Original Article

Lower respiratory tract microbiome is associated with checkpoint inhibitor pneumonitis in lung cancer patients

Dongming Zhang[1#], Junping Fan[1#], Xiaoyan Liu[1], Xiaoxing Gao[1], Qing Zhou[1], Jing Zhao[1], Yan Xu[1], Wei Zhong[1], In-Jae Oh[2], Minjiang Chen[1], Mengzhao Wang[1]

[1]Department of Respiratory and Critical Care Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China; [2]Department of Internal Medicine, Chonnam National University Medical School and Hwasun Hospital, Jeonnam, Republic of Korea

Contributions: (I) Conception and design: M Chen, W Zhong; (II) Administrative support: M Wang; (III) Provision of study materials or patients: All authors; (IV) Collection and assembly of data: D Zhang, J Fan; (V) Data analysis and interpretation: D Zhang, J Fan; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Minjiang Chen, MD; Wei Zhong, MD. Department of Respiratory and Critical Care Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, No. 1 Shuaifuyuan Wangfujing, Dongcheng District, Beijing 100730, China. Email: minjiangchen@163.com; zw_pumch@126.com.

Background: The gut microbiome is associated with the occurrence and severity of immune-related adverse events (irAEs) in cancer patients undergoing immunotherapy. However, the relationship between the lower respiratory tract (LRT) microbiome and checkpoint inhibitor pneumonitis (CIP) in lung cancer patients who underwent immunotherapy is unclear. The aim of the present study was to investigate the associations between the LRT microbiome and CIP in lung cancer patients receiving immunotherapy.

Methods: This retrospective study included lung cancer patients who received immunotherapy and had metagenomic next-generation sequencing (mNGS) results of LRT specimens [bronchoalveolar lavage fluid (BALF)]. Based on their final diagnosis, the patients were allocated to either the CIP group or the non-CIP group. We conducted an exploratory analysis of the LRT microbiome in the CIP and non-CIP patients, delineating the microbial composition, and comparing the differences between the two groups.

Results: In total, 52 lung patients were included in the study, of whom 33 were allocated to the CIP group and 19 to the non-CIP group. The alpha- and beta-diversity analyses revealed no significant differences between the two groups. In the CIP group, the dominant phyla were *Firmicutes* (41.7%), *Acinetobacter* (18.2%), and *Proteobacteria* (16.3%). In the non-CIP group, the dominant phyla were *Firmicutes* (38.2%), *Acinetobacter* (18.4%), and *Proteobacteria* (17.8%). Notably, the relative abundance of the *Proteobacteria* phylum (P<0.001) and *Firmicutes* phylum (P=0.01) was significantly higher in the CIP group than the non-CIP group.

Conclusions: The elevated relative abundance of the *Proteobacteria* and *Firmicutes* phyla in the LRT samples is associated with CIP in lung cancer patients.

Keywords: Lower respiratory tract microbiome (LRT microbiome); checkpoint inhibitor pneumonitis (CIP); metagenomic next-generation sequencing (mNGS); immunotherapy

Submitted Sep 19, 2024. Accepted for publication Nov 14, 2024. Published online Nov 27, 2024.
doi: 10.21037/tlcr-24-853
View this article at: https://dx.doi.org/10.21037/tlcr-24-853

Introduction

The advent of immune checkpoint inhibitors (ICIs) revolutionized the treatment landscape of lung cancer, providing significant survival benefits by harnessing the body's immune system (1,2). However, in addition to their remarkable efficacy, ICIs also have a distinct spectrum of immune-related adverse events (irAEs), among which checkpoint inhibitor pneumonitis (CIP) stands out as a significant concern (3-5). CIP, which is characterized by inflammation of the lung parenchyma secondary to immune activation, poses a formidable challenge in the management of lung cancer patients undergoing immunotherapy (6-10).

The human microbiome is associated with the development and progression of various diseases (11-15). Studies have shown that the gut microbiota is also associated with irAEs in cancer patients receiving immunotherapy (16-18). For instance, in patients with hepatobiliary cancers, a higher relative abundance of *Prevotellamassilia timonensis* in the gut microbiota was found to be correlated with more severe immunotherapy-related colitis (16). Similarly, in patients with melanoma, a higher abundance of *Bacteroides intestinalis* was linked to severe irAEs (17). Additionally, in non-small-cell lung cancer (NSCLC) patients, *Bacteroides dorei* was found to be enriched in those experiencing ICI-related skin toxicity (18).

The predominant phyla in healthy lungs are *Bacteroidetes* and *Firmicutes* (19). The disruption of the lung microbiome is associated with various lung diseases such as asthma, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, and lung cancer (20-29). Moreover, the lung microbiome is believed to be associated with the prognosis of lung cancer patients receiving immunotherapy (30,31). However, the relationship between the lung microbiome and irAEs, particularly CIP, remains unclear. This relationship is crucial to understand the onset of irAEs from the microbial perspective and for the early prediction of such events.

Hence, our study sought to address this gap by analyzing of the lower respiratory tract (LRT) microbiome in lung cancer patients undergoing immunotherapy and experiencing CIP using metagenomic next-generation sequencing (mNGS) (32). Our analysis shed light on the microbial differences between CIP and non-CIP patients, providing insights into the association between the LRT microbiome and the occurrence of CIP in the context of lung cancer immunotherapy. We present this article in accordance with the STROBE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-853/rc).

Highlight box

Key findings

- The relative abundance of the *Proteobacteria* phylum (P<0.001) and *Firmicutes* phylum (P=0.01) was significantly higher in the checkpoint inhibitor pneumonitis (CIP) group than the non-CIP group.

