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Predisposing and prognostic factors of Pneumocystis jirovecii pneumonia diagnosed by metagenomic next-generation sequencing in patients with malignancies: a single-center retrospective study

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Research Article

Keywords: Pneumocystis jirovecii pneumonia, metagenomic next-generation sequencing, bronchoalveolar lavage fluid, cancer, intensive care unit

Posted Date: November 24th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11286931/v1

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Additional Declarations: No competing interests reported.

Abstract

Background: *Pneumocystis jirovecii* pneumonia (PJP) is rising in patients with malignancies and is associated with higher morbidity and mortality. This study aimed to assess the diagnostic performance of metagenomic next-generation sequencing (mNGS) for PJP and to identify risk factors for PJP occurrence and mortality in cancer patients admitted to the intensive care unit (ICU).

Methods: We retrospectively reviewed the medical records of 136 cancer patients with severe pneumonia admitted to the ICU of Jiangsu Cancer Hospital from September 2020 to September 2025. *Pneumocystis jirovecii* (*P. jirovecii*) and co-pathogens in bronchoalveolar lavage fluid (BALF) were detected via mNGS. Logistic regression analyses were performed to identify risk factors for PJP occurrence and 30-day mortality.

Results: Of the 136 patients enrolled in this study, 25 were positive for PJP and 111 were negative for PJP based on BALF-mNGS results. Mixed infections were more prevalent in PJP than non-PJP patients (*P*=0.024), particularly cytomegalovirus co-infection (*P*=0.001). PJP occurrence was independently associated with radiotherapy (OR=3.867, 95% CI=1.274–11.737, *P*=0.017), immune checkpoint inhibitor (ICI) therapy (OR=6.643, 95% CI=1.662–26.548, *P*=0.007), and malignant solid tumor (OR=0.107, 95% CI=0.019–0.611, *P*=0.012). Overall 30-day mortality was significantly higher in the PJP group than that in the non-PJP group (60.00% vs. 32.43%, *P*=0.013). In PJP patients, univariate analysis identified malignant solid tumor (OR=14.000, 95% CI=1.299–150.889, *P*=0.030), ICI therapy (OR=11.000, 95% CI=1.603–75.502, *P*=0.015), invasive mechanical ventilation (OR=6.000, 95% CI=1.003–35.908, *P*=0.050), and lower oxygenation index (OR=0.981, 95% CI=0.962–1.000, *P*=0.045) as mortality predictors, though none remained significant in multivariate analysis.

Conclusions: Clinicians should maintain high suspicion for PJP in cancer patients with severe pneumonia, particularly those receiving radiotherapy and ICI therapy, as mNGS enables rapid and comprehensive detection of *P. jirovecii* and co-infecting pathogens.

Introduction

Pneumocystis jirovecii pneumonia (PJP) is a life-threatening opportunistic fungal infection caused by *Pneumocystis jirovecii* (*P. jirovecii*), primarily affecting immunocompromised populations. Historically, PJP was recognized as an acquired immunodeficiency syndrome-defining disease in patients infected with human immunodeficiency virus (HIV). However, the widespread implementation of highly active antiretroviral therapy and chemoprophylaxis has led to a marked decline in PJP incidence among HIV-infected patients in recent years[1]. In contrast, the incidence of PJP among non-HIV-infected populations, including oncology patients, has progressively increased alongside the expanding use of intensive immunosuppressive therapies[2]. Notably, the clinical characteristics of PJP are distinctly different between patients with and without HIV infection. In non-HIV-infected patients, PJP tends to rapidly progress to severe respiratory failure within a short period, with a mortality rate of 30–60%, greatly exceeding the 10–20% observed in HIV-infected patients[3, 4]. The increasing incidence and high

mortality of PJP in non-HIV-infected patients underscore the importance of heightened clinical awareness. Nevertheless, the risk factors for the development and prognosis of PJP in this population have not been fully elucidated, partly due to the diversity and complexity of underlying diseases.

Beyond conventional radiotherapy and chemotherapy, novel molecular targeted agents and immune checkpoint inhibitors (ICIs) have transformed the therapeutic paradigm for cancer, substantially improving survival while also increasing the proportion of immunocompromised patients, consequently elevating the risk of PJP. In a meta-analysis encompassing 867 non-HIV-infected PJP patients, malignancy was the most prevalent underlying disease, accounting for 35% of the study cohort[5]. A recent retrospective study of 420 PJP patients showed that individuals with hematological malignancies tended to receive established high-risk medications, whereas patients with solid cancers were more likely to be prescribed suspected high-risk medications and exhibited a relatively poorer prognosis[6]. These findings underscore that emerging oncology-related therapeutic paradigms modulate host immunity through mechanisms distinct from conventional agents, thereby generating non-traditional risk profiles. Nevertheless, the impact of underlying malignancy and anticancer treatment on the incidence and prognosis of PJP remains incompletely understood.

