CHEST

Clinical Characteristics and Prognosis of Patients With Severe Pneumonia With *Pneumocystis jirovecii* Colonization

A Multicenter, Retrospective Study

Yongpo Jiang, MD; Xiaohan Huang, MD; Huili Zhou, MD; Mingqiang Wang, MD; Shengfeng Wang, MD; Xindie Ren, MD; Guojun He, MD; Jun Xu, MD; Qianqian Wang, MD; Muhua Dai, MD; Yonghui Xiong, MD; Lin Zhong, MD; Xuwei He, MD; Xuntao Deng, MD; Yujie Pan, MD; Yinghe Xu, MD; Hongliu Cai, MD; Shengwei Jin, MD; Hongyu Wang, MD; and Lingtong Huang, MD

BACKGROUND: For decades, the incidence and clinical characteristics of *Pneumocystis jirovecii* colonization in patients with severe pneumonia was unclear.

RESEARCH QUESTION: What are the clinical features and outcomes associated with *P jirovecii* colonization in individuals diagnosed with severe pneumonia?

STUDY DESIGN AND METHODS: In this multicenter, retrospective, matched study, patients with severe pneumonia who underwent bronchoalveolar lavage clinical metagenomics from 2019 to 2023 in the ICUs of 17 medical centers were enrolled. Patients were diagnosed based on clinical metagenomics, pulmonary CT scans, and clinical presentations. Clinical data were collected retrospectively, and according to propensity score matching and Cox multivariate regression analysis, the prognosis of patients with *P jirovecii* colonization was compared with that of patients who were *P jirovecii*-negative.

RESULTS: A total of 40% of *P jirovecii*-positive patients are considered to have *P jirovecii* colonization. The *P jirovecii* colonization group had a higher proportion of patients with immunosuppression and a lower lymphocyte count than the *P jirovecii*-negative group. More frequent detection of cytomegalovirus, Epstein-Barr virus, human herpesvirus-6B, human herpesvirus-7, and torque teno virus in the lungs was associated with *P jirovecii* colonization than with *P jirovecii* negativity. By constructing two cohorts through propensity score matching, we incorporated codetected microorganisms and clinical features into a Cox proportional hazards model and revealed that *P jirovecii* colonization was an independent risk factor for mortality in patients with severe pneumonia. According to sensitivity analyses, which included or excluded codetected microorganisms, and patients not receiving trimethoprim-sulfamethoxazole treatment, similar conclusions were reached.

INTERPRETATION: Immunosuppression and a reduced lymphocyte count were identified as risk factors for *P jirovecii* colonization in patients with non-Pneumocystis pneumonia. More frequent detection of various viruses was observed in patients colonized with *P jirovecii*, and *P jirovecii* colonization was associated with an increased 28-day mortality in patients with severe pneumonia.

CHEST 2025; 167(1):54-66

KEY WORDS: clinical metagenomics; colonization; *Pneumocystis jirovecii*; severe pneumonia; trimethoprim-sulfamethoxazole

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Take-home Points

Study Question: What are the clinical features and outcomes associated with *Pneumocystis jirovecii* colonization in individuals diagnosed with severe pneumonia?

Results: In this multicenter retrospective cohort study, there were differences in the proportion of patients with immunosuppression and lower lymphocyte count between the *P jirovecii* colonization group and the *P jirovecii*-negative group. More frequent detection of cytomegalovirus, Epstein-Barr virus, human herpesvirus-6B, human herpesvirus-7, and torque teno virus in the lungs was associated with *P jirovecii* colonization than with *P jirovecii* negativity. There was a significant difference in 28-day mortality between the *P jirovecii* colonization group than with the *P jirovecii*-negativity group after adjusting for patient background characteristics.

Interpretation: These results highlight that *P jirovecii* colonization is an independent risk factor for mortality in patients with severe pneumonia.

Pneumocystis pneumonia (PCP) is a life-threatening opportunistic fungal infection.[1,2] Although PCP often has occurred in patients with HIV, an increasing number of non-HIV-infected individuals are being diagnosed with PCP, with hospital mortality reaching as high as 50% to 75%.[3-5] *Pneumocystis jirovecii* colonization, detecting the organism or its DNA without typical pneumonia symptoms, is increasingly recognized as clinically significant.[6] A multicenter, international, retrospective study revealed that PCP predominantly occurs in non-HIV-infected critically ill patients admitted to the ICU.[5] Notably, approximately 40% of *P jirovecii* polymerase chain reaction (PCR)-positive patients were not diagnosed with PCP, and these patients were widely considered to have *P jirovecii* colonization.[5] However, the clinical significance of *P jirovecii* colonization remains unclear. In 2006, the American Thoracic Society[7] published a document posing questions related to *P jirovecii* colonization. However, it seems that critical care medicine research on this issue has remained stagnant for more than a decade.[8]

Clinical metagenomics is an unbiased method for detecting lung microbial information, allowing for the discovery of many potential microbial details, which is widely used for the diagnosis of infected or suspected infected patients.[9-11] With the development and improvement of monitoring methods (eg, clinical metagenomics), the detection of *P jirovecii* continues to increase.[12] In this study, we analyzed a multicenter severe pneumonia cohort comprising 1,897 patients who underwent clinical metagenomics to elucidate the clinical characteristics of *P jirovecii* colonization and the association of *P jirovecii* colonization with patient outcomes.

Study Design and Methods

Population and Data Collection

This multicenter, retrospective cohort study was conducted in adult ICUs at 17 medical centers in China from January 2019 to June 2023 and was approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine (No. IIT20230222A). The collection of bronchoalveolar lavage fluid (BALF) follows the standardized operating

ABBREVIATIONS: BALF = bronchoalveolar lavage fluid; BDG = (1,3)-beta-D-glucan; CMV = cytomegalovirus; HR = hazard ratio; PCP = Pneumocystis pneumonia; PCR = polymerase chain reaction; PSM = propensity score matching; SOFA = Sequential Organ Failure Assessment; TMP-SMX = trimethoprim-sulfamethoxazole

