

Phenotypes and Lung Microbiota Signatures of Immunocompromised Patients with Pneumonia-Related Acute Respiratory Distress Syndrome

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Objective: We aim to identify the clinical phenotypes of immunocompromised patients with pneumonia-related ARDS, to investigate the lung microbiota signatures and the outcomes of different phenotypes, and finally, to develop a machine learning classifier for a specified phenotype.

Methods: This prospective study included immunocompromised patients with pneumonia-related ARDS. We identified phenotypes using hierarchical clustering to analyze clinical variables and serum cytokine levels. We then compared outcomes and lung microbiota signatures between phenotypes. Based on lung microbiota markers, we developed a random forest classifier for a specified phenotype with worse outcomes.

Results: This study included 92 patients, who were divided into three phenotypes, namely “type α ” (N = 33), “type β ” (N = 12), and “type γ ” (N = 47). Compared to type α or type β , patients with type γ had no obvious inflammatory presentation and had significantly lower IL-6 levels and more severe oxygenation failure. Type γ was also related to higher 30-day mortality and lower ventilator free days. The microbiota signatures of type γ were characterized by lower alpha diversity and distinct compositions than those of other patients. We developed a lung microbiota-derived random forest model to differentiate patients with type γ from other phenotypes.

Conclusion: Immunocompromised patients with pneumonia-related ARDS can be clustered into three clinical phenotypes, namely type α , type β , and type γ . Phenotypes were distinguished from each other with different outcomes and lung microbiota signatures. Type γ , which was characterized by insufficient inflammation response and worse outcomes, can be detected with a random forest model based on lung microbiota markers.

Keywords: immunocompromised host, respiratory failure, microbiota

Introduction

With the progression of oncology and rheumatology, the prognosis of patients with various malignancies or auto-immune diseases has been greatly improved.¹⁻³ Yet many patients are in an immunocompromised state after chemotherapy or immunosuppressive treatments, and their risk of opportunistic infections increases significantly.^{4,5} Life-threatening infectious complications occur frequently, and patients need to be transferred to Intensive Care Units (ICU) for advanced life supports.⁶

Acute respiratory distress syndrome (ARDS) is a common complication that accompanies pulmonary infection in immunosuppressed patients.^{7,8} Previous studies have shown that, for immunosuppressed patients with ARDS, the ICU mortality rate is 50–75%, which is much higher than for immunocompetent patients.^{9,10} On the other hand, there is significant heterogeneity in the results of studies that focused on the evaluation of outcome and treatment methods: the

study by Hilbert et al. suggested that, when compared to traditional oxygen therapy, non-invasive mechanical ventilation can significantly reduce ICU mortality in these patients,¹¹ while subsequent studies did not reach a consistent conclusion.^{12–14} Some studies have suggested that glucocorticoid treatment may improve outcomes in selected patients,^{15,16} but this result could not be replicated in immunocompromised hosts¹⁷ or other patients.^{18,19} This evidence suggests that novel methods are necessary to predict patient prognosis and their response to specific treatments to ensure appropriate support for specified patients.

Recent studies on ARDS patients have shown that latent class analysis or supervised learning like clustering can be used to classify patients into different phenotypes based on their basic clinical variables and their serum cytokine levels. Calfee's research suggests that ARDS patients can be divided into hyperinflammatory (high IL-6, IL-8, TNF α , etc.) and hypoinflammatory phenotypes.²⁰ These two phenotypes were characterized by different clinical outcomes and responses to steroids and fluid managements.^{21,22} For immunocompromised hosts, no studies investigated whether such phenotypes existed or were clinically relevant.

On the other hand, based on next-generation sequencing technology (NGS), recent research on the lung microbiome provides a new perspective for the prognostic assessment and inflammatory response classification of ARDS. Dickson et al. analyzed ICU hospitalized patients requiring mechanical ventilation support and found that the enrichment of gut bacteria in the lungs was associated with high patient mortality.²³ Kyo et al reported that the diversity of lung microorganisms in patients with ARDS was significantly reduced, and the enrichment of specific microorganisms can lead to a high inflammatory state and higher ICU mortality.²⁴ For immunosuppressed patients, there are no relevant studies that focus on the relationship between the patient's lung microbiota and inflammatory response.

Based on current knowledge, we hypothesized that by analyzing clinical variables, the immunocompromised ARDS patients can be divided into sub-phenotypes that characterized by different lung microbiota signatures and clinical outcomes.

Therefore, in this prospective observational study, we aim to identify the clinical phenotypes of immunocompromised patients with pneumonia-related ARDS, to investigate the lung microbiota signatures and the outcomes of different phenotypes, and finally, to develop a machine learning classifier for a specified phenotype.

Methods

Ethics Statement

This study was approved by the ethics committee of Peking University International Hospital (PKUIH, 2021-KY-0026-02) and was conducted under the principles of the Helsinki Declaration. Patients or their relatives signed informed consent before participation (please check the details in the Declarations).

Subject Recruitment

From January, 2021, to December, 2022, we recruited patients who were transferred to the ICUs of Peking University International Hospital and Peking University People's Hospital with the diagnosis of immunosuppression and ARDS.

The criteria for immunocompromisation were ① neutropenia ($<1.5 \times 10^9/L$) caused by chemotherapy or hematopoietic stem cell transplantation, ② drug-induced immunosuppression in organ transplant recipients, ③ immunosuppression caused by corticosteroid or cytotoxic therapy in non-malignant diseases, or ④ diagnosed of acquired immunodeficiency syndrome. ARDS was diagnosed with reference to Berlin Definition.²⁵

The exclusion criteria were ① <18 years old, ② in pregnancy, or ③ having a decision that precludes intubation.