Severe PJP patients often require intensive care unit (ICU) management, and the superimposition of compromised host defenses and critical illness results in a substantially elevated mortality rate[7, 8]. Thus, a rapid and accurate etiological diagnosis is essential to guide precise anti-infective treatment and improve prognosis. Since *P. jirovecii* is extremely difficult to culture in vitro and frequently occurs in mixed infections with other pathogens, establishing a microbiological diagnosis in PJP patients remains a major challenge[9]. Metagenomic next-generation sequencing (mNGS) is a high-throughput nucleic acid sequencing technique that can theoretically detect virtually all pathogens in a single assay. This state-of-the-art technology features high sensitivity, high accuracy, and short turnaround time, and is regarded as a promising approach for microbial identification[10]. Bronchoalveolar lavage fluid (BALF) is the current gold standard specimen for the diagnosis of PJP and is recognized as the highest quality respiratory sample[11]. Multiple studies have demonstrated that BALF-mNGS possesses high diagnostic capability for PJP and exhibits excellent performance in the detection of co-infecting pathogens[12–14]. However, the application of BALF-mNGS for diagnosing PJP in patients with malignancies has rarely been explored.

In the present study, we systematically analyzed the clinical characteristics of cancer patients with severe pneumonia admitted to the ICU, based on a diagnosis confirmed by BALF-mNGS, aiming to evaluate the utility of BALF-mNGS in detecting *P. jirovecii* and to identify risk factors associated with PJP incidence and mortality in this specific population.

Methods

Study participants

This single-center retrospective study included cancer patients with severe pneumonia admitted to the ICU of Jiangsu Cancer Hospital from September 2020 to September 2025. All enrolled patients underwent fiberoptic bronchoscopy, and BALF samples were collected for mNGS analysis. Patients with the following conditions were excluded: (1) Age < 18 years; (2) HIV infection; (3) death or discharge within 24 h of ICU admission; (4) incomplete clinical data.

PJP definition

The diagnosis of PJP requires the simultaneous fulfillment of clinical, radiographic, and microbiological criteria[15]. In this study, patients who met the following criteria were diagnosed with PJP: (1) clinical symptoms relevant to PJP (cough, fever, or dyspnea) ; (2) radiological findings compatible with PJP (pulmonary infiltrates on chest radiograph or diffuse ground-glass opacity on computed tomography) ; (3) a positive result for *P. jirovecii* by mNGS in BALF specimens.

Microbiological investigations

After the patients had provided written informed consent to undergo fiberoptic bronchoscopy and mNGS testing, BALF specimens were collected by experienced clinicians in accordance with previous consensus and guideline[16]. Briefly, all patients underwent bedside bronchoscopy under either combined intravenous anesthesia or topical anesthesia with 2% lidocaine. Based on chest imaging, the most severe lesion sites were selected for lavage. If diffuse lesions were present, the right middle lobe or the left lingual lobe was selected as the lavage site. Subsequently, equal volumes of sterile normal saline were fractionally instilled into the target bronchial segments and BALF was aspirated under negative pressure after a brief interval.

The collected BALF specimens were aliquoted into sterile tubes and submitted to Dinfectome Inc. (Nanjing, China) for mNGS analysis. The mNGS workflow for BALF specimens included nucleic acid extraction, library construction, high-throughput sequencing, and bioinformatic analysis, as detailed in our previous study.[17].

Clinical data collection

Clinical data of each patient were acquired from electronic medical records and analysed anonymously. We collected clinical data regarding demographic characteristics, underlying malignancies, oncologic treatment modalities, comorbidities, prior corticosteroid use, respiratory support mode, laboratory parameters, Acute Physiology and Chronic Health Evaluation Ⅱ (APACHE Ⅱ) score, Sequential Organ Failure Assessment (SOFA) score, PJP treatment, and clinical outcomes.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 27.0 (IBM Corp., Armonk, NY, USA). Normally distributed continuous variables were presented as mean ± standard deviation (SD) and compared using the Student's t-test, while non-normally distributed variables were presented as median (interquartile range, IQR) and compared using the Mann-Whitney U test. Categorical variables were presented as number (percentage) and compared using the chi-square test or Fisher's exact test. The Kaplan-Meier survival curves were plotted to estimate 30-day survival rate, and the log-rank test was used for an unadjusted comparison of survival curves between patients with and without PJP. The stepwise binary logistic regression analysis was performed to identify the risk factors for the occurrence and 30-day mortality of PJP in cancer patients. Variables with $P < 0.05$ in univariable analysis were entered into the multivariable logistic regression model. All results were expressed as odds ratios (ORs) and 95% confidence intervals (95% CIs). A two-tailed $P < 0.05$ was considered statistically significant.

Results

Detection results of bronchoalveolar lavage fluid-metagenomic next-generation sequencing

BALF-mNGS was performed in the 136 enrolled cancer patients with severe pneumonia. *P. jirovecii* was detected in 25 (18.38%) patients and not detected in 111 (81.62%) patients. Among the 25 patients with PJP, BALF-mNGS identified 155 pathogens, including 62 (40.00%) bacteria, 43 (27.74%) fungi, and 50 (32.26%) viruses (Table S1 and Figure S1). Apart from *P. jirovecii*, the most frequently detected fungus was *Candida albicans* (9 cases), followed by *Aspergillus* species (7 cases). The main bacteria detected were *Staphylococcus aureus* (7 cases), *Streptococcus pneumoniae* (7 cases), *Corynebacterium striatum* (7 cases), *Pseudomonas aeruginosa* (5 cases), and *Acinetobacter baumannii* (5 cases). The viruses detected were primarily human betaherpesvirus 5 (16 cases) and human gammaherpesvirus 4 (14 cases) (Fig. 1).

