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Original Article

Differences in immunity and survival among non-small cell lung cancer patients: a gut microbiota perspective

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Abstract: Objective: This study aimed to investigate the relationship between key gut microbiota [Enterococcus, Escherichia coli (E. coli), Bifidobacterium, and Lactobacillus] and immune function in Chinese patients with non-small cell lung cancer (NSCLC). Methods: This study included 208 patients with NSCLC enrolled between March 2021 and June 2023. Fecal samples were collected from patients for quantitative analysis of Enterococcus, E. coli, Bifidobacterium, and Lactobacillus. Additionally, peripheral blood samples were collected from patients, and levels of T lymphocyte subsets ($CD3^+$, $CD4^+$, and $CD8^+$) were measured using flow cytometry. Analysis was conducted based on 6-month immune checkpoint inhibitor (ICI) efficacy and survival outcomes to examine the relationship between gut microbiota, immune function, and prognosis in NSCLC patients. Pearson correlation coefficients were used to investigate the interrelationships between microbial abundance and immune variables. Results: In the immune checkpoint inhibitor (ICI) responders (R group), higher proportions of Bifidobacteria and Lactobacilli were observed, whereas non-responders (NR group) exhibited increased proportions of Enterococcus and E. coli. Furthermore, Bifidobacteria and Lactobacilli showed positive correlations with T cell counts but negative correlations with inflammatory cytokine levels. Opposing relationships were seen for Enterococcus and E. coli, which correlated negatively with T cells and positively with IL-6 and TNF-α ($P < 0.05$). Conclusion: In NSCLC, Bifidobacterium and Lactobacillus promote beneficial immune feedback loops by activating T cells and exerting anti-inflammatory effects, thereby supporting antitumor immunity.

Keywords: NSCLC, gut microbiota, immune cells, inflammatory factors, prognosis

Introduction

Lung cancer (LC) is the leading cause of cancer death worldwide, of which non-small cell lung cancer (NSCLC) accounts for about 85% [1, 2]. Although immune checkpoint inhibitors (ICIs) can significantly prolong survival in some patients with advanced NSCLC, only 20-30% of patients achieve long-term survival [3, 4]. We believe that identifying key factors influencing the efficacy of ICIs and patient outcomes is crucial for improving patient prognosis [5].

As a collective "second genome", the gut microbiota interacts with the tumor immune microenvironment through the gut-lung axis, and changes in its composition are directly related to immune outcomes [6]: beneficial genera (e.g., Bifidobacterium, Lactobacillus) bolster anti-tumor immunity by producing short-chain fatty acids (SCFAs) that stimulate dendritic cells [7]. Conversely, overgrowth of opportunistic pathogens like Enterococcus and Escherichia coli (E. coli) can impair T-cell function, fuel inflammation, and undermine immunotherapy effectiveness [8]. The gut microbiota's role in NSCLC remains incompletely characterized, especially the four key flora: Enterococcus, E. coli, Bifidobacterium, and Lactobacillus. Although some previous studies have carried out preliminary analysis on the relationship between intestinal flora and immune function, such as the study by Huang et al. and Chen et al. [9, 10], their research mainly focused on a single species of flora. Additionally, international studies are mainly aimed at European and American populations [11], and few studies are aimed at Chinese people. Studies have shown

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that different races, dietary habits, environment and many other factors may cause significant differences in gut microbiota, for example, HLA gene polymorphisms in Asian populations affect the pattern of immune response, which may amplify the immune-enhancing effect of bacteria such as Bifidobacterium [12]. At the same time, the high rate of antibiotic use in the Chinese population may lead to a lower diversity of bacterial flora than in Europe and the United States, strengthening the key role of a single bacterial flora (such as Bifidobacterium) [13]. A high-fiber Chinese diet promotes the production of SCFAs, while awestern high-fat diet may promote the proliferation of E. coli [14]. Therefore, targeted studies for specific populations are necessary.

This study focused on four key bacterial genera-Enterococcus, E. coli, Bifidobacterium, and Lactobacillus-to systematically compare their distribution across patient groups stratified by immune response status and survival outcomes. Concurrently, monitored peripheral blood T-cell subset ratios and serum inflammatory cytokine levels. By analyzing correlations between the abundance of these microbiota and immune data, we aimed to clarify their distinct roles in modulating the immune microenvironment in order to provide a reference and guidance for the treatment of NSCLC patients in China.

