

# Lung Microbiota Signature and Corticosteroid Responses in Pneumonia-Associated Acute Respiratory Distress Syndrome in Hematological Patients

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**Objective:** In this study, we aim to classify hematological patients with the pneumonia-associated acute respiratory syndrome (ARDS) into different groups that were characterized by distinct early responsiveness to corticosteroids, describe the microbiota signatures of the non-responders and responders, and compare the prognosis of the two groups.

**Methods:** Hematological patients with ARDS were included and treated with mechanical ventilation and corticosteroid. According to the early improvement to the corticosteroid therapy, patients were classified as non-responders and responders. The lung microbiota signatures and the outcomes of the non-responders and responders were compared.

**Results:** Fifty patients were included in this study. Twenty-eight patients were classed as non-responders and 22 as responders. Compared to the non-responders, responders had higher serum levels of IL-6, IL-8, TNF- $\alpha$  and CRP, their lung microbiota was with lower alpha diversity and enriched with virus species. The responders had an overall higher ventilator free days than the non-responders [4 (0–6) vs 6 (0–10),  $p=0.034$ ], for survivors the difference was more significant [5 (3–6) vs 8 (3–10),  $p=0.012$ ]. Survival analysis showed that there was no difference in survival rate between the two groups over time (Log-rank  $p=0.073$ ). When non-responders were stratified into subgroups of patients with infection or co-infection, those non-responders with co-infection had significantly lower survival rate than other patients (Log-rank  $p=0.028$ ).

**Conclusion:** For hematological patients with pneumonia-associated ARDS, the responders of corticosteroids had higher ventilator free days at day 28 than the non-responders. The microbiota signatures were distinct in the two groups. The non-responders with coinfections had the lowest survival rate when compared to the non-responders with no coinfections and the responders.

**Keywords:** microbiota, acute respiratory distress syndrome, corticosteroid, hematologic neoplasms

## Introduction

Acute respiratory failure, especially the Acute Respiratory Distress Syndrome (ARDS) following lung infection contributed to a large proportion of admissions of hematological patients to the Intensive Care Units (ICU).<sup>1,2</sup> As a result of the therapeutic progress in critical care medicine and as a result of the advancements in immunological therapies and chemotherapies, the prognosis of these patients has been improved over the years.<sup>3</sup> However, ARDS in hematological patients still has relatively high mortality and responded poorly to various treatments when compared to immunocompetent hosts.<sup>4,5</sup>

Lots of studies have evaluated the therapeutic effect of corticosteroids on ARDS. Although not all the previous studies have justified the routine use of this immune-modulation agent,<sup>6,7</sup> more emerging evidence have supported the low to

moderate dosage application of corticosteroids, especially in ARDS caused by bacterial pneumonia and sepsis.<sup>8,9</sup> In a recent study by Sinha et al., patients with coronavirus disease (COVID-19) and ARDS were clustered into subgroups that responded differently to corticosteroids – patients with higher inflammatory markers had decreased mortality after corticosteroid therapy<sup>10</sup> – which indicates that an important factor in corticosteroid therapy was selecting the right subgroup of patients.

The metagenomic next-generation sequencing (mNGS) has provided a new perspective to the understanding of pathogen-host interactions. By analyzing sequencing data of bronchial alveolar lavage fluid (BALF), Wang et al. reported that the bio-diversity and dominant species varies significantly from patients with adenoviral or mycoplasma pneumoniae.<sup>11</sup> In immunocompromised hosts (ie, HIV patients with pneumonia), different microbiomes were correlated with distinct host immune-responses.<sup>12</sup> Improvement of oxygenation within 1 to 2 days of corticosteroids therapy has been proved to be correlated with reduced ICU mortality in pediatric patients with ARDS.<sup>13</sup> On the other hand, side effects like secondary infections may take weeks to occur,<sup>14</sup> which imply that it is possible and crucial to predict the prognosis of corticosteroid-treated patients based on their early response to the drug. Besides, timely withdrawn of the corticosteroids for non-responsive patients may avoid side effects of the drug. Thus, it is rational to hypothesize that the early response of hematological patients with ARDS to corticosteroids was also correlated with diverse lung microbiome signatures and different prognosis.

In this prospective study, we aim to 1) classify hematological patients with pneumonia-associated ARDS into different groups that are characterized with distinct early responsiveness to corticosteroids. 2) describe the microbiota signatures of the non-responders and responders. 3) compare the prognosis of the non-responders and responders.

## Methods

### Ethics Statement

This prospective study was approved by the Ethics and Research board of Peking University People's Hospital (2020PHB400-09) was conducted under the principles of the Helsinki Declaration. Informed consent was collected from patients or relatives before the inclusion of patients.

### Inclusion of Patients

Hematological patients with pneumonia that admitted (From June 2020 to August 2021) to the two study centers were screened for their possibility of inclusion in the study. The inclusion criteria were as follows: 1) meeting the diagnosis of ARDS.<sup>15</sup> 2) consent on BAL and NGS tests. We exclude patients who were 1) <18 years old. 2) In pregnancy. 3) have received hematopoietic stem cell transplantation. 4) with pulmonary diseases that may benefit from corticosteroid therapy, including asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, diffuse alveolar hemorrhage, bronchiolitis obliterans, etc. 5) have received corticosteroid therapy before the onset of ARDS. 6) declined invasive mechanical ventilation. 7) with intracranial bleeding, status epilepticus and refractory hypoxemia that cannot be alleviated by non-invasive ventilation, which required invasive mechanical ventilation (IMV) or extracorporeal membrane oxygenation (ECMO) in the first 24 hours after ICU admission.

### Baseline Variables

On the day of ICU admission, baseline characteristics of patients (eg, age, sex and primary malignancy) were recorded. Blood samples were collected from included patients, regular laboratory tests including whole blood count, chemistry tests (ie, creatine, lactate), the function of coagulation (ie, prothrombin time), serum cytokine (IL- 6, IL- 8, INF-  $\gamma$  and TNF-  $\alpha$ ), C- reactive protein, procalcitonin levels, Brain natriuretic peptide (BNP) levels and arterial blood gas (ie, PO<sub>2</sub> /FiO<sub>2</sub>, PCO<sub>2</sub>, pH value) were performed in the hospital laboratory.

