.95	2.49	0.122
Monocytes (10^9/L)	0.50 ± 0.16^a	0.45 ± 0.18^b	0.31 ± 0.11^c	9.8	0.003
Hemoglobin (g/L)	142.73 ± 9.06^a	108.51 ± 11.92^b	104.87 ± 14.14^c	9.16	<0.001
Platelets (10^9/L)	254.01 ± 0.54^a	199.01 ± 65.97^b	155.72 ± 46.39^c	6.03	0.019
Albumin (g/L)	45.06 ± 3.08^a	40.73 ± 2.98^b	38.39 ± 2.47^c	8.40	<0.001
Serum creatinine (μmol/L)	62.35^a (74.00, 85.19)	811.88^b (778.25, 938.25)	899.97^b (786.05, 988.75)	–	<0.001$^\#$
Parathyroid hormone (pg/ml)	–	219.72 ± 90.06^a	301.00 ± 112.91^b	6.67	0.013
Urea (mmol/L)	4.87 ± 8.32^a	28.36 ± 7.67^b	29.60 ± 7.64^b	0.28	<0.001
Uric acid (μmol/L)	240.34 ± 97.5^a	431 ± 70.52^b	449.07 ± 68.83^b	10.16	<0.001
Triglyceride (mmol/L)	1.46 ± 0.53	1.89 ± 1.87	1.52 ± 0.76	0.727	0.399
Total cholesterol (mmol/L)	3.67 ± 0.54	3.50 ± 0.87	3.39 ± 0.76	0.21	0.653

Note: *Fisher exact test, #Kruskal-Wallis H test; abc different letters indicate statistically significant differences, $p<0.05$ for comparison between the three groups, $p<0.017$ for two-by-two comparison between groups.

Table 2. Comparison of the proportions of T lymphocyte subpopulations in hemodialysis patients with different immunity and healthy controls.

	HC group ($n=30$人)	HD-NLI group ($n=21$)	HD-LI group ($n=24$)	F/Z	p
CD3+(%)	76.90 ± 4.98^a	71.11 ± 7.27^b	67.61 ± 5.51^b	−7.861	<0.001
CD4+(%)	49.67 ± 5.43^a	40.70 ± 5.29^b	30.52 ± 3.28^c	−9.891	<0.001
CD8+(%)	29.83^a (27.42, 32.1)	28.13^a (24.95, 30.99)	36.68^b (33.70, 39.33)	7.005	<0.001
CD4+/CD8+	1.69^a (1.53, 1.82)	1.43^b (1.22, 1.64)	0.83^c (0.77, 0.92)	−4.251	<0.001

Note: Different abc letters indicate statistically significant differences, $p<0.05$ for comparison between the three groups and $p<0.017$ for two-by-two comparison between groups.

alterations in gut microbiota composition among the groups. The increased abundance of taxa such as *Escherichia-Shigella* and *Blautia* may be associated with the dialysis process, while reductions in *Prevotella_copri* and *Bacteroides_vulgatus* likely indicate disruptions in gut microbial homeostasis. These observations provide critical insights for further exploration of underlying mechanisms.

3.3. Sequencing depth and diversity analysis of gut microbiota

Rarefaction curves were employed to evaluate whether the sequencing data adequately captured species diversity and abundance within the samples. Comparison of ASV rarefaction curves across the three groups showed that all curves plateaued with increasing sequencing depth (Supplementary Figure 1A), indicating sufficient sequencing depth to represent species diversity in each sample. Analysis of α-diversity revealed no statistically significant differences in the Chao, Shannon, or Simpson indices among the HC, HD-NLI, and HD-LI groups ($p>0.05$) (Supplementary Figure 1B-D). β-diversity was evaluated through PCoA using weighted UniFrac distances, revealing distinct clustering of sample points for each group. However, partial overlap was noted between the HC group and the HD-NLI and HD-LI groups. Additionally, some overlap occurred between the HD-NLI and HD-LI groups, indicating compositional differences in gut microbiota between the HC group and the hemodialysis groups, with similarities in community structure between the HD-NLI and HD-LI groups (Figure 2A).