According to the BALF-mNGS results, all patients with PJP manifested mixed infections with other pathogens. The most frequent patterns were *P. jirovecii* co-infection with bacteria and viruses (9 cases) and *P. jirovecii* co-infection with bacteria, viruses, and other fungi (9 cases) (Fig. 2). Notably, mixed infections with multiple pathogens were also common among patients without PJP, accounting for 92 of 111 cases (82.88%), predominantly bacterial–viral co-infection (34 cases) and bacterial–fungal–viral co-infection (30 cases) (Figure S2). These findings suggested that cancer patients with severe pneumonia were more susceptible to mixed infections, and BALF-mNGS exhibited strong diagnostic performance for *P. jirovecii* and mixed infections.

Comparison of clinical characteristics between patients with and without PJP

A total of 136 cancer patients with severe pneumonia admitted to the ICU were included in the study and none had received PJP prophylaxis. According to PJP diagnostic criteria, 25 patients (18.38%) were classified as PJP and 111 (81.62%) as non-PJP. The detailed clinical characteristics are presented in Table 1.

Among all patients, 119 (87.50%) were male and 17 (12.50%) were female, with a mean age of 68.66 ± 9.05 years and a mean body mass index (BMI) of 21.74 ± 3.11 kg/m². These baseline demographic characteristics were similar between patients with and without PJP. The underlying malignancies comprised malignant solid tumors in 123 (90.44%) patients and malignant lymphomas in 13 (9.56%) patients. The proportion of patients with malignant solid tumors was significantly lower in the PJP group than in the non-PJP group (P = 0.015). Lung cancer was more frequent in the PJP group (12 cases, 48.00%), while esophageal cancer was more common in the non-PJP group (45 cases, 40.54%) (Figure S3). Regarding other comorbidities, myelosuppression was more common in the PJP group compared with the non-PJP group (P = 0.049), whereas diabetes mellitus and hypertension showed no significant difference between the two groups. All patients had received prior oncologic treatment including chemotherapy, radiotherapy, surgery, ICI therapy, molecular targeted therapy, or combination therapy (Figure S4). Radiotherapy (P = 0.006), ICI therapy (P = 0.034), and molecular targeted therapy (P = 0.024) were more common in the PJP group, whereas surgery alone (P = 0.031) was more common in the non-PJP group. Chemotherapy did not differ significantly between the two groups. None of the patients had received prolonged high-dose corticosteroid therapy prior to PJP diagnosis, defined as a daily dose of ≥ 20 mg prednisone equivalent for ≥ 3 weeks.

Mixed infections were very prevalent in cancer patients with pneumonia, particularly in PJP patients compared with non-PJP patients (P = 0.024). The most frequently detected co-infecting pathogen was cytomegalovirus (CMV), with a significantly higher detection rate than that in the non-PJP group (P = 0.001). For laboratory parameters at the time of mNGS testing, the PJP group had lower neutrophil count, lymphocyte count, platelet count, and hemoglobin level than the non-PJP group, which might be explained by the higher proportion of patients with myelosuppression in the PJP group. However, only neutrophil count (P = 0.027) and hemoglobin level (P = 0.010) showed a statistically significant difference between the two groups. Moreover, lactate dehydrogenase (LDH) level was significantly elevated in the PJP group compared with the non-PJP group (P = 0.028). In contrast, there was no significant difference in albumin, C-reactive protein (CRP), and procalcitonin (PCT) levels between the two groups.

At ICU admission, the enrolled patients were generally critically ill, with a median oxygenation index of 127.30 mmHg, a median APACHE Ⅱ score of 19, and a median SOFA score of 6.5. Furthermore, 39 (28.68%) patients had septic shock and 105 (77.21%) patients required invasive mechanical ventilation. However, no significant differences were observed in these variables between the PJP group and the non-PJP group. Even with aggressive treatment, the overall 30-day mortality remained as high as 37.50%. Notably, the PJP group had a 30-day mortality rate of 60.00%, significantly higher than the 32.43% in the non-PJP group (P = 0.013). Similarly, the Kaplan-Meier survival curve indicated a higher 30-day survival

probability in non-PJP patients than in PJP patients following definitive diagnosis by mNGS (log-rank *P* = 0.010) (Fig. 3).

Risk factors for PJP occurrence in cancer patients

To identify patients at high risk of PJP among the malignant tumor population, we performed stepwise binary logistic regression analysis to explore potential risk factors for PJP, focusing on underlying malignancies and oncologic treatment modalities. The results of univariate and multivariate analyses are displayed in Table 2.