What is known, and what is new?

- The gut microbiome is associated with the occurrence and severity of immune-related adverse events in cancer patients undergoing immunotherapy.
- The lower respiratory tract (LRT) microbiome is associated with CIP in lung cancer patients.

What is the implication, and what should change now?

- The relative abundance of the *Proteobacteria* and *Firmicutes* phyla in the LRT may hold potential as a biomarker for CIP in lung cancer patients receiving immune checkpoint inhibitors. Studies with more patients are needed to confirm these results.

Methods

Patients

This retrospective study included a total of 52 patients diagnosed with lung cancer who underwent immunotherapy at Peking Union Medical College Hospital between October 1, 2020, and July 1, 2024. All patients had undergone mNGS analysis of LRT specimens, specifically bronchoalveolar lavage fluid (BALF). The patients were allocated to the CIP and non-CIP groups based on their final diagnosis determined via multidisciplinary discussion.

A diagnosis of CIP was considered if all three of the following criteria were met: (I) the patient had received treatment with ICIs; (II) imaging studies revealed new lung shadows; and (III) other potential causes such as lung infection, lung tumor progression, interstitial lung disease, pulmonary vasculitis, pulmonary embolism, and pulmonary edema were excluded.

mNGS

BALF specifically refers to BALF obtained through bronchoalveolar lavage (BAL) procedures. All BALF samples were collected via BAL procedures at sites of

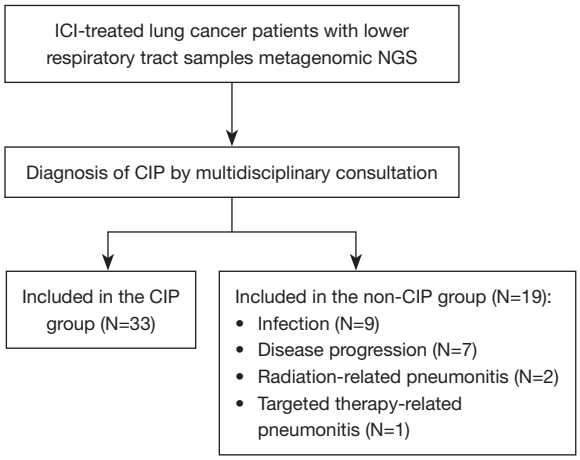

Figure 1 Flow chart of study design. ICI, immune checkpoint inhibitor; NGS, next-generation sequencing; CIP, checkpoint inhibitor pneumonitis.

suspected pneumonia identified through imaging. To ensure sample quality, we defined a qualified BALF sample as one obtained by instilling 100 mL of sterile saline solution and recovering at least 35 mL of fluid.

Samples were then transported to the laboratory under cold-chain conditions to ensure rapid and secure delivery. Subsequently, next-generation sequencing (NGS) analysis and bioinformatics services were provided by KingMed Diagnostics (Tianjin, China). After sample homogenization, nucleic acids were extracted using the 2005-01 Nucleic Acid Extraction and Purification Kit (Genseq, Shanghai, China), and the extracted concentrations were quantified with a Qubit 4.0 fluorometer (Thermo Scientific, Waltham, MA, USA). For library preparation, 5 ng of extracted DNA was used with the 2102 Sequencing Reaction Preparation Universal Kit (Genseq), with adaptor primers added during the process. Library quality was assessed again using the Qubit 4.0 fluorometer before sequencing, which was performed on the MGISEQ-200 platform (MGI, Shenzhen, China) with a single-end 50 bp read length.

The reference pathogen database included all species cataloged in sources such as the Manual of Clinical Microbiology, Clinical Microbiology Diagnosis and Interpretation, and the NCBI RefSeq Genome Database (https://www.ncbi.nlm.nih.gov/refseq/). Initial sequencing results were filtered to exclude species with <1% genome coverage and >2× depth or any species deemed background contamination by comparison to the negative control, using historical fluctuation data. For bacteria, viruses, and parasites, the top 10 organisms by read count were reported and interpreted alongside clinical data to identify likely pathogens.

Statistical analysis

We conducted the microbial diversity analysis using the R package vegan (version 2.6-4). The alpha-diversity analysis included calculations of abundance-based coverage estimator (ACE), Chao1, Simpson, Shannon, and observed species indexes. For the beta-diversity analysis, we used the Bray-Curtis distance metric and a principal coordinates analysis. The Anosim analysis was employed to determine if there were statistically significant differences in the microbial distribution between the CIP and non-CIP groups. Additionally, we performed a linear discriminant analysis (LDA) effect size (LEFse) analysis to identify any microbes that differed significantly between the CIP and non-CIP patients using R software (version 4.1.1; R Foundation for Statistical Computing, Vienna, Austria).

The categorical variables were compared using either the χ^2 test or Fisher's exact test, while the continuous variables were compared using either the independent *t*-test or Mann-Whitney *U* test. All the statistical analyses were two-tailed, and a P value <0.05 was considered statistically significant.

Ethical approval

This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the Ethics Committee of Peking Union Medical College Hospital (approval number: I-24PJ1079). The patients provided their written informed consent to participate in this study.