Baseline Data, Laboratory Tests and Cytokine Analysis

The patient's baseline demographic data (gender, age, primary disease diagnosis, etc.) and basic laboratory tests (complete blood count, blood biochemistry, C-reactive protein, procalcitonin, arterial blood gas, etc.) and serum cytokine levels (IL-17a, IL-2, IL-4, IL-5, IL-6, IL-10, IFN- γ , TNF- α) were collected within 24 hours after the patient was transferred to the ICU. Please refer to [Table S1](#) for instruments and reagents used in the tests.

Mechanical Ventilation Support

All patients were supported by non-invasive ventilation on ICU admission. Ventilators were set to maintain a minimal SPO₂ of 93%, a tidal volume of 6 mL/kg (predicted body weight), and a respiratory rate lower than 30 per minute. If these parameters cannot be maintained through non-invasive ventilation, patients will be intubated and supported with invasive mechanical ventilation (IMV). Sedation and analgesia were applied to improve patient-ventilator synchronization. Other adjunctive therapies for ARDS (eg, prone positioning, neuro-muscular blocking, extracorporeal membrane oxygenation) were performed at the discretion of the physicians.

Clustering of Phenotypes, Variables, and Outcomes of Phenotypes

Variables were normalized, and Ward's hierarchical clustering was performed to minimize the variance within clusters (using the “ggdendro” package in R). The optimal number of clusters was assessed with the “Nbclust” package by calculating the average silhouette width. Visualization of clustering results was performed with the “factoextra” package. Outcomes, including 30-day mortality, ventilator free days, and ICU stay were compared between phenotypes. Then, using Cox regression statistics to estimate the 60-day survival of patients, the survival curve was stratified by phenotypes, or the SOFA (sequential organ failure assessment) score was conducted and visualized with the “survminer” package.

Bronchial Alveolar Lavage and RNA-Based Metagenomic Next-Generation Sequencing

All included patients received a bronchoscope examination with bronchial alveolar lavage within 48 hours of ICU admission. At least 5 mL of BALF was collected for each patient. BALF samples were analyzed with RNA-based metagenomic next-generation sequencing (mNGS) to ensure the detection of microbes, including bacteria, fungus and viruses.²⁶ Briefly, RNA was extracted from BALF samples, and through reverse transcription, cDNA libraries were constructed and sequenced with the NextSeq 1000 System (150-bp paired-end reads; Illumina) to produce sequencing data for downstream analysis. BALF samples were also sent for culture, and the results were recorded.

After cleaning the human sequences and adapter sequences, we identified the microbes in BALF samples and calculated the abundances (including alpha and beta diversity) of different phenotypes. The constitution of microbes between phenotypes was compared. Please refer to [Table S2](#) for our bioinformatic protocol.

Etiological Diagnosis

After the metagenomic analysis and culture of the BALF samples. The etiological diagnoses were made based on the following criteria: ① positive in culture or ② in mNGS, RPM (reads per million) higher than 100 for virus, 20 for bacteria, fungus, or other pathogens.

Develop Prediction Tools for Phenotype

To test whether lung microbiota constitution can distinguish a specified phenotype, we trained a random forest model at the genus level based on random sampling with replacement (using the R package “randomForest”, with the number of decision trees = 1000). The number of marker genera and clinical variables was identified using 10-fold cross-validation with five repeats. The variable importance by mean decrease of accuracy was calculated and visualized. The classification efficacy of the random forest model was evaluated and compared with Receiver Operating Characteristic (ROC) curves.

Statistical Analysis

All statistical analyses were performed on R (version 4.3.2). Missing variables were replaced using multiple imputation chained equations (“MICE” package). Continuous variables were presented as medians (interquartile range), categorical variables were presented as numbers (percentage). A comparison of categorical variables (ie, 30-day mortality) was achieved using χ^2 test. Comparison of two groups of continuous variables was performed with the Kruskal–Wallis *H*-test. All tests were two-sided, and $p < 0.05$ was considered statistically significant.

Results

Patient Recruitment and Clustering of Phenotypes

A total of 112 patients who met the inclusion criteria were admitted to the two ICUs in the study period, and 20 patients were excluded (7 under 18 years old and 13 refused to intubate). There were finally 92 patients included in this study (Figure 1, downstream analyses based on phenotypes were also illustrated). Baseline characteristics including clinical variables, laboratory tests, and serum cytokine levels were used for phenotype clustering.

With the average silhouette width method, the optimal cluster number was set to 3, the clusters were defined as type α (33 patients), type β (12 patients), and type γ (47 patients) (Figure 2A and B). As reported in Table 1, when compared to type α and type β , type γ was characterized by lower SOFA score, temperature, white blood cell count, lymphocyte count, neutrophil count, hemoglobin, platelets, C-reactive protein, procalcitonin, and IL-6 level [type γ vs type β vs type α : 0(0–0) vs 22.38(0–34.2) vs 97.60(59.05–382.60), $p < 0.001$] but higher mean arterial pressure and INF- γ level and was characterized with more significant lower PaO₂/FiO₂ levels [type γ vs type β vs type α : 202(180–220) vs 245(155–286) vs 227(188–259), $p = 0.004$]. In other words, unlike the other two types, type γ was lacking typical signs of inflammation.