Materials and methods

Study participants

This study enrolled NSCLC patients who were admitted to the Second Affiliated Hospital, Hengyang Medical School, University of South China between March 2021 and June 2023. After screening for inclusion and exclusion criteria, 208 patients were included. All patients provided informed consent.

Sample size calculation

The sample size was calculated using the log-rank test formula for survival analysis, with the primary endpoint being the association between the abundance of four key bacterial genera and overall survival (OS). The calculation assumed a hazard ratio (HR) of 0.5, representing a clinically significant survival benefit between comparison groups (long-term vs. short-term survivors). With a two-sided alpha of 0.05 and 80% power (beta = 0.2), and based on effect sizes from prior similar research [15], an initial sample of 180 patients was required. In addition, we expected a dropout rate of 15%.

Inclusion criteria: (1) age from 18 to 75 years, regardless of gender; (2) a pathologic diagnosis of stage IIIb-IV NSCLC; (3) designated to receive or already receiving ICI treatment; (4) a projected survival of ≥ 3 months; (5) no administration of broad-spectrum antimicrobials, probiotic supplements, or fecal microbiota transfer in the 4 weeks preceding enrollment; (6) provision of signed informed consent.

Exclusion criteria: (1) concurrent severe impairment of major organ systems (cardiac, hepatic, renal); (2) active autoimmune or immunodeficiency syndromes; (3) being pregnant or lactating; (4) a diagnosis of another malignancy within the past 5 years; (5) undergoing bowel preparation or experiencing acute diarrhea within 72 h of baseline stool sampling.

Treatment and follow-up

All patients received ICI regimens, such as pembrolizumab or nivolumab, either as monotherapy or combined with chemotherapy/anti-angiogenic agents. Following treatment completion, they were monitored for at least 24 months through monthly follow-up assessments. The follow-up period concluded upon patient death or by the cutoff date of August 2025.

Grouping

Treatment regimen: only receiving PD-1/PD-L1 inhibitors (such as pembrolizumab monotherapy) as a single drug group; Patients who received ICIs combined with chemotherapy or antiangiogenic drugs were divided into the combined drug group.

Immune response: Treatment response to ICIs was evaluated at the 6-month mark [16]. The responder group (R) included patients demonstrating complete response (CR), partial response (PR), or sustained SD (≥ 6 months). The non-responder group (NR) encompassed those with progressive disease (PD) or SD lasting < 6 months.

Table 1. Baseline data of the study subjects

Item	n	Percentage
Age	62.06±5.38	-
Male	134	64.42
Female	74	35.58
BMI (kg/m^2)	23.13±2.69	-
Stage IIIb	92	44.23
Stage IV	116	55.77
Smoking	161	77.40
Don't smoke	47	22.60

Survival status: Patients who died during follow-up were assigned to the deceased group, while survivors constituted the survival group.

Survival duration: Using the mean survival time (survival of patients who did not die during follow-up was recorded as 24 months) of the entire cohort as a threshold, patients were dichotomized into long-term survival (L, survival ≥ mean) and short-term survival (S, survival < mean) groups.

Sample detection

Following 3 months of treatment, both fasting blood samples (3 mL) and fecal samples (4-6 g) were acquired from every patient. DNA was extracted from the fecal material using a kit designed for stool genomic DNA isolation. The bacterial 16S rRNA gene V4 segment was PCR-amplified with universal primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The amplicons, once purified and quantified, were subjected to paired-end sequencing (2×250 bp) on the Illumina NovaSeq 6000 platform. Using the QIIME2 platform (v2023.2), low-quality sequences (quality score < 20), primers, and chimeras were removed via the DADA2 plugin to generate Amplicon Sequence Variants (ASVs). Taxonomic classification of ASVs was then performed against the SILVA database (release 138.1), with a 99% confidence threshold. For the bacterial genera of interest, relative abundance data were acquired and subsequently converted to absolute abundance (log CFU/g) for statistical evaluation.

