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ORIGINAL RESEARCH

Prognostic Significance of BALF Neutrophil Percentage and Hypoxemia in Non-HIV *Pneumocystis jirovecii* Pneumonia: A Retrospective Cohort Study

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Purpose: The rising incidence of *Pneumocystis jirovecii* pneumonia (PJP) in patients without human immunodeficiency virus (HIV) infection, coupled with high mortality rates, highlights growing challenges in disease management. However, mortality risk factors in non-HIV PJP remain incompletely defined, particularly in large cohorts. Thus, we aimed to explore the clinical characteristics and mortality risk factors in these patients.

Patients and Methods: This study included non-HIV patients with PJP who were hospitalized at Peking University Third Hospital between January 2014 and May 2023. We collected relevant clinical data and performed logistic regression analysis to identify mortality risk factors.

Results: This study included 207 participants (127 male and 80 female) with a median age of 57 (interquartile range: 45–68) years. The 90-day all-cause mortality rate was 15%. Renal transplant recipients accounted for the highest proportion of patients with underlying diseases (34.8%), followed by those with immune-mediated inflammatory diseases (23.7%). In multivariate logistic regression analysis, the percentage of neutrophils in bronchoalveolar lavage fluid (BALF) and a ratio of arterial oxygen partial pressure to fraction of inspired oxygen (PaO_2/FiO_2) <300 mmHg were independently associated with 90-day mortality. A neutrophil percentage ≥41.5% in BALF effectively identified patients at high risk of death.

Conclusion: In non-HIV patients with PJP, the percentage of neutrophils in BALF and PaO_2/FiO_2 ratio were strong predictors of adverse outcomes. These markers may help clinicians identify high-risk patients and initiate early intensive care intervention.

Keywords: neutrophil percentage in BALF, PaO_2/FiO_2, non-HIV patients with PJP, 90-day mortality

Introduction

Pneumocystis jirovecii pneumonia (PJP), predominantly associated with human immunodeficiency virus (HIV) infection, has become increasingly common among individuals receiving immunosuppressive therapies and those who have undergone organ transplantation. This trend parallels the rising incidence of PJP in patients without HIV infection observed in recent years.[1,2] In contrast to patients with HIV-associated PJP, individuals with non-HIV PJP tend to experience more rapid disease progression and have a greater likelihood of developing respiratory failure and death, as well as higher intensive care unit (ICU) admission rates.[2–5] Multiple investigations have indicated that the mortality rate associated with non-HIV patients with PJP is relatively high, varying between 25.6% and 50%.[6,7] Moreover, research indicates that the mortality rate of non-HIV patients with PJP admitted to the ICU may reach as high as 53% to 75.6%.[8–12]

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Collectively, these findings highlight the increasing challenges of managing non-HIV patients with PJP. Therefore, considering the high mortality rate and complexity of clinical management of these patients, developing more specific and sensitive predictive markers is essential to aid clinicians in the early detection of high-risk patients and facilitate the implementation of more intensive treatment approaches. To achieve this objective, we aimed to conduct a retrospective clinical analysis to explore the clinical features and prognostic factors of patients with PJP, to identify biomarkers with improved predictive value.

Materials and Methods

Population

This retrospective single-center study included non-HIV patients with PJP who were hospitalized at Peking University Third Hospital between January 2014 and May 2023. The study was approved by the Ethics Committee of Peking University Third Hospital (approval number: IRB00006761-M2023339), with the requirement for informed consent waived due to the retrospective nature of the study. All patient data were anonymized and handled confidentially. The study was performed in accordance with the Declaration of Helsinki.

Inclusion Criteria: Patients were eligible for inclusion if they exhibited new-onset respiratory symptoms—such as fever, cough, and shortness of breath—along with radiological findings on chest computed tomography (CT) indicative of PJP[13,14] and detection of *Pneumocystis jirovecii* in induced sputum, tracheal aspirates, or bronchoalveolar lavage fluid (BALF). Detection methods included identification of *Pneumocystis jirovecii* cysts by direct microscopic examination, as well as positive findings from real-time polymerase chain reaction (qPCR) and metagenomic next-generation sequencing (mNGS) of BALF samples. Patients without etiological evidence or with positive PJP sequencing results but no corresponding clinical manifestations were not considered to have confirmed PJP, as a positive molecular test alone may reflect colonization rather than active infection. Owing to the limitations of conventional diagnostic techniques, mNGS is increasingly used to diagnose infectious diseases.[15] In the diagnosis of PJP and identification of co-pathogens in complex pulmonary infections, mNGS has demonstrated high sensitivity and specificity.[16,17] However, its specificity may be influenced by *P. jirovecii* colonization, particularly in immunocompromised hosts. Therefore, distinguishing colonization from active infection requires integration of mNGS results with clinical manifestations, radiological features, and host immune status, rather than reliance on pathogen detection alone. PJP was diagnosed based on concordant clinical features and pathogen examination.