Results

Baseline characteristics

A total of 52 patients were included in the study. Among these patients, 33 were confirmed to have CIP, while 19 were classified as non-CIP (of whom, nine had infections, seven had disease progression, two had radiation-related pneumonitis, and one had targeted therapy-related pneumonitis) (*Figure 1*). In the CIP group, 69.7% of the patients were male and 48.5% had squamous cell carcinoma. Previous smokers accounted for 66.7% of the

patients, and 33.3% of the patients had a family history of tumors. Additionally, 78.8% of the patients in the CIP group received programmed cell death protein 1 (PD-1) therapy. There were no differences between the CIP and non-CIP groups in terms of clinical characteristics such as age, gender, pathology, smoking history, family history of tumors, and ICI type (*Table 1*).

Besides, the radiologic patterns observed in 33 CIP patients were as follows: organizing pneumonia (OP) in 16 patients (48.5%), nonspecific interstitial pneumonia (NSIP) in 13 patients (39.4%), hypersensitivity pneumonitis (HP) in 3 patients (9.1%), and acute interstitial pneumonia (AIP) in 1 patient (3.0%). In the cohort of 33 CIP patients, 45.5% were classified as having severity grades 3–4. A total of 87.9% of CIP patients received glucocorticoids therapy, 18.2% received intravenous immunoglobulin (IVIG), 18.2% received interleukin-6 (IL-6) inhibitors, and 6.1% received Janus kinase (JAK) inhibitors.

Microbial diversity between the CIP and non-CIP groups

The alpha-diversity analysis showed that there were no significant differences in the ACE (P=0.33), Chao1 (P=0.96), Simpson (P=0.30), Shannon (P=0.61), and observed species

Table 1 Clinical characteristics

Items	All patients (n=52)	Non-CIP (n=19)	CIP (n=33)	P value
Age (years)				0.15
≤65	26 (50.0)	7 (36.8)	19 (57.6)	
>65	26 (50.0)	12 (63.2)	14 (42.4)	
Gender				0.76
Female	15 (28.8)	5 (26.3)	10 (30.3)	
Male	37 (71.2)	14 (73.7)	23 (69.7)	
ECOG PS				0.89
0–1	28 (53.8)	10 (52.6)	18 (54.5)	
≥2	24 (46.2)	9 (47.4)	15 (45.5)	
Treatment lines				0.41
First line	43 (82.7)	14 (73.7)	29 (87.9)	
Second line	5 (9.6)	3 (15.8)	2 (6.1)	
Third line	4 (7.7)	2 (10.5)	2 (6.1)	
TNM staging				0.76
III	15 (28.8)	5 (26.3)	10 (30.3)	
IV	37 (71.2)	14 (73.7)	23 (69.7)	
Pathology				0.13
Adenocarcinoma	22 (42.3)	10 (52.6)	12 (36.4)	
Squamous cell carcinoma	20 (38.5)	4 (21.1)	16 (48.5)	
SCLC	6 (11.5)	2 (10.5)	4 (12.1)	
Others	4 (7.7)	3 (15.8)	1 (3.0)	
Smoking history				0.60
No	16 (30.8)	5 (26.3)	11 (33.3)	
Yes	36 (69.2)	14 (73.7)	22 (66.7)	

Table 1 (*continued*)

Table 1 (*continued*)

Items	All patients (n=52)	Non-CIP (n=19)	CIP (n=33)	P value
Family history of tumor				0.17
No	38 (73.1)	16 (84.2)	22 (66.7)	
Yes	14 (26.9)	3 (15.8)	11 (33.3)	
Type of ICI				0.76
PD-1 inhibitor	43 (82.7)	17 (89.5)	26 (78.8)	
Pembrolizumab	27 (51.9)	11 (57.9)	16 (48.5)	
Tislelizumab	11 (21.2)	5 (26.3)	6 (18.2)	
Camrelizumab	3 (5.8)	1 (5.3)	2 (6.1)	
Sintilimab	2 (3.8)	0 (0.0)	2 (6.1)	
PD-L1 inhibitor	9 (17.3)	2 (10.5)	7 (21.2)	
Grading of CIP	NA	NA		NA
1–2			18 (54.5)	
3–4			15 (45.5)	
Use of glucocorticoids	NA	NA		NA
No			4 (12.1)	
Yes			29 (87.9)	
Use of IVIG	NA	NA		NA
No			27 (81.8)	
Yes			6 (18.2)	
Use of IL-6 inhibitors	NA	NA		NA
No			27 (81.8)	
Yes			6 (18.2)	
Use of JAK inhibitors	NA	NA		NA
No			31 (93.9)	
Yes			2 (6.1)	

Data are presented as n (%). CIP, checkpoint inhibitor pneumonitis; ECOG PS, eastern cooperative oncology group performance status; TNM, tumor-node-metastasis; SCLC, small-cell lung cancer; ICI, immune checkpoint inhibitor; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; NA, not applicable; IVIG, intravenous immunoglobulin; IL-6, interleukin-6; JAK, Janus kinase.

indexes (P=0.98) between the CIP and non-CIP groups (*Figure 2*). The beta-diversity analysis also showed that there was no statistically significant difference in the microbial distribution between the two groups as determined by the Anosim test (P=0.58).