Using flow cytometry, the proportions of $CD3^+$, $CD4^+$, and $CD8^+$ T cells were assessed, and the $CD4^+/CD8^+$ ratio was derived. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation (e.g. Ficoll-Paque method) and resuspended in PBS to a concentration of 1×10^6 cells/mL for later use. $CD3^+$ antibody: labeled total T cells (ab68235, Abcam). $CD4^+$ antibody: labeled helper T cell (ab238798, Abcam) $CD8^+$ antibody: labeled cytotoxic T cells (ab217344, Abcam). Isotype control: IgG antibody of the same species was used as a negative control (ab172730, Abcam). 100 μL suspension of PBMCs (1×10^6 cells) was collected and fluorescently labeled antibody was added (recommended working concentration 1:100, incubation time 30 min, dark, 4°C). Unbound antibodies were removed by washing twice with PBS. The cells were resuspended in 500 μL PBS and immediately tested on the machine.

The serum levels of IL-6, TNF-α, and IFN-γ were detected by enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

The statistical analysis was carried out using SPSS 34.0. Descriptive statistics for categorical data (e.g., gender and pathology stage) are shown as n (%). Normally distributed continuous data (e.g., microbial counts, IL-6 levels), verified by Shapiro-Wilk testing, are reported as (χ±s). Correlations were determined by Pearson's method. Logistic regression analysis was used to analyze the influencing factors. A *P*-value of less than 0.05 was considered significant.

Results

Baseline characteristics of participants

The study enrolled 208 participants with a mean age of (62.06±5.38) years, comprising 134 males and 74 females (**Table 1**).

Relationship between gut microbiota and treatment regimens

Compared to the single drug group, the number of probiotics (Bifidobacterium and Lactobacillus) in the combined drug group increased, while Enterococcus was decreased ($P < 0.05$). However, there was no significant difference in E. coli between the two groups ($P > 0.05$) (**Table 2**).

Table 2. Relationship between gut microbiota and treatment regimens

Group	n	Bifidobacterium (lgCFU/g)	Lactobacillus (lgCFU/g)	Enterococcus (lgCFU/g)	E. coli (lgCFU/g)
Single drug group	120	7.31±1.36	6.19±1.29	6.39±1.22	7.13±1.53
Combined drug group	88	8.64±1.20	6.76±1.35	5.75±1.45	6.77±1.47
t		7.363	3.089	3.425	1.719
P		< 0.001	0.002	< 0.001	0.087

Table 3. Relationship between gut microbiota and immune response

Group	n	Bifidobacterium (lgCFU/g)	Lactobacillus (lgCFU/g)	Enterococcus (lgCFU/g)	E. coli (lgCFU/g)
NR group	65	7.07±1.07	5.93±1.10	6.61±1.46	7.56±1.39
R group	143	8.24±1.45	6.65±1.38	5.89±1.24	6.71±1.50
t		5.807	3.693	3.677	3.883
P		< 0.001	< 0.001	< 0.001	< 0.001

Table 4. Effects of Gut microbiota on immune responses

Microbiota	B	S.E,	Wald χ^2	P	OR	95% CI (lower limit)	95% CI (upper limit)
Bifidobacterium	-0.517	0.146	12.55	0	0.596	0.448	0.794
Lactobacillus	-0.143	0.145	0.975	0.323	0.867	0.652	1.151
Enterococcus	0.152	0.142	1.138	0.286	1.164	0.881	1.538
E. coli	0.18	0.13	1.936	0.164	1.198	0.929	1.545

Table 5. Association between gut microbiota and prognostic survival

Group	n	Bifidobacterium (lgCFU/g)	Lactobacillus (lgCFU/g)	Enterococcus (lgCFU/g)	E. coli (lgCFU/g)
Died	45	6.43±0.92	5.90±1.18	6.67±1.37	7.90±1.21
Survived	163	8.27±1.31	6.57±1.35	5.97±1.31	6.72±1.49
t		8.877	3.029	3.134	4.884
P		< 0.001	0.003	0.002	< 0.001

Association between gut microbiota and immune response

Patients were stratified by their response to ICIs, resulting in 65 non-responders (NR group) and 143 responders (R group). The R group showed elevated populations of Bifidobacterium and Lactobacillus, and lower counts of Enterococcus and E. coli compared to the NR group ($P < 0.05$) (**Table 3**).