3.4. Differential gut microbial composition and key taxa identified by LEfSe analysis

LEfSe analysis was conducted to identify region-specific bacterial taxa and their dominance characteristics among the groups, with results visualized using cladograms and LDA score bar plots (Figure 2B–C). In total, 39 differential taxa were detected, showing notable changes at the phylum, family, and order levels within the HD-LI group. Thirteen taxa, including *Proteobacteria*, *Gammaproteobacteria*, *Enterobacterales*, and *Enterobacteriaceae*, were enriched in the HD-LI group, potentially reflecting dialysis-related inflammation or metabolic disturbances. In the HD-NLI group, nine taxa such as *Bacilli*, *Blautia*, *Peptostreptococcales Tissierellales*, and *Peptostreptococcaceae* were enriched, defining its characteristic microbiota. Conversely, 17 taxa, including *Bacteroidota*, *Bacteroidia*, *Bacteroidales*, *Prevotellaceae*, and *Agathobacter*, were enriched in the HC group, indicative of a microbiota conducive to gut homeostasis in healthy individuals. These differences were statistically significant ($p<0.05$).

At the family level, *Eggerthella* and *Blautia* demonstrated the highest relative abundance in the HD-LI group ($p=0.001$), highlighting their potential importance in the gut ecosystem of dialysis patients. *Erysipelatoclostridium* exhibited the highest relative abundance in the HD-NLI group. Compared to the HC group, *Lachnospira*, and *Butyricicoccus* had the lowest relative abundance in both HD-LI and HD-NLI groups, suggesting compromised gut microbial functionality in dialysis patients (Figure 3A–E). At the genus level, *Citrobacter*, *Intestinibacter*, and *Faecalitalea* were most abundant in the HD-LI group, while *Tyzzerella* showed reduced abundance compared to the HC and HD-NLI groups (Figure 3F–I). These