Exclusion Criteria: All patients underwent HIV testing. Individuals were excluded if they tested positive for HIV, were younger than 18 years, or lacked complete clinical data.

Methods

Data Collection

Data on the following parameters were collected from patients' medical records: age, sex, weight, height, and underlying diseases. We also recorded the use of glucocorticoids and other immunosuppressive medications before the diagnosis of PJP. Clinical manifestations at the time of PJP diagnosis, including fever, cough, and dyspnea, were recorded along with laboratory test results and chest CT findings. Information on treatment regimens, respiratory support requirements, and ICU admissions were also collected, along with the timeline from symptom onset to PJP diagnosis, hospitalization, and initiation of treatment. The 90-day all-cause mortality rate was tracked by categorizing patients into survivors and non-survivors based on whether they died within 90 days of hospital admission.

Statistical Methods

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 17.0 (IBM Corporation, Armonk, NY, USA). Quantitative data that followed a normal distribution were expressed as mean ± standard deviation (x ± s) and compared between two groups using the Least Significant Difference *t*-test. For quantitative data that did not follow a normal distribution, the median (interquartile range [IQR]) was used, and groups were compared using the non-parametric Mann–Whitney *U*-test. Categorical data were presented as frequencies (proportions) and analyzed using the chi-square test or Fisher's exact test, as appropriate. Univariate logistic regression identified factors associated with 90-

day mortality. Significant factors at the $p<0.05$ threshold were then included in a multivariate logistic regression model. Stepwise regression was used to construct the final model. Variables representing fewer than ten events were excluded from the regression analyses. In cases of collinearity among covariates, only the variable with the highest odds ratio (OR) was retained in the multivariate model. No missing data were imputed. For specific analyses, we excluded patients with missing values for the required variables (eg: BALF differential cell counts) from the corresponding analyses (listwise deletion). Regarding diagnostic criteria, we included only patients who met predefined clinical and radiological features of pneumonia and had microbiological confirmation by microscopy, qPCR, or mNGS from bronchoalveolar lavage fluid or endotracheal aspirate as confirmed PJP cases. Cases with positive microbiological results in the absence of clinical disease were considered colonized and excluded. The optimal cutoff values were determined using receiver operating characteristic (ROC) curve analysis. We used Kaplan–Meier survival analysis to estimate survival functions for groups stratified by these cutoff values and applied the Log rank test to compare the survival curves between groups.

Results

Clinical Characteristics

General Information

Over a decade, 371 individuals suspected of having PJP were screened for the causative pathogen, with 131 cases lacking etiological evidence. Following a thorough evaluation of clinical and radiological features, 23 patients were identified as colonized rather than infected, leaving 217 patients with a confirmed diagnosis of PJP. After excluding 1 patient younger than 18 years, 6 with HIV infection, and 3 with incomplete data—207 patients were recruited for the study (Figure 1). The study population included 127 male and 80 female participants, with a median age of 57 (IQR: 45–68) years. A total of 197 patients (95.2%) underwent bronchoalveolar lavage for pathogen detection; details are provided in Table 1. Detection methods revealed that 11 patients had *Pneumocystis* cysts identified through direct microscopic examination, whereas 99 and 97 tested positive for *Pneumocystis jirovecii* by qPCR and mNGS, respectively. Detailed baseline characteristics of the enrolled patients are presented in Table 2.

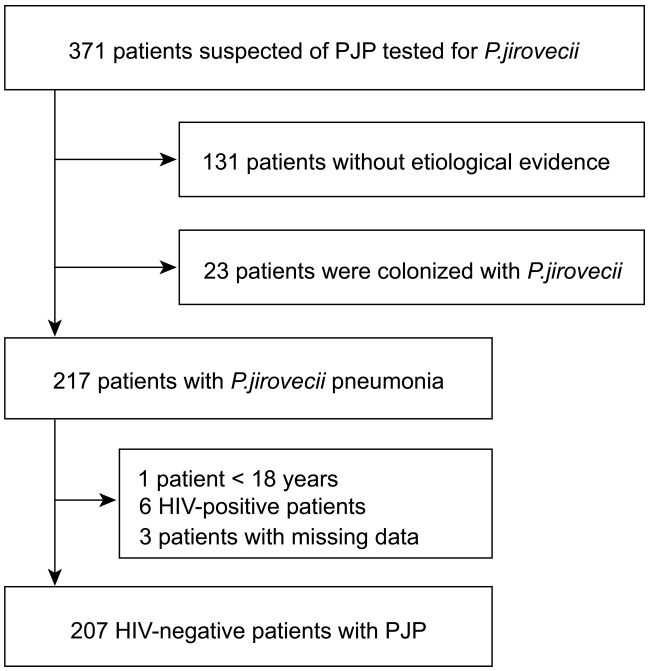

Figure 1 Flowchart of case selection process.
Abbreviations: PJP, *Pneumocystis jirovecii* pneumonia; HIV, human immunodeficiency virus.