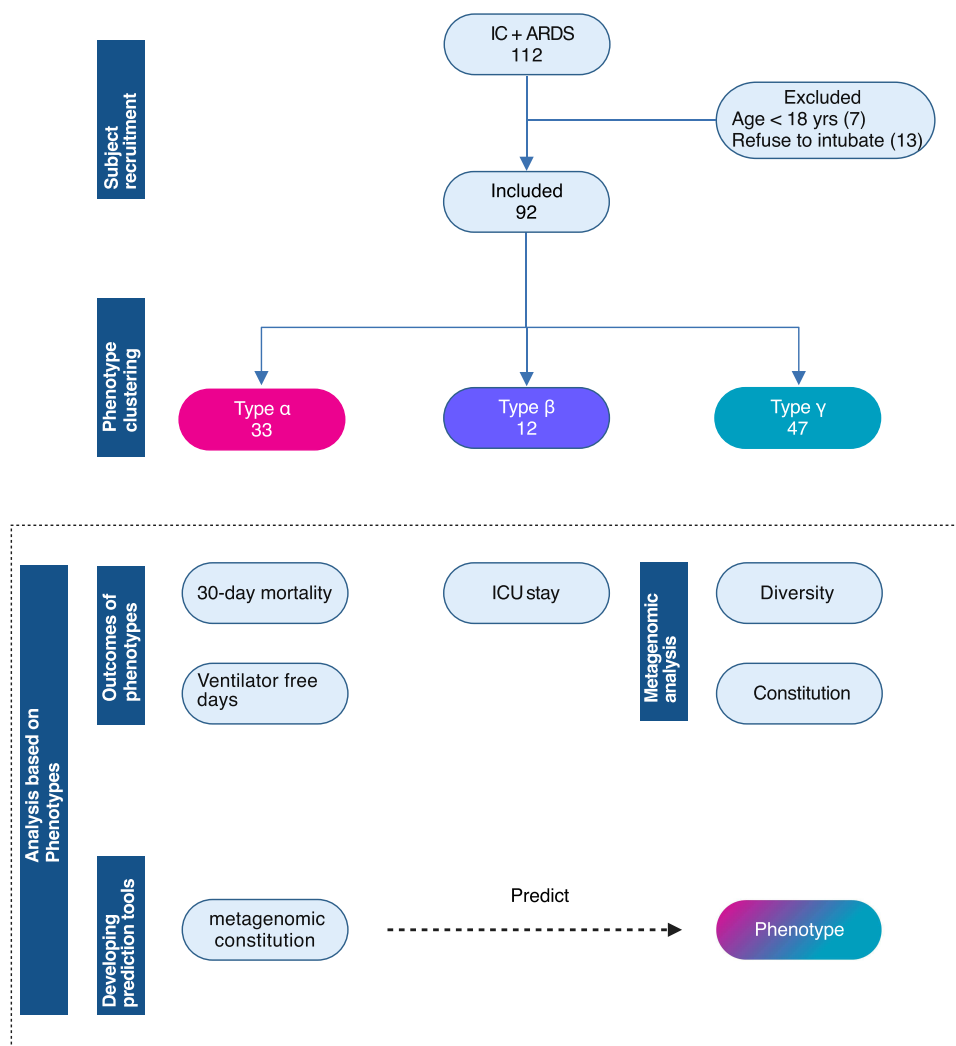


Figure 1 Flow chart of this study. All included patients were divided into three phenotypes using hierarchical clustering. And the following analyses were performed: comparison of outcomes, metagenomic analysis of bronchoalveolar lavage fluids, and development of a prediction tool for a specified phenotype.

