Infection and Drug Resistance

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ORIGINAL RESEARCH

Clinical characteristics, treatment outcomes, and prognostic factors of *Pneumocystis* pneumonia in non-HIV-infected patients

This article was published in the following Dove Press journal:
Infection and Drug Resistance

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Objectives: The incidence of *Pneumocystis* pneumonia (PCP) has been increasing among non-HIV-infected patients. Here, we investigated the clinical characteristics, treatment outcomes, and prognostic factors of PCP in non-HIV-infected patients.

Patients and methods: Information on clinical characteristics, treatment outcomes, and prognostic factors of PCP patients who were treated at a medical center in northern Taiwan from October 2015 to October 2016 were retrieved from medical records and evaluated.

Results: Among the patients with PCP included in the study, 84 were non-HIV-infected and 25 were HIV-infected. Non-HIV-infected patients with PCP had a longer duration between radiographic findings and treatment ($P<0.001$), and a higher rate of hospital-associated PCP ($P<0.001$), hypoxia ($P=0.015$), respiratory failure ($P<0.001$), and mortality ($P=0.006$) than HIV-infected patients with PCP. Among non-HIV-infected patients, non-survivors had a higher fungal burden (46.2% vs 22.2%, $P=0.039$), higher requirement for adjunctive steroid treatment (94.9% vs 71.1%, $P=0.011$), and higher rate of pneumothorax (17.9% vs 2.2%, $P=0.038$) than survivors. Multiple logistic regression revealed that lymphopenia (odds ratio [OR] =3.24, 95% confidence interval [CI] =1.07–9.79; $P=0.037$), adjunctive steroid use (OR =6.23, 95% CI =1.17–33.14; $P=0.032$), and pneumothorax (OR =10.68, 95% CI =1.00–113.93; $P=0.050$) were significantly associated with increased 60-day mortality among non-HIV-infected PCP patients.

Conclusion: Lymphopenia, adjunctive steroid therapy, and pneumothorax were significantly associated with higher mortality in non-HIV-infected patients with PCP.

Keywords: *Pneumocystis* pneumonia, *Pneumocystis jirovecii*, immunocompromised host, non-HIV-infected patients

Introduction

Pneumocystis pneumonia (PCP) is a life-threatening opportunistic pulmonary fungal infection caused by *Pneumocystis jirovecii* (PJ) among immunocompromised patients.[1–3] Although the incidence of PCP in human immunodeficiency virus (HIV)-infected patients has gradually declined, owing to the availability of highly active antiretroviral therapy,[4,5] its incidence has been increasing among non-HIV-infected patients with malignancy, hematologic disorders, or autoimmune diseases.[6–8] Besides the change in incidence, the clinical course of PCP also differs between HIV- and non-HIV-infected patients. In non-HIV-infected patients, PCP is a fulminant disease that is associated with a higher risk of respiratory failure and mortality.[9] The disparities in clinical course and outcome between PCP in HIV-infected and non-HIV-infected patients raise questions about the generalizability of the data between these two groups.

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http://doi.org/10.2147/IDR.S199761

In the HIV-infected population with PCP, poor prognostic factors have been well identified, and include old age, anemia, hypoxemia, high alveolar-arterial oxygen difference, high serum lactic dehydrogenase (LDH) levels, low serum albumin levels, and concomitant positivity for cytomegalovirus (CMV) in bronchoalveolar lavage.[10,11] Data for non-HIV-infected patients, however, are very limited and reveal inconsistent results.[10,12,13] Some important factors, such as PJ fungal load and hospital-associated PCP, have not yet been evaluated. Besides, the reasons for the inconsistent prognosis factors among non-HIV-infected patients with PCP are still not clear; however, differences in PCP diagnostic criteria might play a crucial role. Previous studies have used immunofluorescence to detect the pathogen; however, the yield rate of microscopy-based diagnosis may be suboptimal, owing to a relatively low number of PJ cysts in respiratory specimens from non-HIV-infected patients.[2] Other studies have used molecular diagnostic methods, such as conventional polymerase chain reaction (PCR) or nested PCR, which possess higher detection sensitivities than conventional staining methods[14,15] for PCP diagnosis. Nevertheless, when using such techniques, the possibility of including patients with PJ colonization alone cannot be eliminated. According to recent studies, quantitative real-time PCR (qPCR) could semi-quantitate the PJ fungal burden, which could help differentiate colonization from infection.[16,17] Therefore, in this study, we used qPCR for the diagnosis of PCP, and aimed to investigate the treatment outcomes and predictors of mortality among non-HIV-infected patients with PCP.