In the univariate analyses, PJP occurrence was significantly associated with radiotherapy (OR = 3.727, 95% CI = 1.512–9.186, *P* = 0.004), ICI therapy (OR = 2.674, 95% CI = 1.103–6.484, *P* = 0.029), molecular targeted therapy (OR = 3.018, 95% CI = 1.228–7.417, *P* = 0.016), and myelosuppression (OR = 3.208, 95% CI = 1.113–9.250, *P* = 0.031). Conversely, PJP occurrence was inversely associated with malignant solid tumor (OR = 0.213, 95% CI = 0.065–0.704, *P* = 0.011) and surgery (OR = 0.262, 95% CI = 0.074–0.931, *P* = 0.038). Multivariate analysis revealed that radiotherapy (OR = 3.867, 95% CI = 1.274–11.737, *P* = 0.017) and ICI therapy (OR = 6.643, 95% CI = 1.662–26.548, *P* = 0.007) were independent predictors of PJP occurrence, whereas the inverse association with PJP occurrence remained significant for malignant solid tumor (OR = 0.107, 95% CI = 0.019–0.611, *P* = 0.012).

Comparison of clinical characteristics between PJP survivors and non-survivors

A total of 25 PJP patients were enrolled during the study period and all received trimethoprim-sulfamethoxazole (TMP-SMX) for PJP treatment, with an overall 30-day mortality rate of 60.00%. The comparison between survivors (10 cases) and non-survivors (15 cases) is summarized in Table 3.

There were no statistically significant differences in baseline demographic characteristics such as gender, age, and BMI between survivors and non-survivors. However, with respect to underlying malignancies, malignant lymphomas were more prevalent in non-survivors, while malignant solid tumors were more common in survivors (*P* = 0.023). Regarding prior oncologic treatment modalities, non-survivors were more likely to receive ICI therapy (*P* = 0.015), whereas chemotherapy, radiotherapy, molecular targeted therapy, and surgery did not differ significantly between the two groups. Laboratory parameters were similar between survivors and non-survivors, except for the PCT level, which was higher in non-survivors (*P* = 0.048). Non-survivors tended to have more severe disease, as reflected by higher APACHE Ⅱ and SOFA score, lower oxygenation index, higher lactate level, and higher incidences of concurrent myelosuppression and septic shock, as well as a greater likelihood of requiring invasive mechanical ventilation. However, none of these variables reached statistical significance. Adjunctive corticosteroids were administered to 9 (36.00%) patients, comprising 3 (30.00%) survivors and 6 (40.00%) non-survivors, with no significant difference between the two groups.

Risk factors associated with 30-day mortality in cancer patients with PJP

To identify prognostic factors in cancer patients with PJP, clinical data were compared between survivors and non-survivors. The results of the logistic regression analysis are shown in Table 4.

Malignant solid tumor (OR = 14.000, 95% CI = 1.299–150.889, *P* = 0.030), ICI therapy (OR = 11.000, 95% CI = 1.603–75.502, *P* = 0.015), invasive mechanical ventilation (OR = 6.000, 95% CI = 1.003–35.908, *P* = 0.050), and lower oxygenation index (OR = 0.981, 95% CI = 0.962–1.000, *P* = 0.045) were associated with poor outcome in the univariate analysis. However, none of these factors was statistically associated with 30-day mortality in the multivariate analysis.

Discussion

Although advances in treatment paradigms have substantially prolonged survival in patients with malignancies over recent decades, immunosuppression related to underlying malignancy and anticancer therapy has led to a rising incidence of opportunistic infections. Among the various infections encountered in patients with malignancies, PJP is a life-threatening opportunistic infection of particular concern. Evidence indicates that delayed initiation of anti-PJP therapy is associated with poor prognosis[18], emphasizing the importance of rapid and accurate pathogen identification. Owing to the inability to culture *P. jirovecii*, the diagnosis of PJP traditionally relied on microscopic visualization of *P. jirovecii* cysts in respiratory specimens following Gomori methenamine silver (GMS) staining. However, the low sensitivity of microscopic staining examination and the relatively low microbial burden in non-HIV-infected patients may yield false-negative results, rendering the method inadequate for ruling out the diagnosis of PJP[19]. As a component of the cyst wall of *P. jirovecii*, 1,3-β-D-glucan (BDG) is widely used in the auxiliary diagnosis of PJP. Nevertheless, due to its susceptibility to interference from other fungal infections or certain intravenous medications (e.g., immunoglobulins and albumin preparations), BDG is not specific for PJP and is prone to false-positive results, requiring further testing to confirm the diagnosis[20, 21]. More recently, polymerase chain reaction (PCR) has emerged as the preferred method for PJP detection. Despite its superior sensitivity and specificity, PCR has limited utility in diagnosing mixed infections and has difficulty in distinguishing colonization from active infection[22]. Notably, with the advent of mNGS, pathogen detection has become more diverse, providing new diagnostic insights for PJP as well.