### Mechanical Ventilation Support

All included patients were supported by non-invasive ventilation at the time of ICU admission, the parameters of mechanical ventilation were set to maintain a minimal SPO<sub>2</sub> of 93%, a tidal volume around 6 mL/Kg (predicted body weight) and a respiratory rate lower than 30 per minutes. If these requirements cannot be maintained through non-invasive ventilation, the patient will be intubated and supported with IMV (those intubated in the first 24 hours after

admission were excluded from this study). The IMV was performed with the same target as the non-invasive ventilation. Sedation and analgesia will be used to improve patient-ventilator synchronization. Other adjunctive therapies of ARDS (eg, ECMO, prone positioning, Neuro-muscular blocking) were performed at the discretion of the physician in charge.

## Corticosteroid Therapy

Patients will receive methylprednisolone with a dosage of 2mg/kg per day for 3 days. The dosage will be tapered every three days, with the total dosage downregulated 40mg every time (dosage lower than 40mg will be stopped in the following days). For example, a patient with body weight of 70kg will receive the following dosages day by day: 140mg, 140mg, 140mg, 100mg, 100mg, 100mg, 60mg, 60mg, 60mg, 20mg, 20mg, 20mg. The corticosteroid therapy will be stopped in case of gastrointestinal bleeding suspected of being caused by methylprednisolone.

## Classification of Patients as Non-Responders or Responders

The arterial blood gas was tested at the time of ICU admission and before the first dosage of corticosteroid and re-tested 24 hours later, before the application of corticosteroid. The changes of  $PO_2/FiO_2$  between these two time points were recorded as  $\Delta PO_2/FiO_2$ , patients with  $\Delta PO_2/FiO_2 \geq 150$ mmHg were classified as responders, other patients were classified as non-responders (Figure 1A).

## Bronchial Alveolar Lavage Sampling and RNA-Based mNGS

All included patients received a bronchoscope examination with bronchial alveolar lavage within the first 24 hours of admission. At least 5mL of bronchial alveolar lavage fluid (BALF) was collected in the procedure. BALF samples were analyzed with an RNA-based mNGS approach to ensure the detection of RNA viruses with DNA viruses, fungus and bacteria. The mNGS was performed as previously described.<sup>16</sup> Briefly, RNA was extracted from BALF. Through reverse transcription, cDNA libraries were constructed and sequenced with NextSeq 550 System (150-bp paired-end reads; Illumina) to generate sequencing data for downstream analysis.

## Bioinformatic Analysis of Sequencing Data

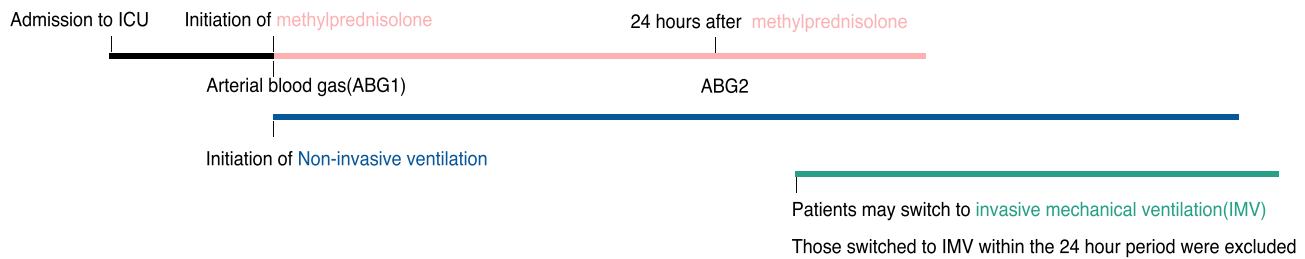
Sequencing data were processed with “Kneaddata” (v0.9.0) to remove host contaminations.<sup>17</sup> Cleaned data were analyzed with the “MetaPhlan2” function in “HUMAnN2” (v2.8.1) to assign taxonomy of the species in the BALF sample.<sup>18</sup> The visualization of alpha and beta diversity were performed with the “EazyAmplicon” pipeline<sup>19</sup> in R. Heatmap of the relative abundances of different families were presented with the “pheatmap” package. Linear discriminant analysis effect size (LDA with LEfSe, v1.1.1) was applied to the abundance output file from MetaPhlan2 to identify species or families as markers associated with each group.<sup>20</sup>

## Etiological Diagnosis for the Non-Responders and Responders

The etiological diagnoses of patients were made based on taxonomy results from MetaPhlan2. The overall threshold of reads (positive reads per ten million) of different microbes were: viruses- 100 reads, bacteria- 20 reads (mycobacteria- 5 reads), fungus –20 reads (Mucoraceae- 1 reads), those microbes that had reads over their thresholds will be considered as pathogens, with reference to culture results of bronchoalveolar lavage fluids, and with reference to chest radiological examinations. Two physicians (JS and YH) will identify the etiological organism of the patient. Generally, organisms with the highest abundance will be considered as the primary etiological organism, however, if another organism was presented with high abundance and correlated with the clinical characteristics of the patients, it will be identified as the secondary etiological organism – in this case, a diagnosis of coinfection will be considered. If the two physicians had disagreements on the diagnosis, the issue (SL) will be referred to a senior physician for the final decision.