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Table 1 Diagnostic Methods for 207 Non-HIV Patients with PJP

Specimen Type	**Number of Cases**
BALF	197 (95.2%)
Endotracheal aspirate	10 (4.8%)
Diagnostic Methods	**Number of Positive Cases**
Direct microscopic examination	11 (5.3%)
qPCR	99 (47.8%)
mNGS	97 (46.9%)

Abbreviations: BALF, bronchoalveolar lavage fluid; qPCR, polymerase chain reaction; mNGS, next-generation sequencing.

Table 2 Clinical Characteristics of 207 Non-HIV Patients with PJP

Characteristic	**All Patients, n=207**	**Non-Survivor, n=31**	**Survivors, n=176**	**p value**
Sex, male	127 (61.4%)	18 (58.1%)	109 (61.9%)	0.638
Age, y	57 (45–68)	67 (52–72)	54 (43–67)	**0.002**
BMI, kg/m^2	22.7 (19.8–25.1)	22.8 (19.7–25.6)	22.5 (19.8–25.0)	0.696
Underlying diseases				
Kidney transplantation	72 (34.8%)	5 (16.1%)	67 (38.1%)	**0.018**
Immune-mediated inflammatory diseases	49 (23.7%)	16 (51.6%)	32 (18.2%)	**<0.001**
Hematologic malignancies	39 (18.8%)	5 (16.1%)	34 (19.3%)	0.675
COVID-19	16 (7.7%)	4 (12.9%)	12 (6.8%)	0.421
Solid tumors	8 (3.9%)	1 (3.2%)	7 (4.0%)	1.000
Other diseases	23 (11.11%)	0 (0.0%)	23 (13.1%)	0.068
Immunosuppressive therapy				
Use of glucocorticoids	138 (66.7%)	26 (83.9%)	112 (63.6%)	**0.028**
Duration of glucocorticoids use, mo	3.0 (2.0–6.0)	2.0 (2.0–8.0)	3.0 (2.0–6.0)	0.222
Glucocorticoids dose, mg/d	20.0 (10.0–30.0)	30.0 (10.0–35.0)	15.0 (10.0–30.0)	0.068
Duration of prednisone ≥20 mg/d or equivalent glucocorticoids use, mo	2.0 (2.0–3.8)	2.0 (2.0–3.0)	2.5 (1.9–4.0)	0.551
Use of other immunosuppressive agents	136 (65.7%)	19 (61.3%)	117 (66.5%)	0.575
Combination of glucocorticoids and other immunosuppressive agents	67 (32.4%)	14 (45.2%)	53 (30.1%)	0.099
Symptoms				
Dyspnea	139 (67.1%)	28 (90.3%)	134 (76.1%)	0.077
Fever	162 (78.3%)	24 (77.4%)	138 (78.4%)	0.902
Cough	108 (52.2%)	19 (61.3%)	89 (50.6%)	0.27

(*Continued*)

Table 2 (Continued).