In our study, BALF-mNGS exhibited favorable diagnostic capability for PJP and offered distinct advantages in identifying mixed infections. Consistent with previous studies, mixed infections were prevalent among PJP patients[23]. All 25 PJP patients in our cohort manifested mixed infections, a significantly higher rate than that in non-PJP patients, highlighting the need for heightened attention to mixed infections in cancer patients with PJP. Previous studies have shown that viral co-infections, especially CMV, are the most common in mixed infections[24] and are associated with greater PJP severity[25, 26]. Interestingly, our data likewise showed that mNGS detected CMV in more than half of

the PJP patients, but the CMV detection rate did not differ significantly between survivors and non-survivors. This finding may reflect the fact that cancer patients with PJP have profound immunosuppression, leading to an increased risk of CMV replication, whereas CMV itself does not influence the prognosis of PJP. Thus, clinicians should perform CMV diagnostic testing in PJP patients and remain vigilant for CMV reactivation. In addition, our data revealed that mNGS detected *Aspergillus* species in 6 PJP patients. Although *Aspergillus* has not been widely recognized as a co-pathogen of *P. jirovecii*, the positivity rate for *Aspergillus* is slightly higher in non-HIV PJP patients[27]. Evidently, the pathogenic spectrum of PJP patients is highly complex and mNGS is well suited for the etiological diagnosis of PJP due to its significant advantages in detecting rare and mixed pathogens.

PJP patients without HIV generally present with severe symptoms necessitating intensive care, and the prognosis remains unfavorable even with aggressive treatment[28]. In line with previous studies, the overall 30-day mortality rate of PJP patients in this study was 60%, significantly higher than that in non-PJP patients, highlighting the need for PJP prophylaxis. Nevertheless, there is a lack of clinical efficacy data to support routine implementation of PJP prophylaxis in non-HIV patients, particularly those with malignancies. Concerningly, previous literature indicated that a substantial proportion of immunocompromised patients did not receive PJP prophylaxis[29]. Indeed, no patient in our study received appropriate PJP prophylaxis prior to disease onset. Hence, this study evaluated the potential risk factors for PJP occurrence in patients with malignancies to identify individuals who may benefit from PJP prophylaxis.

In our study, malignant solid tumors were inversely associated with PJP occurrence, consistent with previous research on PJP in cancer patients[30]. According to epidemiological data, the incidence of PJP in solid cancers was < 25 cases per 100,000 patient-years, compared with > 45 cases per 100,000 patient-years in hematologic malignancies[31]. A possible explanation for the higher incidence of PJP in hematologic malignancies is the severe immune dysfunction secondary to intensive chemotherapy. However, the majority of our study cohort consisted of patients with solid malignant tumors, which may have introduced selection bias. Furthermore, a recent study reported a marked epidemiological shift in non-HIV PJP, with solid cancers now exceeding hematologic malignancies as the most common predisposing factor[6]. This shift potentially reflects the changes in oncology therapeutic paradigms, including the widespread adoption of molecular targeted agents and ICIs, which may alter host immunity via mechanisms distinct from those of traditional agents.

Based on our data, ICI therapy and radiotherapy were independent predictors of PJP development in patients with malignant tumors. As an emerging therapeutic strategy, ICIs have dramatically transformed the treatment landscape for many types of malignant tumors. Although a few case reports have described PJP as a possible complication of ICI therapy, the association between ICIs and the risk of PJP remains controversial. In a recent large cohort study, exposure to ICIs did not increase risk of PJP in lung cancer patients[32]. In contrast, another study based on the FDA Adverse Event Reporting System (FAERS) database revealed that ICIs may be associated with a safety signal for PJP, particularly in males aged > 65 years[33], which is similar to our findings. The contradiction between the occurrence and poor

outcome of PJP (an immunodeficiency-related disease) and the administration of ICIs (agents that enhance antitumor immune responses) suggests that PJP associated with ICIs may be independent of immunosuppression. A recent study revealed that the underlying mechanism may involve immune reconstitution inflammatory syndrome, whereby immune checkpoint blockade could induce an exaggerated immune response to *P. jirovecii* colonization and facilitate *P. jirovecii* proliferation[34]. Additional studies are required to confirm the association between PJP and ICIs and to elucidate the underlying mechanisms of ICIs-related PJP. On the other hand, as a conventional treatment modality, although radiotherapy can enhance immune responses against tumors, it may also induce T cell exhaustion, thereby increasing susceptibility to opportunistic infections[35]. Indeed, previous studies have demonstrated that radiotherapy is a risk factor for the development of PJP[36, 37]. Taken together, for patients with malignancies undergoing antitumor therapy, especially radiotherapy and ICI therapy, implementation of PJP prophylaxis appears reasonable.

Corticosteroid exposure has long been recognized as a major risk factor for PJP development in a dose-dependent manner. However, the threshold daily dose and cumulative dose that confer PJP risk remain undefined[38]. Some investigators reported that a daily prednisolone equivalent dose of 20 mg for ≥ 3 weeks was independently associated with PJP development[39], while other researchers proposed that high-dose intermittent corticosteroid therapy was also an important risk factor for PJP occurrence[40]. In our study cohort, none of the participants received long-term high-dose corticosteroid therapy. Therefore, more prospective studies are needed to clarify the relationship between corticosteroid exposure and PJP occurrence in patients with malignant tumors. Moreover, adjunctive corticosteroid therapy is a well-established practice in HIV-infected PJP patients, but its role in non-HIV-infected PJP patients remains controversial. A meta-analysis demonstrated that adjunctive corticosteroid therapy was associated with better prognosis in PJP patients without HIV infection[41], whereas a recent multicenter prospective cohort study reported no significant difference in 6-month mortality between recipients and non-recipients of adjunctive corticosteroid therapy. Subgroup analysis further revealed that adjunctive corticosteroids use was significantly associated with accelerated mortality in non-HIV-infected patients[18]. In our study, only 9 PJP patients received adjunctive corticosteroid therapy, and there was no difference between the survivors and non-survivors. Therefore, there is still no definitive answer to the question of adjunctive corticosteroid therapy for PJP in non-HIV patients, and the decision to administer corticosteroids in this patient population should be individualized.