Patients and methods

Study participants

We identified adult patients with PCP in the National Taiwan University Hospital from October 2015 to October 2016. The patients who met the following criteria were considered definitively diagnosed with PCP: (1) clinical symptoms or signs relevant to PCP (cough, fever, or shortness of breath); (2) imaging findings compatible with PCP; and (3) positive PJ qPCR from respiratory samples (sputum, bronchial washing, or bronchoalveolar lavage). Patients with PJ colonization (defined as positive qPCR with very low PJ fungal burden, and no relevant symptoms or radiographic changes) were excluded. The Institutional Review Board of the National Taiwan University Hospital (201802082RIND) approved this study. To maintain confidentiality and anonymity, we did not collect identifying information of participants and only the investigators of the research team can assess the data. Informed consent was waived by IRB due to the retrospective nature and study was performed according to Declaration of Helsinki.

We recorded patient data regarding demographics, underlying diseases, use of immunosuppressants, use of PCP prophylaxis agents (trimethoprim/sulfamethoxazole with 80/400 mg or 160/800mg daily or three times weekly), PCP-associated symptoms/signs, and laboratory tests. Immunosuppressive agents were classified into three categories: steroids, chemotherapeutic agents, and immunomodulatory agents. For steroids, the average dosage over the prior four weeks was presented as a prednisolone-equivalent dose. Immunomodulatory agents were classified into four categories: calcineurin inhibitors (cyclosporine and tacrolimus), mechanistic target of rapamycin (mTOR) inhibitors (sirolimus and everolimus), antiproliferative agents (azathioprine, mycophenolate mofetil, cyclophosphamide, and methotrexate), and monoclonal antibodies (rituximab and obinutuzumab). Adjunctive steroids were divided into three groups according to prednisolone-equivalent dosage: no steroid use; low dose (<1 mg/kg/day); or high dose (≥1 mg/kg/day). Hospital-associated PCP was defined as PCP-relevant clinical symptoms occurring >48 h after hospitalization.

Radiological findings

Radiological abnormalities were listed on the basis of chest computed tomography (CT) findings at the time of PCP diagnosis, and included ground glass opacity, reticular opacity, consolidation, nodules, and pleural effusion. We evaluated disease severity by chest radiographic score[18] according to plain chest X-ray at the time of diagnosis. Briefly, each lung was divided into three areas, and each area was rated on a four-point scale of 0–3 for the extent of infiltration. The maximum radiographic score was 18, with higher scores indicating a greater extent of disease involvement. Radiographic scores of 0–6, 7–12, and 13–18 were further defined as mild, moderate, and severe, respectively.

Microbiological investigations

Expectorated sputum or bronchoscopic samples (washing or bronchoalveolar lavage) were examined by qPCR in an automated Becton Dickinson MAX real-time PCR platform (Becton Dickinson, Diagnostic Systems, Sparks, MD, USA).[16] The primer sequences for the PJ target

gene, major surface glycoprotein (*MSG*), were MSG-fw, 5′-GAATGCAAATCCTTACAGACAG-3′, and MSG-rv, 5′-AAATCATGAACGAAATAACCATTGC-3′. A dual-labeled fluorescence resonance energy transfer (FRET) hydrolysis probe (MSG-probe 5′-FAM-AGACATCGACA CACACAAGCACGTCT-BHQ1-3′) was used for detection. The cycle threshold (*Ct*) value was checked for positive samples, and defined as the replicated cycle number at which the fluorescence generated within a reaction crossed the fluorescence threshold.[17] A lower *Ct* value correlates with a higher PJ fungal burden. According to previous studies, a qPCR *Ct* value higher than 35, correlates with a clinically low PJ fungal burden and colonization.[16,17] Thus, patients with a *Ct* value above 35 were excluded from this study. For all included patients with PCP, *Ct* values were classified into tertiles, and a high fungal burden was defined as that with a *Ct* value within the first tertile, which represented values ≤24.8.