AFFILIATIONS: From the Department of Critical Care Medicine (Y. J. and Y. Xu), Taizhou Hospital of Zhejiang Province affiliated to Wenzhou Medical University, Taizhou; the Department of Critical Care Medicine (X. H., H. Z., X. R., G. H. J. X., H. C., and L. H.), The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou; the Department of Critical Care Medicine (M. W.), Henan Key Laboratory for Critical Care Medicine, Zhengzhou Key Laboratory for Critical Care Medicine, Henan Provincial People's Hospital, Zhengzhou; the Zhengzhou University People's Hospital (M. W.), Henan University People's Hospital, Zhengzhou; the Department of Critical Care Medicine (S. W.), The Second Affiliated Hospital of Zhengzhou University, Zhengzhou; the Department of Critical Care Medicine (Q. W.), The First Hospital of Jiaxing, Jiaxing; the Department of Critical Care Medicine (M. D.), Tongde Hospital of Zhejiang Province, Hangzhou; the Department of Critical Care Medicine (Y. Xiong), Lanxi Hospital of Traditional Chinese Medicine, Lanxi; the Department of Critical Care Medicine (L. Z.), The First People's Hospital of Pinghu, Pinghu; the Department of Critical Care Medicine (X. H. and X. D.), Lishui People's Hospital, Lishui; the Department of Critical Care Medicine (Y. P.), Wenzhou Central Hospital, Wenzhou; the Department of Anesthesia and Critical Care (S. J.), The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou; and the Department of Emergency Intensive Care Unit (H. W.), The Fifth Clinical Medical College of Henan University of Chinese Medicine, Zhengzhou, China.

Y. Jiang, X. Huang, H. Zhou, and M. Wang contributed equally to this manuscript.

CORRESPONDENCE TO: Lingtong Huang, MD; email: lingtonghuang@zju.edu.cn

DOI: https://doi.org/10.1016/j.chest.2024.07.140

procedures of local hospitals. The attending physician determines whether to conduct a BALF examination for the patient. The exclusion criteria were as follows: (1) < 18 years of age and (2) lost to follow-up or who abandoned treatment within 28 days after ICU admission. Because the ICUs involved in the study rarely admit patients with HIV, patients with HIV were not included in this research. The following demographic and medical history data were collected from the patients' medical records: age, sex, comorbidities, immunosuppressive status, laboratory test results (eg, WBC, lymphocyte, and neutrophil counts; C-reactive protein level; β-D-glucan level), and Sequential Organ Failure Assessment (SOFA) scores. For patients who tested positive for *P jirovecii*, we collected the following additional data: previous treatments, including immunosuppressive agents, IV immunoglobulin, blood transfusions, albumin therapy, PCP prophylaxis, chemotherapy, and radiotherapy, and chest CT scan results. The missing data were imputed using multiple imputation.

Definitions

Severe pneumonia was defined as previously described.[13] The different types of pneumonia were classified as follows: (1) community-acquired pneumonia (pneumonia occurring before or within 48 hours after admission, irrespective of the necessity for mechanical ventilation), (2) hospital-acquired pneumonia (pneumonia acquired at least 48 hours after admission and without tracheal intubation or tracheotomy after 48 hours), and (3) ventilator-associated pneumonia (pneumonia occurring in patients at least 48 hours after tracheal intubation or tracheotomy). Immunosuppression was defined as previously described.[14]

The diagnosis of PCP or *P jirovecii* colonization was independently determined by two physicians without blinding. In case of discrepancy, a third physician participated in the final assessment. Specifically, if clinical metagenomic testing for *P jirovecii* yielded negative results, the patient was categorized into the negative group. The diagnostic criteria for PCP were as follows: (1) positive clinical metagenomic test for *P jirovecii*, (2) presence of consistent clinical manifestations of PCP, and (3) chest CT scan results consistent with PCP. After these conditions were met, we established the following exclusion criteria: (1) patients with other conditions causing CT features similar to those of PCP, including confirmed COVID-19 and invasive aspergillosis, which was described previously[5]; and (2) patients in the PCP group who lacked host risk factors and had continuous negative (1,3)-beta-D-glucan (BDG) test results. The remaining patients were classified as having *P jirovecii* colonization. According to these definitions, patients were divided into the following three groups: *P jirovecii* colonization group, PCP group, and *P jirovecii*-negative group.

Clinical Metagenomics

All clinical metagenomic tests followed the previously reported protocol and yielded results within 36 hours of sample submission.[15] During the clinical metagenomic examination, all patients or family members were informed and signed informed consent forms to perform the examination as permitted by Chinese law. If clinical metagenomics was performed multiple times, the results obtained from the first instance were included in our analysis. The procedure for the clinical metagenomics is provided in e-Appendix 1.

Data Analysis and Propensity Score Matching

One-way analysis of variance, Student *t* test for continuous variables, and χ^2 test or Fisher exact test for categorical variables were used. We used two propensity score matching (PSM) methods to construct the final analysis cohort. The MatchIt package in R software (v1.0-14) was used to perform PSM. PSM analysis with the full matching algorithm was conducted with a caliper width of 0.05, whereas PSM analysis with nearest-neighbor matching was conducted with a ratio of 1:4 and a caliper width of 0.05. The matched baseline parameters of the *P jirovecii* colonization group and control group included all clinical features and codetected microbes with $P < .10$, excluding trimethoprim-sulfamethoxazole (TMP-SMX) treatment.

The risk factors associated with mortality within 28 days after ICU admission were estimated using the Cox proportional hazards regression model, as historical confounder definition with purposeful variable selection, along with the status of *P jirovecii* colonization, TMP-SMX treatment, and sex. Stepwise model selection was then conducted as previously described.[16] To evaluate pneumonia-related death, we conducted a competing risk model analysis of the fully matched cohort, considering extubation as the competing risk factor for patients who died after intubation. For univariate

analysis, we used the Fine and Gray model. The mstate package in R software (v4.2.3) was used to construct a semiparametric model with transition-specific covariates. This model incorporated all parameters with a significance level of $P < .10$ in the Cox univariate model. All statistical analyses were performed using R software (v4.2.3), and $P < .05$ (two-tailed) was used to indicate statistical significance.

Sensitivity Analysis

We conducted several sensitivity analyses. First, we included or excluded other microbial information detected by clinical metagenomics in the model (e-Fig 1). Second, to weaken the possibility of PCP in patients colonized with *P jirovecii*, patients treated without TMP-SMX were analyzed as sensitivity analysis. Additionally, a backward elimination procedure instead of a stepwise elimination procedure was used for variables screening in the full matching cohort.