Effects of gut microbiota on immune responses

Subsequently, we performed Logistic regression analysis with the patient's immune response status as the dependent variable (R = 1, NR = 2) and gut microbiota as the independent variable. The output results showed that Lactobacillus, Enterococcus, and E. coli were not the key factors affecting the immune response of patients ($P > 0.05$). However, the decrease of Bifidobacterium determined the NR of patients (OR = 0.596, 95% CI = 0.448-0.794, $P < 0.001$) (**Table 4**).

Impact of gut microbiota on survival status

Of the 208 patients followed, 163 survived and 45 died. Survivors were associated with a greater abundance of Bifidobacterium and Lactobacillus, whereas non-survivors showed higher counts of Enterococcus and E. coli ($P < 0.05$) (**Table 5**).

Effect of gut microbiota on survival status

Similarly, the survival status of the patients was analyzed (survival = 1, death = 2), and the results also showed that Bifidobacterium was a key strain affecting the survival of the patients (OR = 0.264, 95% CI = 0.162-0.428, $P < 0.001$).

Table 6. Effect of gut microbiota on survival status

Microbiota	B	S.E,	Wald χ^2	P	OR	95% CI (lower limit)	95% CI (upper limit)
Bifidobacterium	-1.332	0.247	29.018	< 0.001	0.264	0.162	0.428
Lactobacillus	0.113	0.189	0.359	0.549	1.12	0.773	1.622
Enterococcus	-0.047	0.191	0.06	0.806	0.954	0.657	1.387
E. coli	0.372	0.174	4.565	0.033	1.451	1.031	2.042

Table 7. Association between gut microbiota and prognostic survival

Group	n	Bifidobacterium (lgCFU/g)	Lactobacillus (lgCFU/g)	Enterococcus (lgCFU/g)	E. coli (lgCFU/g)
S group	44	6.42±0.93	5.91±1.19	6.67±1.39	7.89±1.22
L group	164	8.26±1.31	6.57±1.35	5.97±1.31	6.73±1.17
t		8.767	2.936	3.089	4.723
P		< 0.001	0.004	0.002	< 0.001

Table 8. Effects of gut microbiota on survival cycles

Microbiota	B	S.E,	Wald χ^2	P	OR	95% CI (lower limit)	95% CI (upper limit)
Bifidobacterium	-1.329	0.248	28.732	< 0.001	0.265	0.163	0.43
Lactobacillus	0.131	0.19	0.476	0.49	1.14	0.786	1.654
Enterococcus	-0.038	0.191	0.04	0.842	0.963	0.662	1.399
E. coli	0.346	0.174	3.951	0.047	1.413	1.005	1.986

In addition, the increase of E. coli also increased the risk of death (OR = 1.451, 95% CI = 1.031-2.042, P = 0.033). Lactobacillus and Enterococcus had no effect on the survival of patients (P > 0.05) (**Table 6**).

Relationship between gut microbiota and survival duration

The average survival time of all patients was 21.43 months. Patients were stratified by median survival into an L group (n = 164) and an S group (n = 44). The gut microbiota composition differed significantly between groups, with the L group exhibiting enriched Bifidobacterium and Lactobacillus, and reduced Enterococcus and E. coli (P < 0.05) (**Table 7**).

Effects of gut microbiota on survival cycles

Consistent with the results of survival status analysis, in the survival cycle analysis (> 21 months = 1, ≤ 21 months = 2), Bifidobacterium (OR = 0.265, 95% CI = 0.163-0.430, P < 0.001) and E. coli (OR = 1.413, 95% CI = 1.413, P < 0.001) were positively correlated with the survival cycle. (95% CI = 1.005-1.986, P = 0.047) were the key flora in determining the survival of patients (**Table 8**).

Correlation of gut microbiota with immune cell profiles

After testing, the $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ levels of the patient were (42.53±6.71) %, (32.61±5.51) % and (1.08±0.31), respectively. Pearson's correlation analysis demonstrated that Bifidobacterium and Lactobacillus levels correlated positively with $CD3^+$, $CD4^+$ T-cell counts, and the $CD4^+/CD8^+$ ratio. Conversely, Enterococcus and E. coli showed a negative correlation with the same immunological indicators (**Figure 1**).