Previous studies have shown that risk factors for mortality in non-HIV-infected PJP patients are complex[5, 42]. Our findings revealed that malignant solid tumor, ICI therapy, invasive mechanical ventilation, and lower oxygenation index were associated with poor outcome in the univariate analysis. However, none of these factors remained statistically associated with 30-day mortality in the multivariate analysis, which may be at least partly attributable to the relatively small number of PJP patients. Among the PJP patient cohort, non-survivors had a significantly higher prior ICI exposure rate compared with survivors, suggesting that ICI therapy may be a poor prognostic factor in PJP. Previous studies have also reported that lung cancer patients receiving ICI therapy had a higher mortality rate following the onset of PJP than those not receiving ICI therapy[43]. Additionally, lymphocytopenia[44]

and elevated serum LDH levels[45] have been noted in patients with PJP. However, these laboratory markers were not identified as independent predictors of 30-day mortality in our cohort, suggesting that they are not specific predictors of mortality in PJP patients with malignant tumors.

There are still certain limitations in this study. Firstly, this is a single-center retrospective study focusing on ICU patients, which may introduce selection bias and limit the generalizability of the results. Therefore, the applicability of our findings to all PJP patients requires further validation in future studies. Secondly, the sample size of our cohort is relatively small, particularly among patients diagnosed with PJP. Thus, larger-scale prospective studies are needed to identify the risk factors associated with the incidence and mortality of PJP in patients with malignant tumors. Lastly, like other highly sensitive test methods, mNGS can also yield false-positive results. Hence, despite the combined use of clinical manifestations, radiological findings, and mNGS testing for the diagnosis of PJP to reduce the likelihood of *P. jirovecii* colonization as far as possible, the results of mNGS should be interpreted cautiously and additional diagnostic modalities should be integrated to ensure an accurate diagnosis.

Conclusion

In summary, PJP in cancer patients is a lethal disease that is highly prone to mixed infections with multiple pathogens, particularly CMV, while mNGS can provide rapid and comprehensive pathogen detection for such patients. According to our study, malignant lymphoma, radiotherapy, and ICI therapy were independent predictors of PJP occurrence. In addition, cancer patients who received ICI therapy appeared to have a higher mortality after PJP onset. In clinical practice, for patients undergoing antitumor therapy, particularly those receiving radiotherapy and ICI therapy, greater attention should be given to PJP prophylaxis.

Abbreviations

PJP
Pneumocystis jirovecii pneumonia
mNGS
Metagenomic next-generation sequencing
BALF
bronchoalveolar lavage fluid
ICI
immune checkpoint inhibitor
ICU
intensive care unit
HIV
human immunodeficiency virus
APACHE II score
Acute Physiology and Chronic Health Evaluation II score

SOFA
Sequential Organ Failure Assessment score
BMI
body mass index
CMV
cytomegalovirus
LDH
lactate dehydrogenase
CRP
C-reactive protein
PCT
procalcitonin
TMP-SMX
trimethoprim-sulfamethoxazole
BDG
1,3-β-D-glucan
PCR
polymerase chain reaction.

Declarations

Author contributions

CW and SM conceptualized and designed the study. CW, YJ, XW, NM and CZ collected and summarized the clinical data. CW, LC, and ZX analyzed the data and performed the statistical analysis. CW drafted the manuscript. SM and ZX reviewed and revised the manuscript. All authors have read and approved the final version of the manuscript.

Funding

This study was supported by the Noncommunicable Chronic Diseases-National Science and Technology Major Project (2024ZD0526000).

Acknowledgments

The authors wish to thank all research staff and patients for participating in this study. We also thank Dinfectome Inc. (Nanjing, China; https://www.dinfectome.com) for providing technical support in metagenomic next-generation sequencing and bioinformatics analysis.

Availability of data and materials

The pathogen reads of our study were deposited in the Genome Warehouse in the National Genomics Data Center (https://ngdc.cncb.ac.cn/) under project PRJCA066417.

Ethical approval statement

This study was approved by the Ethics Committee of Jiangsu Cancer Hospital (approval number: 2023-050) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was waived by the Ethics Committee due to the retrospective and anonymized nature of the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Clinical trial number

Not applicable.