## Outcomes of the Non-Responders and Responders

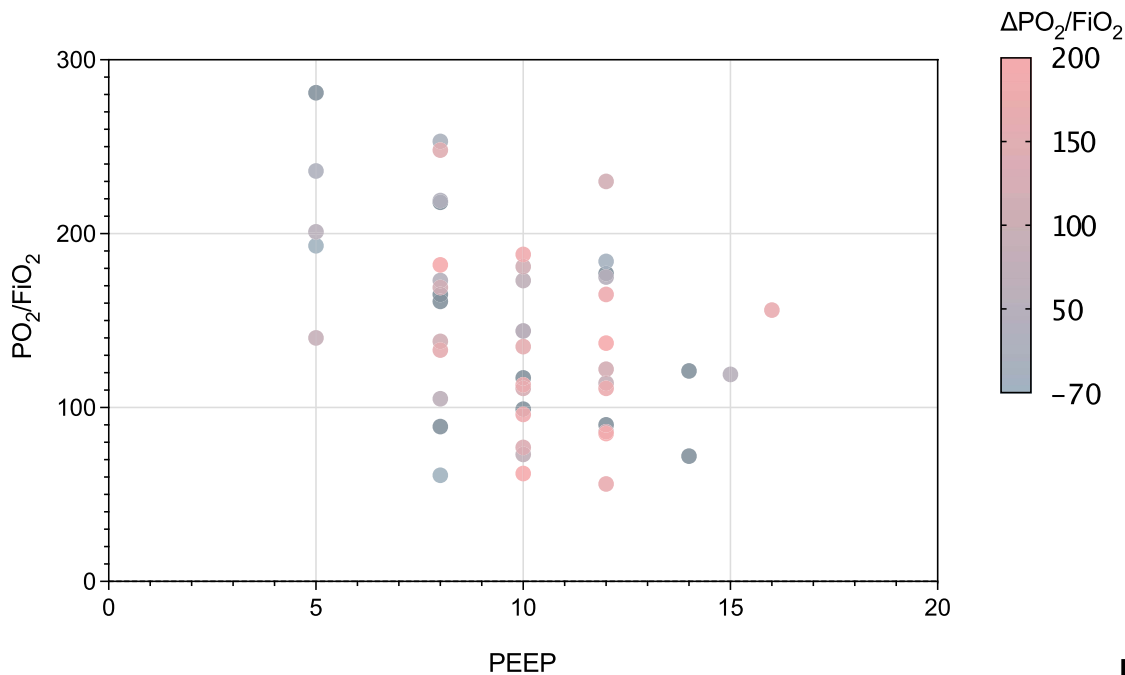
The primary outcome of this study was the 30-day mortality. Secondary outcomes include the rate of IMV after the first 24 hours, ICU length of stay, ventilator free days (at day 28), ventilator free days in survivors (at day 28), new-onset viremia or bacteremia. Kaplan-Meier statistics were used to estimate the 30-day survival of patients stratified by groups



Responder:  $\Delta PO_2/FiO_2 \geq 150$  mmHg between ABG2 and ABG1

Non-responder:  $\Delta PO_2/FiO_2 < 150$  mmHg between ABG2 and ABG1

**A**



**B**

**Figure 1** Study design and distribution of  $\Delta PO_2/FiO_2$  for all included patients. **(A)** Schematic diagram of study design. **(B)** Distribution of  $\Delta PO_2/FiO_2$  of all included patients. **Abbreviation:** PEEP, Positive End Expiratory Pressure.

was conducted with “survival” and “survminer” packages in R. The clinical course of patients in the two groups and the constitution of non-responders and responders (by patients with or without coinfection) were demonstrated with Sankey plots in Originlab (v2021b, OriginLab Corporation, Northampton, MA, USA).

## Statistical Analysis

All statistical analyses were performed with R (v4.1.1). Continuous variables were presented as median (interquartile range), categorical variables were presented as numbers (percentage). Comparison of two groups of continuous variables was performed with the Mann–Whitney  $U$ -test. Comparison of categorical variables was achieved using  $\chi^2$  test. The correlations between two variables were evaluated using the Pearson correlation coefficient. All tests were two-sided and  $p < 0.05$  was considered statistically significant.

## Results

### Patients

58 hematological patients with a diagnosis of ARDS were admitted into the two ICUs during the study period. 8 patients met the exclusion criteria (3 patients were intubated in the first 24 hours after admission), other 50 patients were included, all patients received the full course of corticosteroid therapy. 28 patients were classified as non-responders while 22 patients were classified as responders. The baseline characteristics of the two groups were presented in Table 1. Compared to their counterparts, the responders tend to have lower level of  $PO_2/FiO_2$  (169 mmHg vs 175 mmHg,  $p=0.041$ ), but higher levels of  $\Delta PO_2/FiO_2$  [73.52 (29.63–86.55) mmHg vs 165 (111.03–195.52) mmHg,  $p<0.001$ ], C-reactive protein (87.21 mg/L vs 58.32 mg/L,  $p<0.001$ ) and cytokines including IL-6 [220.3 (96.37–371.01) pg/mL vs 62.52 (12.26–93.11) pg/mL,  $p<0.001$ ], IL-8 [114.27 (67.55–240.22) pg/mL vs 18.44 (13.22–20.56) pg/mL,  $p<0.001$ ]

