Original Article

Gut microbiome in advanced non-small cell lung cancer: effect of chemotherapy and impact on efficacy

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Background: While evidence linking the gut microbiome (GM) to cancer immunotherapy is growing, data regarding its role in chemotherapy remains limited. This study aims to investigate the effect of chemotherapy on GM composition and its potential as a predictive biomarker for treatment outcomes in advanced non-small cell lung cancer (NSCLC).

Methods: Advanced NSCLC patients treated with chemotherapy at Ramathibodi Hospital were prospectively enrolled. Clinical data and stool samples were collected at three time points: baseline, post-evaluation, and at progression of disease (PD). Fecal bacterial DNA was extracted, followed by PacBio Sequel II sequencing and comprehensive bioinformatic analysis. Clinical data were summarized using descriptive statistics. Progression-free survival (PFS) and overall survival (OS) were estimated by the Kaplan-Meier method, and predictive factors were identified using Cox-regression analysis.

Results: This study analyzed 54 stool samples from 27 NSCLC patients treated with platinum-doublet chemotherapy. The median PFS and OS were 5.3 months [95% confidence interval (CI): 2.4–8.4] and 13.8 months (95% CI: 5.2–not reached), respectively. Post-chemotherapy changes (n=20 paired samples) showed a significant decrease in microbial richness, as evidenced by reduced Abundance-based Coverage Estimator (ACE) (P=0.02) and Chao1 (P=0.03) alpha diversity indices. Taxonomically, the relative abundance of *Enterobacter* was significantly decreased post-chemotherapy (P=0.03). Regarding treatment response (n=26 evaluable patients; 13 PD, 13 clinical benefit), baseline alpha diversity was not predictive of outcome. However, the relative abundance of *Akkermansia* was notably higher in the clinical benefit group, approaching statistical significance (P=0.07).

Conclusions: Chemotherapy significantly reduced GM by decreasing species richness (as measured by the ACE and Chao1 index), while species diversity (as measured by the Shannon and Simpson index) remained unchanged. Therefore, confirming the definitive role of the GM as a predictive biomarker in chemotherapy-treated NSCLC patients necessitates further investigation in a larger, more robustly powered cohort.

Keywords: Gut microbiome (GM); chemotherapy; non-small cell lung cancer (NSCLC)

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Introduction

Lung cancer is one of the most common cancers and a leading cause of cancer-related mortality worldwide. In 2022, 2.4 million new cases of lung cancer were diagnosed, resulting in approximately 1.8 million deaths (1), In Thailand, lung cancer accounted for a mortality rate of 15 per 100,000 population, making it the second leading cause of cancer-related deaths after liver cancer (2).

Chemotherapy remains the major treatment for cancer patients in Thailand, despite novel treatments, including targeted therapy and immunotherapy, that have demonstrated superior efficacy. Response rates for lung cancer treated with targeted therapy reach 60–70%, while immunotherapy achieves 20–50%, and combination immunotherapy and chemotherapy achieve 40–60%. In contrast, chemotherapy alone provides response rates of only 20–40% (3-8). However, the high costs of targeted therapies and immunotherapy limit their accessibility for many patients, making chemotherapy the mainstay of treatment in Thailand.

Highlight box

Key findings

- In chemotherapy-treated non-small cell lung cancer (NSCLC) patients, there is a significant reduction in alpha diversity post-chemotherapy.

What is known and what is new?

- The gut microbiome (GM) is known to affect the effectiveness and side effects of cancer treatments, but most studies focus on immunotherapy or gastrointestinal cancers.
- Platinum-based chemotherapy significantly alters GM richness [Abundance-based Coverage Estimator (ACE) and Chao1 indices] and causes a notable decrease in Enterobacter abundance. Higher relative abundances of *Akkermansia* were observed in patients with clinical benefit response, although this was not statistically significant.

What is the implication, and what should change now?

- The presence of *Akkermansia* indicates a promising trend as a predictor for favorable treatment response. These findings highlight the importance of larger studies to confirm microbial biomarkers while accounting for confounding clinical factors, such as concomitant medication use.

The microbiome is the community of microorganisms in a particular environment, such as skin, respiratory tract, and gastrointestinal (GI) tract. These microorganisms outnumber human cells approximately 10-fold and have coevolved with humans, playing essential roles in various biological processes that directly affect human health (9). Key functions of the microbiome include food and drug metabolism, vitamin synthesis, stimulation of immune responses, and inhibition of external pathogenic microorganisms. An imbalance in the microbiome, known as dysbiosis, often disrupts internal physiological states and has been linked to the development of numerous diseases, including cancer (10).

Microbiome research has become increasingly widespread with the use of 16S rRNA sequencing, a technique that analyzes the genetic sequences of bacterial ribosomal RNA. Studies in animal models have revealed that certain bacterial species influence the side effects of chemotherapy. For instance, a reduction in gut microbiota in experimental mice has been associated with intestinal inflammation caused by methotrexate (11,12), Similarly, the presence of β-glucuronidase-producing bacteria in the gut has been linked to side effects of irinotecan (13). Gammaproteobacteria produce cytidine deaminase, an enzyme that degrades gemcitabine, leading to resistance to chemotherapy. Probiotics, including *Lactobacillus* and *Bifidobacterium*, have been shown to enhance the efficacy of alkylating agents such as cisplatin (14), further highlighting the therapeutic potential of the microbiome.

In addition to chemotherapy, specific bacterial strains also influence cancer's response to immunotherapy. This hypothesis has been substantiated by studies in germ-free mice, in which the transfer of microbiota from immunotherapy responders led to tumor regression (15-17). Research on the microbiome in cancer patients has been increasing rapidly, with most studies focusing on patients receiving immunotherapy. A study conducted in China on patients with lung cancer treated with nivolumab, an immune checkpoint inhibitor, found that those with higher gut microbial diversity had better treatment responses and longer progression-free survival (PFS) than those with lower microbial diversity. Furthermore, patients who responded well to nivolumab showed an increased abundance of

Alistipes putredinis, *Bifidobacterium longum*, and *Prevotella copri*. In contrast, patients who did not respond to treatment exhibited higher levels of *Ruminococcus* species. These findings highlight the potential of the gut microbiome as a biomarker for predicting immunotherapy response and for modulating treatment efficacy (18).