Link between Gut microbiota and inflammatory cytokines

Finally, a negative correlation was observed between the counts of beneficial bacteria (Bifidobacterium and Lactobacillus) and pro-inflammatory cytokines (IL-6 and TNF-α) (P < 0.05). On the other hand, the presence of Enterococcus and E. coli was positively associated with IL-6 and TNF-α concentrations (P < 0.05). In addition, only Bifidobacterium was positively correlated with IFN-γ (P < 0.05), but Lactobacillus, Enterococcus and E. coli were not significantly correlated with IFN-γ (P > 0.05) (**Figure 2**).

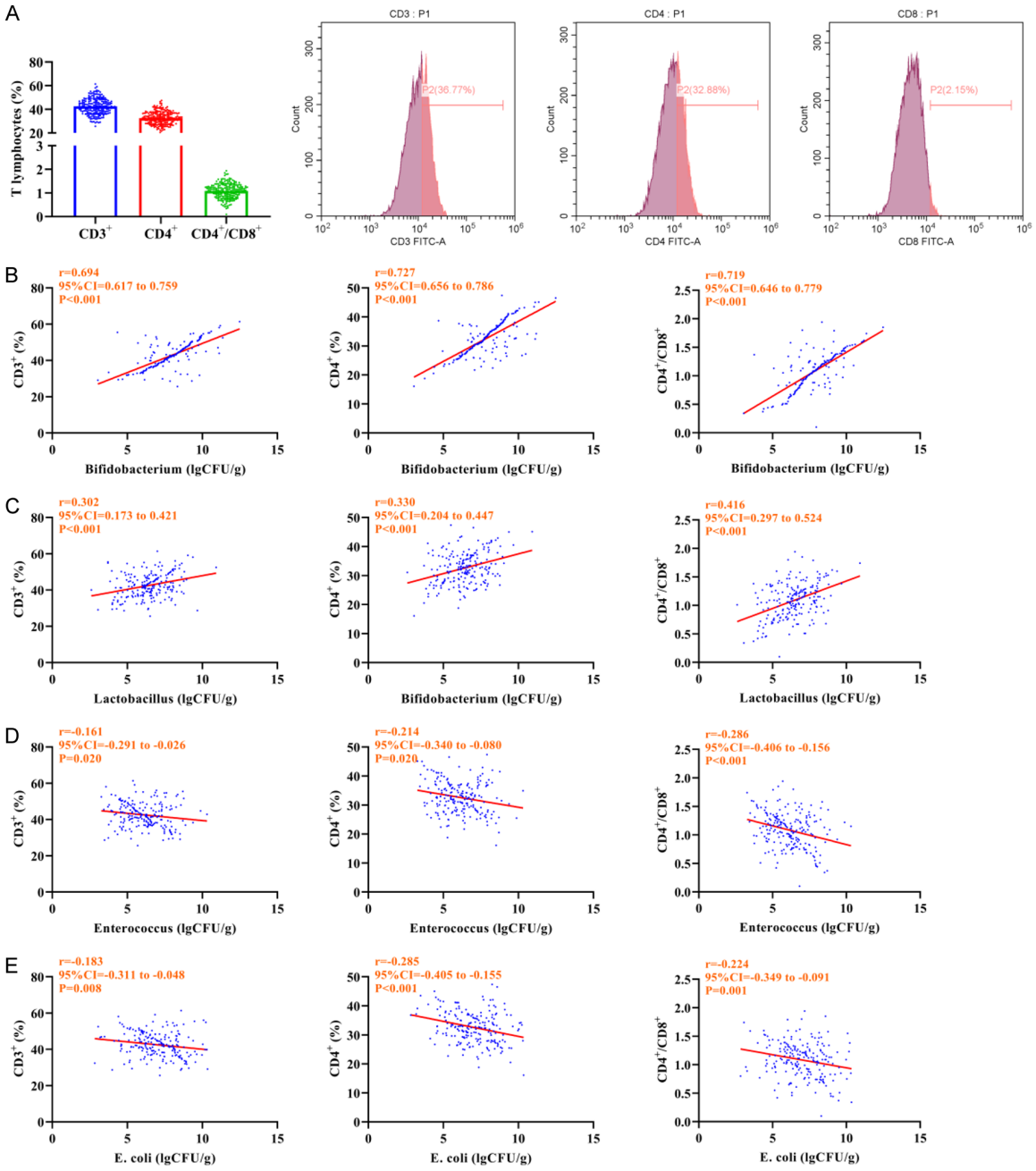

Figure 1. Correlation between gut microbiota and immune cells ($CD3^+$, $CD4^+$, $CD4^+/CD8^+$ T cells). A. Test results of $CD3^+$, $CD4^+$ and $CD8^+$. B. Correlation analysis between Bifidobacterium and immune cells. C. Correlation analysis between Lactobacillus and immune cells. D. Correlation analysis between Enterococcus and immune cells. E. Correlation analysis between E. coli and immune cells.

Discussion

In this first systematic investigation conducted in Chinese NSCLC patients, we evaluated the relationship of four principal gut microbiota-Enterococcus, E. coli, Bifidobacterium, and Lactobacillus-with immunotherapy efficacy and survival. Our findings indicate significant enrichment of Bifidobacterium and Lactobacillus in ICI responders and long-term survivors, whereas Enterococcus and E. coli were associated with non-response and poorer survival. Mechanistic exploration further revealed that these microbiota modulated host immunity,