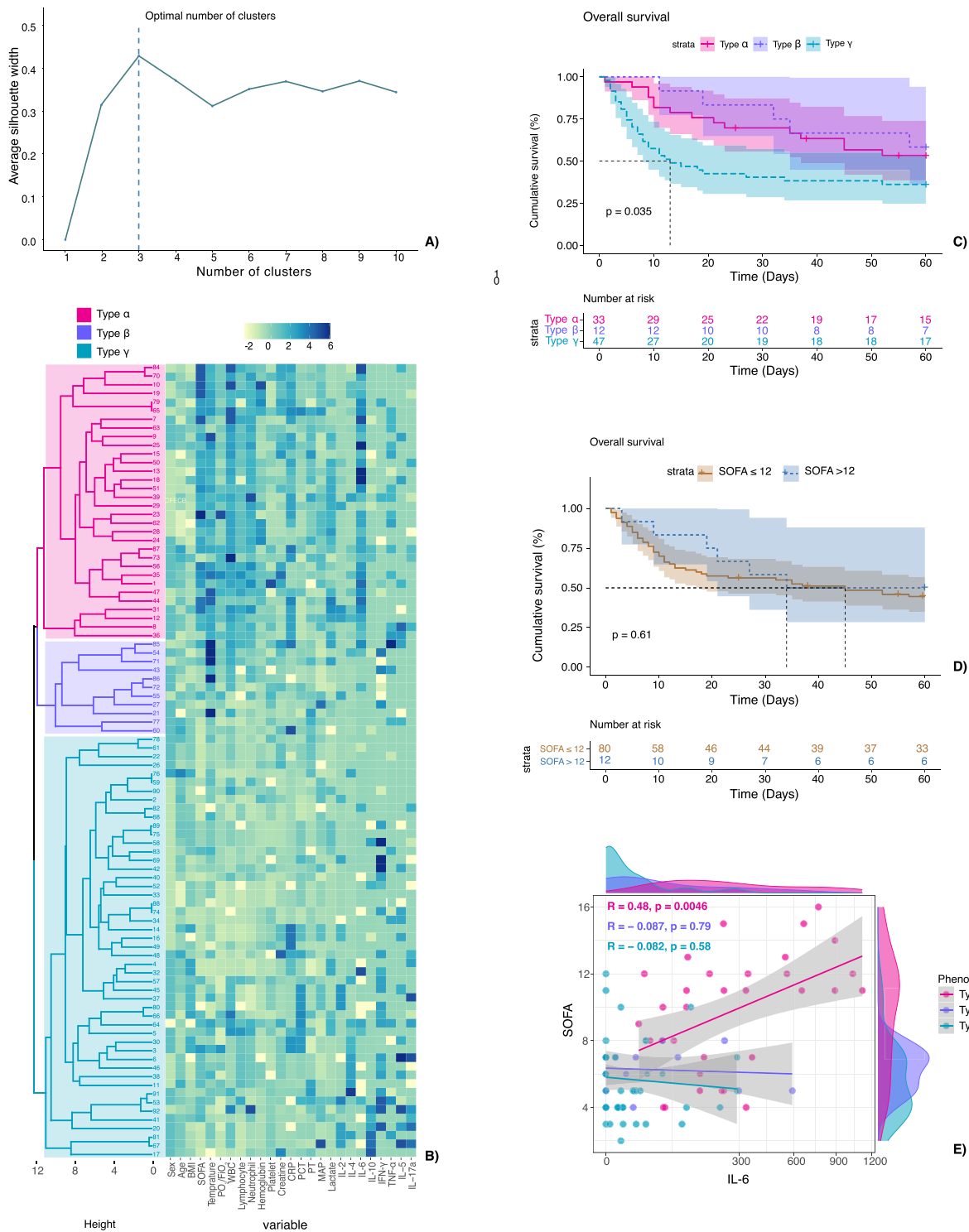


Figure 2 Identification of clinical phenotypes and estimation of survival between phenotypes. **(A)** Calculation of the optimal cluster number. The optimal cluster number was 3 as evaluated with average silhouette width. **(B)** Hierarchical clustering of phenotypes. Based on the optimal cluster number, clinical variables, and cytokine levels, we defined three phenotypes: α , β and γ . Rows: patients. Columns: clinical variables. First column: dendrogram in red: "type α ", dendrogram in blue: "type β ", dendrogram in green: "type γ ". **(C)** Cox survival curve to 60 days for patients stratified with phenotypes. Type γ had a significantly lower survival rate than patients in other phenotypes $p = 0.035$, adjusted for age, SOFA score, and PaO₂/FiO₂. **(D)** Cox survival curve to 60 days for patients stratified by SOFA score. Patients stratified with SOFA score (≤ 12 or >12 points) did not show a difference of survival over time. $p = 0.610$, adjusted for age, and PaO₂/FiO₂. **(E)** Scatter plot between IL-6 levels and SOFA score for patients with different phenotypes. The correlation was evaluated with the Pearson correlation coefficient (R). The correlation of IL-6 level and SOFA score only existed in type α (Pearson's $R = 0.48, p = 0.0046$).

Abbreviations: SOFA, Sequential Organ Failure Assessment.

Table 1 Characteristics and Outcomes of Patients of Different Phenotypes

	Type α n=33	Type β n=12	Type γ n=47	P
Age (y)	39(22–49)	36(24–57)	32(27–53)	0.127
Female (%)	13(39.40)	7(58.33)	22(46.81)	0.511
SOFA score	9(5–10)	8(4–9)	5(4–7)	0.032
Highest Temperature, °C	38.6(37.8–38.8)	38.2(37.4–38.8)	37.2(36.5–37.6)	0.028
PaO ₂ /FiO ₂ , mmHg	227(188–259)	245(155–286)	202(180–220)	0.004
WBC count, ×10 ⁹ /L	12.5(6.3–16.5)	10.5(4.8–12.5)	3.6(2.5–5.5)	<0.001
Lymphocyte count, ×10 ⁹ /L	1.4(0.8–1.7)	1.0(0.6–2.1)	0.1(0–0.5)	<0.001
Neutrophil count, ×10 ⁹ /L	4.2(2.1–7.0)	3.7(2.0–6.4)	1.2(0.2–2.3)	<0.001
Hemoglobin, g/dL	73(62–89)	68(67–80)	62(58–84)	0.452
Platelets, ×10 ⁹ /L	78(32–117)	92(45–110)	23(17–41)	<0.001
Creatine, mmol/L	89(58–110)	86(75–115)	82(62–93)	0.342
CRP, mg/L	199(65.25–270.00)	152.59(65.80–183.00)	50.50(25.00–83.00)	<0.001
PCT, ng/mL	10.07(5.51–20.76)	7.32(2.43–9.76)	0(0–0)	<0.001
PT, s	11.00(10.50–13.25)	11.50(10.50–13.50)	10.25(10.00–15.50)	0.732
MAP, mmHg	62(55–76)	71(58–79)	82(68–89)	<0.001
Lactate, mmol/L	7.21(5.30–10.72)	5.10(4.21–8.50)	1.10(0.40–1.75)	<0.001
Cytokines				
IL-2, pg/mL	0(0–0)	0(0–0)	0(0–0)	0.882
IL-4, pg/mL	0(0–2.2)	0(0–2.7)	0(0–0)	0.863
IL-5, pg/mL	0(0–2.81)	0(0–3.59)	0(0–5.42)	0.722
IL-6, pg/mL	97.60(59.05–382.60)	22.38(0–34.20)	0(0–0)	<0.001
IL-10, pg/mL	0(0–1.83)	0(0–0)	0(0–0)	0.813
IFN- γ , pg/mL	0(0–0)	0(0–0)	11.60(5.11–23.40)	<0.001
TNF- α , pg/mL	0(0–0)	0(0–0)	0(0–0)	0.537
IL-17a, pg/mL	0(0–0)	0(0–2.2)	0(0–3.1)	0.244
First symptom to ICU admission (days)	3(2–8)	4(2–6)	4(3–6)	0.193
Types of immunosuppression				
Neutropenia after CTx/HSCT	8(24.24)	2(16.67)	29(61.70)	0.004
Solid organ transplantation	12(36.36)	4(33.33)	10(21.28)	
Non-malignant disease	13(39.40)	6(50.00)	8(17.02)	
Outcomes				
30-day mortality (%)	11(33.33)	2(16.67)	30(63.83)	0.002
Ventilator Free Days	5(2–8)	5(3–11)	2(0–7)	0.012
ICU stay	16(9–22)	14(5–18)	15(4–19)	0.135

Notes: Values are given as medians (interquartile range) or number (%).