Studies on the impact of chemotherapy on alterations in the microbiome in cancer patients remain limited. Previous research has reported the effects of chemotherapeutic agents such as cyclophosphamide, doxorubicin, 5-fluorouracil (5-FU), and irinotecan on the microbiome, as well as the associations between specific bacterial taxa and chemotherapy-induced side effects (19). However, there is a lack of studies focusing on the effects of chemotherapy agents commonly used in lung cancer treatment—such as carboplatin, paclitaxel, gemcitabine, pemetrexed, and docetaxel on the microbiome. This study aims to evaluate the effects of chemotherapy agents on alterations in the microbiome in patients with lung cancer. Additionally, the study also investigates whether specific bacterial taxa are associated with treatment response, thereby providing insights into the potential role of the microbiome in influencing chemotherapy outcomes. We present this article in accordance with the STROBE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-2026-1-0209/rc).

Methods

Study design and sample collection

A prospective cohort study was conducted. Patients diagnosed with advanced non-small cell lung cancer (NSCLC) who were treated with first-line chemotherapy in the Faculty of Medicine, Ramathibodi Hospital, Mahidol University between November 2021 and January 2024 were included. Patients with locally advanced disease receiving curative treatment, including surgery and/or definite radiotherapy or concurrent chemoradiotherapy, or those treated with targeted therapy, immunotherapy, or palliative care were excluded. For each patient, clinical data and fecal samples were collected at multiple time points: at baseline (before treatment initiation), during the first treatment evaluation (within 2–4 months after treatment initiation), and upon disease progression for patients whose best response was not progression of disease (PD). Demographic characteristics, laboratory results, and adverse events (AEs) were recorded. Treatment response was assessed using RECIST criteria v1.1 (20). The primary objectives were to evaluate the effect of chemotherapy on the gut microbiome and to explore the microbiome's potential as a predictive biomarker for treatment response and AEs.

After obtaining informed consent, a fecal sample of about 50–100 mg was collected from each participant. The samples were immediately preserved in StoolFiX™ (Isohelix, Cell Projects, Harrietsham, United Kingdom) collection tubes and kept at room temperature until DNA extraction.

The study was conducted in accordance with the Declaration of Helsinki and its subsequent amendments. The study was approved by the Human Research Ethics Committee, Faculty of Medicine, Ramathibodi Hospital, Mahidol University (approval No. COA.MURA2021/956). Written informed consent was obtained from all patients prior to sample collection.

DNA extraction, library preparation, and sequencing

Bacterial DNA was isolated from the samples using the QIAamp Power Fecal DNA Kit (QIAGEN, Hilden, Germany) at the Center for Medical Genomics, Ramathibodi Hospital. Following isolation, DNA purity was assessed using a NanoDrop One spectrophotometer (Thermo Fisher Scientific, Waltham, United States), and quantification was performed with a Qubit 4.0 fluorometer (Thermo Fisher Scientific, Waltham, United States). Subsequently, the full-length (V1-V9 regions) 16S rRNA gene was amplified. The resulting amplicons were used to construct a SMRTbell® library for sequencing on a PacBio Sequel II System. This approach was selected to generate high-quality, long-read data, thereby ensuring enhanced taxonomic resolution.

Statistical analysis

Raw sequencing data underwent a rigorous bioinformatics pipeline. High-fidelity Circular Consensus Sequencing (CCS) reads were initially generated from raw subreads using SMRT Link (version 8.0), which requires at least 5 full passes and a predicted accuracy of at least 90%. The CCS reads were subsequently demultiplexed using the lima tool (v1.7.0). Subsequent quality control involved primer removal via cutadapt (v2.7), length-based filtration (retaining sequences between 1,200–1,650 bp), and chimera removal utilizing the UCHIME algorithm (v8.1). The final set of high-quality reads was then processed using the DADA2 method within the QIIME2 framework (v2020.06) to

generate a feature table of Amplicon Sequence Variants (ASVs). Taxonomic assignment was performed against the SILVA database (Release 138) employing the classify-sklearn Naive Bayes classifier with a confidence threshold of 0.7.

Baseline patient characteristics were summarized using descriptive statistics. Survival outcomes, including PFS and overall survival (OS), were defined as the time from chemotherapy initiation to PD or death and were estimated using the Kaplan-Meier method. Clinical statistical analysis was undertaken using Stata software version 17 (StataCorp, College Station, Texas, USA). Alpha diversity [Shannon, Simpson, Abundance-based Coverage Estimator (ACE), and Chao1 index] was calculated to assess within-sample richness and evenness. Beta diversity was computed using the Bray-Curtis metric and visualized with Principal Coordinates Analysis (PCoA). A Permutational Multivariate Analysis of Variance (PERMANOVA) was used to test for significant differences in community structure between clinical groups. The Kruskal-Wallis or Wilcoxon rank-sum test was used to identify differentially abundant taxa. All microbiome-related statistical analyses were conducted in R software. A P value <0.05 was considered statistically significant for all tests.

Results

Patient characteristics

A total of 27 advanced NSCLC patients were included, with a mean age of 66 years. Seventeen (63%) patients were male. Mostly, they were former smokers (45%). Eastern Cooperative Oncology Group performance status (ECOG-PS) score of 0–1 in 17 (63%) patients. Adenocarcinoma was the most common histology (62%), followed by squamous cell carcinoma and other histology (each at 19%). All except two patients got platinum-doublet chemotherapy as first-line systemic therapy; only 2 patients got single gemcitabine and a combination of carboplatin/paclitaxel/bevacizumab for treatment (see *Table 1*). Response evaluation with a computed tomography scan was done in 26 patients; one patient died from cancer 1 month after chemotherapy initiation without disease evaluation. Complete response (CR) was 1 (4%) patient, partial response (PR) was 6 (22%), stable disease (SD) was 6 (22%), and PD was 13 (48%) from 27 patients. The median follow-up time was 10.2 months (range, 1.3–28.4 months). The median PFS [95% confidence interval (CI)] was 5.3 (2.4–8.4) months, and the median OS (95% CI) was 13.8 (5.2–not reach) months.