Microbial composition in the CIP and non-CIP groups

In the CIP group, the dominant phyla were *Firmicutes* (41.7%), *Acinetobacter* (18.2%), *Proteobacteria* (16.3%), *Bacteroidetes* (12.1%), and *Ascomycota* (3.4%). The dominant genera included *Streptococcus* (17.8%), *Prevotella* (9.8%), *Veillonella* (6.4%), *Rothia* (5.7%), *Actinomyces* (5.3%), *Haemophilus* (5.3%), *Neisseria* (4.9%), *Staphylococcus* (4.9%), *Gemella* (3.8%), and *Corynebacterium* (3.4%). In the non-CIP group, the dominant phyla were *Firmicutes* (38.2%), *Acinetobacter* (18.4%), *Proteobacteria* (17.8%), *Bacteroidetes* (11.2%), and *Ascomycota* (5.3%). In the non-CIP group,

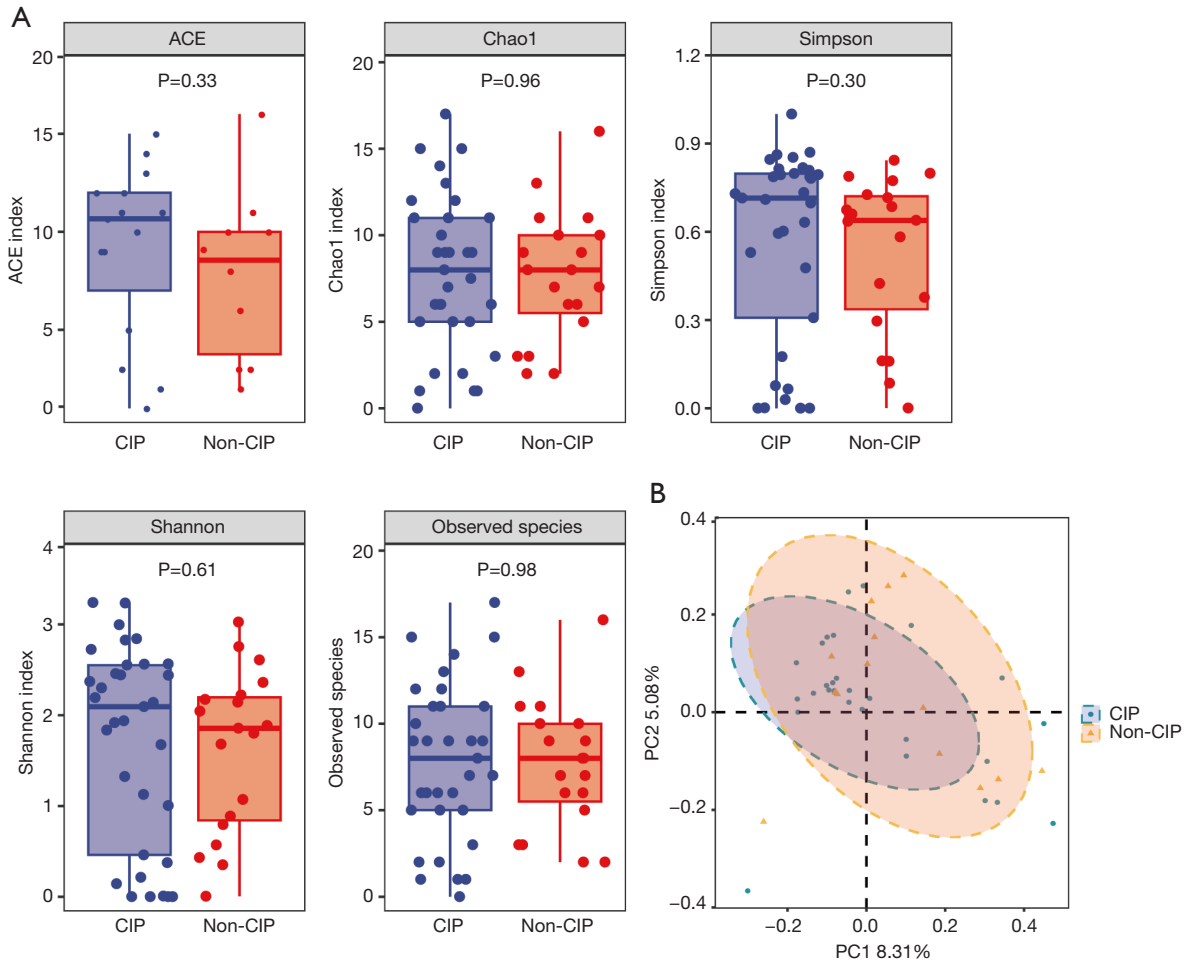

Figure 2 Comparison of microbial diversity between the CIP and non-CIP groups. (A) Alpha-diversity based on ACE, Chao1, Simpson, Shannon, and observed species indexes. (B) Beta-diversity performed by Bray-Curtis distance. ACE, abundance-based coverage estimator; CIP, checkpoint inhibitor pneumonitis; PC, principal component.

the dominant genera were *Streptococcus* (19.1%), *Prevotella* (10.5%), *Rothia* (7.9%), *Veillonella* (7.2%), *Haemophilus* (7.2%), *Actinomyces* (6.6%), *Neisseria* (5.3%), *Corynebacterium* (3.3%), *Staphylococcus* (2.6%), and *Candida* (2.6%). The distribution of the dominant phyla and genera in both groups is presented in *Figure 3*.

Subsequently, we conducted a detailed analysis of the top 50 species in the CIP and non-CIP groups (*Figure 4*). Among the top 50 species in both groups, 29 species were detected in common, including 24 bacteria, two fungi (*Candida albicans* and *Candida parapsilosis*), and three viruses [cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human betaherpesvirus]. Further, 21 species were exclusively found in the CIP group, comprising 20 bacteria and one virus (human herpesvirus 1). Additionally, 21 species were exclusive to the non-CIP group, including 18 bacteria, two fungi (*Pneumocystosis jiroveci* and *Aspergillus fumigatus*), and one virus (human betaherpesvirus 6B).