Results

Clinical Characteristics and Codetected Microorganisms of the Three Groups

A total of 1,897 patients were screened for the study (Fig 1), and 1,737 patients were included in the analysis. Among the 67 patients in the *P jirovecii* colonization group identified after the screening, two patients were ultimately included in the PCP group. The discrepancies between the diagnoses made by the two physicians included three patients without typical CT scan features and six patients with similar PCP imaging features. A final diagnosis was reached after analysis by a third physician. In patients with suspicious CT scan findings of PCP in the colonization group, continuous monitoring of BDG in most of these patients with suspected PCP CT scan manifestations was negative, with only two patients diagnosed with invasive aspergillosis showing mild BDG elevation (e-Table 1). One patient did not receive PCP treatment and exhibited a good prognosis. Finally, among these patients, 95 had PCP, 65 had *P jirovecii* colonization, and 1,577 had *P jirovecii* negativity.

Regarding clinical characteristics, when comparing the three groups of patients, significant differences were observed in clinical characteristics, including chronic kidney disease, connective tissue disease, hematologic malignancy, transplantation, immunosuppressive status, type of pneumonia, WBC count, lymphocyte count, and proportion of patients with respiratory failure and liver dysfunction (Table 1). Among the patients in the PCP group who did not receive TMP-SMX, eight patients were administered caspofungin due to an inability to tolerate TMP-SMX, three patients were misdiagnosed and did not use TMP-SMX, and one patient died on the day of receiving clinical metagenomic results; therefore, the administration of the medication was a missed opportunity (e-Table 2). Most of the patients in this cohort were administered corticosteroids (negative vs colonization vs infection, 53.5% vs 78.5% vs 88.4%, respectively; $P < .001$). Compared with *P jirovecii*-negative patients, a higher proportion of those with *P jirovecii* colonization had an immunosuppressive status (38.5% vs 21.6%, $P = .002$) and a lower lymphocyte count (0.5 vs 0.6, $P = .041$), respectively.

In addition, we reported other codetected microorganisms based on clinical metagenomics (Table 2). In the context of the three groups, statistically significant differences were detected for the presence of various viruses, bacteria, and fungi, including cytomegalovirus (CMV) ($P < .001$), Epstein-Barr virus ($P < .001$), torque teno virus ($P < .001$), human herpes virus-7 ($P = .044$), human herpes virus-6B ($P = .01$), *Acinetobacter* species ($P < .001$), *Klebsiella* species ($P < .001$), *Pseudomonas* species ($P = .01$), and *Aspergillus* species ($P < .005$). Furthermore, when comparing the *P jirovecii* colonization group with the *P jirovecii*-negative group, the former exhibited a higher prevalence of *Candida* species (41.5% vs 30.5%), CMV (40.0% vs 13.8%), Epstein-Barr virus (27.7% vs 15.3%), torque teno virus (20.0% vs 8.2%), human herpes virus-7 (10.8% vs 4.9%), *Corynebacterium* species (9.2% vs 6.1%), and human herpes virus-6B (6.2% vs 1.9%) and a lower frequency of *Acinetobacter* species (18.5% vs 32%), respectively. We provided the results of culture in e-Table 3.

When assessing PCP or *P jirovecii* colonization, it was found that, compared with those with PCP, those with *P jirovecii* colonization received less immunosuppressive therapy in the month before diagnosis (32.3% vs 78.9%, $P < .001$), less IV immunoglobulin treatment (1.5% vs 11.6%, $P = .028$), and fewer blood transfusions (13.8% vs 33.7%, $P = .008$), respectively (e-Table 4). Additionally, in the *P jirovecii* colonization group, no patients received PCP prophylaxis, whereas 15.8% of patients in the PCP group did ($P = .002$). Radiologic examinations indicated that the *P jirovecii* colonization group had fewer typical PCP imaging changes, including bilateral ground-glass opacities (5.5% vs 37.2%, $P < .001$) and