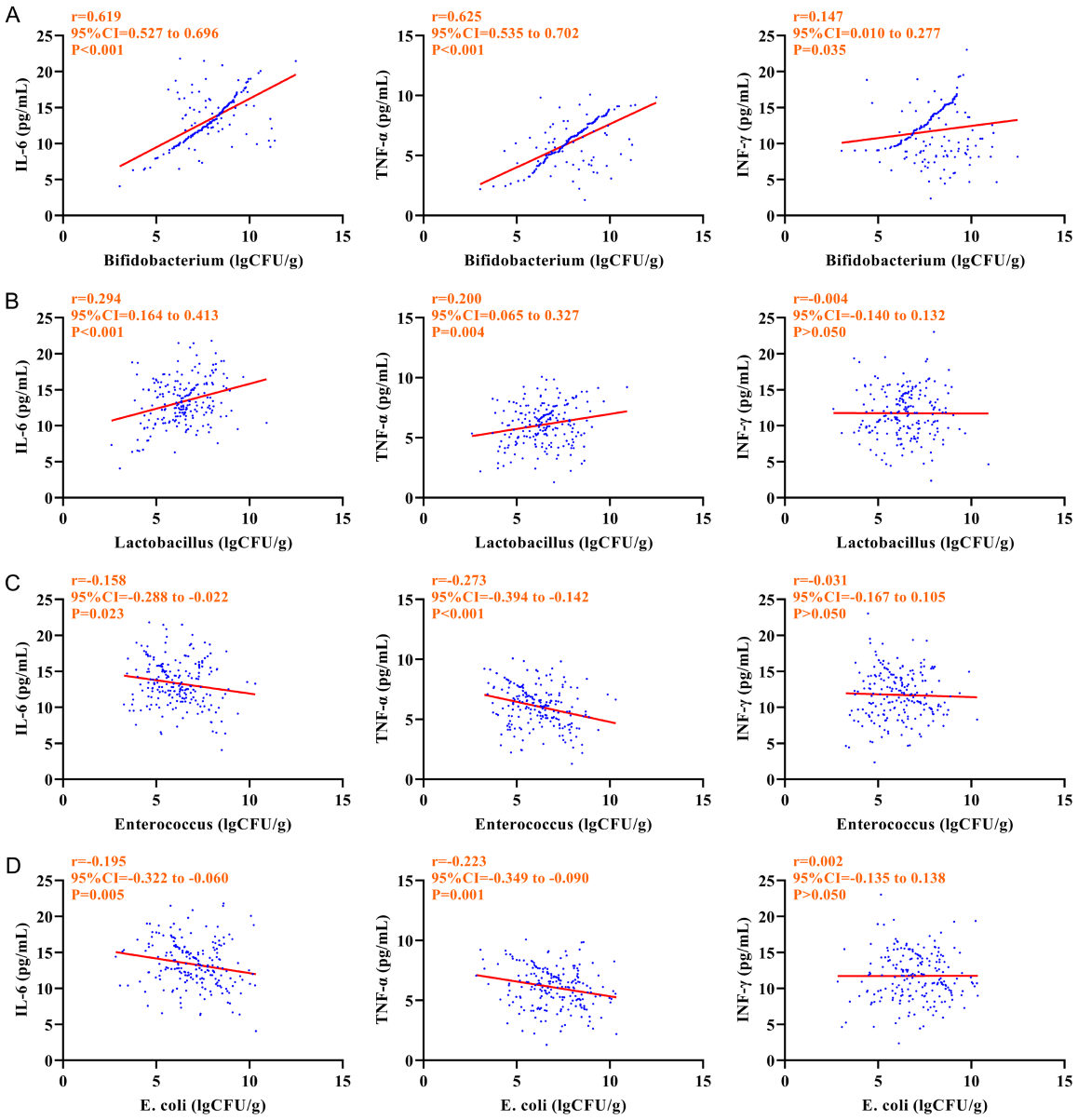

Figure 2. Correlation between gut microbiota and inflammatory factors (IL-6, TNF-α, IFN-γ). A. Correlation analysis between Bifidobacterium and inflammatory factors. B. Correlation analysis between Lactobacillus and inflammatory factors. C. Correlation analysis between Enterococcus and inflammatory factors. D. Correlation analysis between E. coli and inflammatory factors.

possibly by adjusting T-cell composition and inflammatory signaling.

In an analysis of treatment regimen, Bifidobacterium and Lactobacillus in the combined drug group were higher than those of the single drug group, which was related to the synergistic effect of chemotherapy on immunomodulation to enhance the beneficial effect of bacteria [17]. Previous studies have repeatedly verified this point of view [18, 19], so this paper does not repeat the analysis. According to our results, Enterococcus and E. coli may drive immunosuppression, in contrast to Bifidobacterium and Lactobacillus, which appear to function as key activators of immune responses. Also, significant enrichment of Enterococcus and E. coli was detected among non-responders and deceased individuals, corroborating earlier reports. As demonstrated by Long et al., E. coli-derived lipopolysaccharide (LPS) can trigger myeloid-derived suppressor

cell (MDSC) activation through the TLR4/NF-κB pathway, resulting in reduced infiltration of $CD8^+$ T cells [20]. Meanwhile, Enterococci-produced enterocins may interfere with dendritic cells' ability to present antigens [21]. Subsequently, using regression analysis, we found that only a decrease in Bifidobacterium independently predicted immune nonresponse. This is consistent with the significant enrichment of Bifidobacterium in the R group in the above comparison, but the regression analysis further highlights its uniqueness as the "core of immune response driver". In addition, Lactobacillus