**Table 1** Characteristics of Corticosteroid Non-Responders and Responders

	Non-Responders (n=28)	Responders (n=22)	P
Age (y)	37 (24–49)	34 (20–53)	0.167
Female sex (%)	16 (57.14)	9 (54.5)	0.254
BMI, Kg/m <sup>2</sup>	20.65 (18.23–22.42)	21.48 (19.27–23.42)	0.315
Primary Malignancy			0.982
AML/CML/MDS	11 (39.29)	9 (40.91)	
ALL	5 (17.86)	3 (13.64)	
Lymphoma	4 (14.28)	4 (18.18)	
MM	6 (21.43)	5 (22.72)	
Others	2 (7.14)	1 (4.55)	
Time from the diagnosis of pneumonia to BAL, hours	42 (36–94)	39 (32–78)	0.072
SOFA score	7 (4–8)	6 (4–9)	0.263
Prothrombin Time, s	11.3 (10.2–13.5)	10.8 (10.5–11.3)	0.364
Lactate, mmol/L	2.3 (1.5–2.8)	1.9 (1.3–2.5)	0.762
Creatine, $\mu$ mol/L	97 (59–99)	95 (68–105)	0.624
Tmax, °C	38.4 (36.8–38.9)	38.6 (37.2–38.6)	0.532
WBC, $\times 10^9/L$	7.62 (1.82–10.57)	6.23 (2.21–9.63)	0.152
Lymphocyte count, $\times 10^9/L$	0.42 (0.19–0.58)	0.33 (0.24–0.69)	0.055
C-reactive protein, mg/L	87.21 (35.75–111.60)	58.32 (22.73–75.13)	0.027
Procalcitonin, ng/mL	0.47 (0.13–1.49)	0.51 (0.11–1.45)	0.258
Cytokines			
IL-6, pg/mL	62.52 (12.26–93.11)	220.30 (96.37–371.01)	<0.001
IL-8, pg/mL	18.44 (13.22–20.56)	114.27 (67.55–240.22)	<0.001
IFN- $\gamma$ , pg/mL	29.17 (16.64–44.87)	32.53 (12.10–37.98)	0.158
TNF- $\alpha$ , pg/mL	11.54 (10.22–26.42)	118.27 (87.63–142.45)	<0.001
BNP, pg/mL	273 (150–570)	316 (189–624)	0.158
$PO_2/FiO_2$ , mmHg	175 (88.5–193.2)	169 (96.1–188.4)	0.041
$\Delta PO_2/FiO_2$ , mmHg	73.52 (29.63–86.55)	165 (111.03–195.52)	<0.001
$PCO_2$ , mmHg	37 (32–42)	35 (33–44)	0.381

(Continued)

**Table I** (Continued).

	Non-Responders (n=28)	Responders (n=22)	P
PEEP, cmH <sub>2</sub> O	10 (8–12)	10 (8–12)	0.856
pH	7.37 (7.34–7.51)	7.39 (7.35–7.49)	0.733
Respiratory rate, breath/mins	24 (22–31)	22 (19–29)	0.188
Tidal volume, mL/Kg	7.3 (6.6–8.5)	6.8 (6.2–7.9)	0.522
Adjunctive therapies of ARDS			0.505
ECMO	0	1	
Neuro-muscular blocking	2	1	
Prone positioning	5	6	

**Abbreviations:** BMI, Body Mass Index; BNP, Brain Natriuretic Peptide; ECMO, Extra Corporal Membrane Oxygenation; FiO<sub>2</sub>, Fraction of inspired oxygen; LRTI, Lower Respiratory Tract Infection; PaO<sub>2</sub>, Partial pressure of Oxygen; PCO<sub>2</sub>, Partial pressure of Carbon Dioxide; PEEP, Positive End Expiratory Pressure; SOFA, Sequential Organ Failure Assessment; WBC, White Blood Cell.

and TNF-  $\alpha$  [11.54 (10.22–26.42) pg/mL vs 118.27 (87.63–142.45) pg/mL,  $p < 0.001$ ]. Other characters were comparative between the two groups. The distribution of  $\Delta PO_2/FiO_2$  of all included patients was depicted in [Figure 1B](#) (Also see [Table S1](#)), for those with lower baseline  $PO_2/FiO_2$  value, their  $\Delta PO_2/FiO_2$  tend to be higher, and the level of baseline PEEP was not strongly correlated with  $\Delta PO_2/FiO_2$  (Pearson correlation coefficient = 0.21,  $p = 0.15$ ), evaluated with simple linear regression, R squared for the model was 0.042, with a p-value of 0.152, indicating the PEEP and  $\Delta PO_2/FiO_2$  did not had a linear relationship (See [Figure S1](#)).

## Microbiota Signatures and Marker Species of Non-Responders and Responders

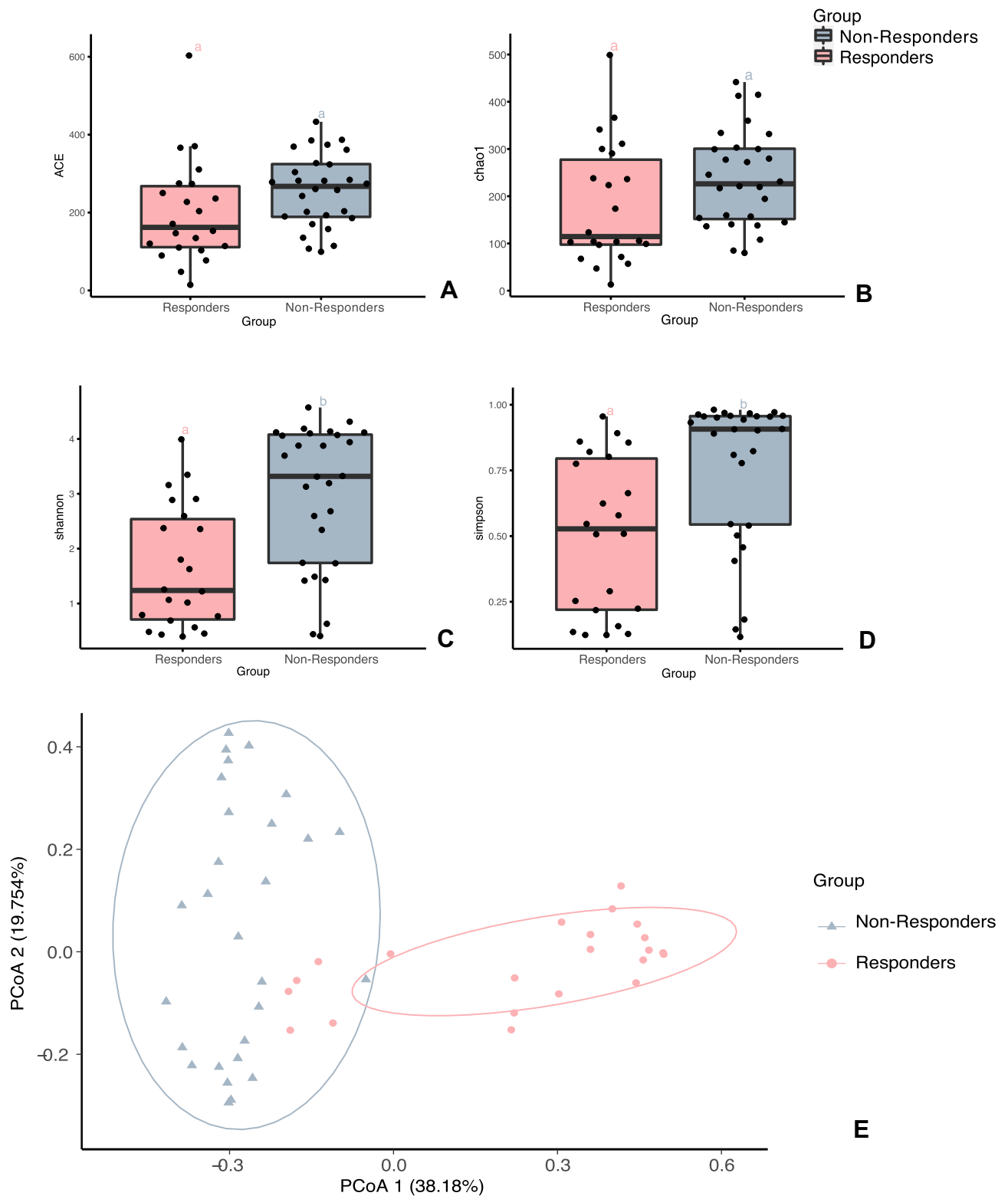
There was no difference in species richness (evaluated with ACE index and Chao1 index) in the lung microbiota of patients from the two groups (As depicted in [Figure 2A](#) and [B](#)). The Shannon's Diversity Index and Simpson's Diversity Index were used to evaluate the alpha diversity of the microbiotas, as presented in [Figure 2C](#) and [D](#), the non-responders had a higher diversity of species than the responders. An apparent pattern of clustering between the two groups was presented after the principal coordinate analysis (PCoA) of the Bray-Curtis dissimilarity metric of the lung microbiota from included patients ([Figure 2E](#)).