Abbreviations: CRP, C-reactive protein; CTx, Chemotherapy; FiO₂, Fraction of inspired oxygen; HSCT, Hematopoietic Stem Cell Transplantation; ICU, Intensive Care Unit; IL, Interleukin; IFN, Interferon; MAP, Mean Arterial Pressure; PaO₂, Partial Pressure of Oxygen; PCT, Procalcitonin; PT, Prothrombin Time; SOFA, Sequential Organ Failure Assessment; TNF, Tumor Necrosis Factor; WBC, White Blood Cell.

Worse Outcomes for Patients with Type γ

The type γ was related to higher 30-day mortality (type γ vs type β vs type α : 63.83% vs 16.67% vs 33.33%, $p = 0.002$) and ventilator free days [type γ vs type β vs type α : 2(0–7) vs 5(3–11) vs 5(2–8), $p = 0.012$] (Table 1). In Cox regression for survival analysis (Figure 2C), patients with type γ had a significantly lower survival rate than patients with other phenotypes ($p = 0.035$, adjusted for age, SOFA score, and PaO₂/FiO₂), while patients stratified by SOFA score (≤ 12 or > 12 points) did not show a difference in survival over time (Figure 2D, $p = 0.61$). The correlation between IL-6 level and SOFA score only existed in type α (Figure 2E, Pearson's $R = 0.48$, $p = 0.0046$).

Type γ Was Characterized by Low Alpha Diversity and a Distinct Microbiome Composition

When evaluated with the Shannon index and Chao1 index, type γ presented with a significantly lower alpha diversity than type α or type β . In other words, lung microbiotas in type γ had a lower richness and diversity (Figure 3A and B).