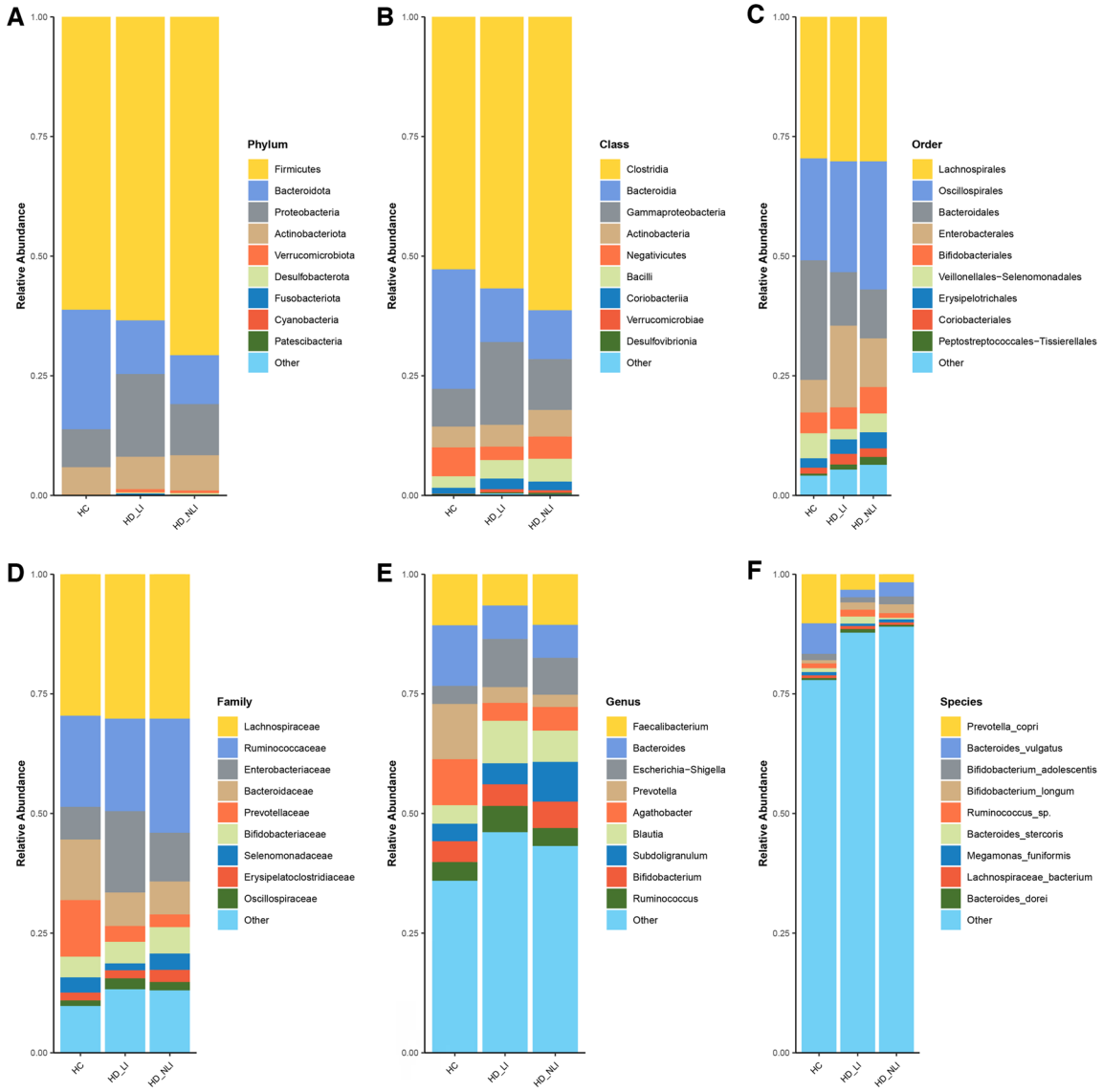

Figure 1. Species annotation and compositional analysis. (A–F) Bar charts of species distribution at the phylum, class, order, family, genus, and species levels in HC, HD-LI, and HD-NLI groups.

results underscore the profound impact of dialysis on gut microbial composition and offer valuable insights into potential clinical biomarkers and intervention strategies for restoring gut balance in dialysis patients.

3.5. Inflammatory cytokines, immune parameters, and their impact on gut microbiota composition

Compared to the HC group, patients in the HD-NLI and HD-LI groups exhibited significantly elevated levels of IL-6 [5.56 (4.49, 6.80), 13.68 (9.81, 16.76), and 17.05 (15.04, 19.13)], IL-10 [7.84 ± 1.86, 14.27 ± 5.13, and 18.83 ± 4.56], TNF-α [45.40 ± 14.21, 55.71 ± 12.96, and 74.01 ± 10.56], and IFN-γ [5.76 ± 1.39, 6.76 ± 1.61, and 7.20 ± 1.17]. IL-12 levels were significantly higher in the HD-LI group [7.76 ± 2.45, 8.92 ± 1.45, and 10.46 ± 2.10] compared to both the HC and HD-NLI groups ($p < 0.017$). Additionally, comparisons between HD-LI and HD-NLI patients revealed significant increases in IL-6, IL-10, TNF-α, and IFN-γ levels in the HD-LI group ($p < 0.017$) (Table 3). RDA identified CD4+, CD4+/CD8+ ratio, IL-12, CD3+, and TNF-α as the primary clinical indicators influencing gut microbial community structure ($p < 0.05$). Correlation analysis further showed strong associations between CD4+ levels, CD4+/CD8+ ratios, and gut microbiota distribution patterns. Specifically, CD4+ levels and CD4+/CD8+ ratios were positively correlated with *Lachnospira* abundance but negatively correlated with *Eggerthella* abundance ($p < 0.05$) (Table 4 and Figure 4). These results highlight that inflammatory cytokines and immune parameters vary significantly across patient groups and play a pivotal role in shaping gut microbiota composition. The observed immune-microbiota interactions suggest that these factors may critically influence host health, underscoring their potential impact at multiple physiological levels.

4. Discussion

The alterations in gut microbiota composition among HD patients are still under discussion. Nevertheless, most