At the bacterial level, the most frequently detected species in the CIP group were *Streptococcus pneumoniae* (42.4%), *Rothia mucilaginosa* (42.4%), *Actinomyces spp.* (42.4%), *Haemophilus parainfluenzae* (39.4%), and *Prevotella melaninogenica* (36.4%). Similarly, in the non-CIP group, the dominant bacterial species included *Rothia mucilaginosa* (57.9%), *Prevotella melaninogenica* (52.6%), *Actinomyces spp.* (52.6%), *Streptococcus pneumoniae* (42.1%), and Veillonella atypica (36.8%). In both the CIP and non-CIP groups, the most commonly encountered fungi were *Candida albicans* and *Candida parapsilosis*. The most frequently detected viruses were EBV and CMV.

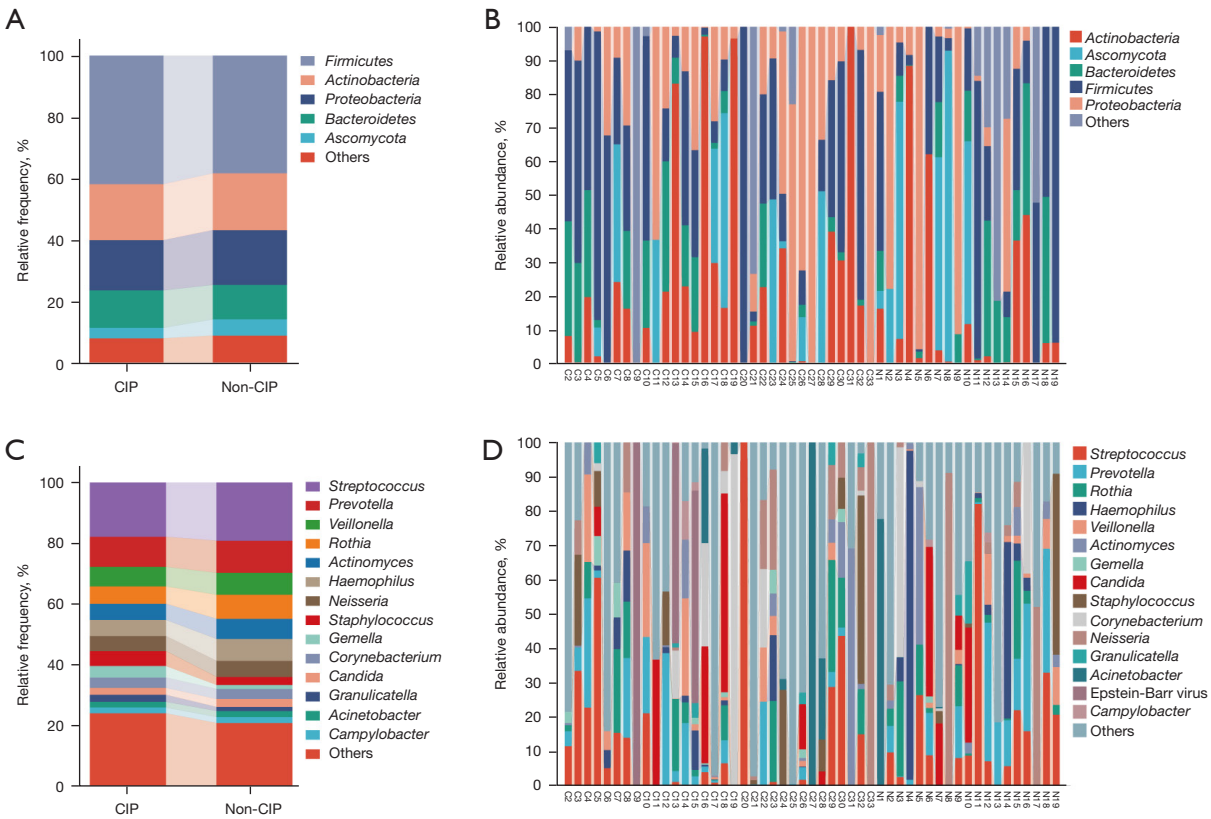

Figure 3 Composition of the microbiome community between the CIP and non-CIP groups. (A) Dominant phyla. (B) Relative abundance of different phyla in all patients. (C) Dominant genera. (D) Relative abundance of different genera in all patients. No microorganisms were detected by NGS in patient C1, and the results for this patient are not shown in (B,D). CIP, checkpoint inhibitor pneumonitis; C, CIP group; N, non-CIP group; NGS, next-generation sequencing.

Microbial differences between the CIP and non-CIP groups

We further analyzed the differences in the relative abundance of the top five phyla between the CIP and non-CIP groups (*Figure 5A*). Notably, the relative abundance of the *Proteobacteria* phylum (P<0.001) and *Firmicutes* phylum (P=0.01) was significantly higher in the CIP group than the non-CIP group. However, there were no significant differences in the relative abundance of the *Actinobacteria*, *Ascomycota*, and *Bacteroidetes* phyla between the two groups. To further explore potential microbiome differences across radiologic patterns within CIP cohort, we compared the microbial composition between the two most prevalent patterns, OP and NSIP. Our analysis showed no significant differences in the relative abundance of various phyla between these two groups (Figure S1).