Characteristic	All Patients, n=207	Non-Survivor, n=31	Survivors, n=176	p value
Laboratory Tests				
White blood cell count, $\times 10^9$/L	7.0 (4.9–9.5)	8.2 (6.0–10.0)	6.8 (4.7–9.3)	0.137
Neutrophil count, $\times 10^9$/L	5.7 (3.6–7.8)	6.7 (4.5–8.3)	5.5 (3.3–7.6)	0.128
Lymphocyte count, $\times 10^9$/L	0.7 (0.4–1.0)	0.4 (0.3–0.8)	0.7 (0.4–1.0)	0.056
CD4 cell count, $\times 10^6$/L	177.8 (97.1–337.3)	120.1 (57.9–181.5)	182.6 (105.4–382.4)	**0.016**
LDH, U/L	346.5 (254.5–492.5)	532.0 (406.5–668.0)	321.5 (240.0–441.5)	**<0.001**
G test	96 (46.4%)	18 (58.1%)	78 (44.3%)	0.157
BALF				
Macrophage percentage	19.7 (10.0–43.0)	11.5 (3.8–35.3)	22.0 (11.0–43.9)	0.052
Lymphocyte percentage	41.5 (17.3–72.8)	9.0 (4.5–30.7)	50.5 (20.8–75.3)	**<0.001**
Neutrophil percentage	17.0 (5.0–37.4)	62.0 (38.5–81.5)	14.0 (4.4–32.0)	**<0.001**
Eosinophil percentage	0.0 (0.0–1.0)	0.0 (0.0–0.4)	0.0 (0.0–1.0)	0.152
PaO_2/FiO_2, mmHg	259.7 (179.8–326.9)	148.8 (110.5–223.9)	283.8 (218.3–338.3)	**<0.001**
Chest CT				
Diffuse ground-glass opacity	177 (85.51%)	26 (83.9%)	151 (85.8%)	0.997
Mediastinal or subcutaneous emphysema	2 (0.97%)	1 (0.6%)	1 (0.6%)	0.278
Pulmonary air cysts	3 (1.45%)	0 (0.0%)	3 (1.7%)	1.000
Reticular shadows	16 (7.73%)	1 (0.6%)	15 (8.5%)	0.513
Nodules	14 (6.76%)	1 (0.6%)	13 (7.4%)	0.644
Consolidation	28 (13.53%)	6 (19.4%)	22 (12.5%)	0.457
CMV coinfection	41 (19.8%)	6 (19.3%)	35 (19.9%)	1.000
Time from symptom onset to hospitalization, d	7 (4.0–14.0)	7 (6.0–14.0)	7 (4.0–14.0)	0.510
Time from symptom onset to diagnosis, d	12.5 (8.0–19.0)	13.5 (9.0–17.8)	12.0 (7.0–19.0)	0.384
Time from symptom onset to treatment, d	10.0 (6.0–16.0)	10.0 (7.0–15.0)	10.0 (6.0–16.0)	0.626

Notes: For continuous data, the median (interquartile range) was used for description, and for categorical data, the number of cases (percentage of the total) was used. Bold values indicate statistical significance ($p<0.05$).

Abbreviations: BALF, bronchoalveolar lavage fluid; BMI, body mass index; CD, cluster of differentiation; COVID-19, coronavirus disease 2019; CT, computed tomography; FiO_2, fraction of inspired oxygen; LDH, lactate dehydrogenase; PaO_2, partial pressure of arterial oxygen; PJP, *Pneumocystis jirovecii* pneumonia; CMV, cytomegalovirus.

Underlying Disease

In this study, renal transplantation was the most common underlying disease (72 patients, 34.8%), followed by immune-mediated inflammatory diseases (IMIDs, 49 patients, 23.7%). Hematological malignancies were noted in 39 patients (18.8%). Notably, 16 patients (7.7%) developed PJP after coronavirus disease (COVID-19); however, none of these patients had other immunosuppressive diseases (Table 2).

Past Immunosuppression

A total of 138 patients (66.7%) had received glucocorticoid therapy before the diagnosis of PJP, with a median dose equivalent to 20.0 mg of prednisone. The median duration of continuous administration of ≥20 mg of prednisone or

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equivalent glucocorticoids was 2 months. High-dose glucocorticoid use was defined as continuous administration of ≥20 mg of prednisone or equivalent glucocorticoids for ≥2 months. Furthermore, 136 patients (65.7%) had received other immunosuppressive agents, and 67 (32.4%) had received combination therapy with glucocorticoids and other immunosuppressive agents (Table 2).

Treatment Status

A total of 201 patients (97.1%) received sulfamethoxazole/trimethoprim. Concurrently, 161 patients (77.8%) received glucocorticoid therapy in addition to anti-infective treatment (Table 3). During the study period, 25 in-hospital fatalities occurred, corresponding to an in-hospital mortality rate of 12.1%. Furthermore, within 90 days post-hospitalization, 31 mortalities were recorded, resulting in a 90-day mortality rate of 15.0% (Table 3).

Prognostic Factors of Included Patients

Comparison of Clinical Characteristics Between Non-Survivors and Survivors

Significant differences were observed between non-survivors and survivors in the median age (67 vs 54 years, p=0.002), prevalence of a history of kidney transplantation (16.1% vs 38.1%, p=0.018), presence of IMIDs as underlying conditions (51.6% vs 18.2%, p<0.001), and glucocorticoid use before PJP diagnosis (83.9% vs 63.6%, p=0.028) (Table 2).

At the time of PJP diagnosis, significant differences in specific laboratory parameters were observed between non-survivors and survivors, specifically the cluster of differentiation 4 cell count (120.1×10^6/L vs 182.6×10^6/L, p=0.016), lactate dehydrogenase (LDH) levels (532.0 U/L vs 321.5 U/L, p<0.001), and the PaO_2/FiO_2 ratio (148.8 mmHg vs 283.8 mmHg, p<0.001). Furthermore, significant differences were observed in the composition of the BALF, particularly in lymphocyte (9.0% vs 50.5%, p<0.001) and neutrophil percentages (62.0% vs 14.0%, p<0.001) (Table 2).