The relative abundance of top 20 families in the BAL samples of included patients were presented in [Figure 3A](#), xanthomonadaceae had the highest abundance among all families. The relative abundances of the top 20 families within the two groups were shown in [Figure 3B](#). The family of higher abundances of the responders is Polyomaviridae, Anelloviridae, Herpesviridae and Pneumocystidaceae, while for the non-responders, Xanthomonadaceae, Debaryomycetaceae, Moraxellaceae, Enterococcaceae and Pseudomonadaceae are more abundant than other families.

As determined by the LEfSe analysis, the species profiles of the two groups were depicted in [Figure 4A](#) and [B](#). In the responders, the marker species (LDA score  $> 2$ ) are mostly viruses including BK polyomavirus, Torque teno virus 10, Human herpesvirus 6, Human herpesvirus 5, Torque teno virus 1, Torque teno virus 4, Pneumocystis jirovecii. For the non-responders, the enriched marker species are bacteria-dominant, including Stenotrophomonas maltophilia, Candida albicans, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterococcus faecium.

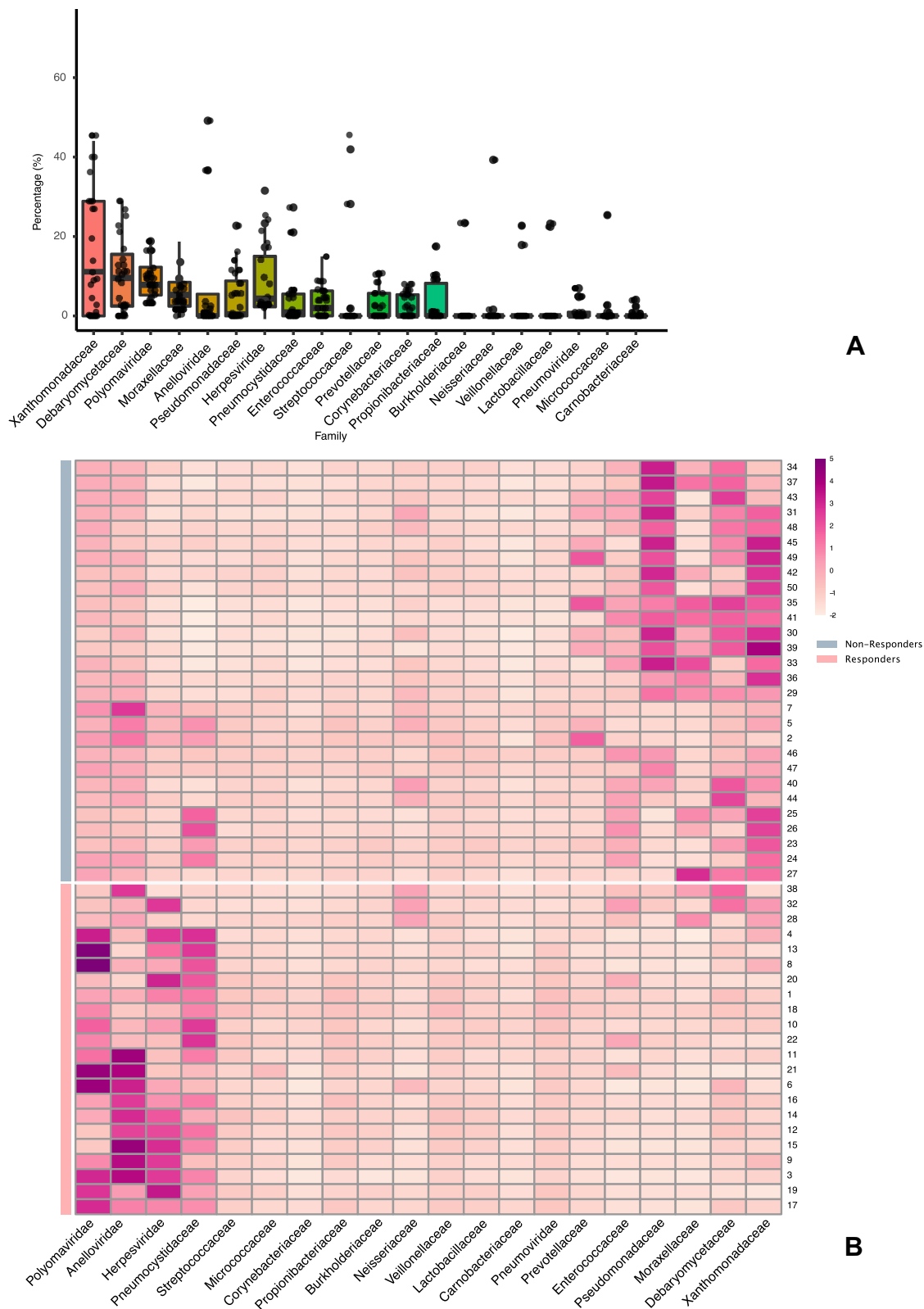
## Etiological Diagnosis of Non-Responders and Responders