A LEFse analysis was conducted to further evaluate the differences between the CIP and non-CIP groups (*Figure 5B*). The level of *Neisseria mucosa*, which belongs to the *Proteobacteria* phylum, was significantly higher in the CIP group (P=0.02). The *Proteobacteria* phylum had the most substantial effect on the differences observed between the CIP and non-CIP groups with an LDA score of 4.2 for *Neisseria mucosa*. Additionally, the *Mogibacterium genus* and *Streptococcus pseudopneumoniae*, which are part of the *Firmicutes* phylum, were also significantly more abundant in the CIP group than the non-CIP group (P=0.02 and P=0.047, respectively). The *Firmicutes* phylum contributed to the differences between the groups, with LDA scores of 3.6 for *Mogibacterium* and 3.1 for *Streptococcus pseudopneumoniae*.

Discussion

In this study, we conducted an exploratory analysis of the LRT microbiome among CIP and non-CIP patients

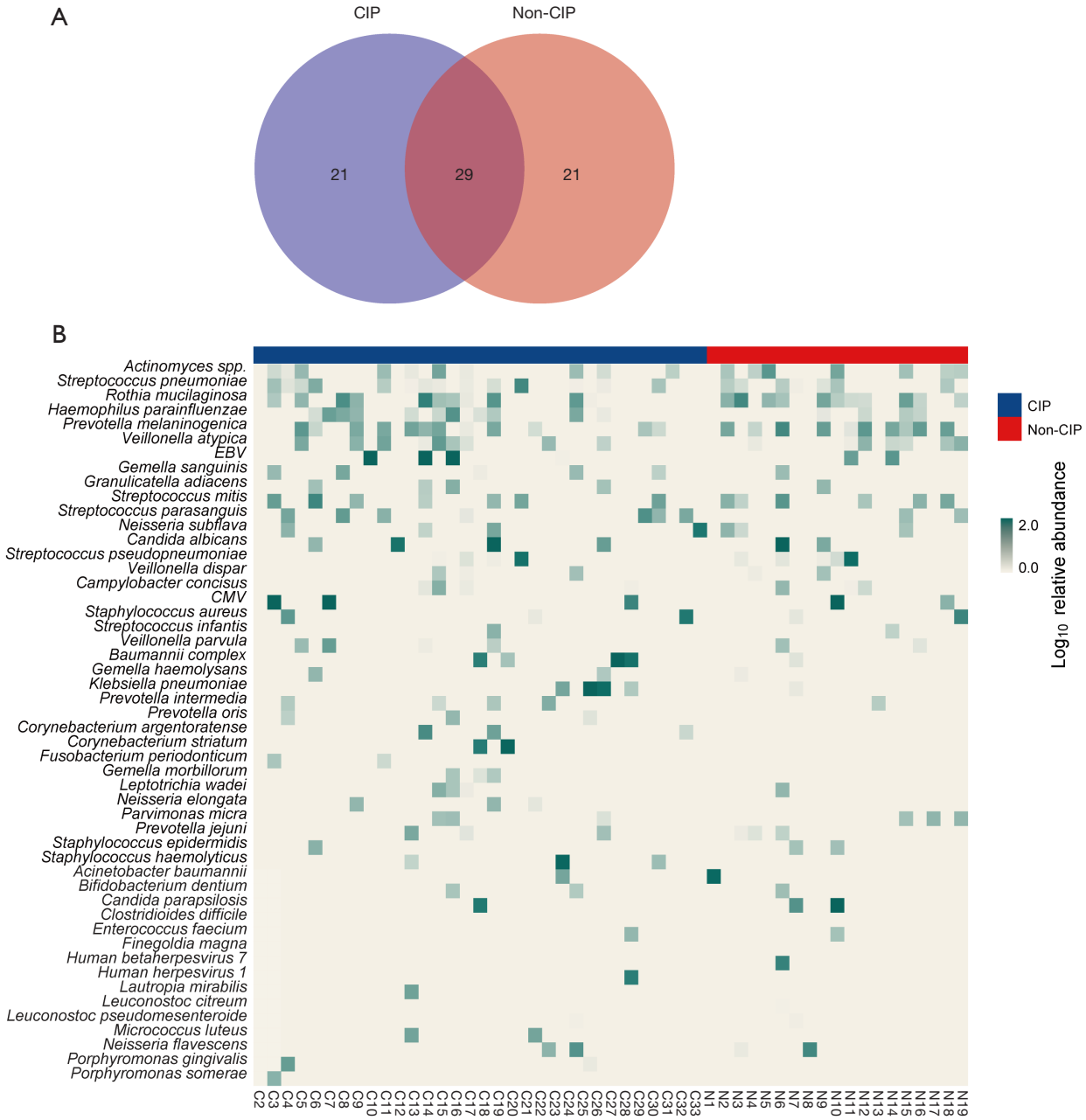

Figure 4 Composition of the top 50 species between the CIP and non-CIP groups. (A) Venn diagram of the top 50 species between the CIP and non-CIP groups. (B) Heatmap of the $\log_{10}$-transformed relative abundance of the top 50 species, with color intensity representing abundance levels. Darker colors indicate higher relative abundance of the species. CIP, checkpoint inhibitor pneumonitis; EBV, Epstein-Barr virus; CMV, cytomegalovirus; C, CIP group; N, non-CIP group.

using mNGS, delineated the microbial composition, and compared differences between the CIP and non-CIP groups. Our findings revealed a notable distinction in the relative abundance of the *Proteobacteria* and *Firmicutes* phyla, both of which were significantly higher in the CIP group than the non-CIP group.