lost significance by the multivariate model, implying that its effect may be dependent on the co-existence of Bifidobacterium or indirectly through the same metabolic pathway, such as SCFAs. The results of survival analysis showed that Bifidobacterium and E. coli were independent factors affecting the survival of patients. This result again suggested that the "protective effect" of Lactobacillus may depend on the synergy of Bifidobacterium. The negative effect of Enterococcus on survival may be masked by E. coli. The proinflammatory properties of E. coli may be the key to affecting the survival of patients [22]. Unlike in the study by Chatterjee et al., only Bifidobacterium was found to be associated with IFN-γ in our study, and this difference may be due to gut metabolic characteristics or antibiotic exposure differences in different ethnic groups [23]. We also determined elevated Bifidobacterium and Lactobacillus counts in the R and L groups. These findings were also consistent with the findings of Lu D et al. [24]. Finally, the L group exhibited increased abundances of Bifidobacterium and Lactobacillus, along with decreased levels of Enterococcus and E. coli. This compositional shift is consistent with longitudinal findings reported by Huang et al. [25], reaffirming the pro-inflammatory roles of Enterococcus and E. coli in oncologic contexts. Another point worth our attention: smoking has been considered as one of the key factors affecting gut microbiota and ICI in many studies [26, 27]. Tobacco toxin reduces probiotic colonization and increases the load of pro-inflammatory bacteria, thereby aggravating TNF-α/IL-6 driven immunosuppression [28]. However, since more than 90% of the patients in our study smoked, we did not perform subgroup analyzes according to smoking status. Future studies are recommended to incorporate smoking history into microbiota prognostic models to optimize risk stratification.