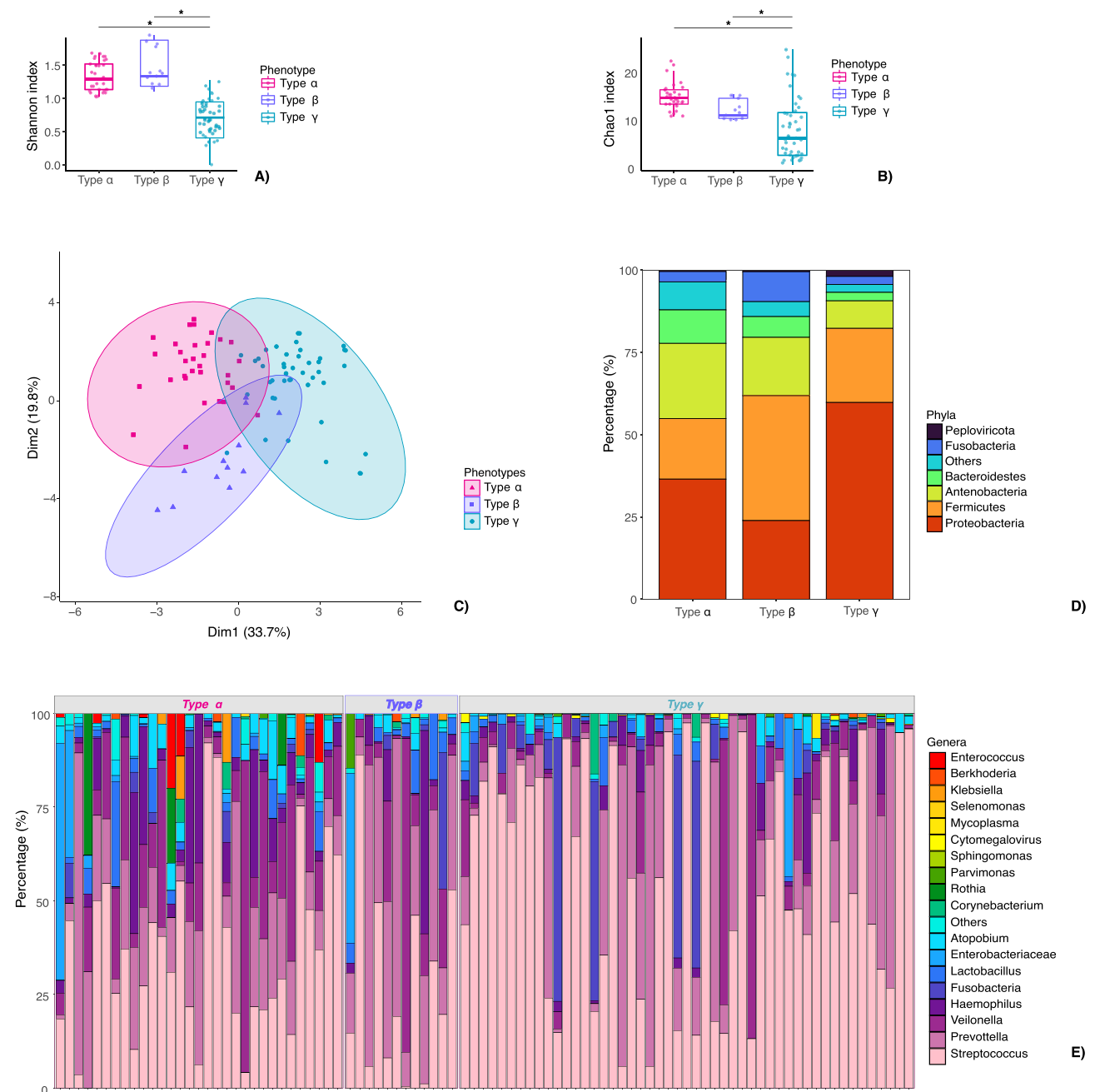


Figure 3 Microbiota signatures for patients with different phenotypes. **(A and B)** Alpha diversity of lung microbiota from patients with different phenotypes. Alpha diversity was evaluated with the Shannon index and the Chao1 index. Patients with phenotype γ had significantly lower alpha diversity than the other two phenotypes. * $P < 0.05$. **(C)** Beta diversity as evaluated with the Bray-Curtis dissimilarity metric plotted in a principal coordinate analysis (PCoA). The analysis showed separation of species in phenotypes and revealed an apparent pattern of clustering. **(D)** Average microbial compositions of phenotypes at the phylum level. In type γ , the most abundance of phylum were *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Fusobacteria*, and *Pepliviricota*, their proportion were slightly different from type α or type β . **(E)** Microbial compositions of different samples within phenotypes at the genus level. On the genus level, the most abundant genera were *Streptococcus*, *Prevotella*, *Veillonella*, and *Haemophilus* across phenotypes.

The beta diversity among phenotypes was analyzed by PCoA with the Bray-Curtis dissimilarity metric, which showed the separation of species in phenotypes and revealed an apparent pattern of clustering (Figure 3C). The contribution of phylum and genus to the lung microbiota of phenotypes were depicted in Figure 3D and E. In type γ , the most abundant phylum were *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Fusobacteria*, and *Pepliviricota*, their proportions were slightly different from those in type α or type β .

On the genus level, the most abundant genera (Figure 3E) were *Streptococcus*, *Prevotella*, *Veillonella*, and *Haemophilus* across phenotypes. Figure 4 depicts significant differences of species in phenotypes. Most prominently, *Human betaherpesvirus 5*, *Fusobacterium necrophorum*, *Streptococcus pneumoniae*, *Human alphaherpesvirus 1*, *Staphylococcus cohnii*, and *Veillonella parvula* were significantly enriched in type γ when compared to type α or type β . In type α , the significantly enriched genera were *Stenotrophomonas maltophilia*, *Enterococcus faecium*, and *Klebsiella pneumoniae*, while in type β , *Staphylococcus haemolyticus* and *Candida albicans* were with high abundance.

The etiological results of the patient were reported in Figures S1 and S2. In general, mNGS plus the standard method identified pathogens in 72.09% of the included patients. In 43.48% of the cases, the tmNGS and standard method had the same results (Figure S1). mNGS had an obvious advantage in detecting virus (for viruses cannot be cultured by the standard method) and rarely were the cases that mNGS missed the pathogen, while culture showed positive results (in 7 cases, Figure S2).

Lung Microbiota as Markers for Type γ

We next explored whether lung microbiota could be used as markers for specified phenotypes. Using the random forest method, we built a random forest model that correlated microbiota composition (on the genus level) with type γ . We used a 10-fold cross-validation with five repeats to evaluate the importance of predictor genera. The cross-validation error stabilized with 17 genera were used as markers (Figure 5A). In these markers, 11 genera had higher abundance in type γ than the other two types (positive contribution), while 6 markers had lower abundance in type γ (Figure 5B, negative contribution). In the study cohort, the random forest model achieved an AUC (area under curve) of 0.793 (Figure 5C).

Discussion

In the present study, we identified three clinical phenotypes in immunocompromised patients with ARDS. Type γ presents with no significant inflammation but more severe oxygenation failure and worse outcomes and is characterized by distinct microbiota signatures and outcomes. We also developed a lung microbiota-derived random forest model to

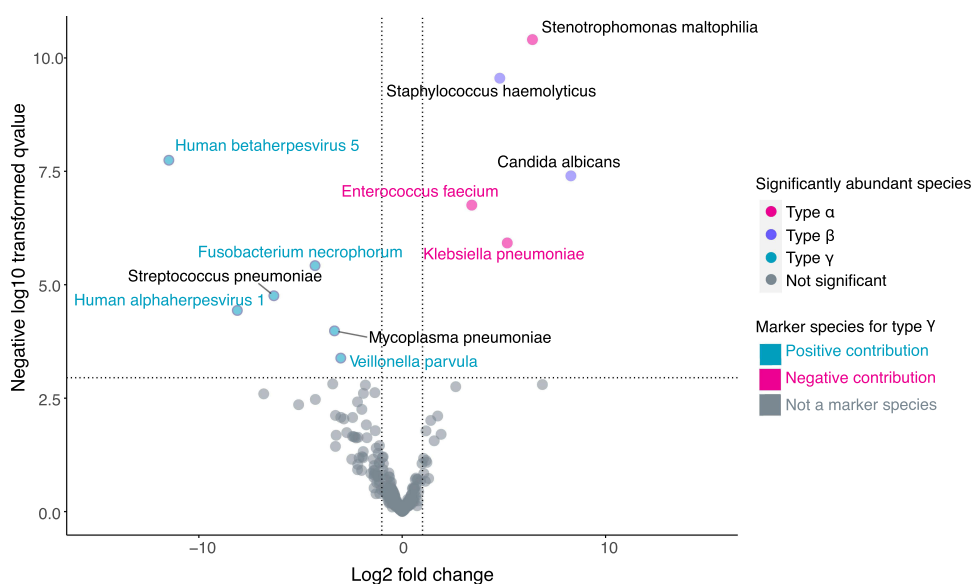


Figure 4 Enrichment of species between type γ and other types. Lung microbiota composition data was processed by edgeR to test enriched species between type γ and the other two phenotypes. Most prominently, *Human betaherpesvirus 5*, *Fusobacterium necrophorum*, *Streptococcus pneumoniae*, *Human alphaherpesvirus 1*, *Staphylococcus cohnii*, and *Veillonella parvula* were significantly enriched in type γ when compared to type α or type β . In type α , the significantly enriched genera were *Stenotrophomonas maltophilia*, *Enterococcus faecium*, and *Klebsiella pneumoniae*. While in type β , *Staphylococcus haemolyticus* and *Candida albicans* were in high abundance. Green dots represent significantly differentially abundant species in type γ . Red and blue dots represent significantly differentially abundant species in type α and type β . Species with name colored in green: within the genus that was selected by a random forest model for type γ , positive contributed to the detection of type γ . Species with names colored in red: within the genus were selected by a random forest model for type γ , negatively contributed to the detection of type γ . Black colored species were not marker species.