The role of microbiota in the development and prognosis of tumors, as well as the occurrence of adverse reactions in cancer patients undergoing immunotherapy, has been an area of increasing interest. However, much of the previous

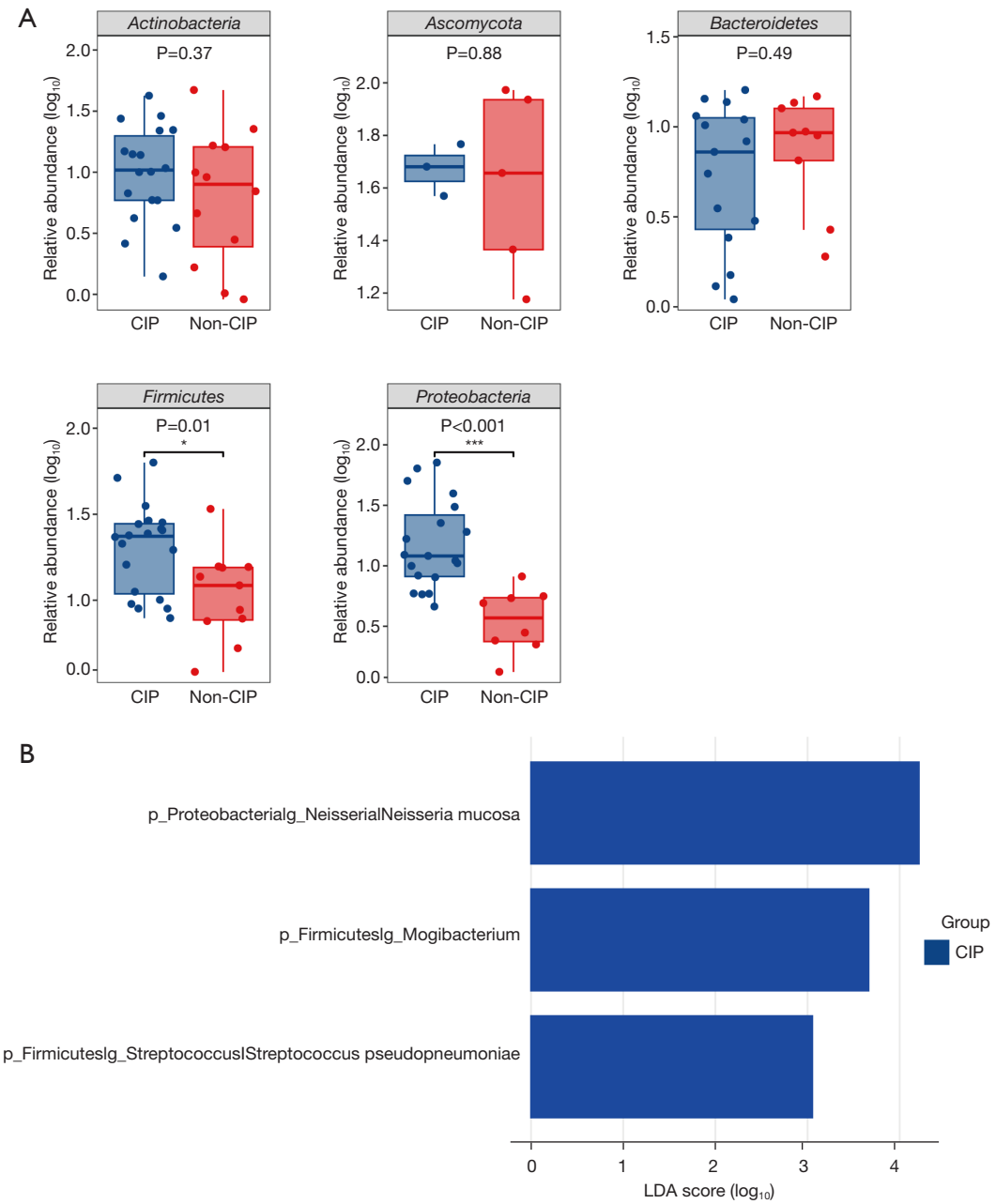

Figure 5 Differential relative abundances between the CIP and non-CIP groups. (A) Differential relative abundance (log_{10}) of the top five phyla between the CIP and non-CIP groups. (B) LEFse analysis of the dominant microbes between CIP and non-CIP groups. Only microbes meeting an LDA score >2.0 are shown. The blue bars indicate microbes with a higher relative abundance in the CIP group. Since no significantly enriched microbes were identified in the non-CIP group, they are not shown in (B). *, P<0.05; ***, P<0.001. CIP, checkpoint inhibitor pneumonitis; LDA, linear discriminant analysis; LEFse, LDA effect size.

research in this field has predominantly focused on the gut microbiota (13,33,34). In recent years, attention has shifted toward investigating the relationship between the lung microbiota and lung cancer (22-24). For instance, Cheng *et al.* conducted a study in which they analyzed BALF samples from 32 lung cancer patients and 22 individuals with benign lung diseases using 16S ribosomal RNA amplicon sequencing. They observed a significant enrichment of six genera (i.e.,

TM7-3, *Capnocytophaga*, *Sediminibacterium*, *Gemmiger*, *Blautia*, and *Oscillospira*) in the BALF of lung cancer patients compared to those with benign lung diseases (35). Similarly, Kim *et al.* reported significant differences in the alpha- and beta-diversity of the BALF microbiota between lung cancer patients and those with benign lung diseases. Notably, the presence of *unclassified_SAR202_clade*, which belongs to the phylum *Chloroflexi*, was a prominent microbial difference between the two groups (24). These findings collectively suggested that lung cancer is associated with dysbiosis of the local microbiota, characterized by shifts in bacterial composition and alterations in diversity.