The primary etiological diagnoses of non-responders and responders, and the related culture results and radiological features were recorded in [Tables S2](#) and [S3](#). Similar to the result of the LEfSe analysis, more viruses were identified as the pathogen in the responders, while bacteria contributed the largest part of pathogens in non-responders. The details of the primary and secondary etiological species were depicted in [Figure 5A](#). Patients with coinfections were more likely to be non-responders to corticosteroids than patients absent of coinfections (82.35% vs 57.5%,  $p = 0.007$ ).



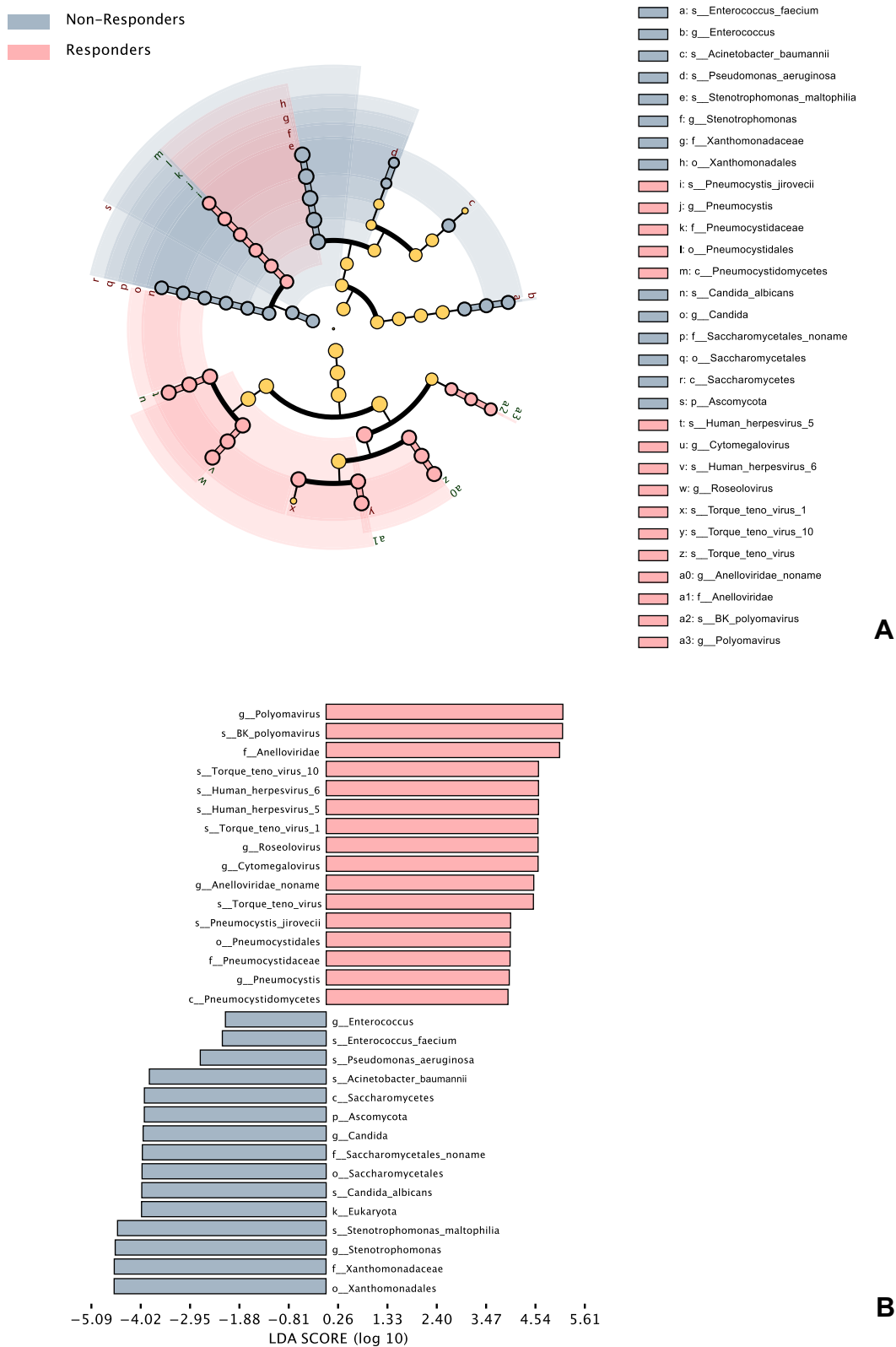
**Figure 2** Alpha diversity and beta diversity of lung microbiota for the non-responders and responders. **(A and B)** The non-responders and responders had no difference in the richness of species (evaluated with ACE index and chao1 index). **(C and D)** The responders had lower diversity in lung microbiota than the non-responders (evaluated with Shannon index and Simpson index). **(E)** The beta diversity was evaluated with Bray-Curtis Dissimilarity metric plotted in a principal coordinate analysis (PCoA), revealing an apparent pattern of clustering.