Sequencing overview and baseline microbiome characteristics

A total of 54 stool samples from 27 patients were extracted and underwent DNA sequencing. Of these, 7 patients provided only a baseline stool sample, while 20 patients provided follow-up samples, resulting in 13 paired (doublet) and 7 triplet sample sets. (*Figure 1*). Full-length 16S rRNA gene sequencing of these samples generated an initial 2,007,354 raw CCS reads. Following a rigorous bioinformatics pipeline that included quality filtering and chimera removal, a total of 1,630,795 high-quality, non-chimeric reads were retained for subsequent analysis. The median read length was 1,457 bp, confirming the successful sequencing of the full-length gene. On average, each sample yielded 27,292 effective reads, which clustered into an average of 721 unique ASVs. Rarefaction analysis confirmed that the sequencing depth was sufficient. The Shannon diversity curves reached saturation for all samples at approximately 10,000 reads, indicating that the microbial community was robustly characterized.

We next assessed whether baseline gut microbiome diversity was associated with key clinical factors. No significant differences in alpha or beta diversity were observed when patients were stratified by gender, histological subtype, or smoking status. However, a significant association was identified with baseline serum albumin levels. Patients with low albumin levels (albumin <35 g/L) exhibited significantly lower microbial diversity compared to those with normal albumin levels (P=0.01 and 0.009 for Shannon and Simpson index, respectively) (*Figure 2*).

Effect of chemotherapy on to gut microbiome

The effect of chemotherapy on the gut microbiome was assessed. Alpha diversity was significantly decreased in post-chemotherapy stool (P=0.02 and 0.03 for ACE and Chao1, respectively). However, Shannon and Simpson indices did not differ between baseline and post-chemotherapy stool (*Figure 3A*). We explored the top 10 taxa and found that *Enterobacter* showed a significant decrease (rank sum test, P=0.03), while *Fusobacterium* showed an increase (P value = non-significant) after chemotherapy (*Figure 3B*). Beta diversity, assessed by the Bray-Curtis method, showed no significant difference between the

Table 1 Baseline characteristics

Characteristics	Value (N=27)
Gender	
Male	17 [63]
Female	10 [37]
Age (years)	66.2±9.2
Smoking status	
Never smoker	9 [33]
Former smoker	12 [45]
Current smoker	6 [22]
ECOG PS	
0	5 [19]
1	12 [44]
2	8 [30]
Unknown	2 [7]
Histology	
Adenocarcinoma	17 [62]
Squamous cell carcinoma	5 [19]
Others	5 [19]
First line treatment	
Platinum doublet	25 [93]
Other[†]	2 [7]

Data are presented as mean ± standard deviation or n [%]. [†], 1 patient got single gemcitabine and 1 patient got carboplatin/paclitaxel/bevacizumab. ECOG PS, Eastern Cooperative Oncology Group performance status.

baseline and post-chemotherapy groups (*Figure 4*).

Gut microbiome as a predictive factor of treatment efficacy or adverse events

The patients were categorized into two groups [PD and clinical benefit response (CBR), which included CR, PR, and SD] according to best response evaluation. Baseline microbiomes were explored to identify an association with treatment response. The relative abundance was shown in *Figure 5A*. *Proteus* and *Morganella* were relatively increased in the PD group, while unclassified *Muribaculaceae*, *Ligilactobacillus*, and *Dubosiella* were increased in the CBR group (*Figure 5B*). Top 10 taxa of baseline relative abundance were explored. There was a trend of higher abundance of *Shingella* and *Akkermansia* and lower abundance of *Klebseilla* in CBR compared to the PD group, but the statistical test by the Kruskal-Wallis test was not significant (P=0.22, 0.07, and 0.10, respectively) (*Figure 5C*). There was no significant difference in alpha nor beta diversity between PD and CBR (*Figure 6*).

AEs related to the stool sample at each time point were analyzed. Any AEs occurred in 20 patients, which were related to 27 stool samples. AEs of interest were neutropenia or febrile neutropenia, diarrhea, and neuropathy. Incidence of each AEs was reported in *Table 2*. There was no significant difference in alpha diversity in any AEs and AEs of interest. The relative abundance showed more diversity in patients without diarrhea (*Figure 7*).

There was a trend of higher Chao1 and Shannon index in patients who developed any AEs, and a trend of higher ACE, Shannon index, and Simpson index in patients who did not have diarrhea during the treatment period, but the differences were not significant. The confounding factors

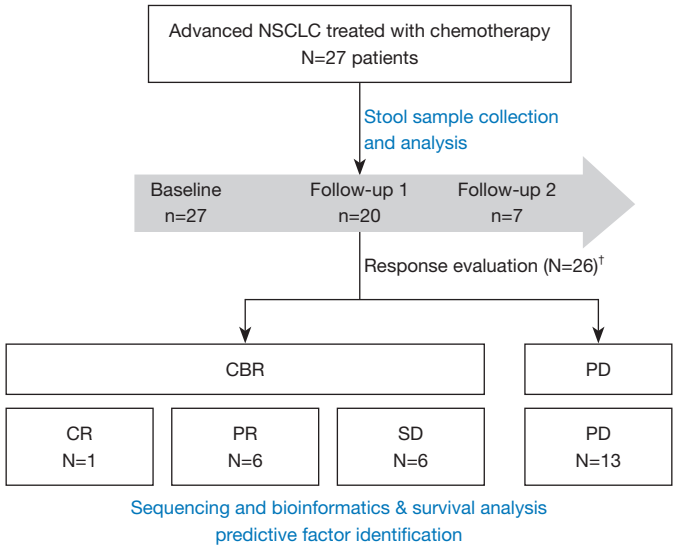


Figure 1 Flowchart of sample collection and data analysis. †, one patient died before disease evaluation. CBR, clinical benefit response; CR, complete response; NSCLC, non-small cell lung cancer; PD, progression of disease; PR, partial response; SD, stable of disease.

associated with diarrhea and gut microbiome results were considered in a multivariate logistic regression analysis. The history of antibiotics, proton pump inhibitors, and pre- and probiotics use was adjusted for diarrhea, but the result remained non-significant (see *Table 3*).