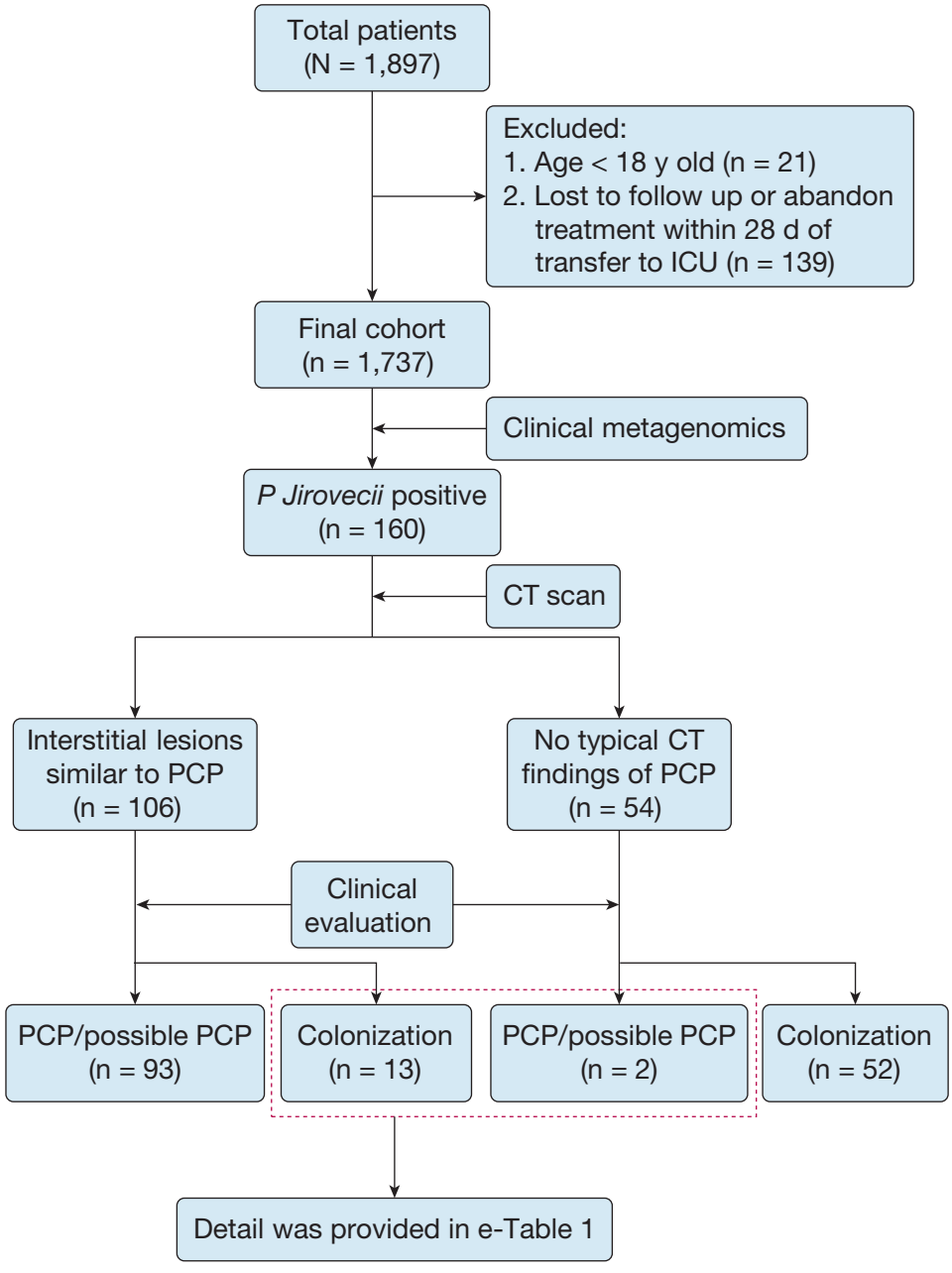

Figure 1 – *Flowchart of the study. PCP = Pneumocystis pneumonia.*

bilateral ground-glass opacities and consolidations (14.5% vs 61.7%, $P < .001$). Among *P jirovecii* colonization patients, 50.8% received TMP-SMX treatment, whereas this proportion was 87.4% in the PCP group ($P < .001$). As for BDG, 40% patients were positive in the *P jirovecii* colonization group, whereas 60.8% were positive in the PCP group ($P = .044$). The concentration of BDG was also higher in the PCP group than the *P jirovecii* colonization group (185.2 vs 49.93 pg/mL, respectively; $P = .005$). We reported the diagnostic criteria for several potentially controversial *P jirovecii* colonization patients. To determine why physicians chose to administer TMP-SMX in the *P jirovecii* colonization group, we reported the clinical characteristics of patients who were or were not treated with TMP-SMX (e-Table 5). We found that immunosuppression differed between the two groups. However, even if the SOFA scores of the two groups of patients differed by nearly 1 point, their mortality rates were similar (48.5% vs 53.1%).

TABLE 1] Differences in Clinical Characteristics Among the Three Groups of Patients

Characteristic	Negative (n = 1,577)	Colonization (n = 65)	Infection (n = 95)	*P* Value
Age, y	67 (55-75)	70 (59-77)	64.0 (54-74)	.071
Male	1109 (70.3)	43 (66.2)	64 (67.4)	.654
Comorbidities				
Diabetes mellitus	390 (24.7)	14 (21.5)	23 (24.2)	.839
Myocardial infarction	96 (6.1)	4 (6.2)	3 (3.2)	.500
Chronic pulmonary disease	318 (20.2)	13 (20.0)	19 (20.0)	.999
Liver disease	102 (6.5)	5 (7.7)	11 (11.6)	.151
Chronic kidney disease	178 (11.3)	11 (16.9)	30 (31.6)	**< .001**
Solid tumor	234 (14.8)	10 (15.4)	17 (17.9)	.718
Hematologic malignancy	74 (4.7)	6 (9.2)	11 (11.6)	**.005**
CTD	56 (3.6)	4 (6.2)	14 (14.7)	**< .001**
Transplantation	69 (4.4)	3 (4.6)	16 (16.8)	**< .001**
Cerebrovascular disease	258 (16.4)	13 (20.0)	8 (8.4)	.084
Immunosuppression	340 (21.6)	25 (38.5)[a]	65 (68.4)	**< .001**
Type of pneumonia				**< .001**
CAP	931 (59.0)	47 (72.3)	73 (76.8)	
HAP	387 (24.5)	12 (18.5)	20 (21.1)	
VAP	259 (16.4)	6 (9.2)	2 (2.1)	
Respiratory support				.084
IMV	1379 (87.4)	57 (87.7)	74 (77.9)	
NIMV	44 (2.8)	3 (4.6)	5 (5.3)	
Others[b]	154 (9.8)	5 (7.7)	16 (16.8)	
Duration of mechanical ventilation within 28 d, d	8 (3-16)	7 (3-12)	7 (1.5-11)	**.025**
Laboratory tests				
WBC count, 10^9/L	11.2 (7.0-15.9)	10.3 (6.7-14.9)	9.6 (5.6-13.4)	**.022**
Lymphocyte, 10^9/L	0.6 (0.3-1.0)	0.5 (0.3-0.8)[a]	0.4 (0.2-0.7)	**< .001**
Neutrophil, 10^9/L	9.7 (5.7-14.2)	8.7 (5.1-12.6)	8.8 (4.9-12.5)	.149
C-reactive protein, mg/L	87.5 (28.4-159.0)	86.7 (42.9-149.7)	100.5 (57.9-172.9)	.072
Procalcitonin, ng/mL	0.9 (0.2-5.4)	0.9 (0.3-6.1)	0.8 (0.3-3.7)	.924
PCP prophylaxis	…[c]	0 (0)	15 (15.8)	**.002**
β-D-glucan				
Positive	…[c]	18 (40)	45 (60.8)	**.044**
Concentration, pg/mL	…[c]	49.93 (0-173.1)	185.2 (0-279.2)	**.005**
Missing	…[c]	20 (30.8)	21 (22.1)	.294
SOFA, mean ± SD	7.8 ± 4.0	8.6 ± 4.1	8.0 ± 3.3	.191
Organ dysfunction				
Respiratory	1,319 (83.6)	56 (86.2)	91 (95.8)	**.006**
Coagulation	573 (36.3)	23 (35.4)	33 (34.7)	.942
Hepatic	257 (16.3)	14 (21.5)	6 (6.3)	**.016**
Cardiovascular	776 (49.2)	39 (60.0)	52 (54.7)	.146
Neurologic	587 (37.2)	22 (33.8)	27 (28.4)	.201
Kidney	344 (21.8)	20 (30.8)	27 (28.4)	.087
TMP-SMX treatment	175 (11.1)	33 (50.8)	83 (87.4)	**< .001**
Corticosteroid treatment	844 (53.5)	51 (78.5)	84 (88.4)	**< .001**

(Continued)