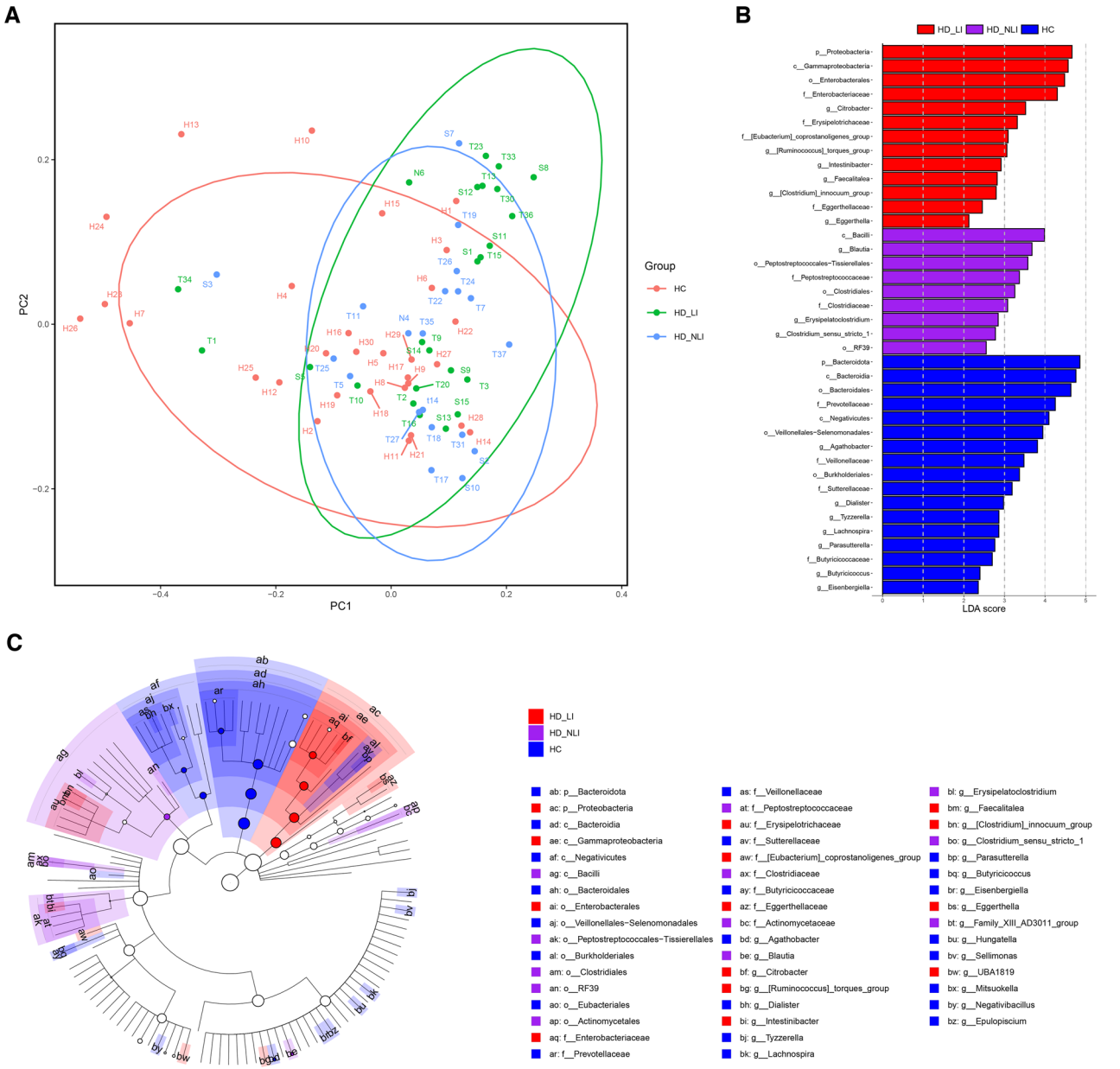

Figure 2. β-Diversity analysis and differential species abundance. (A) PCoA. (B) Histogram of LDA value distribution. (C) Evolutionary branching diagram.

research agrees that the gut microbiota of HD patients differs markedly from that of healthy individuals in terms of both α-diversity and β-diversity. Lin et al. [18] reported that HD patients taking β-blockers exhibited higher α-diversity and distinct β-diversity compared to those not taking β-blockers. Similarly, Li Sheng et al. [19] reported that HD patients exhibited lower α-diversity compared to healthy individuals, though the difference was not statistically significant. In contrast, β-diversity showed significant separation between HD patients and healthy controls. Contrarily, an international study [20] found that gut microbiota diversity in HD patients was significantly increased compared to healthy individuals. These inconsistencies suggest that variations in study populations, sample characteristics, or analytical methods may contribute to the differing conclusions. This study focused on exploring the connection between immune function and gut microbiota in HD patients. The findings revealed that, compared to the HC group, HD patients exhibited a trend of increased α-diversity, although the difference was not statistically significant. β-diversity analysis revealed significant separation among the three groups (HC, HD-LI, and HD-NLI), reflecting marked differences in gut microbiota composition and structure. Stratified analysis based on immune function further showed that the α-diversity in the low-immunity HD-LI group was numerically lower than in the high-immunity HD-NLI group, suggesting a potential link between reduced immune function and alterations in gut microbiota composition and distribution. This finding differs from some previous reports, which could be explained by variations in study populations, geographic regions, or study design. For example, medication use, especially β-blockers, may affect gut microbiota composition and could explain