**Abbreviation:** ACE, Abundance-based Coverage Estimators.



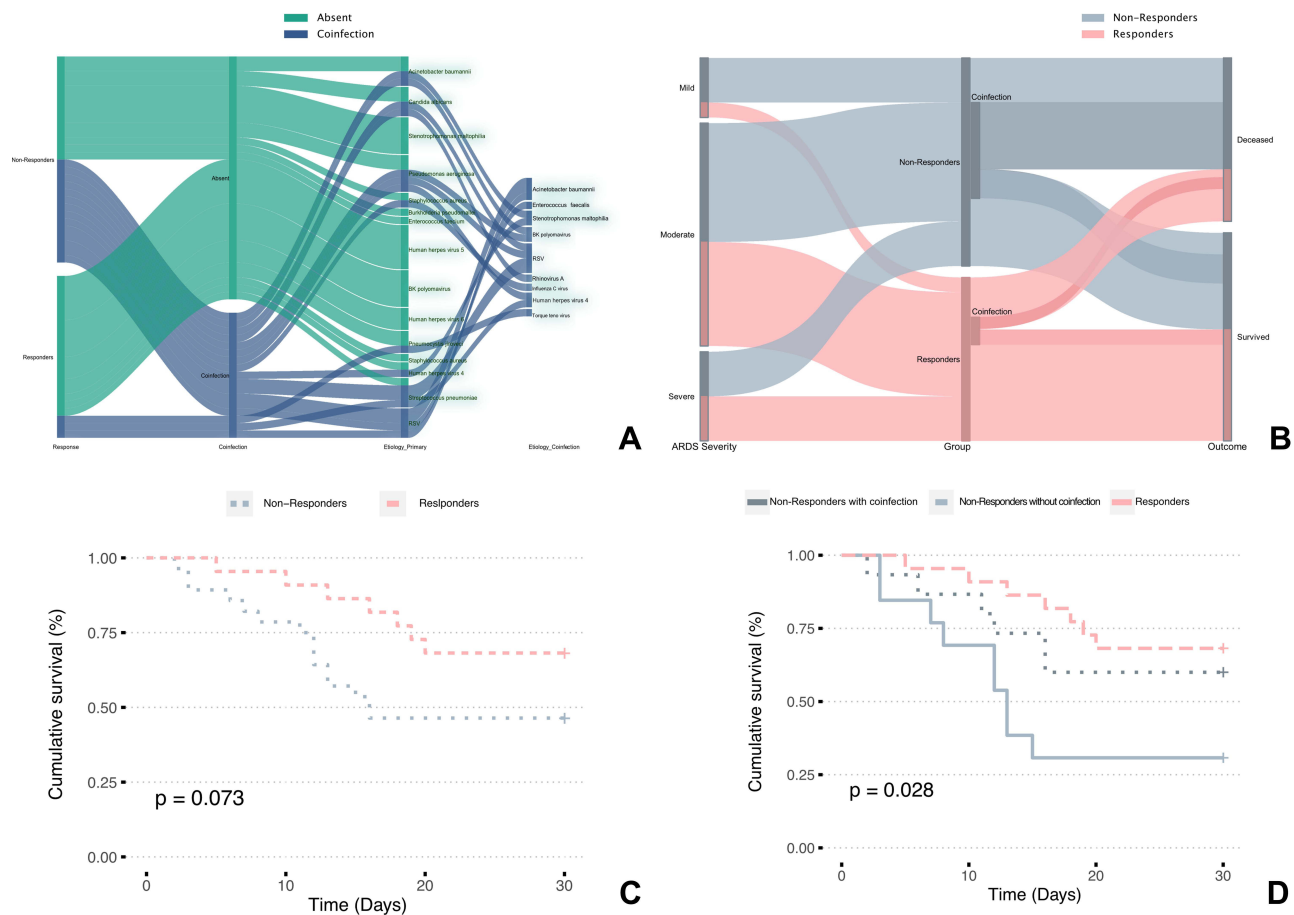
**Figure 3** Constitution of microorganisms in the lung microbiota of non-responders and responders. **(A)** The relative abundance of top 20 families in the BAL samples of included patients. **(B)** The relative abundances of the top 20 families within the groups of non-responders and responders.





**Figure 4** Taxonomic difference of lung microbiota in non-responders and responders. **(A)** Cladogram using the LEfSe method indicating the phylogenetic distribution of lung microbiota of non-responders and responders, only the units with difference  $s$  were presented. **(B)** LDA effect size analysis revealed significant microbial differences in the two groups. LDA score ( $\log_{10} > 2$ ) and  $p < 0.05$  are presented.

**Abbreviations:** LDA, linear discriminant analysis; LEfSe, LDA effect size analysis.



**Figure 5** The etiological microbiome, clinical course and outcomes of non-responders and responders. **(A)** The primary and secondary etiological species of non-responders and responders. **(B)** Clinical courses of non-responders and responders. **(C)** The Kaplan-Meier survival curve of non-responders and responders (Log-rank  $p = 0.073$ ). **(D)** The Kaplan-Meier survival curve of non-responders with or without coinfection and responders (Log-rank  $p = 0.028$ ).

## Outcomes of Non-Responders and Responders

As depicted in Figure 5B, when compared to responders, there were more patients with coinfection in non-responders, and a large proportion (11/17, 64.71%) of these patients deceased at day 30. The outcomes of patients were recorded in Table 2, the non-responders and responders had no difference in 30-day mortality (53.5% vs 31.8%,  $p=0.124$ ), need for IMV after the first 24 hours (28.6% vs 22.7%,  $p=0.640$ ), ICU length of stay [17 (12–22) vs 16 (10–21),  $p=0.416$ ] and New-onset viremia or bacteremia (7.14% vs 4.15%,  $p=0.701$ ). The responders had higher ventilator free days than the

**Table 2** Outcomes of Corticosteroid Non-Responders and Responders

	Non-Responders (n=28)	Responders (n=22)	P
30-day mortality	15 (53.5%)	7 (31.8%)	0.124
Invasive Mechanical Ventilation after the first 24 hours	8 (28.6%)	5 (22.7%)	0.640
ICU length of stay	17 (12–22)	16 (10–21)	0.416
Ventilator free days (at day 28)	4 (0–6)	6 (0–10)	0.034
Ventilator free days in survivors (at day 28)	5 (3–6)	8 (3–10)	0.012
New-onset viremia or bacteremia	2 (7.14%)	1 (4.15%)	0.701

non-responders [4 (0–6) vs 6 (0–10),  $p=0.034$ ], in the survivors, the difference of ventilator free days was more significant [5 (3–6) vs 8 (3–10),  $p=0.012$ ]. Survival analysis (Figure 5C) showed that there was no difference of survival rate between the two groups over time (Log-rank  $p=0.073$ ). However, when non-responders were stratified into subgroups of patients with infection or co-infection, those non-responders with co-infection had significantly lower survival rates than other patients (Log-rank  $p=0.028$ ).