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Figures

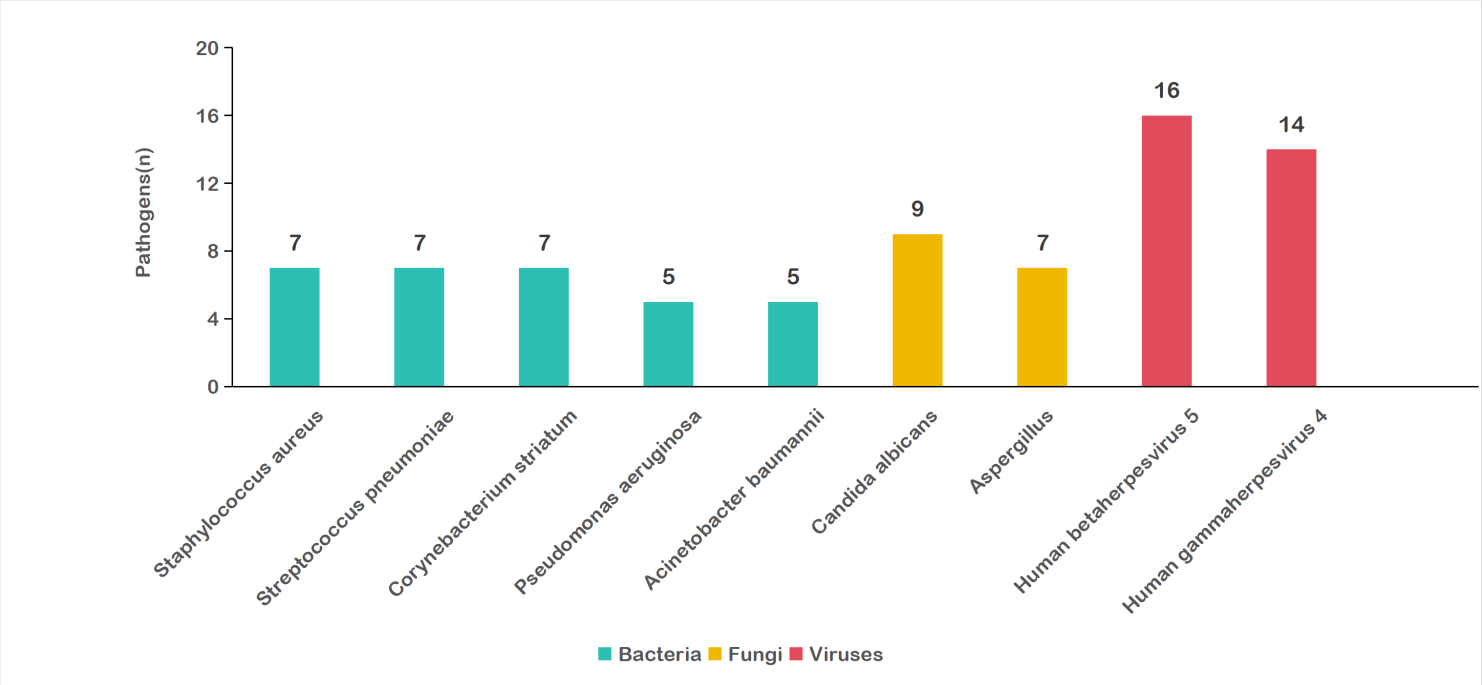


Figure 1

Distribution of major pathogens identified in BALF from cancer patients with PJP detected by mNGS.