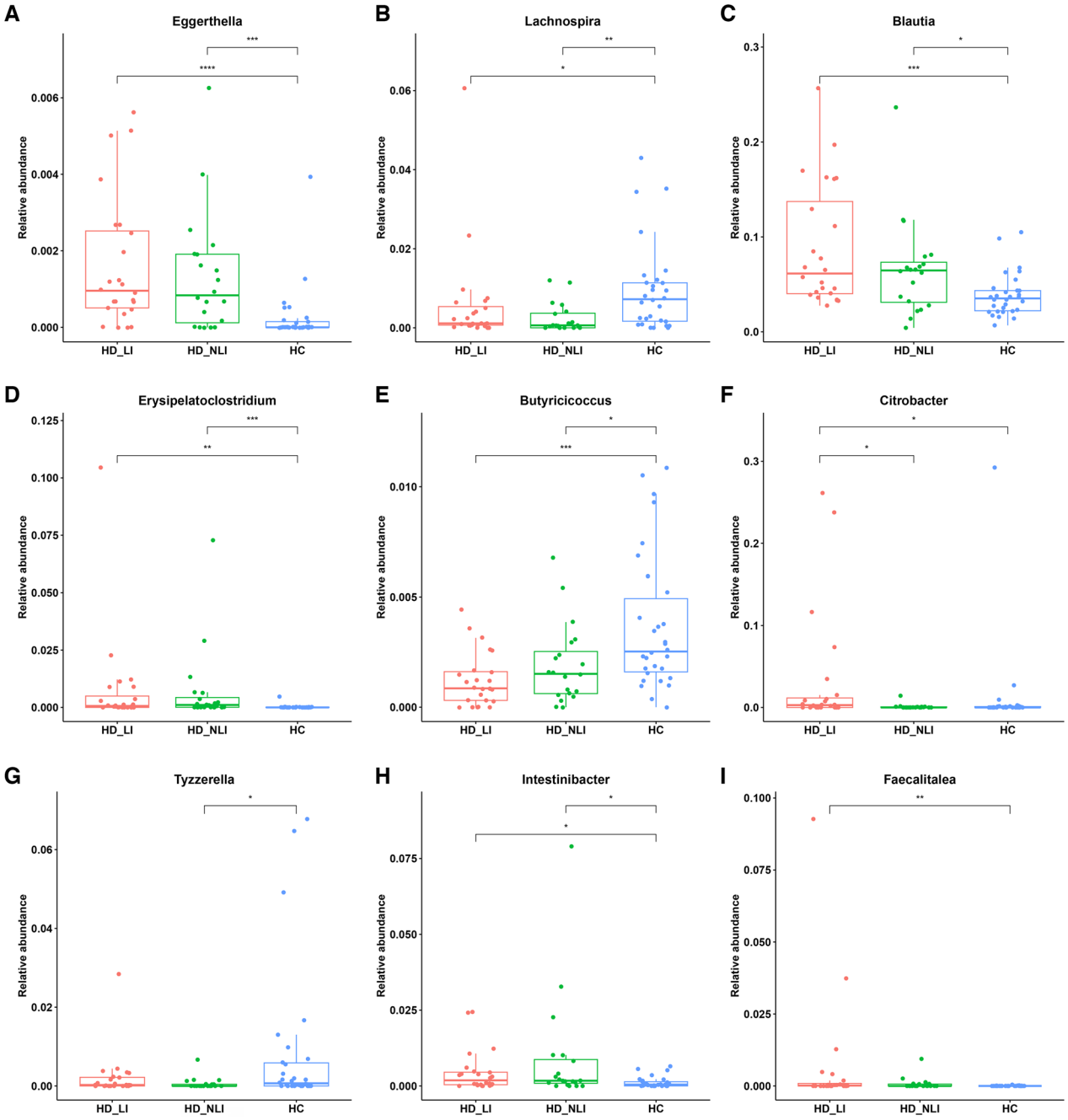

Figure 3. Differential species analysis. (A–E) Differential species at the family level. (F–I) Differential species at the genus level.

Table 3. Comparison of cytokines in hemodialysis patients with different immunity and healthy controls.