Discussion

Advanced NSCLC patients treated with chemotherapy had a poorer prognosis compared to patients with genomic alteration who can access targeted therapy or those without genomic alteration who can access immunotherapy. Patients in our cohort, including advanced NSCLC treated with chemotherapy, had a median PFS of 5.3 months and a median OS of 13.8 months, comparable to historical studies (8). The association of gut microbiome and chemotherapy in advanced NSCLC is still under investigation. Our study explored the effect of chemotherapy on gut microbiome change and the role of gut microbiome as a predictive biomarker for efficacy or AEs. We found that alpha diversity was reduced afterwards. Chemotherapy was not significantly associated with beta diversity. We did not see significant predictive ability of gut microbiome for treatment response or AEs.

We also explored the gut microbiome between each baseline characteristic variable; only albumin level was significantly associated with gut microbiome diversity, with lower diversity in patients with low albumin levels. In contrast to previous studies on different medical conditions, which suggest a high-protein diet is associated with low gut microbiome diversity (21). Previous studies reporting a relationship between protein levels and gut microbiome have suggested that this association is likely mediated by the mechanism of protein metabolism, which elevates short-chain fatty acid (SCFA) levels, consequently affecting immune modulation (21). However, our study did not collect baseline data and stool samples prior to cancer onset; therefore, the duration of hypoalbuminemia in the patients remains unknown. Since albumin is an acute-phase reactant that can decrease rapidly during severe illness, it is difficult to determine why our gut microbiome findings diverge from earlier reports. To gain a more comprehensive understanding, future research should investigate stool metabolites, such as SCFA levels, butyric acid, or L-tryptophan.

Regarding the effects of chemotherapy on gut microbiome, our study observed a decrease in alpha diversity post-chemotherapy, while beta diversity remained unchanged. At the genus level, *Enterobacter* abundance decreased, whereas *Fusobacterium* increased. These results partially align with existing literature but also present significant discrepancies. Specifically, Papanicolas *et al.* (22), investigating the impact of chemotherapy on the gut microbiome in solid tumors, reported that bacterial richness increased 7–10 days post-treatment and remained significantly elevated after the first chemotherapy cycle. This contrasts with our findings, where the ACE and

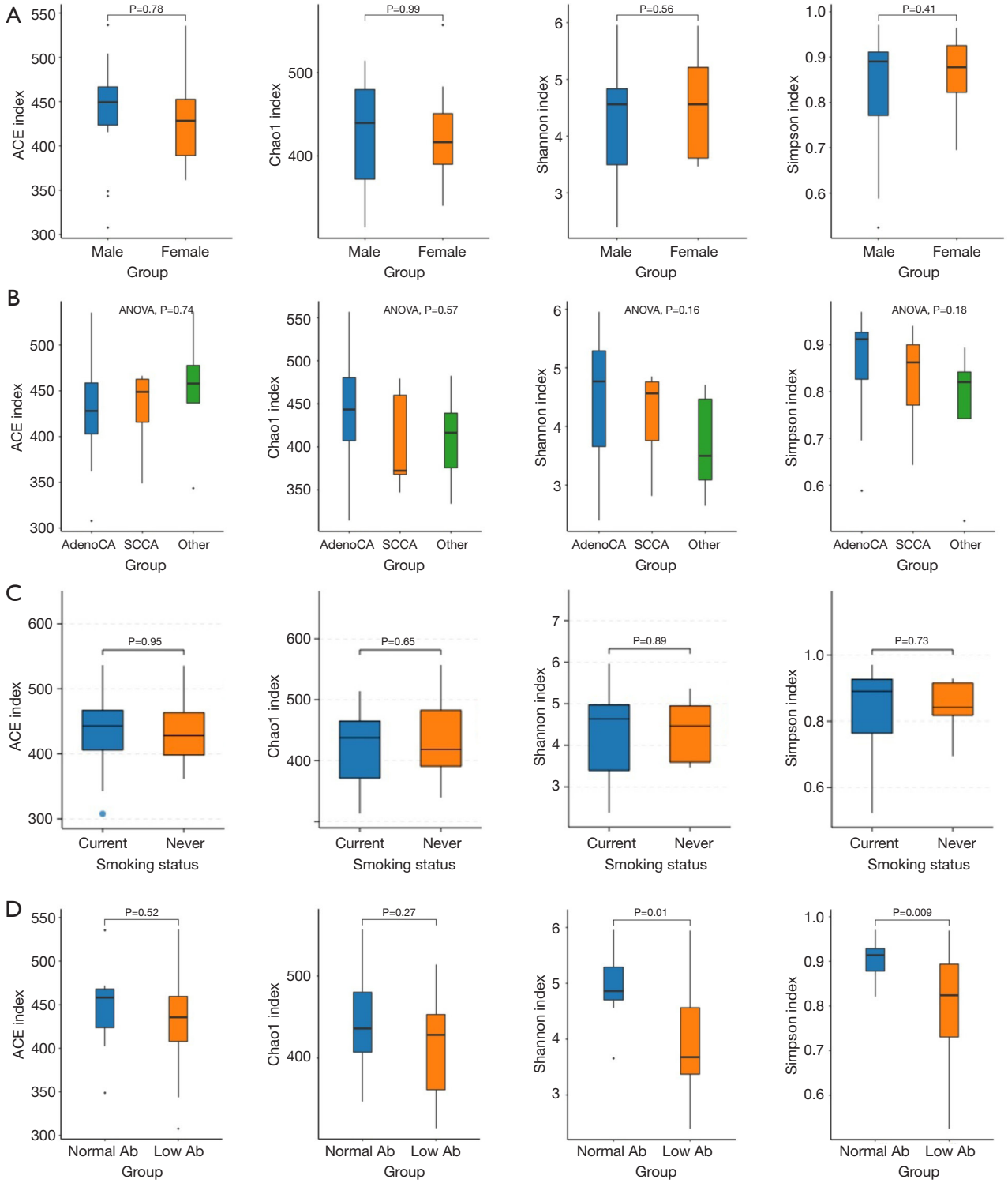


Figure 2 Alpha diversity in baseline stool samples by clinical subgroup. (A) Gender; (B) histologic subtype; (C) smoking status; (D) albumin level. Ab, albumin; ACE, abundance-based coverage estimator; AdenoCA, adenocarcinoma; ANOVA, analysis of variance; ns, non-significant; SCCA, squamous cell carcinoma.