Further, the lung microbiome played a pivotal role in the prognosis of lung cancer patients. A study indicated that the preoperative LRT microbiome composition differed significantly between patients with postoperative recurrence and those without, suggesting a potential association between the preoperative LRT microbiome composition and early recurrence of NSCLC (36). Additionally, patients with higher diversity and abundance of the microbiome in lung tumor-distal normal tissue exhibited shorter disease-free survival and lower recurrence-free survival rates (37). For lung cancer patients receiving immunotherapy, the respiratory microbiota also played a role in predicting treatment efficacy. Chu *et al.* suggested that patients with pre-existing enrichment of *Fusobacterium* in the airway had a poorer response to immunotherapy (30). Jang *et al.* found that lung cancer patients with enrichment of *Haemophilus influenzae* and *Neisseria perflava* in BALF had a worse response to immunotherapy (31).

Mechanistically, the lung microbiome regulates both innate and adaptive immunity (25,38,39). Commensal bacteria in the upper respiratory tract were shown to defend against influenza virus infection in mice by polarizing M2 macrophages and secreting anti-inflammatory mediators such as interleukin-10 (IL-10) and transforming growth factor-β (38). The enrichment of the oropharyngeal microbiota in the lungs, such as *Veillonella* and *Prevotella*, was shown to be associated with an inflammatory phenotype, characterized by an increase in T helper 17 cell lymphocytes, the upregulation of inflammatory cytokines, and the decreased expression of Toll-like receptor 4 in alveolar macrophages (40). Thus, the lung microbiome is involved in the development and prognosis of lung cancer by shaping the tumor microenvironment and modulating the activity of tumor-infiltrating immune cells.

However, research on the relationship between respiratory microbiota and irAEs in lung cancer patients is limited. Our study revealed a significant increase in the relative abundance of the *Proteobacteria* and *Firmicutes* phyla in the LRT samples of the CIP group compared to pneumonia caused by other etiologies. Research has shown that an elevated abundance of *Proteobacteria* in the gut microbiome is associated with a poor prognosis in patients with solid tumors (41). Additionally, Liu *et al.* found that individuals with increased levels of *Proteobacteria* in the gut are more likely to experience serious adverse effects from immunotherapy, which is consistent with our findings (42).

In terms of the *Firmicutes* phylum, previous studies have consistently shown a favorable correlation between the *Firmicutes* phylum in the gut and the improved prognosis of cancer patients receiving immunotherapy (16,43,44). However, research on the relationship between the *Firmicutes* phylum and irAEs has yielded conflicting results. Mao *et al.* analyzed the mNGS results of fecal samples from 65 patients with advanced hepatobiliary carcinoma receiving PD-1 therapy and found enrichment of the *Firmicutes* phylum in patients with mild diarrhea, which suggests that *Firmicutes* phylum may exert a protective effect against immunotherapy-related toxicity (16). Similarly, Hakozaki *et al.* prospectively collected fecal samples from patients with advanced NSCLC undergoing immunotherapy and observed that patients with enrichment of the *Firmicutes* phylum had lower grades of irAEs (43). Conversely, Chaput *et al.* reported an increased incidence of ipilimumab-induced colonitis in patients with enriched *Firmicutes* phylum among those receiving ipilimumab treatment for metastatic melanoma (44). Further studies are warranted to examine the relationship between the *Firmicutes* phylum and irAEs, along with their underlying mechanisms.

This study provided preliminary insights into the associations between the LRT microbiome and CIP in lung cancer patients receiving immunotherapy. As it was not feasible to collect BAL samples from patients without respiratory symptoms, all patients included in this study had suspected pneumonitis and underwent BAL, which meant our findings might not apply as a predictive biomarker for all ICIs-treated patients. However, microbiome analysis might hold clinical significance in differentiating CIP from other causes of pneumonitis in ICIs-treated patients. This could support clinicians in making more informed decisions regarding the etiology of pneumonitis in such contexts.

Our study has several limitations. First, the relatively small sample size may limit the generalizability of our findings to a broader population. A larger, prospective multicenter study involving diverse patient groups would

be valuable to confirm these results. Second, while some patients received antibiotics prior to sample collection, we did not separate patients based on antibiotic treatment status. This information could have provided more insight into how antibiotic usage may influence microbial composition (45). Finally, our study lacked paired BALF samples from the same patients before and after treatment, which restricted our ability to assess microbial changes associated with the onset of CIP over time. A future study design including matched pre- and post-treatment samples from each patient would enable a more thorough analysis of treatment-induced microbiome changes.

Conclusions

The relative abundance of the *Proteobacteria* and *Firmicutes* phyla was significantly higher in the CIP group than the non-CIP group, which suggested that the relative abundance of the *Proteobacteria* and *Firmicutes* phyla in the LRT may hold potential as a biomarker for CIP in lung cancer patients receiving ICIs.

Acknowledgments

Funding: This work was supported by funding from the National High-Level Hospital Clinical Research Funding (Nos. 2022-PUMCH-C-054 and 2022-PUMCH-B106).

Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-853/rc

Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-853/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-853/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the Ethics Committee of Peking Union Medical College Hospital (approval number: I-24PJ1079). The patients provided their written informed consent to participate in this study.

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(English Language Editor: L. Huleatt)

Cite this article as: Zhang D, Fan J, Liu X, Gao X, Zhou Q, Zhao J, Xu Y, Zhong W, Oh IJ, Chen M, Wang M. Lower respiratory tract microbiome is associated with checkpoint inhibitor pneumonitis in lung cancer patients. Transl Lung Cancer Res 2024;13(11):3189-3201. doi: 10.21037/tlcr-24-853