TABLE 1] *(Continued)*

Characteristic	Negative (n = 1,577)	Colonization (n = 65)	Infection (n = 95)	*P* Value
Metagenomics after IMV	1,338 (84.8)	54 (83.1)	70 (73.7)	**.015**
Time from IMV to metagenomics, d	2.0 (1.0-5.0)	1.0 (0.0-2.0)[a]	1.0 (0.0-1.0)	**< .001**
Time from ICU admission to clinical metagenomics, d	3.0 (2.0-7.0)	3.0 (2.0-4.0)	3.0 (2.0-4.0)	**< .001**
Hospital LOS, d	21.0 (12.0-36.0)	18.0 (8.0-32.0)	19.0 (10.5-37.5)	.077
ICU LOS, d	13.0 (8.0-24.0)	10.0 (6.0-16.0)[a]	10.0 (7.0-20.0)	**.003**
28 d-mortality	637 (40.4)	33 (50.8)	48 (50.5)	**.043**

Values are No. (%), median (interquartile range), or as otherwise indicated. *P* values set in boldface font are considered statistically significant ($P < .05$). CAP = community-acquired pneumonia; CTD = connective tissue disease; HAP = hospital-acquired pneumonia; IMV = invasive mechanical ventilation; LOS = length of stay; NIMV = noninvasive mechanical ventilation; PCP = Pneumocystis pneumonia; SOFA = Sequential Organ Failure Assessment; TMP-SMX = trimethoprim-sulfamethoxazole; VAP = ventilator-associated pneumonia.
[a]Negative vs colonization, $P < .05$.
[b]Other types of respiratory support include high-flow nasal cannula, Venturi mask, and so forth.
[c]Data not collected.

TABLE 2] Characteristics of Major Microbial Detection in Three Groups of Patients

Genus or Species	Domain	Total (N = 1,737)	Negative (n = 1,577)	Colonization (n = 65)	Infection (n = 95)	*P* Value
Pneumocystis	Fungi	160 (9.2)	0 (0)	65 (100)	95 (100)	**< .001**
Candida	Fungi	532 (30.6)	481 (30.5)	27 (41.5)[a]	24 (25.2)	.085
Acinetobacter	Bacteria	529 (30.5)	505 (32.0)	12 (18.5)[a]	12 (12.6)	**< .001**
Klebsiella	Bacteria	498 (28.7)	472 (29.9)	15 (23.1)	11 (11.6)	**< .001**
HSV-1	Viruses	466 (26.8)	421 (26.7)	21 (32.3)	24 (25.3)	.569
CMV	Viruses	309 (17.8)	218 (13.8)	26 (40.0)[a]	65 (68.4)	**< .001**
Enterococcus	Bacteria	299 (17.2)	270 (17.1)	10 (15.4)	19 (20)	.712
EBV	Viruses	289 (16.6)	242 (15.3)	18 (27.7)[a]	29 (30.5)	**< .001**
Pseudomonas	Bacteria	281 (16.1)	267 (16.9)	9 (13.8)	5 (5.3)	**.010**
Aspergillus	Fungi	274 (15.8)	235 (14.9)	14 (21.5)	25 (26.3)	**.005**
Stenotrophomonas	Bacteria	274 (15.8)	255 (16.2)	12 (18.5)	7 (7.4)	.061
Staphylococcus	Bacteria	166 (9.6)	152 (9.6)	8 (12.3)	6 (6.3)	.420
Torque teno virus	Viruses	164 (9.4)	130 (8.2)	13 (20)[a]	21 (22.1)	**< .001**
Burkholderia	Bacteria	161 (9.3)	153 (9.7)	4 (6.2)	4 (4.2)	.136
Streptococcus	Bacteria	151 (8.7)	144 (9.1)	5 (7.7)	2 (2.1)	.059
Corynebacterium	Bacteria	107 (6.2)	96 (6.1)	6 (9.2)[a]	5 (5.3)	.547
Nakaseomyces	Fungi	95 (5.5)	89 (5.6)	3 (4.6)	3 (3.2)	.558
HHV-7	Viruses	92 (5.3)	77 (4.9)	7 (10.8)[a]	8 (8.4)	**.044**
Haemophilus	Bacteria	83 (4.8)	75 (4.8)	4 (6.2)	4 (4.2)	.844
Escherichia	Bacteria	83 (4.8)	77 (4.9)	3 (4.6)	3 (3.2)	.745
Elizabethkingia	Bacteria	58 (3.3)	54 (3.4)	3 (4.6)	1 (1.1)	.386
Achromobacter	Bacteria	48 (2.8)	43 (2.7)	3 (4.6)	2 (2.1)	.609
Serratia	Bacteria	46 (2.6)	45 (2.9)	1 (1.5)	0 (0)	.207
Enterobacter	Bacteria	44 (2.5)	43 (2.7)	0 (0)	1 (1.1)	.250
HHV-6B	Viruses	39 (2.2)	30 (1.9)	4 (6.2)[a]	5 (5.3)	**.010**

Values are No. (%) or as otherwise indicated. The species or genus with the top 25 detection frequencies are shown. For bacteria and fungi, genus is shown, and for viruses, species is shown. There is statistical significance of *Faecalis* species in the negative group and colonization group in all other detected species except for in this table. *P* values set in boldface font are considered statistically significant ($P < .05$). CMV = cytomegalovirus; EBV = Epstein-Barr virus; HHV-6B = human herpes virus-6B; HHV-7 = human herpes virus-7; HSV-1 = herpes simplex virus 1.
[a]Codetected species or genus with statistical differences ($P < .05$) between the colonization group and the negative group.

P jirovecii *Colonization and the Prognosis of Patients With Severe Pneumonia*

The association between *P jirovecii* colonization and mortality in patients with severe pneumonia was assessed. Full matching was performed to reduce the heterogeneity of the cohort independently (e-Figs 2A, 2B; e-Table 6). Despite achieving a balance in baseline characteristics through full matching, significant differences persisted in immunosuppressive status ($P < .007$), lymphocyte count ($P = .028$), corticosteroid use ($P < .001$), and detection of various microorganisms.

Cox analysis was subsequently conducted. Given that our study involved the simultaneous detection of various other microorganisms, a sensitivity analysis was performed. The results indicate that in both models, *P jirovecii* colonization is an independent risk factor for mortality (adjusted model 1: hazard ratio [HR], 1.518; 95% CI, 1.023-2.253; $P = .038$; adjusted model 2: HR, 1.503; 95% CI, 1.011-2.235; $P = .044$) (Table 3). To further eliminate the influence of patients with suspected PCP who received TMP-SMX treatment, additional sensitivity analysis was conducted (Table 4). Among patients who did not receive TMP-SMX, in models including or excluding microorganisms as covariates, *P jirovecii* colonization was an independent risk factor for patient mortality (adjusted model 1: HR, 1.691; 95% CI, 1.007-2.837; $P = .047$; adjusted model 2: HR, 1.789; 95% CI, 1.067-3.000; $P = .027$).