	HC group ($n=30$)	HD-NLI group ($n=21$)	HD-LI group ($n=24$)	F/Z	p
IL-6 (pg/ml)	5.56[a] (4.49, 6.80)	13.68[b] (9.81, 16.76)	17.05[c] (15.04, 19.13)	18.781	<0.001
IL-10 (pg/ml)	7.84 ± 1.86[a]	14.27 ± 5.13[b]	18.83 ± 4.56[c]	15.747	<0.001
IL-12 (pg/ml)	7.76 ± 2.45[a]	8.92 ± 1.45[a]	10.46 ± 2.10[b]	4.92	<0.001
TNF-α (pg/ml)	45.40 ± 14.21[a]	55.71 ± 12.96[b]	74.01 ± 10.56[c]	5.889	<0.001
IFN-γ (pg/ml)	5.76 ± 1.39[a]	6.76 ± 1.61[b]	7.20 ± 1.17[b]	7.651	<0.001

Note: Different abc letters indicate statistically significant differences, $p<0.05$ for comparison between the three groups and $p<0.017$ for two-by-two comparison between groups.

the diversity observed in other studies [21]. Additionally, dietary and lifestyle differences, including the use of antibiotics and other immunosuppressants, could play a significant role in shaping the gut microbiota in HD patients. While our study did not control for all these factors, future studies should consider these variables to better understand the dynamics between immune function and gut microbiota composition in HD patients.

Comparative analysis of clinical characteristics revealed significantly elevated serum creatinine, urea, and uric acid levels in HD patients. The accumulation of these metabolic waste products may impair digestive and absorptive functions, resulting in the retention of proteins and amino acids in the intestinal lumen. These retained substrates provide a favorable environment for bacterial enzymatic activity, promoting the overgrowth of specific bacterial populations, particularly *Escherichia coli*. This imbalance further disrupts the gut microbial composition in HD patients, marked by a significant decline in beneficial bacteria and a rise in the relative abundance of potentially harmful bacteria [20]. This mechanism likely contributes to the abnormal distribution of gut microbiota in HD patients and may exacerbate clinical symptoms and complications. Previous studies have provided additional insights into specific changes in the gut microbiota of HD patients. Sun Weiqian et al. [22] reported significantly reduced levels of *Bifidobacterium* and *Lactobacillus acidophilus* in HD patients compared to healthy individuals, accompanied by elevated levels of *Escherichia coli* and *Enterococcus faecalis*.

Table 4. Association of intestinal flora with clinical indicators.

	RDA1	RDA2	r^2	*p*
CD3+	0.998	−0.063	0.119	0.018
CD4+	0.989	0.146	0.242	0.001
CD8+	−0.958	0.285	0.004	0.875
CD4/CD8	0.949	0.315	0.196	0.001
IL-6	−0.695	0.719	0.001	0.956
IL-10	0.708	0.706	0.014	0.605
IL-12	0.765	−0.644	0.136	0.009
TNF-α	0.088	−0.996	0.129	0.012
IFN-γ	0.178	−0.984	0.024	0.455