Table 3 Therapeutic Regimens for 207 Non-HIV Patients with PJP

Treatment	All Patients, n=207	Non-Survivors, n=31	Survivor, n=176
Pharmacological therapy			
Sulfamethoxazole/trimethoprim	201 (97.1%)	29 (93.5%)	172 (97.7%)
Caspofungin	75 (36.23%)	19 (61.3%)	56 (31.8%)
Clindamycin	55 (26.57%)	15 (48.4%)	40 (22.7%)
Glucocorticoids	161 (77.8%)	24 (77.4%)	137 (77.8%)
Respiratory support			
High-flow nasal cannula	30 (14.49%)	12 (38.7%)	18 (10.2%)
Non-invasive ventilation	36 (17.39%)	25 (80.6%)	11 (6.3%)
Mechanical ventilation	18 (8.7%)	15 (48.4%)	3 (1.7%)
Intensive care			
ICU admission	49 (23.67%)	21 (67.7%)	26 (14.8%)
ICU length of stay, d	12 (6–19.5)	14 (8.5–20.0)	8.5 (6.0–17.8)
In-hospital mortality	25 (12.1%)		
90-day mortality	31 (15.0%)		

Abbreviations: ICU, intensive care unit; PJP, *Pneumocystis jirovecii* pneumonia.

Prognostic Factors for 90-Day Mortality

In the univariate logistic regression analysis, several factors were found to be significantly associated with 90-day mortality, including advanced age, presence of IMIDs, prior glucocorticoid use, exposure to high-dose glucocorticoids, elevated LDH ≥400 U/L, PaO_2/FiO_2 <300 mmHg, and the relative percentages of lymphocytes and neutrophils in BALF. In multivariate logistic regression analysis, the percentage of neutrophils in BALF (OR: 1.03; 95% confidence interval [CI]: 1.01–1.05; p=0.003) and PaO_2/FiO_2 <300 mmHg (OR: 10.82; 95% CI: 1.23–95.3; p=0.032) emerged as independent predictors of 90-day mortality post-hospital admission (Table 4).

ROC curve analysis revealed an optimal cutoff threshold of 41.5% for neutrophil percentage in BALF to distinguish non-survivors from survivors (Figure 2). This threshold yielded a sensitivity of 77.8% and a specificity of 84.9%. Furthermore, Kaplan–Meier survival analysis with Log rank testing confirmed significant differences in the survival outcomes between groups stratified by this cutoff (p<0.0001), underscoring its robust discriminative power (Figure 3).

Discussion

Based on a cohort of 207 patients, we provide clinically significant findings on non-HIV PJP. The 90-day all-cause mortality was 15%. COVID-19 accounted for 7.7% of underlying conditions, suggesting a potential role in increasing PJP risk through immune impairment. A BALF neutrophil proportion ≥41.5% and PaO_2/FiO_2 ratio <300 mmHg were identified as independent predictors of 90-day mortality. These findings meet the study aim and offer valuable prognostic indicators for non-HIV patients with PJP. Compared with previous studies that largely excluded BALF data, we used extensive clinical experience and the published literature to identify all prognostic indicators—BALF findings included. The cohort of 207 patients significantly strengthens the clinical validity and applicability of our results.

In this study, confirmation of PJP relied primarily on molecular testing (qPCR or mNGS) of high-quality lower respiratory tract specimens, specifically BALF (95.2%) or endotracheal aspirates (4.8%). This approach aligns with current recommendations, as these invasive specimens provide higher sensitivity than expectorated sputum for detecting *P. jirovecii*.[18,19] A pertinent consideration is whether our cohort represents all clinically suspected cases or is limited to patients able to tolerate invasive sampling. Reassuringly, screening data (Supplementary Table 1) confirm that all 131 patients evaluated but excluded from the PJP cohort also underwent diagnostic testing via BALF or endotracheal aspirates, thereby minimizing selection bias related to specimen type.

Table 4 Logistic Regression Analysis Results for 207 Non-HIV Patients with PJP

Parameter	Univariate Analysis				Multivariate Analysis			
	OR	95% CI		p value	OR	95% CI		p value
Sex, male	1.04	1.01	1.07	**0.004**				
Immune-mediated inflammatory diseases	4.59	2.06	10.21	**<0.001**				
Use of glucocorticoids	3.00	1.10	8.19	**0.032**				
High-dose glucocorticoid use	4.00	1.77	8.94	**0.001**				
CD4 cell count <200×10^6/L	4.58	0.94	22.48	0.061				
LDH ≥400 U/L	8.19	3.11	21.54	**<0.001**				
PaO_2/FiO_2 <300 mmHg	24.23	3.21	182.65	**0.002**	10.82	1.23	95.37	**0.032**
BALF								
Lymphocyte percentage	0.96	0.94	0.99	**0.001**				
Neutrophil percentage	1.04	1.02	1.06	**<0.001**	1.03	1.01	1.05	**0.003**

Notes: Bold values indicate statistical significance (p<0.05).