## Discussion

In this study, we classified corticosteroid-treated hematological patients with pneumonia-associated ARDS into two groups: the non-responders and the responders. The responders had higher ventilator free days (For all included patients or the survivors only) than the non-responders. The signatures of the lung microbiotas in the two groups were described. For the non-responders and the responders, their lung bio-diversities and marker species differ from each other. Besides, the non-responders with coinfection had the lowest survival rate when compared to the non-responders with no coinfections and the responders.

As we know, this is the first study that reported the association of the signatures of lung microbiota and the response to corticosteroid in hematological patients with pneumonia-associated ARDS. Using latent class analysis for ARDS patients in clinical trials, Calfee et al., discovered that ARDS patients can be clustered into subgroups with distinct inflammation phenotypes,<sup>21</sup> moreover, the COVID-19 patients with a hyperinflammation phenotype can benefit from corticosteroid therapy,<sup>10</sup> while previous studies of non-COVID patients had heterogenous results.<sup>6,9,22</sup> Similarly, the responders in the present study- who expressed higher levels of inflammatory biomarkers (eg, IL-6, IL-8, TNF- $\alpha$ ) and CRP- had a higher degree of improvements in oxygenation and higher ventilator free days, indicating that patients with overreactive inflammatory response are potential candidates of corticosteroid therapy.

This study had also linked up the lung microbiota signature and the response to corticosteroid therapy. We found the enrichment of viruses (and *Pneumocystis jirovecii*) as marker species for the responders. Bacteria (and *Candida albicans*) were marker species for the non-responders. On the contrary, in our previous study on patients after hematopoietic stem cell transplantation (HSCT), the marker species for patients with reactive inflammatory responses were all bacteria, viruses were more abundant in patients with insufficient inflammatory response.<sup>23</sup> The possible cause of the phenomenon is that host response differs in non-HSCT or HSCT hematological patients, the slow reconstitution of adaptive immunity after HSCT made these patients more liable to various infections. For example, time for the full reconstruction of B cells and T cells will take up to 2 years,<sup>24,25</sup> while for non-HSCT patients, newly-developed transitional B cells and naive B and T cells appeared within months after chemotherapy.<sup>26</sup> In fact, the cytokine levels in the present cohort were much higher than in the HSCT cohort, which suggested that the competence of the immune system may shape the host response to various pathogens.

Another finding in this study was that the non-responders with coinfecting pathogens showed the lowest rate of survival, and patients with coinfections were more likely to be non-responders (Figure 5A), the non-responsiveness could be attributed to the influence of the coinfections. In a previous study, coinfections in patients with community-acquired pneumonia have been proved to be associated with hospital death and longer duration of mechanical ventilation.<sup>27</sup> Jamieson et al, reported that in a mouse model, endogenous corticosteroid production triggered by the influenza virus led to a suppression of the innate immune response to the coinfecting *L. monocytogenes*.<sup>28</sup> Likewise, in the present cohort, the coinfection usually consists of a virus and a bacterium (Figure 5A). Thus, the supplement of exogenous corticosteroids to patients with coinfections may eventually cause an over-suppression of the immune system and lead to a poor prognosis for patients with coinfections.

Low alpha diversity in the lung microbiota have been reported to be related to worse outcomes: O'Dwyer et al. reported that in patients after hematopoietic stem cell transplantation, the decrease of lung alpha diversity predicts lung complications and mortality,<sup>29</sup> which was also observed in our previous study.<sup>23</sup> In the present cohort of hematological patients, the responders had lower alpha diversities than the non-responders, however, they had higher ventilator free days than the non-responders, no other differences of outcomes were observed. One possible explanation is that the application of corticosteroids may re-shape the constitution of lung microbiotas, which were not further followed up in our study.

This study is limited by several issues. First, even though the non-responders and responders have distinct microbiota signatures and their ventilator free days were different, no difference in survival was observed. This may be resulted from a limited sample size, as the trend of higher survival rate was observed on the Kaplan-Meier plot. Second, the setting of the ventilator and other therapeutic interventions may also influence the oxygenation of patients. To mitigate the influence of ventilator settings, we set a rather conserved goal for ventilator support. And according to the study design, only the change of P/f ratio in the first 24 hours were analyzed, in this period, no patients were intubated (those intubated within 24 hours were excluded) or received prone positioning. As for diuretics or antibiotics, their influence on P/F cannot be directly evaluated by this study. However, since the serum BNP level was not significantly different in the two groups, which indicate that the fluid overload may not be the main factor that influenced the P/F ratio. A following clinical study with more sufficient sample size and rigorous methodology is still needed to further test the conclusions. Another concern of the findings was that we still do not know how different marker species interacted with the hematological hosts, in other words, why patients infected with specified pathogen will present as responders or non-responders of corticosteroid. On the other hand, the etiological diagnosis based on NGS results may not be accurate despite that we integrated BAL culture and radiological features, and commensal microbes might be identified as pathogens. The future study integrates host responses (eg, single-cell sequencing and meta-transcriptome studies) and microbiome characters (eg, meta-genomic studies) will help to elucidate the mechanism of host-microbe interaction and improve the precision of etiological diagnosis.

## Conclusion

For hematological patients with pneumonia-associated ARDS, the responders of corticosteroids had higher ventilator free days at day 28 than the non-responders. The microbiota signatures were distinct in the two groups. The non-responders with coinfections had the lowest survival rate when compared to the non-responders with no coinfections and the responders.

## Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Disclosure

The authors report no conflicts of interest in this work.

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