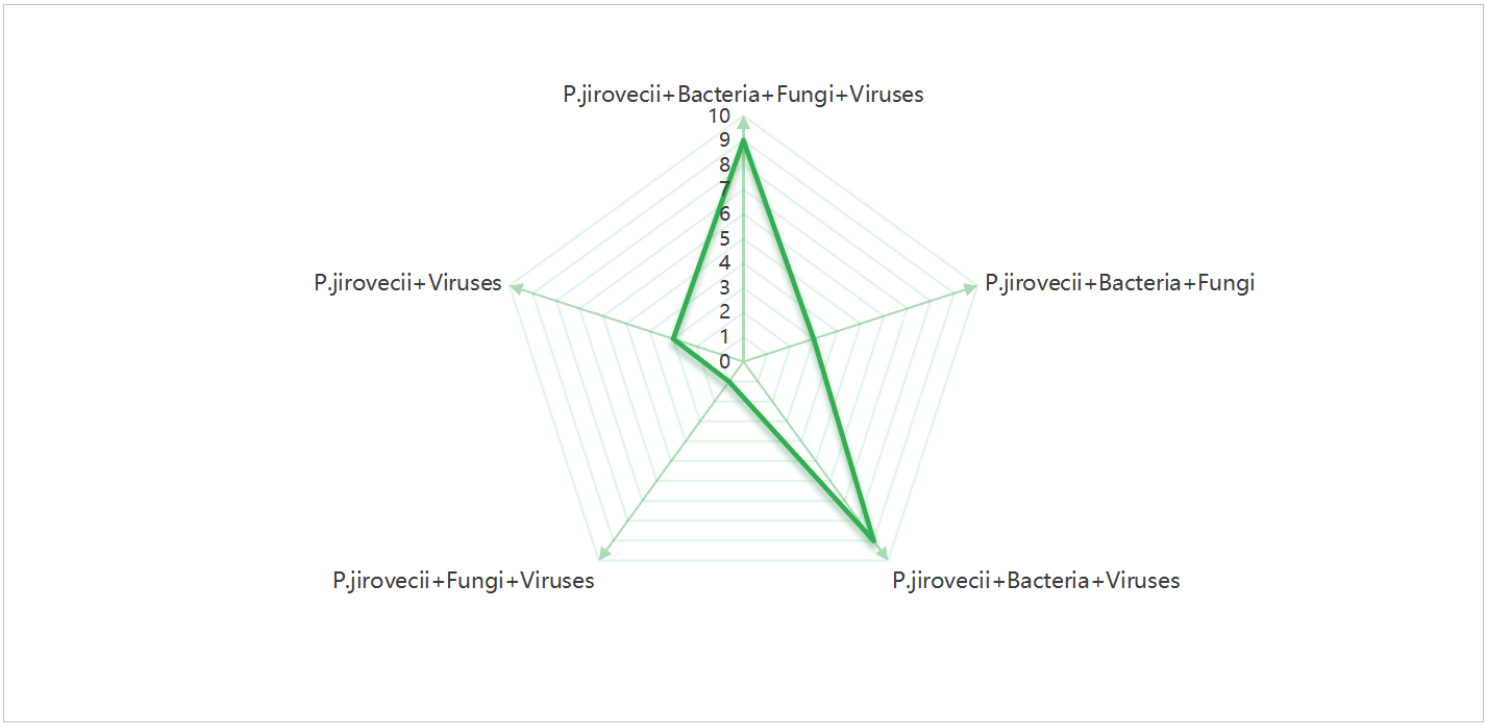


Figure 2

Distribution of co-infecting pathogen types in cancer patients with PJP detected by BALF-mNGS.

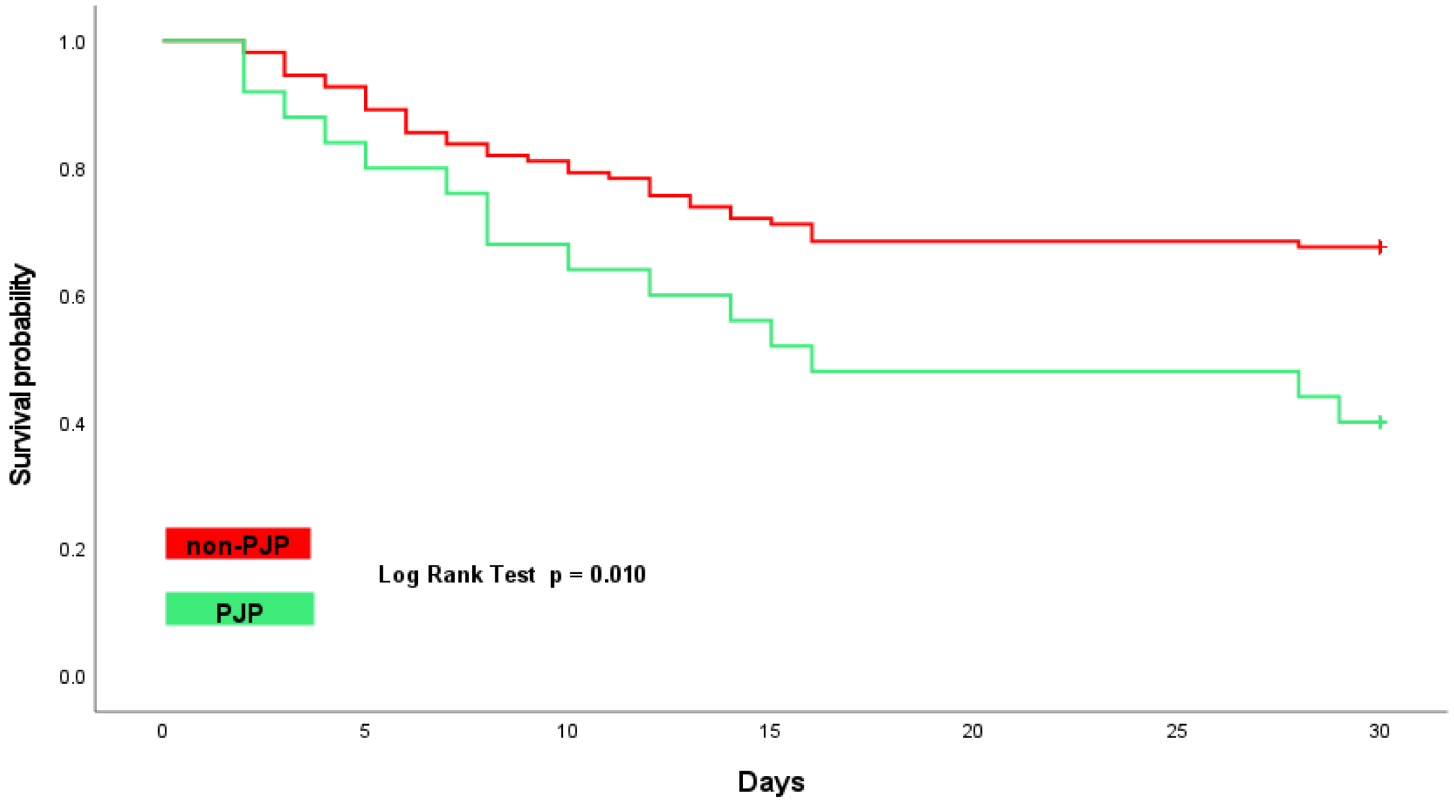


Figure 3

Survival curves of 30-day survival probability in cancer patients with PJP vs. without PJP following mNGS testing, with statistical comparison by log-rank test (P = 0.010).

Supplementary Files

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