Abbreviations: BALF, bronchoalveolar lavage fluid; CI, confidence interval; LDH, lactate dehydrogenase; OR, odds ratio; PaO_2, partial pressure of arterial oxygen; FiO_2, fraction of inspired oxygen; PJP, *Pneumocystis jirovecii* pneumonia.

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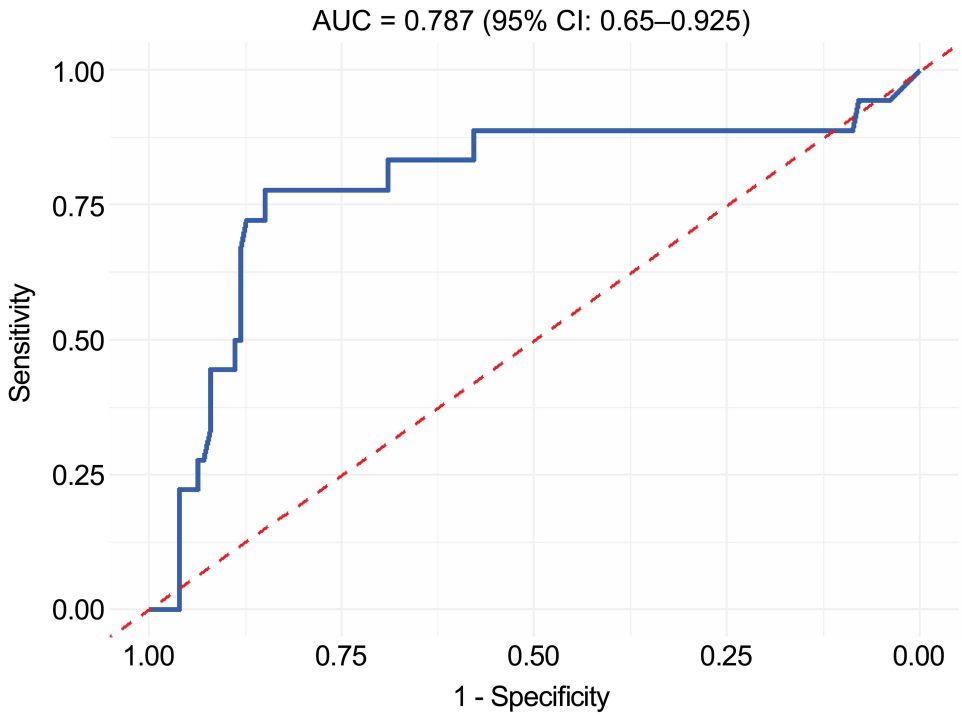

Figure 2 ROC curve of neutrophil percentage in BALF. Cutoff value: 41.5%.
Abbreviations: BALF, bronchoalveolar lavage fluid; ROC, receiver operating characteristic.

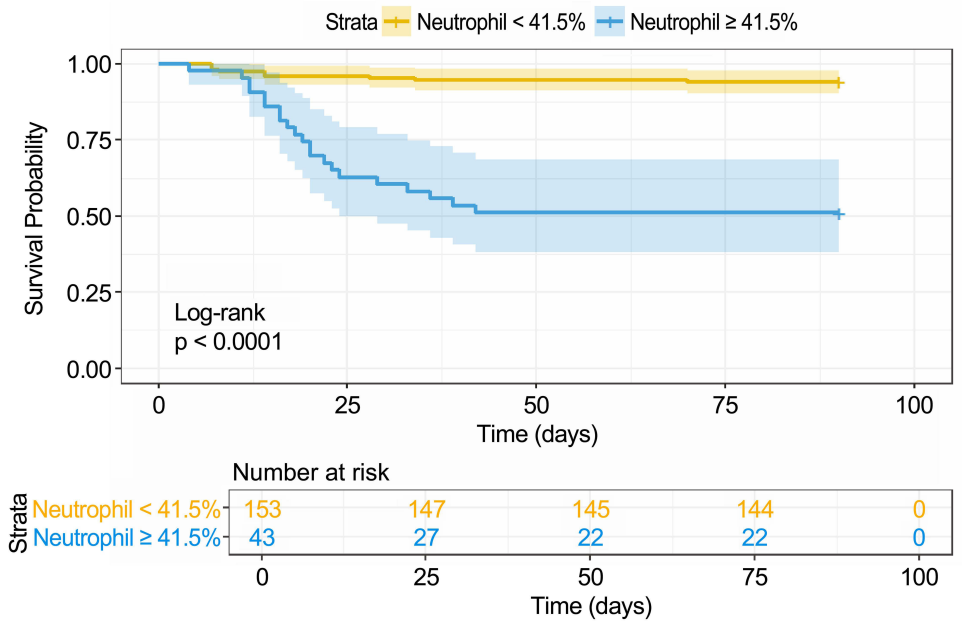

Figure 3 Kaplan-Meier survival analysis according to neutrophil percentage.
Abbreviation: BALF, bronchoalveolar lavage fluid.