Given that the cohort constructed using the full-matching method still exhibited baseline imbalances, we used PSM with nearest-neighbor matching as a sensitivity analysis (e-Figs 2C, 2D; e-Table 7). In the matched cohort, apart from differences in TMP-SMX administration between the two groups, all the baseline data, including codetected species, showed no significant differences. According to the multivariate model, *P jirovecii* colonization was an independent risk factor for patient mortality (HR, 1.820; 95% CI, 1.139-2.907; $P = .012$) (e-Table 8). In the subset of patients who did not receive TMP-SMX, *P jirovecii* colonization was an independent risk factor for patient mortality (HR, 1.868; 95% CI, 1.039-3.359; $P = .037$) (e-Table 9).

Besides, we constructed a competing risk model. In the full matching cohort, after extubation was incorporated as a competing risk factor for patients who died after intubation, *P jirovecii* colonization remained an independent risk factor for 28-day mortality (Fine and Gray model: 95% CI, 1.096-2.282; unadjusted HR, 1.581; $P = .014$; semiparametric model with transition-specific covariates: adjusted HR, 1.84; 95% CI, 1.22-2.77; $P = .0035$) (Fig 2). Survival curve of the full matching cohort is reported in e-Figure 3, and backward elimination procedure for the multivariate model selection in patients without TMP-SMX treatment showed *P jirovecii* colonization was still an independent risk of 28-day mortality (adjusted HR, 1.742; 95% CI, 1.038-2.923; $P = .035$) (e-Table 10).

Discussion

Very few studies have directly noted the incidence of *P jirovecii* colonization in critically ill patients with pneumonia.[5] This study, through unbiased clinical metagenomics, revealed an approximate rate of 3.74% for PCP colonization among critically ill patients with pneumonia in the ICU. Research has shown that in children, the rate of *P jirovecii* colonization is approximately 3%,[17,18] which is close to the data reported for this cohort. In our study, *P jirovecii* colonization was found in 40% of *P jirovecii* DNA-positive patients, whereas it was found in 60% of the patients with PCP. This finding confirms the results reported in another multinational multicenter cohort.[5] Patients with *P jirovecii* colonization exhibited some significant clinical differences compared with those without *P jirovecii* colonization; these differences were similar in patients with and without PCP and included a greater proportion of patients with an immunosuppressive status, with a reduction in lymphocytes, and with an increased rate of lung CMV DNA positivity. Because the clinical characteristics of patients with and without PCP have been extensively reported, we did not include them in this study. Our study focused on the clinical characteristics and prognosis of *P jirovecii* colonization in critically ill patients with pneumonia.

TMP-SMX is a first-line drug for preventing PCP and is widely used in adults and children.[19-21] It is recommended for the risk reduction of PCP.[22] In this study, in the *P jirovecii* colonization group, 50.8% of the physicians chose to administer TMP-SMX for *P jirovecii* clearance. In this study, we did not investigate the direct tissue-invasive role of *P jirovecii* colonization. However, the interplay between fungal colonization and infection may remain inconclusive in the foreseeable future. Due to the ample control group that allowed us to conduct rigorous PSM, we adjusted

TABLE 3] Cox Model of Mortality at Day 28 in Full Matching Cohort

Variables	Unadjusted Model		Adjusted Model I		Adjusted Model 2	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Pneumocystis jirovecii colonization	1.444 (0.995-2.097)	.053	1.518 (1.023-2.253)	.038	1.503 (1.011-2.235)	.044
TMP-SMX treatment	0.820 (0.636-1.056)	.125	0.769 (0.587-1.008)	.057	0.802 (0.612-1.052)	.111
Age	1.015 (1.010-1.021)	< .001	1.013 (1.008-1.019)	< .001	1.014 (1.009-1.020)	< .001
Male	1.130 (0.954-1.339)	.156	0.869 (0.732-1.032)	.109	0.853 (0.719-1.011)	.067
Immunosuppression	1.212 (1.008-1.458)	.042	…[a]	…[a]	…[a]	…[a]
Respiratory support						
NIMV[b]	0.338 (0.168-0.679)	.002	0.382 (0.189-0.773)	.008	0.450 (0.223-0.908)	.026
Others[b]	0.293 (0.193-0.445)	<.001	0.379 (0.248-0.579)	<.001	0.412 (0.270-0.629)	< .001
Type of pneumonia						
HAP[c]	0.738 (0.607-0.898)	.002	0.734 (0.601-0.896)	.002	0.703 (0.576-0.857)	< .001
VAP[c]	0.730 (0.556-0.891)	.004	0.687 (0.540-0.876)	.002	0.685 (0.539-0.871)	.002
Myocardial infarction	1.576 (1.186-2.094)	.002	1.514 (1.128-2.030)	0.006	1.659 (1.241-2.218)	< .001
Hematologic malignancy	2.000 (1.467-2.727)	< .001	1.883 (1.367-2.593)	<0.001	2.088 (1.520-2.868)	< .001
Solid tumor	1.335 (1.087-1.641)	.006	1.420 (1.147-1.759)	0.001	1.351 (1.095-1.667)	.005
WBC count	1.005 (1.001-1.009)	.022	…[a]	…[a]	…[a]	…[a]
Lymphocyte	1.007 (1.001-1.012)	.035	…[a]	…[a]	…[a]	…[a]
Neutrophil	1.009 (1.000-1.019)	.051	…[a]	…[a]	…[a]	…[a]
C-reactive protein	1.001 (1.000-1.002)	.015	…[a]	…[a]	…[a]	…[a]
Procalcitonin	1.004 (1.001-1.007)	.008	…[a]	…[a]	…[a]	…[a]
SOFA score	1.170 (1.149-1.191)	< .001	1.168 (1.146-1.191)	< .001	1.171 (1.149-1.194)	< .001
Candida species	1.200 (1.016-1.417)	.032	…[a]	…[a]	…[a]	…[a]
Klebsiella species	0.850 (0.713-1.014)	.072	0.793 (0.662-0.951)	.012	…[a]	…[a]
Enterococcus species	1.325 (1.089-1.613)	.005	1.296 (1.057-1.589)	.013	…[a]	…[a]
Achromobacter species	1.559 (1.028-2.365)	.037	1.747 (1.143-2.671)	.010	…[a]	…[a]
Aspergillus species	1.935 (1.596-2.346)	< .001	1.874 (1.536-2.287)	< .001	…[a]	…[a]
Nakaseomyces	1.200 (1.016-1.417)	.032	1.546 (1.126-2.123)	.007	…[a]	…[a]