Abbreviation: FDR, False Discovery Rate.

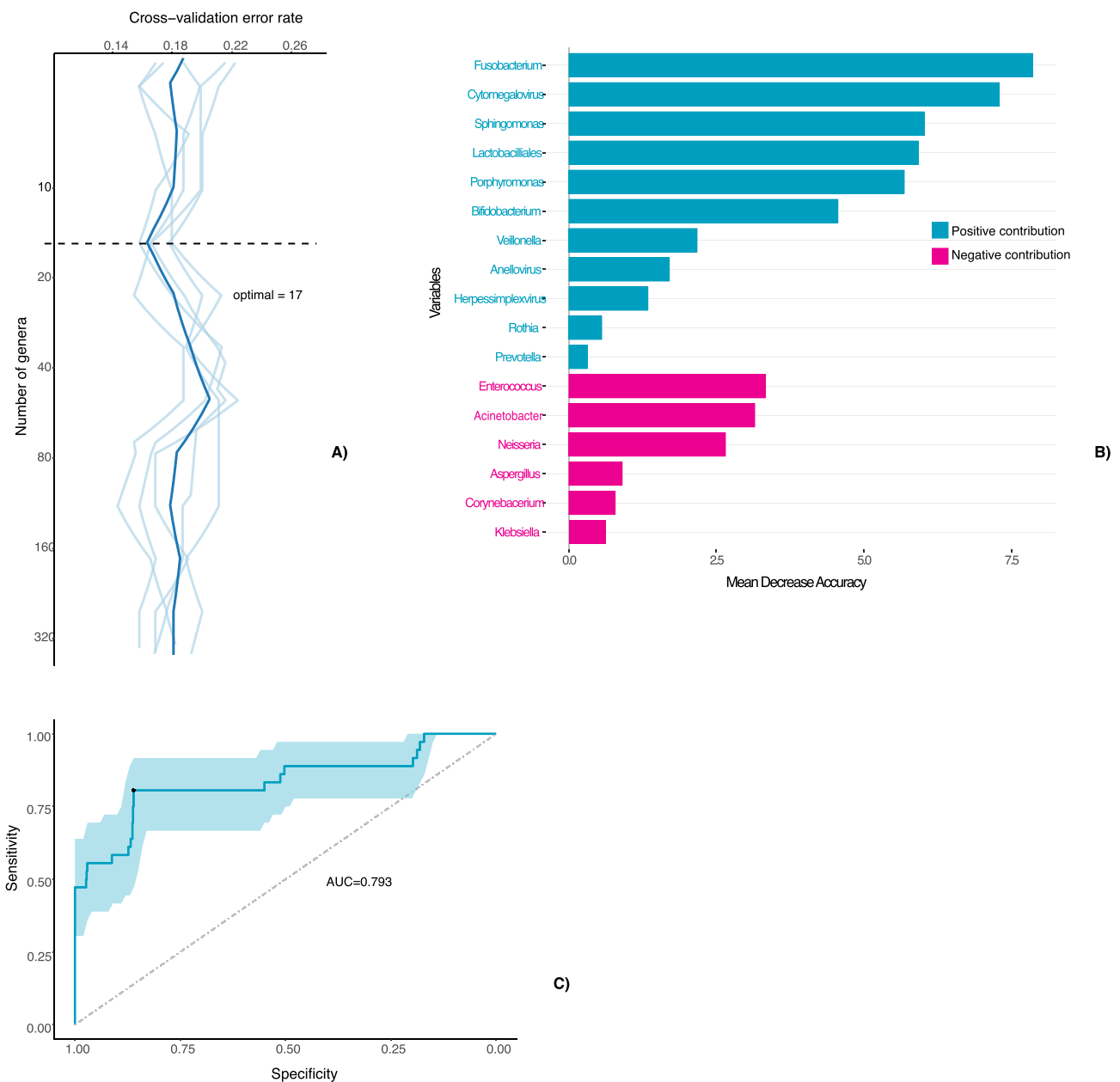


Figure 5 Random forest model for detection of type γ using lung microbiota data. **(A)** Ten-fold cross-validation error as a function of the number of genera used to differentiate type γ and other phenotypes in order of variable importance. The calculated optimal number of genera was 17. **(B)** The top 17 microbe genera were identified by random-forest classification of the abundance of lung microbiota in different phenotypes. Biomarker genera are ranked in descending order of importance to the accuracy of the model. Green: positively contributed to the detection of type γ . Red: Negatively contributed to the detection of type γ . **(C)** Performance of random forest model in detecting type γ , evaluated with ROC curve the random forest model achieved an AUC of 0.793.

Abbreviations: AUC, Area Under the Curve; ROC, the Receiver Operating Characteristic.

differentiate patients with type γ from other phenotypes. These findings will facilitate prognosis evaluation in clinical practice and possible mechanism exploration for ARDS in future studies.