In this study, we demonstrated that the 90-day all-cause mortality rate among non-HIV patients with PJP following hospital admission was 15%, which is significantly lower than the 25.6% all-cause mortality rate reported in a recent study.[7] This discrepancy could be attributed to the underlying diseases. Notably, renal transplantation is frequently conducted at our institution, and in this study, patients with a history of renal transplantation comprised the largest

proportion (34.8%). Previous research has indicated that patients who underwent renal transplantation and subsequently developed PJP at our hospital generally presented with milder symptoms and had a more favorable prognosis.[20] In the aforementioned study, the mortality rate of PJP in organ transplant recipients was also the lowest compared with those with hematologic malignancies, solid tumors, and IMID.[7]

We also found that, among the underlying diseases in non-HIV patients with PJP, COVID-19 accounted for 7.7%. Several studies have reported that COVID-19 can impair host immune function, thereby increasing the risk of secondary bacterial and fungal infections, including *P. jirovecii*.[21–24] COVID-19 may serve as a risk factor for PJP, and additional clinical investigations are warranted to elucidate the association between COVID-19 and PJP prognosis. It is important to note that, in cases of COVID-19 and PJP coinfection, distinguishing the primary contributor to respiratory failure or death is clinically challenging, as both pathogens can cause severe pneumonia. In our cohort, PJP diagnosis and treatment decisions were based on comprehensive microbiological and clinical assessment. However, the potential contribution of severe COVID-19 to mortality in this subgroup cannot be excluded.

We demonstrate that the proportion of neutrophils in BALF serves as a stronger predictor of mortality, compared with other factors. In multivariate analysis, a higher proportion of neutrophils in BALF was found to be independently associated with 90-day mortality. Furthermore, a BALF neutrophil proportion of ≥41.5% exhibited a relatively high sensitivity and specificity for predicting death. These findings suggest that the neutrophil percentage in BALF may serve as a valuable prognostic indicator for non-HIV patients with PJP.

Recently, several studies have examined the association between the BALF cell ratios and prognosis of non-HIV patients with PJP. Some investigators have suggested that a low lymphocyte count in BALF may predict mortality in non-HIV patients with PJP.[25] Kim et al reported that a BALF lymphocyte count ≤45% was associated with reduced effectiveness of trimethoprim/sulfamethoxazole as primary treatment.[26] In our study, univariate analysis showed that both lower lymphocyte and higher neutrophil percentages in BALF were significantly associated with mortality ($p=0.001$ and $p<0.001$, respectively), consistent with prior reports linking lymphopenia to severe PJP. However, in multivariate analysis, only neutrophil percentage remained an independent predictor (OR: 1.03; 95% CI: 1.01–1.05; $p=0.003$), whereas lymphocyte percentage did not remain significant. Nevertheless, our findings indicate that neutrophil proportion in BALF is a more significant prognostic indicator, compared with lymphocyte proportion. Moreover, previous research has shown that BALF neutrophilia correlates with increased mortality in non-HIV patients with PJP.[27] A study conducted by Lee et al demonstrated that the proportion of neutrophils in BALF is an independent predictor of both 30-day and 60-day mortality, with mortality rates increasing by 15% and 21%, respectively, for every 10% increase in BALF neutrophil levels.[28] These findings are corroborated by the results of our study, suggesting that neutrophils play a crucial role in the inflammatory response to severe PJP. French and Japanese research teams studied 39 and 29 patients, respectively, and found that an increase in BALF neutrophils (>15% and ≥31%) may be an important prognostic factor associated with fatal outcomes.[27,29] However, these studies included a relatively small number of patients, which may limit their ability to fully represent the broader population of patients with PJP. Our investigation included 207 patients and identified a specific threshold for the BALF neutrophil percentage (≥41.5%) as an effective discriminator for patients at increased risk of mortality. This finding may provide clinicians with a valuable biomarker for the early identification of patients who could benefit from aggressive therapeutic interventions.