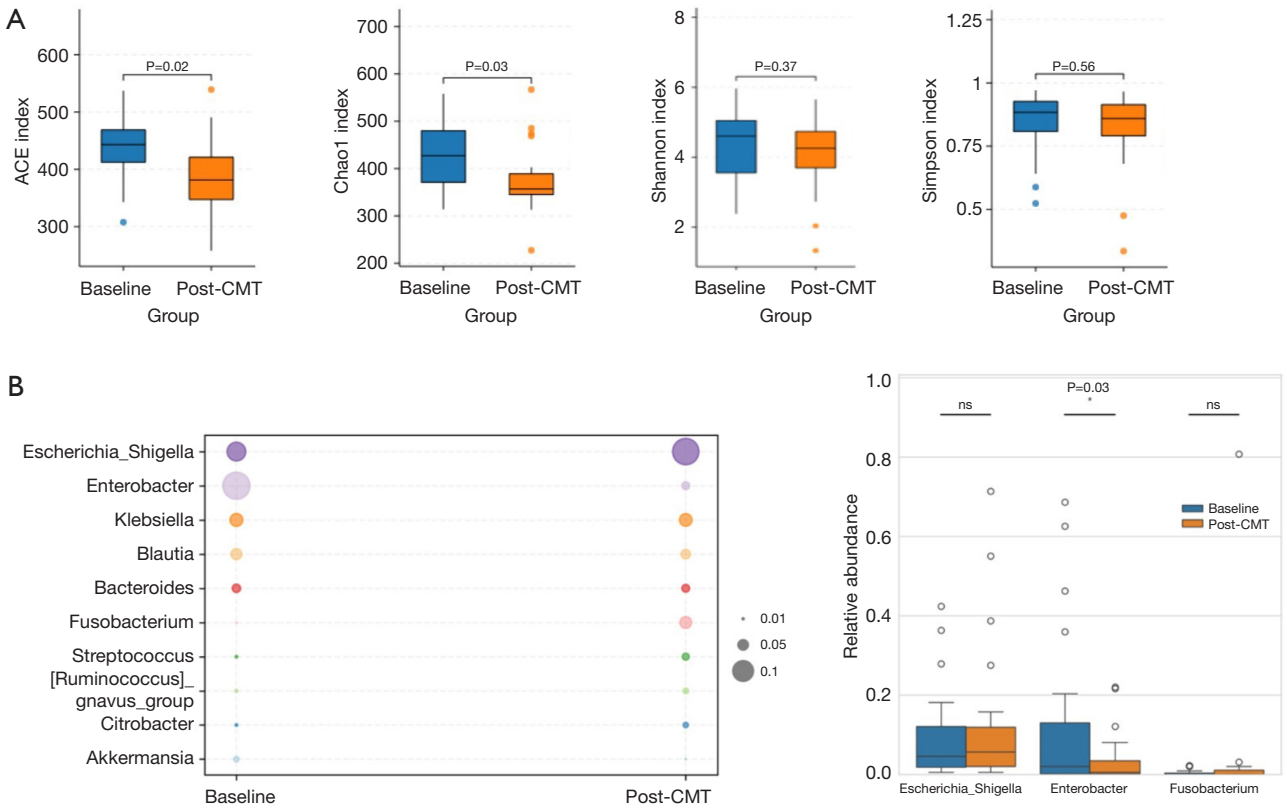


Figure 3 Alpha diversity at baseline compared to post-chemotherapy. (A) Alpha diversity at baseline compared to post-chemotherapy. (B) Relative abundance of top 10 taxa between baseline compare to post-chemotherapy. ACE, abundance-based coverage estimator; ns, non-significant; CMT, chemotherapy.