Unadjusted model is applied to all historical confounder definition with purposeful variable selection, along with the status of *P jirovecii* colonization, TMP-SMX treatment, and sex. In adjusted model 1, other microorganisms detected simultaneously by clinical metagenomics were included. In adjusted model 2, other microorganisms detected simultaneously by clinical metagenomics were not included. Adjusted for sex, TMP-SMX treatment, *P jirovecii* colonization, and all other parameters in the unadjusted model with $P < .10$. Stepwise model selection was adopted. Other types of respiratory support including high-flow nasal cannula, Venturi mask, and so forth. HAP = hospital-acquired pneumonia; HR = hazard ratio; NIMV = noninvasive mechanical ventilation; SOFA = Sequential Organ Failure Assessment; TMP-SMX = trimethoprim-sulfamethoxazole; VAP = ventilator-associated pneumonia.

[a]Data not collected.

[b]Compared with invasive mechanical ventilation.

[c]Compared with community-acquired pneumonia.

TABLE 4] Cox Model of Mortality at Day 28 in the Full Matching Cohort for Patients Without TMP-SMX Treatment

Variables	Unadjusted Model		Adjusted Model 1		Adjusted Model 2	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Pneumocystis jirovecii colonization	1.635 (0.977-2.731)	.061	1.691 (1.007-2.837)	.047	1.789 (1.067-3.000)	.027
Age	1.014 (1.008-1.020)	< .001	1.013 (1.007-1.019)	< .001	1.014 (1.008-1.020)	< .001
Male	0.831 (0.695-0.993)	.042	0.807 (0.674-0.967)	.020	0.799 (0.667-0.956)	.014
Immunosuppression	1.189 (0.970-1.460)	.096	…[a]	…[a]	…[a]	…[a]
Respiratory support						
NIMV[b]	0.389 (0.194-0.782)	.008	0.441 (0.218-0.894)	.023	0.502 (0.249-1.015)	.055
Others[b]	0.304 (0.197-0.471)	< .001	0.401 (0.257-0.625)	< .001	0.425 (0.273-0.663)	< .001
Type of pneumonia						
HAP[c]	0.730 (0.593-0.898)	.003	0.732 (0.592-0.906)	.004	0.722 (0.585-0.891)	.002
VAP[c]	0.707 (0.551-0.908)	.007	0.690 (0.533-0.893)	.005	0.694 (0.538-0.895)	.005
Myocardial infarction	1.671 (1.242-2.247)	< .001	1.604 (1.178-2.185)	.003	1.824 (1.347-2.469)	< .001
Hematologic malignancy	1.980 (1.424-2.753)	< .001	1.795 (1.276-2.524)	< .001	1.992 (1.421-2.793)	< .001
Solid tumor	1.247 (0.999-1.557)	.051	1.372 (1.092-1.725)	.007	1.298 (1.036-1.627)	.023
WBC	1.005 (1.001-1.009)	.028	…[a]	…[a]	…[a]	…[a]
Lymphocyte	1.006 (1.000-1.012)	.041	…[a]	…[a]	…[a]	…[a]
Neutrophil	1.009 (0.999-1.019)	.077	…[a]	…[a]	…[a]	…[a]
C-reactive protein	1.001 (0.999-1.002)	.078	…[a]	…[a]	…[a]	…[a]
Procalcitonin	1.004 (1.001-1.007)	.012	…[a]	…[a]	…[a]	…[a]
SOFA score	1.174 (1.151-1.197)	< .001	1.176 (1.152-1.201)	< .001	1.178 (1.1541-1.2024)	< .001
Klebsiella species	0.834 (0.693-1.005)	.056	0.756 (0.624-0.918)	.005	…[a]	…[a]
Stenotrophomonas species	1.228 (0.983-1.533)	.070	1.335 (1.063-1.676)	.013	…[a]	…[a]
Enterococcus species	1.354 (1.100-1.668)	.004	1.293 (1.041-1.606)	.020	…[a]	…[a]
Enterobacter species	0.627 (0.335-1.171)	.143	…[a]	…[a]	…[a]	…[a]
Aspergillus species	1.957 (1.594-2.402)	< .001	1.852 (1.498-2.289)	< .001	…[a]	…[a]
Nakaseomyces	1.624 (1.193-2.210)	.002	1.663 (1.207-2.292)	.002	…[a]	…[a]
Candida species	1.187 (0.996-1.416)	.056	…[a]	…[a]	…[a]	…[a]

Unadjusted model is applied to all historical confounder definition with purposeful variable selection, along with the status of *P jirovecii* colonization, TMP-SMX treatment, and sex. In adjusted model 1, other microorganisms detected simultaneously by clinical metagenomics were included. In adjusted model 2, other microorganisms detected simultaneously by clinical metagenomics were not included. Adjusted for sex, *P jirovecii* colonization, and all other parameters in the unadjusted model with $P < .10$. Stepwise model selection was adopted. HAP = hospital-acquired pneumonia; HR = hazard ratio; NIMV = noninvasive mechanical ventilation; SOFA = Sequential Organ Failure Assessment; VAP = ventilator-associated pneumonia.

[a]Data not collected.

[b]Compared with invasive mechanical ventilation.

[c]Compared with community-acquired pneumonia.

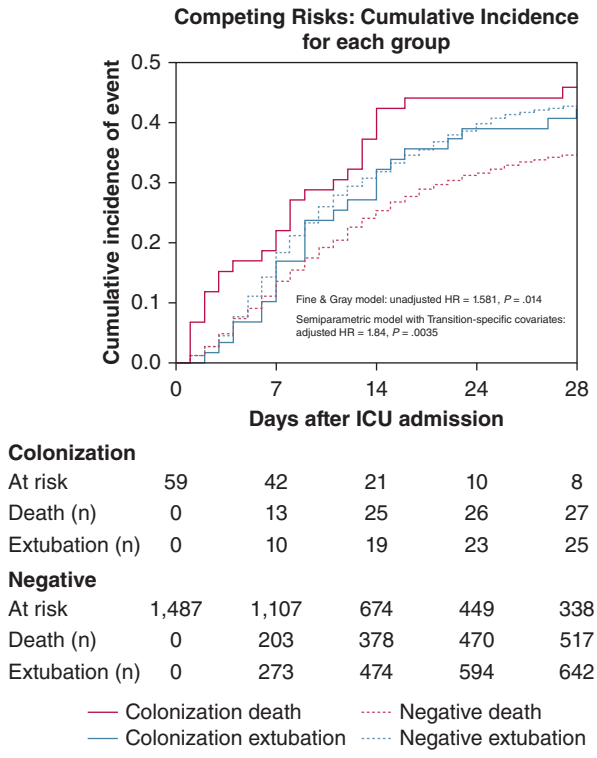

Figure 2 – *Competing risks model analysis on the full matching cohort, considering extubation as the competing risk for patients who died after intubation.*

for parameters known to be independent risk factors for mortality in patients with PCP (eg, comorbidities, SOFA score, severity of organ failure).[23]