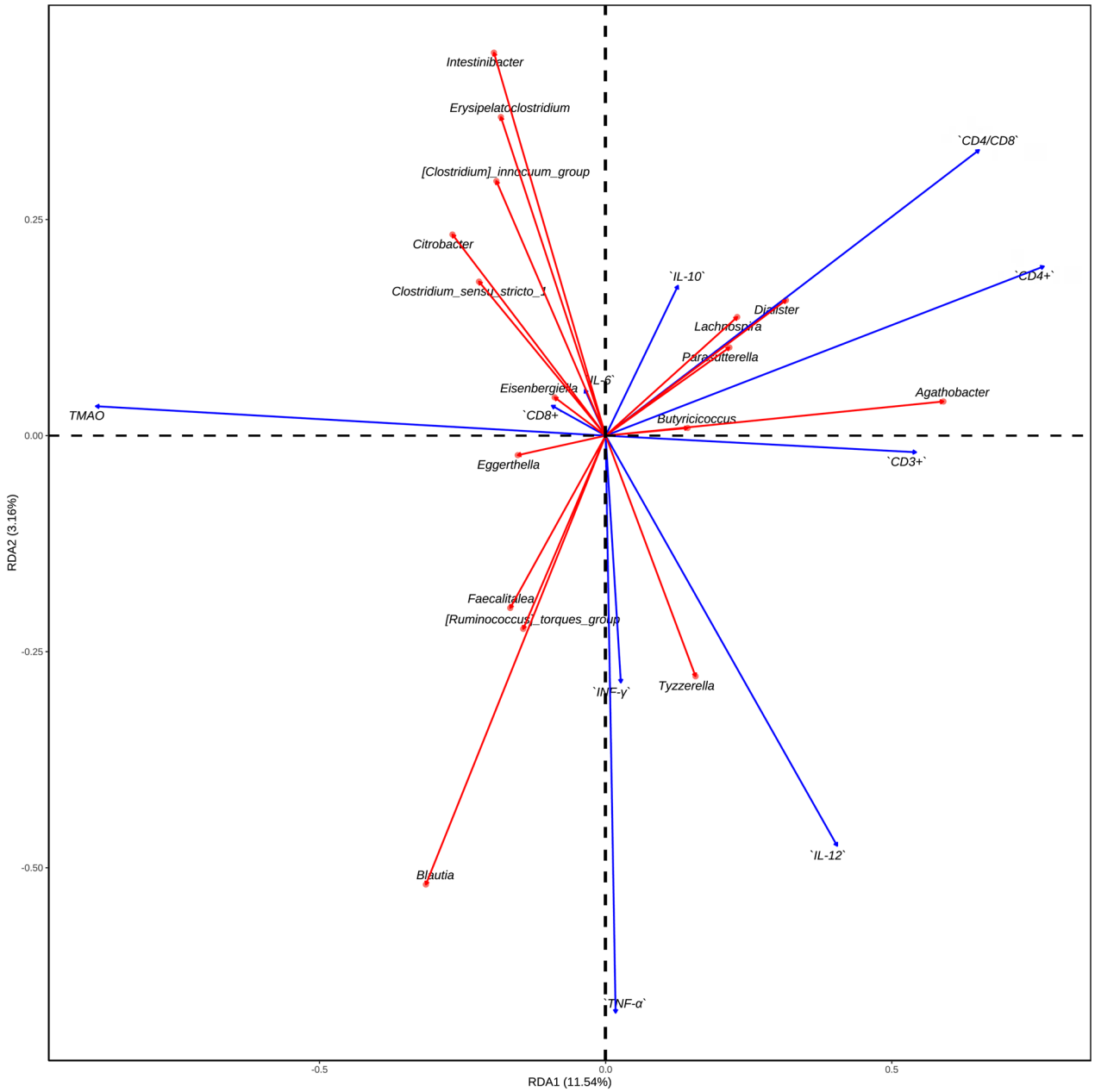

Figure 4. RDA/CCA ordering diagram at the genus level.

Similarly, an international study [23] noted an increased abundance of *Bacteroides fragilis* in HD patients. Importantly, polysaccharide A produced by *Bacteroides fragilis* has been shown to activate T cell-dependent immune responses [24]. Additionally, Luo Dan et al. [25] observed a decrease in *Prevotellaceae* abundance alongside an increase in *Ruminococcaceae* in HD patients. Hu et al. [26] similarly reported a significant reduction in the genus-level abundance of *Prevotella*. The findings of this study align partially with these observations. Compared to healthy controls, HD patients exhibited lower relative abundances of *Prevotellaceae*, *Bacteroidaceae*, *Ruminococcaceae*, and other SCFA-producing bacteria at the family level, alongside a significant increase in Enterobacteriaceae. At the genus level, a decrease in *Prevotella* and *Agarivorans* was detected, whereas *Faecalibacterium prausnitzii*, known for its anti-inflammatory and prebiotic effects, showed a notable increase. Additionally, immune function analysis revealed that the HD-LI group had a significantly greater relative abundance of *Citrobacter spp*. compared to the HD-NLI group. As a pathogenic member of *Enterobacteriaceae*, *Citrobacter* has been implicated in severe intestinal inflammation in animal models [27,28]. In summary, this study highlights significant disruptions in the gut microbial ecosystem of HD patients. The overproliferation of harmful bacteria appears to drive detrimental effects through various mechanisms, occupying ecological niches that would otherwise sustain beneficial bacteria and leading to their diminished abundance. This dysbiosis likely exacerbates metabolic disturbances and impairs immune function, ultimately contributing to disease progression and a heightened risk of complications.