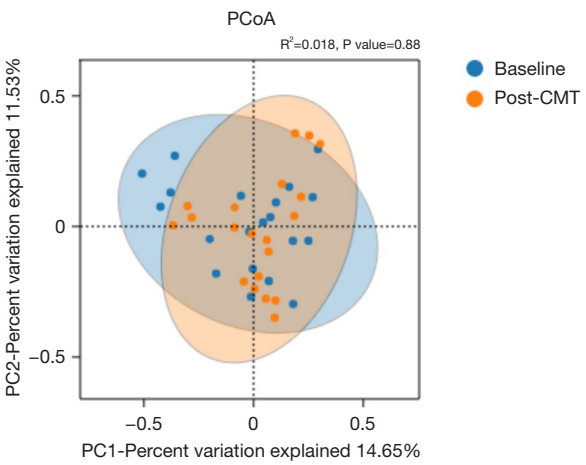
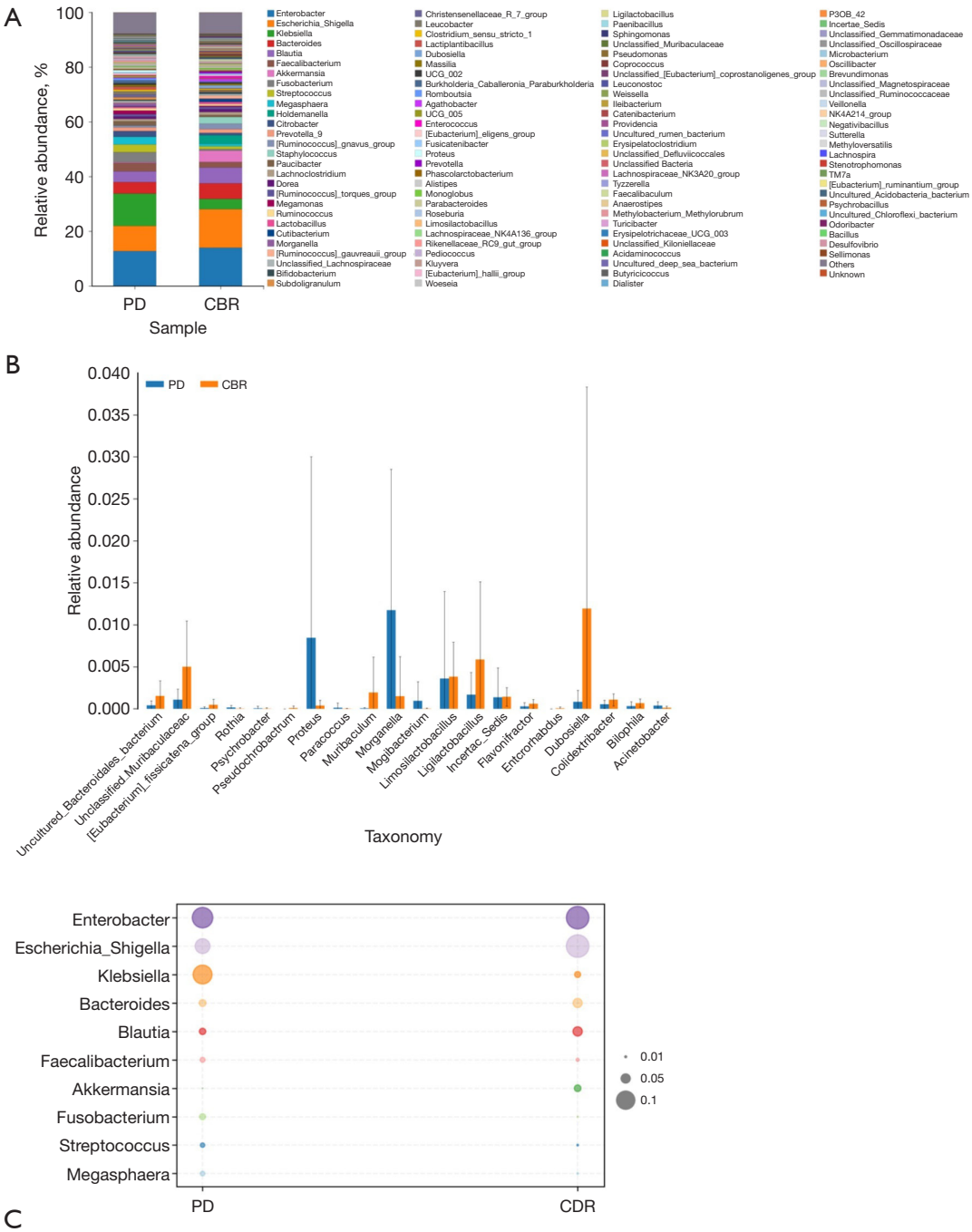


Figure 4 Beta diversity at baseline compared to post-chemotherapy. CMT, chemotherapy; PC, principal component; PCoA, Principal Coordinates Analysis.



Genus	PD, mean ±SEM	CBR, mean ±SEM	H-statistic	P value*
Escherichia_Shigella	0.089258 ± 0.028830	0.142448 ± 0.035633	1.4523	0.22
Klebsiella	0.112806 ± 0.043247	0.035504 ±0.009456	2.6095	0.10
Akkermansia	0.002773 ± 0.001212	0.039876 ±0.019789	3.1302	0.07

*, Kruskal-Wallis test.

Figure 5 Relative abundance of baseline gut microbiome between PD and CBR. (A) relative abundance taxa between PD and CBR; (B) relative abundance of microbial taxa between PD and CBR; (C) relative abundance of top 10 taxa between PD and CBR. CBR, clinical benefit response; PD, progression of disease; SEM, standard error of the mean.

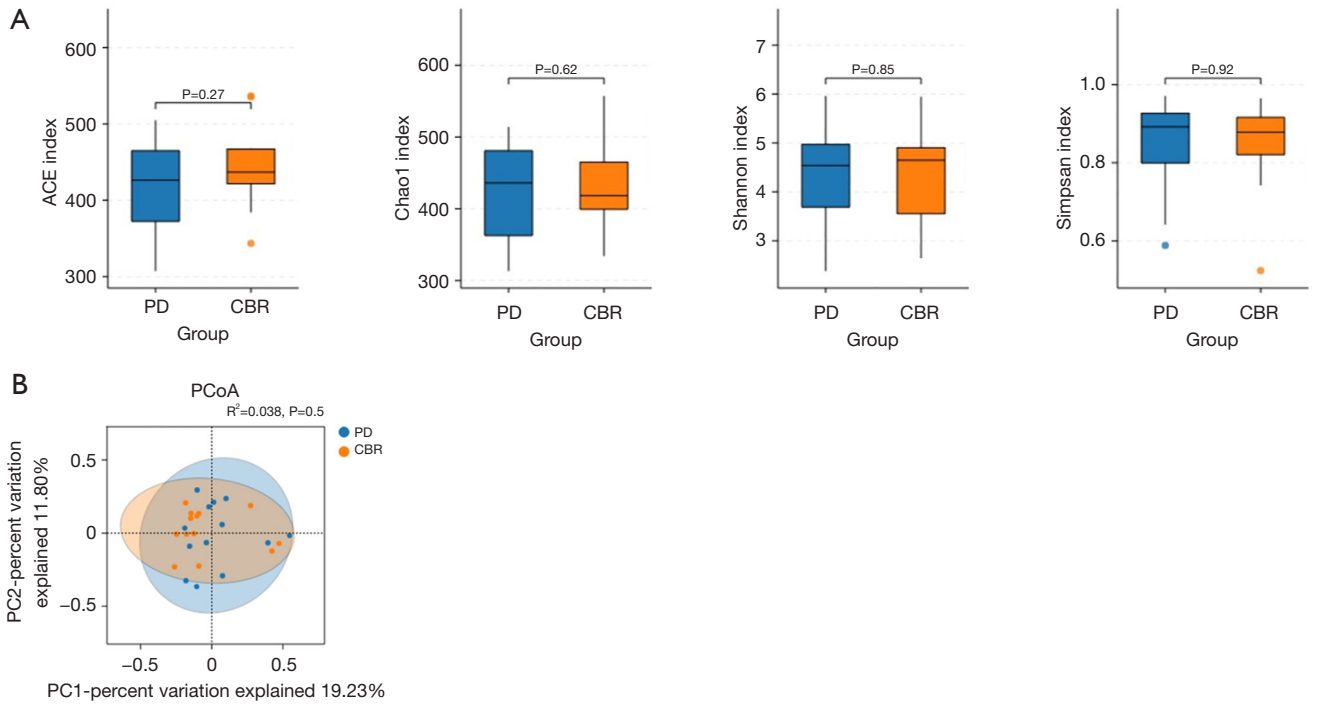


Figure 6 Baseline alpha diversity (A) and beta diversity (B) between PD and CBR. ACE, abundance-based coverage estimator; CBR, clinical benefit response; PC, principal component; PCoA, Principal Coordinates Analysis; PD, progression of disease.