Our study has prompted some contemplations, namely, considering the very low colonization rate of *P jirovecii* and the diagnostic challenges involved, is the consequence more severe due to the excessive use of TMP-SMX, or is it more serious when any patient with PCP is misdiagnosed as a patient with *P jirovecii* colonization? In this real-world study, even when clinicians received early clinical metagenomic results indicating *P jirovecii* positivity, the population that did not receive TMP-SMX may represent individuals globally recognized as those who should not receive TMP-SMX. Therefore, if a portion of the patients in this group who experience PCP are misdiagnosed with *P jirovecii* colonization and, consequently, the opportunity to administer TMP-SMX is missed, this could imply that the opportunity to administer TMP-SMX in a significant number of patients elsewhere might also be missed.

In addition, we reported on other codetected microorganisms according to clinical metagenomics and extensively adjusted for the codetected microorganisms, including torque teno virus, CMV, Epstein-Barr virus, and human herpes virus-7, which are as-yet-unreported microorganisms associated with *P jirovecii* colonization that have been overlooked in nearly all other studies. These DNA viruses are often viewed as reactivation latent viruses as we described before[9,10]; therefore, *P jirovecii* colonization may be a marker and not a causal factor of worse outcome. The microbial characteristics were fully matched in the nearest matching cohort, and full matching served as the primary method in this study, only excluding some extreme cases while retaining most patients. These two matching methods, along with sensitivity analyses that included Cox models both with and without microbial characteristics, further mitigate the hypothesis that our study might be influenced by certain microbes potentially acting as mediator variables.

To our knowledge, our study is the first to assess the epidemiologic characteristics and clinical features of *P jirovecii* colonization in critically ill patients with pneumonia. Most *P jirovecii* PCR tests were performed in patients with high-risk factors for PCP or those with interstitial pneumonia. *P jirovecii* PCR tests are rarely performed in nonimmunosuppressed or noninterstitial pneumonia patients, which can introduce significant bias in the exploration of incidence and clinical characteristics. The advantage of our study is that we identified this group of patients through the incidental construction of a BALF clinical metagenomic cohort. We observed that most patients who underwent clinical metagenomics were nonimmunosuppressed and were not limited to those with interstitial pneumonia. Therefore, the incidental detection of *P jirovecii* through clinical metagenomics appears to better reflect the epidemiology and clinical characteristics of *P jirovecii* in the general population. Through clinical metagenomics, we can also observe a correlation between *P jirovecii* and other species, which is not achievable through other types of testing. In addition, our study shows that the BDG concentration in patients colonized with *P jirovecii* is lower than in patients with PCP, as expected. However, data from some patients with *P jirovecii* colonization were higher than normal values. Studies have shown that elevated BDG in patients without invasive fungal infections (including PCP) is associated with poor prognosis.[24] Whether this increased mortality rate can be partially attributed to *P jirovecii* colonization remains unclear.

Further study is needed, and in our prospective cohort,[25] we will report data on *P jirovecii* colonization and the dynamic changes in *P jirovecii* DNA copies in patients with severe pneumonia to answer the following two questions: (1) will *P jirovecii* proliferate in patients with *P jirovecii* colonization if untreated?; and (2) is the increase of *P jirovecii* DNA copies associated with worse outcomes? The answers to these two questions can drive whether we need to further conduct a randomized controlled trial on TMP-SMX clearance of *P jirovecii* colonization.

Limitations

First, this was a retrospective study, and physicians decided whether different patients underwent BALF clinical metagenomics, which may have resulted in selection bias. Second, as described before, the diagnosis of *P jirovecii* colonization was a gray zone and may not be accurate,[26-31] and treatment with TMP-SMX reflects the uncertainty of the differential diagnosis between colonization and infection. Therefore, in this study, we reported the diagnostic criteria for patients for whom discrepancies might exist and conducted extensive sensitivity analyses. Third, the lack of reports on the significance of *P jirovecii* colonization in critically ill patients has resulted in differences among physicians in choosing to prescribe TMP-SMX for either clearing or not clearing *P jirovecii*. Finally, although many studies have shown that PCR and clinical metagenomic have similar values in the diagnosis of PCP, in this study, we did not perform a quantitative analysis of *P jirovecii*, which is one of the limitations of clinical metagenomics.[32] Patients with *P jirovecii* colonization may indeed have lower concentrations of *P jirovecii* than patients with PCP.

Interpretation

Immunosuppression and a reduced lymphocyte count were identified as risk factors for *P jirovecii* colonization in non-PCP patients. More frequent detection of various viruses was observed in patients with *P jirovecii* colonization, and *P jirovecii* colonization was associated with an increased 28-day mortality in patients with severe pneumonia. Future studies are needed to clarify the role of TMP-SMX treatment in *P jirovecii* clearance among critically ill patients with pneumonia with *P jirovecii* colonization.

Funding/Support

This study was supported by the Zhejiang Provincial Natural Science Fund [Grant LTGY24H190001], National Natural Science Foundation of China [Grant 82202356], the National Key Research and Development Program of China [Grant 2022YFC2504501], the National Key Research and Development Program of Zhejiang Province [Grant 2023C03083], and the Medicines Health Research Fund of Zhejiang [Grant 2022KY435].

Financial/Nonfinancial Disclosures

None declared.

Acknowledgments

Author contributions: L. H., H. W., H. C., and S. J. designed the study. L. H., Y. J., X. H., H. Z., M. W., and S. W. analyzed the data and wrote the manuscript. S. W., H. W., X. R., G. H., J. X., Q. W., M. D., Y. X., L. Z., X. H., X. D., Y. X., and Y. P. participated in data collection. All investigators participated in the discussion and agreed on the final version of manuscript.

Role of sponsors: The sponsor had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript.

Additional information: The e-Appendix, e-Figures, and e-Tables are available online under "Supplementary Data."

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