The health of the gut microbiota is intricately linked to immune function. Studies have shown that a decline in gut microbiota diversity can lead to immune dysfunction and increase the host's susceptibility to intestinal pathogens [29]. Furthermore, the gut microbiota influences the function of antigen-presenting cells, affecting the differentiation of helper T cells (Th1, Th2, Th17) and immunosuppressive Treg cells, thereby maintaining immune homeostasis [30,31]. The gut microbiota exerts a significant impact on immune function through its metabolic products, particularly short-chain fatty acids (SCFAs). SCFAs not only provide energy to intestinal epithelial cells but also enhance intestinal barrier function and exert anti-inflammatory and immunomodulatory effects by promoting the production of Treg [32,33]. The production of SCFAs relies on the metabolic activity of specific symbiotic bacterial communities. For instance, *Prevotellaceae bacteria* possess key enzymes, including phosphotransbutyrylase and butyrate kinase, essential for butyrate synthesis. The abundance of beneficial bacteria such as *Bifidobacteriaceae*, *Prevotellaceae*, and *Lactobacillaceae* significantly influences SCFA production. A decline in these bacterial populations leads to reduced butyrate levels, resulting in insufficient SCFA availability [32]. Butyrate and propionate, as endogenous ligands of GPR41/GPR43 [34], can activate the adenylyl cyclase inhibition pathway, reducing intracellular cAMP levels, thereby suppressing NF-κB nuclear translocation [35]. This process downregulates the expression of pro-inflammatory factors through the PI3K/Akt/mTOR signaling axis, alleviating renal inflammation [36]. Additionally, *Lachnospiraceae*, a protective symbiotic family, ferments dietary fiber to produce SCFAs, playing a vital role in maintaining host health [37]. SCFAs exert their biological effects primarily by binding to G-protein-coupled receptors (GPCRs), inhibiting histone deacetylases, regulating the renin-angiotensin system (RAS), modulating inflammatory responses, and influencing autophagy processes. These mechanisms enable SCFAs to mitigate renal inflammation and fibrosis [38]. Moreover, SCFAs stimulate the production of anti-inflammatory factors such as TGF-β and IL-10, enhance epithelial barrier function, and participate in immunoregulatory processes [39]. In the present study, HD patients demonstrated significantly reduced abundances of *Prevotellaceae*, *Lachnospiraceae*, and other SCFA-producing bacteria at the family level compared to healthy controls. At the genus level, the relative abundances of *Prevotella* and *Agathobacter* were notably lower in HD patients. Agathobacter, a core prebiotic bacterium, is closely associated with immunoregulation and anti-inflammatory functions in various inflammation-related diseases [40]. Previous research has shown that *Lachnospiraceae* and *Agathobacter* species exert significant anti-inflammatory and immunomodulatory effects through SCFA production, which may be essential for maintaining gut microbial balance and systemic immune function.

This study revealed a significant reduction in SCFA-producing gut microbiota in maintenance HD patients compared to healthy controls, which may represent a critical factor underlying impaired immune function in this population. Given the central role of SCFAs in maintaining intestinal epithelial barrier integrity, exerting anti-inflammatory effects, and regulating systemic immunity, their depletion likely exacerbates clinical symptoms and heightens the risk of complications in HD patients. Restoring gut microbial balance to enhance SCFA production capacity emerges as a promising intervention strategy to improve immune function and clinical outcomes for these patients. However, this study has several limitations. First, the small sample size may limit statistical power and the generalizability of the findings. Second, the cross-sectional design precludes establishing causal relationships, only identifying correlations. Future larger-scale, multicenter, longitudinal studies are needed to confirm these preliminary findings and evaluate long-term effects. Additionally, lifestyle factors such as dietary habits, medication use, smoking, and physical activity may significantly impact gut microbiota and immune function. Due to time and resource constraints, this study did not fully account for these factors. Dietary habits play a crucial role in shaping the composition of gut microbiota, with studies indicating that different dietary patterns can lead to significant microbial differences and subsequently affect immune system function [41]. Moreover, medication use, including antibiotics and immunosuppressants, can markedly alter gut microbiota and influence immune responses [21]. Since these factors were not systematically considered in this study, their potential

impact on the results cannot be ruled out. Future research should aim to incorporate more comprehensive data collection and multifactorial analysis to better understand how these lifestyle factors influence the relationship between gut microbiota and immune function.

In conclusion, HD patients exhibit gut microbiota dysbiosis characterized by reduced species diversity and distinct alterations in specific genera. This impaired gut microbial functionality, closely associated with immune dysfunction, may contribute to disease progression and worsen patient outcomes. Interventions such as probiotic supplementation and fecal microbiota transplantation, already employed in treating conditions like irritable bowel syndrome, obesity, diabetes, and Alzheimer's disease, hold potential for application in maintenance hemodialysis patients. Ongoing progress in these therapeutic methods could lead to effective ways to boost immune function and enhance the quality of life for this patient group.

Disclosure statement

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Data availability statement

The data support the findings of this study are available on request from the corresponding author.

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