Table 2 Incidence of AEs

Adverse events	All patients (n=27)	All stool samples (n=54)
Any AEs	20 [74]	27 [50]
Neutropenia or FN	4 [15]	4 [7]
Diarrhea	3 [11]	3 [5]
Neuropathy	9 [33]	9 [17]

Data are presented as n [%]. AE, adverse event; FN, febrile neutropenia.

Chao1 indices decreased following chemotherapy. However, their study similarly found no significant difference in the Shannon index. Furthermore, the relative abundances we observed differed from those reported by Papanicolas *et al.*; our study identified an increase in *Fusobacterium* (Phylum: Bacillota/Firmicutes) and a decrease in *Enterobacter* (Phylum: Pseudomonadota/Proteobacteria), whereas Papanicolas *et al.* reported a decrease in Firmicutes and an increase in Proteobacteria.

In contrast, our results are consistent with those of Zhang *et al.* (23), who investigated changes in gut microbiome in advanced NSCLC patients treated with platinum-doublet chemotherapy, comparing pre- and post-chemotherapy samples, and reported that, at the phylum level, Firmicutes abundance increased and Proteobacteria abundance decreased after chemotherapy. This trend is further supported by Saifon *et al.* (24), which data on EGFR wild-type NSCLC patients demonstrated a reduction in Proteobacteria and an increase in both Bacteroidetes and Firmicutes post-chemotherapy. Currently, there are not many studies investigating the effect of chemotherapy on the gut microbiome, and the data specifically on lung cancer patients is very limited compared to those with GI cancers. Therefore, the interpretation of the findings in this area must be done with caution, as the underlying mechanisms and clinical implications in this specific patient population

Figure 7 Relative abundance of gut microbiome between no diarrhea and diarrhea.

Table 3 Alpha diversity index as predictive factor of diarrhea (logistic regression analysis)

Alpha diversity index	Univariate analysis		Multivariate analysis[†]	
	OR (95% CI)	P value	OR (95% CI)	P value
ACE	1.00 (0.98, 1.01)	0.75	1.00 (0.98,1.02)	0.71
Chao1	1.00 (0.99, 1.02)	0.62	1.00 (0.99, 1.02)	0.83
Shannon index	0.54 (0.21, 1.41)	0.20	0.49 (0.17, 1.42)	0.18
Simpson index	0.02 (0.00, 4.50)	0.15	0.01 (0.00, 4.27)	0.12

[†], adjusted for history use of antibiotics, proton pump inhibitors, and pre-/probiotics use. ACE, abundance-based coverage estimator; CI, confidence interval; OR, odds ratio.

warrant further investigation.

Regarding the role of the gut microbiome as a predictor of chemotherapy response, our study found no significant differences in alpha or beta diversity between the PD and CBR groups. However, the PD group showed an increased relative abundance of *Proteus* and *Morganella*, whereas *Muribaculaceae*, *Ligilactobacillus*, and *Dubosiella* were more prevalent in the CBR group. We also observed a trend toward higher abundance of *Shingella* and *Akkermansia*, alongside a lower abundance of *Klebsiella*, in the CBR group compared to the PD group, although these findings did not reach statistical significance. These findings align with the previous study by Zhang *et al.* (23), which also found a higher relative abundance of *Akkermansia* in responders and higher *Klebsiella* in the PD group. Furthermore, Harberman *et al.* (25) reported that a higher abundance of *Akkermansia muciniphila* is associated with lung cancer patients who achieved durable disease control with chemotherapy. Nevertheless, findings regarding specific bacterial taxa associated with chemotherapy response in lung cancer vary across studies. For example, some studies reported the enrichment of *Streptococcus mutans* and *Enterococcus casseliflavus* in the responder group among NSCLC patients treated with chemotherapy (26). Conversely, others have found a relatively high abundance of *Faecalibacterium*, *Klebsiella*, *Coprococcus*, *Roseburia*, *Lactobacillus*, *Streptococcus*, *Prevotella*, and *Dorea* in advanced NSCLC patients treated with chemotherapy who experienced disease progression (23).

Currently, most of the evidence regarding the gut microbiome's role in cancer response prediction is focused on immunotherapy (18,27). with limited data available for chemotherapy. However, both our cohort and previous studies have identified certain bacteria, especially

Akkermansia, that are related to treatment response. Therefore, acquiring more data and conducting studies on the methods and benefits of manipulating gut microbiome and its impact on tumor response would likely be more beneficial for patients in the future.

For the role of AEs predictor, there was no significant difference in alpha diversity across any AEs. The relative abundance showed greater diversity in patients without diarrhea. This might be due to changes in certain bacteria during diarrhea episodes, or a confounding effect from concomitant antibiotic use during diarrhea, resulting in lower diversity in the diarrhea group compared to the non-diarrhea group. Most of the previous studies concerning the gut microbiome and chemotherapy side effects predominantly focus on GI adverse events. Furthermore, the majority of these studies involve GI cancers or chemotherapy drugs that frequently cause GI symptoms, such as 5-FU, capecitabine, and irinotecan (28-30). Therefore, the interpretation of these findings must be conducted with caution due to multiple confounding factors, including diarrhea itself or the co-administration of various medications used to alleviate the symptoms (e.g., antibiotics, probiotics, prebiotics, and anti-motility drugs). In this study, we addressed concerns about confounding factors by adjusting for them in a multivariate analysis. After this adjustment, no specific gut microbiome index was found to be associated with chemotherapy-induced diarrhea.