Studies on ARDS have been hampered by the heterogeneity of patients.^{27,28} In consequence, trials on potential interventions failed to produce repeatable results.^{29–32} Recently, the phenotype classification of ARDS patients provided a probable solution for this issue: Calfee et al divided ARDS patients into two phenotypes, with the hyper-inflammatory phenotype expressing higher inflammatory cytokines, an increased incidence of shock, and non-pulmonary sepsis.³³ In the following studies, the two phenotypes have been proven to respond differently to treatments including end-expiratory pressure,³³ fluid therapy,²¹ and simvastatin.³⁴ In previous studies on ARDS, about 31% percent of ARDS was triggered

by sepsis, sepsis-induced ARDS was marked by uncontrolled inflammation, multiple organ failures, and high morbidity and mortality.^{35,36} Corresponding to this phenomenon, suppression of inflammation with corticosteroid has improved patient outcomes in a study that included more sepsis-induced ARDS.¹⁶ However, in our study, there was no advantage in survival for the more “hypo-inflammatory” type γ . On the contrary, higher mortality and lower ventilator free days were observed in this group of patients.

In type γ , there were more patients with stem cell transplantation and chemotherapy-induced neutropenia. As the hematopoietic function was suppressed or not successfully reconstructed, patients had low hemoglobin level and low platelet levels. More importantly, they had extremely low levels of white blood cell and cytokine levels. As a result, the unexpectedly poor outcome of type γ may arise from relatively insufficient inflammation responses. Cortigiani et al reported that regardless of ARDS severity, immunocompromised patients had higher mortality rates than the immunocompetent ones.³⁷ This suggested that immunocompromised ARDS patients may be hindered by insufficient inflammatory response to control infection. A similar phenomenon had been observed in end stage sepsis patients, called sepsis-related immunosuppression.³⁸ In these patients, immunosuppression was triggered by macrophage over-activation and T cell disfunction.^{39,40} This may contribute to negative results of glucocorticoid or other inhibition therapies in sepsis studies^{41,42} For patients in a state of insufficient inflammatory response, immune suppression induced by drugs might be catastrophic.

Based on findings from the immunocompromised hosts, we can infer that immunological evaluation for ARDS patients may need to be performed in a quantified manner, including immune cell function assessments, which will inform us whether the patient has adequate inflammatory response. From another perspective, clinical phenotypes but not classical methods (like SOFA) are more valuable in predicting patient outcomes for the subjects of the present study, for positive correlations between inflammation (evaluated with IL-6) and SOFA score only existed within type α (Figure 1E). However, the SOFA score was not correlated with mortality in our cohort.

As far as we know, this is the first study that has investigated lung microbiota signatures in immunocompromised hosts with ARDS. Some of the results we had in this study were consistent with previous reports. First, loss of alpha diversity was observed in the type γ - A phenotype that had worse outcomes. In immunocompetent hosts with ARDS,⁴³ chronic obstructive pulmonary disease (COPD) or idiopathic pulmonary fibrosis (IPF)^{44,45}, the relation between dysbiosis and disease progression also exists. However, whether the dysbiosis is a result of the aggravation of diseases or the cause of the aggravation is not clear.

Second, in type α , which is characterized by more active inflammation, more gut-associated bacteria were enriched. Dickson et al. reported the association of gut bacteria in the lower respiratory tract and intensified systemic inflammation.⁴⁶ Similar results were reported, but no study has proved an association of gut bacteria and higher mortality or lower successful extubation rate.⁴³ On the contrary, we observed a better outcome in gut-bacteria enriched and inflammation-activated immunocompromised hosts (type α) than in the inflammation-inactive hosts (type γ), which indicates that an adequate host response is necessary for infection control. Thus, the enrichment of specified microbes might, to some extent, actually a result of the host immune responses.⁴⁷

We also developed a microbiota data-based random forest model to distinguish type γ from other types. The model has a satisfying classification ability. It proved that microbiota composition is an effective marker for clinical phenotypes. With the advance of rapid NGS analyzing techniques (ie, nanopore-targeted sequencing), the turn-around time for NGS was greatly reduced from around 24 hours⁴⁸ to theoretically 6 hours.⁴⁹ Based on our model, a specified testing panel can be developed for detecting high-risk type γ patients. The microbes in the model should be further evaluated for their role and their functional contribution in host–microbe interaction.

The study has several shortcomings: First, the robustness of the clustering may be limited by the relatively limited sample size.⁵⁰ The results need to be validated in larger cohorts. Second, what we presented in this study was a static description of clinical variables and lung microbiota signatures. The trend of host inflammation response and microbiota transformation over the scale of time and disease state can also provide valuable information,⁵¹ which is worth exploring in further studies.

Conclusion

Immunocompromised patients with pneumonia-related ARDS can be clustered into three clinical phenotypes, namely type α , type β , and type γ . Phenotypes were distinguished from each other with different outcomes and lung microbiota signatures. Type γ , which was characterized by insufficient inflammation response and worse outcomes, can be detected with a random forest model based on lung microbiota markers.

Data Sharing Statement

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

Ethics Approval and Consent to Participate

All participants of this study have signed the informed consent sheet and consented to participate. If the patient lacks capacity to give consent, eg. 1. dementia, 2. delirium 3. shock, stroke, hypoglycemia or sedatives induced unconsciousness. Once the patients regained consciousness, they will be asked to provide their own informed consent to participate in this study.

Consent for Publication

All authors have read this manuscript and consented to publication.

Funding

This project was supported by Peking University International Hospital Research Funds (No. YN2021QN02), The National Natural Science Foundation of China (Grant No. 82202326), Clinical Medicine Plus X – Young Scholars Project, Peking University, The Fundamental Research Funds for the Central Universities (PKU2022LCXQ031), Wu Jieping Medical Foundation Runze Fund for Critical Care Medicine (NO320·6750·2022-2-34) and Peking University People's Hospital Scientific Research Development Funds(No. RDJP2022-54).

Disclosure

The authors report no conflicts of interest in this work.

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