Our findings reinforce the critical role of early BALF acquisition in high-risk non-HIV patients suspected of having PJP, not only for diagnosis but also for prognostication. The strong predictive value of neutrophil percentage supports its routine assessment in the BALF differential cell count. Current adjunctive therapy (eg: corticosteroids) is often guided by oxygenation status; however, our data suggest that a high BALF neutrophil percentage may identify a subset of patients with significant inflammatory burden who could potentially benefit from more tailored immunomodulatory strategies. Future interventional studies are needed to determine whether neutrophil-driven phenotypes respond differently to adjuvant therapies.

Currently, in-depth research into mechanisms by which neutrophil aggregation in the alveoli leads to high mortality rates in PJP remains limited; however, existing theories offer promising avenues for further exploration. Severe PJP is frequently characterized by neutrophilic lung inflammation, which may subsequently lead to diffuse alveolar damage, impaired gas exchange, and ultimately respiratory failure.[30] Furthermore, severe PJP is characterized by acute lung injury

(ALI). A growing body of evidence indicates that neutrophils are critical in the development of various forms of ALI, with the release of neutrophil extracellular traps (NETs) being a common pathogenic mechanism. NETs are a complex mixture of nuclear chromatin, mitochondrial deoxyribonucleic acid, and neutrophil granule proteins. NETs are essential for pathogen control; however, they also contain proinflammatory and cytotoxic molecules that can exacerbate lung tissue injury.[31,32] This association may explain why, in our study, patients with a neutrophil percentage ≥41.5% exhibited a higher 90-day mortality risk. No studies currently address the relationship between NETs and the pathological mechanisms of severe PJP; however, this may represent a mechanism warranting further investigation.

This study also revealed that a PaO_2/FiO_2 ratio <300 mmHg was independently associated with 90-day mortality, consistent with recent research highlighting a strong association between reduced PaO_2/FiO_2 and increased mortality in patients with PJP. A reduced PaO2/FiO2 ratio indicates greater disease severity, including poor general health and a potential need for intensive care. Previous studies have shown that initial hypoxemia is a significant risk factor for mortality, with an elevated alveolar-arterial oxygen gradient associated with poor prognosis.[33,34] Wang et al further demonstrated that a low PaO_2/FiO_2 ratio on admission predicts in-hospital mortality in non-HIV patients with PJP.[10] These findings align with our results and underscore the prognostic value of the PaO2/FiO2 ratio in patients with PJP. In non-HIV patients with PJP, elevated BALF neutrophil levels may be associated with reduced PaO_2/FiO_2 ratio, suggesting that neutrophil-mediated inflammatory responses may contribute to hypoxemia. This hypothesis warrants further validation in clinical and experimental studies.

This was a single-center retrospective study; however, it may not have encapsulated the experiences of patients from other medical facilities or geographical areas, potentially limiting the generalizability of its findings. Nonetheless, the comprehensive inclusion of clinical indicators known to influence the prognosis of non-HIV patients with PJP—and the finding that the neutrophil percentage in BALF and the PaO_2/FiO_2 ratio are independently associated with 90-day mortality after the exclusion of other potential confounding variables—bestows significant clinical relevance to the study's findings. Future research could corroborate these findings through multicenter prospective study designs and larger-scale trials, further exploring the underlying mechanisms and the development of intervention strategies.

Conclusion

In this study, we identified the neutrophil percentage in BALF and the PaO_2/FiO_2 ratio as robust, independent predictors for mortality risk stratification in non-HIV patients with PJP. The derived BALF neutrophil threshold (≥41.5%) provides a clinically actionable biomarker. Furthermore, the notable prevalence of COVID-19 (7.7%) among underlying conditions highlights the evolving epidemiology of non-HIV PJP in the era of emerging immunosuppressive threats. Future studies should validate whether BALF neutrophil percentage independently predicts mortality in non-HIV PJP and establish a precise, widely applicable cutoff across diverse populations.

Abbreviations

ALI, acute lung injury; BALF, bronchoalveolar lavage fluid; COVID-19, coronavirus disease; CT, computed tomography; HIV, human immunodeficiency virus; ICU, intensive care unit; IMIDs, inflammatory diseases; IQR, interquartile range; LDH, lactate dehydrogenase; mNGS, next-generation sequencing; NETs, neutrophil extracellular traps; OR, odds ratio; PJP, *Pneumocystis jirovecii* pneumonia; qPCR, polymerase chain reaction; ROC, receiver operating characteristic.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

Ethics Approval and Informed Consent

The study was approved by the Ethics Committee of Peking University Third Hospital (approval number: IRB00006761-M2023339), and the requirement for informed consent was waived due to the retrospective nature of the study. The study was performed in accordance with the Declaration of Helsinki.

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Author Contributions

All authors made significant contributions to the work reported, including the conception, study design, execution, data acquisition, analysis, and interpretation. All authors participated in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; agreed on the journal to which the article was submitted; and accept accountability for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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