Conclusions

In conclusion, this exploratory study provided a preliminary examination of the associations between chemotherapy and gut microbiome dynamics in patients with NSCLC. Given the limited sample size, the findings did not reach statistical significance and should be interpreted with caution. However, the notable trends observed—specifically regarding the presence of *Akkermansia* and its alignment with favorable treatment responses seen in prior literature—serve a hypothesis-generating purpose. These preliminary observations suggest that *Akkermansia* may warrant further investigation in larger, adequately powered prospective cohorts to determine its potential role as a predictive biomarker or a target for adjunctive therapeutic strategies for chemotherapy treated NSCLC patients.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-2026-1-0209/rc

Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-2026-1-0209/dss

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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. The study was conducted in accordance with the Declaration of Helsinki and its subsequent amendments. The study was approved by the Human Research Ethics Committee, Faculty of Medicine, Ramathibodi Hospital, Mahidol University (approval No. COA.MURA2021/956). Written informed consent was obtained from all patients prior to sample collection.

References

1. Filho AM, Laversanne M, Ferlay J, et al. The GLOBOCAN 2022 cancer estimates: Data sources, methods, and a snapshot of the cancer burden worldwide. Int J Cancer 2025;156:1336-46.
2. Ferlay J, Ervik M, Lam F, et al. Global Cancer Observatory: Cancer Today. Lyon France: International Agency for Research on Cancer; 2024 [cited 2025 June 18]. Available online: https://gco.iarc.who.int/today
3. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
4. Peters S, Camidge DR, Shaw AT, et al. Alectinib versus Crizotinib in Untreated ALK-Positive Non-Small-Cell Lung Cancer. N Engl J Med 2017;377:829-38.
5. Soria JC, Ohe Y, Vansteenkiste J, et al. Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. N Engl J Med 2018;378:113-25.
6. Khunger M, Jain P, Rakshit S, et al. Safety and Efficacy of PD-1/PD-L1 Inhibitors in Treatment-Naive and Chemotherapy-Refractory Patients With Non-Small-Cell Lung Cancer: A Systematic Review and Meta-Analysis. Clin Lung Cancer 2018;19:e335-48.
7. Zhou Y, Chen C, Zhang X, et al. Immune-checkpoint inhibitor plus chemotherapy versus conventional chemotherapy for first-line treatment in advanced non-small cell lung carcinoma: a systematic review and meta-analysis. J Immunother Cancer 2018;6:155.
8. Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002;346:92-8.
9. Zitvogel L, Daillère R, Roberti MP, et al. Anticancer effects of the microbiome and its products. Nat Rev Microbiol 2017;15:465-78.
10. Li W, Deng Y, Chu Q, et al. Gut microbiome and cancer immunotherapy. Cancer Lett 2019;447:41-7.
11. Fijlstra M, Ferdous M, Koning AM, et al. Substantial decreases in the number and diversity of microbiota during chemotherapy-induced gastrointestinal mucositis in a rat model. Support Care Cancer 2015;23:1513-22.
12. Frank M, Hennenberg EM, Eyking A, et al. TLR signaling modulates side effects of anticancer therapy in the small intestine. J Immunol 2015;194:1983-95.
13. Wallace BD, Wang H, Lane KT, et al. Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. Science 2010;330:831-5.
14. Halley A, Leonetti A, Gregori A, et al. The Role of the Microbiome in Cancer and Therapy Efficacy: Focus on Lung Cancer. Anticancer Res 2020;40:4807-18.
15. Routy B, Le Chatelier E, Derosa L, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science 2018;359:91-7.
16. Matson V, Fessler J, Bao R, et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. Science 2018;359:104-8.
17. Gopalakrishnan V, Spencer CN, Nezi L, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science 2018;359:97-103.
18. Jin Y, Dong H, Xia L, et al. The Diversity of Gut Microbiome is Associated With Favorable Responses to Anti-Programmed Death 1 Immunotherapy in Chinese Patients With NSCLC. J Thorac Oncol 2019;14:1378-89.
19. Alexander JL, Wilson ID, Teare J, et al. Gut microbiota modulation of chemotherapy efficacy and toxicity. Nat Rev Gastroenterol Hepatol 2017;14:356-65.
20. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228-47.
21. Wu L, Tang Z, Chen H, et al. Mutual interaction between gut microbiota and protein/amino acid metabolism for host mucosal immunity and health. Anim Nutr 2021;7:11-6.
22. Papanicolas LE, Sims SK, Taylor SL, et al. Conventional myelosuppressive chemotherapy for non-haematological malignancy disrupts the intestinal microbiome. BMC Cancer 2021;21:591.
23. Zhang M, Zhou H, Xu S, et al. The gut microbiome can be used to predict the gastrointestinal response and efficacy of lung cancer patients undergoing chemotherapy. Ann Palliat Med 2020;9:4211-27.
24. Saifon W, Sensorn I, Trachu N, et al. Gastrointestinal microbiota profile and clinical correlations in advanced EGFR-WT and EGFR-mutant non-small cell lung cancer. BMC Cancer 2022;22:963.
25. Haberman Y, Kamer I, Amir A, et al. Gut microbial signature in lung cancer patients highlights specific taxa as predictors for durable clinical benefit. Sci Rep 2023;13:2007.
26. Zhao Z, Fei K, Bai H, et al. Metagenome association study of the gut microbiome revealed biomarkers linked to chemotherapy outcomes in locally advanced and advanced

lung cancer. Thorac Cancer 2021;12:66-78.
27. Del Giudice T, Staropoli N, Tassone P, et al. Gut Microbiota Are a Novel Source of Biomarkers for Immunotherapy in Non-Small-Cell Lung Cancer (NSCLC). Cancers (Basel) 2024;16:1806.
28. Kawasaki Y, Kakimoto K, Tanaka Y, et al. Relationship between Chemotherapy-Induced Diarrhea and Intestinal Microbiome Composition. Digestion 2023;104:357-69.
29. Jiang W, Wu Y, He X, et al. Important Role of Intestinal Microbiota in Chemotherapy-induced Diarrhea and Therapeutics. J Cancer 2025;16:648-59.
30. Fei Z, Lijuan Y, Xi Y, et al. Gut microbiome associated with chemotherapy-induced diarrhea from the CapeOX regimen as adjuvant chemotherapy in resected stage III colorectal cancer. Gut Pathog 2019;11:18.

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