Our correlation analysis demonstrated that Bifidobacterium and Lactobacillus positively correlated with $CD3^+$ and $CD4^+$ T-cell counts, while negatively associating with IL-6 and TNF-α. These findings suggest that probiotic genera may enhance antitumor immunity through multiple mechanisms. First, Bifidobacterium and Lactobacillus produce SCFA (e.g., butyrate), which promote dendritic cell maturation and subsequent $CD4^+$ T helper cell differentiation into Th1 subsets, amplifying IFN-γ production and cytotoxic $CD8^+$ T-cell activity [29]. Second, SCFAs inhibit histone deacetylases, suppressing NF-κB-mediated transcription of pro-inflammatory cytokines (e.g., IL-6, TNF-α) and fostering an anti-inflammatory milieu [30]. Notably, the stronger correlation of Bifidobacterium with $CD4^+$ T cells than with $CD8^+$ T cells implies a preferential impact on helper T-cell responses, potentially by MHC-II antigen presentation. Conversely, Lactobacillus showed balanced effects on both subsets, aligning with its role in modulating mucosal immunity. In contrast, Enterococcus and E. coli were negatively correlated with T-cell counts but positively linked to IL-6 and TNF-α. These opportunistic pathogens may impair immunotherapy by pro-inflammatory pathways: E. coli-derived LPS activates TLR4/NF-κB signaling, fueling IL-6/TNF-α production and myeloid-derived suppressor cell (MDSC) expansion, which dampens $CD8^+$ T-cell infiltration [31]. Enterococcus-produced enterocins may disrupt dendritic cell function, skewing $CD4^+$ T cells toward immunosuppressive Treg phenotypes ($FoxP3^+$), thereby elevating TGF-β and IL-10 levels [32]. Although our study did not directly measure TGF-β/IL-10, prior evidence indicates that E. coli enrichment correlates with elevated TGF-β in NSCLC models, possibly explaining the observed T-cell suppression [33]. The negative correlation between Enterococcus and the $CD4^+/CD8^+$ ratio further hints at its role in disrupting T-cell homeostasis.

Concurrently, adjunctive therapy with specific probiotics (e.g., Bifidobacterium and Lactobacillus strains) during ICI treatment could be a viable approach to remodel the gut microbiome and improve clinical prognosis. Several limitations warrant consideration in this study. First,

the single-center design of the participant cohort might have led to regional microbiota bias, necessitating future multi-center validation. Second, the 24-month median follow-up restricted insight into microbiota dynamics in ultra-long-term survivors (> 36 months). While our study identified robust correlations, causal relationships between the four microbiota and immune outcomes remain to be established. Future work should employ interventional approaches, such as fecal microbiota transplantation (FMT) from responders to germ-free or antibiotic-treated NSCLC models, to test whether Bifidobacterium/Lactobacillus transfer enhances ICI efficacy. Conversely, gavaging mice with Enterococcus/E. coli isolates could recapitulate immunosuppressive phenotypes. *In vitro* co-culture assays of patient-derived microbiota with human peripheral blood mononuclear cells (PBMCs) may further elucidate direct effects on T-cell proliferation and cytokine secretion (e.g., TGF-β/IL-10). Such experiments would clarify whether these genera are drivers or bystanders in immune modulation.

Conclusion

In Chinese NSCLC patients, this study characterizes the profiles of Enterococcus, E. coli, Bifidobacterium, and Lactobacillus and links them to immune modulation and survival outcomes. A reciprocal relationship was observed where probiotic species (Bifidobacterium/Lactobacillus) potentiate immune activation, and opportunistic pathogens (Enterococcus/E. coli) promote an immunosuppressive niche. This evidence supports targeting the gut microbiome for precision immunotherapy. However, correlation does not imply causation; thus, interventional studies (e.g., FMT or *in vitro* assays) are critical to confirm their mechanistic roles. Targeting these microbiota may optimize immunotherapy precision, but translational applications require validated causal links through multi-omics and controlled trials.

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Disclosure of conflict of interest

None.

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