Outcomes

Hypoxia was defined as an oxyhemoglobin saturation <95% under ambient air. Respiratory failure was deemed positive, if the patient needed invasive or noninvasive positive pressure ventilation support to maintain oxygenation and ventilation for more than 24 h. The index date was the earliest date on which the PCP-related radiographic findings were detected. Mortality was defined as death within 60 days of the index date.

Statistical analysis

Data were expressed as either the median (range) or proportions, as appropriate. Continuous variables were compared using the Mann–Whitney test. Categorical variables were compared using the chi-squared or Fisher's exact test, as appropriate. Logistic regression models were used to identify the prognostic factors. Covariates with a *P*-value <0.10 in the univariate analysis were included in the final model of multivariable logistic regression. Survival curves were generated by the Kaplan–Meier estimator and compared using the log-rank test. All *P*-values were two-sided and considered significant if $P<0.05$. The graphs of conditional probabilities of mortality against the variables of interest were plotted, based on the final logistic regression model in which the rest of the predictors were set to their mean values.[19] All statistical analyses were performed using the Stata statistical software version 14.1.

Results

Characteristics of study subjects

During the 13-month study period, 109 patients fulfilled the diagnostic criteria of PCP. Among them, 25 (22.9%) were HIV-infected and 84 (77.1%) were non-HIV-infected. The median age of the study cohort was 53 (21–89) years, and 65.1% of the study participants were male. The common comorbidities were as follows: hematologic malignancy (n=29, 26.6%), HIV infection (n=25, 22.9%), autoimmune diseases (n=21, 19.3%), solid cancers (n=17, 15.6%), and solid organ transplantation (n=7, 6.4%). Within the 4 weeks prior to PCP diagnosis, 68 patients (62.4%) received steroids, 41 (37.6%) took an immunomodulatory drug, and 19 (17.4%) received chemotherapy. Only four patients (3.7%) received PCP prophylaxis within the 4 weeks prior to PCP diagnosis. For respiratory specimen collection, 88 specimens were from expectorated sputum, five were from bronchial washing, and 16 were from bronchoalveolar lavage.

As shown in Table 1, non-HIV-infected patients with PCP were older (60 vs 34 years, $P<0.001$). Compared with HIV-infected patients with PCP, non-HIV-infected patients with PCP had the following: a higher rate of pleural effusion (28.6% vs 0.0%, $P=0.003$); leukopenia (white blood cell count <4000 cells/μL, 34.5% vs 12.0%, $P=0.030$); lymphopenia (lymphocytes <800 cells/μL, 69.0% vs 44.0%, $P=0.023$); anemia (9.9 vs 12.5 g/dL, $P<0.001$); thrombocytopenia (platelet count $<100\times10^3$ cells/μL, 34.5% vs 8.0%, $P<0.011$); higher C-reactive protein (CRP) levels (11.93 vs 5.74 mg/dL, $P<0.001$); higher qPCR *Ct* value (indicating lower fungal load, 26.6 vs 23.3, $P=0.002$); shorter duration between symptom onset and treatment (7 vs 30 days, $P<0.001$); longer duration between radiographic findings and treatment (4 vs 0 days, $P<0.001$); higher rate of hospital-associated PCP (46.4% vs 0%, $P<0.001$); and higher rates of hypoxia (95.2% vs 80%, $P=0.015$); respiratory failure (67.9% vs 28.0%, $P<0.001$); and mortality (46.4% vs 16.0%, $P=0.006$).

Treatment outcomes and prognostic factors of non-HIV-infected patients with PCP

As shown in Table 2, among 84 non-HIV-infected patients with PCP, 39 (46.4%) died within 60 days after the index date. No differences were noted in underlying disease, previous use of immunosuppressive agents, steroid dosage, clinical symptoms, radiographic findings, use of antifungal

Table 1 Clinical characteristics, management, and outcomes of 109 patients with *Pneumocystis* pneumonia

	Number (%) of patients			
Variables	**All (N=109)**	**Non-HIV-infected (N=84)**	**HIV-infected (N=25)**	***P*-value**
Age				<0.001
<50	44 (40.4)	22 (26.2)	22 (88.0)	
50–59	21 (19.3)	18 (21.4)	3 (12.0)	
60–69	21 (19.3)	21 (25.0)	0 (0.0)	
≥70	23 (21.1)	23 (27.4)	0 (0.0)	
Gender				0.001
Male	71 (65.1)	47 (56.0)	24 (96.0)	
Female	38 (34.9)	37 (44.0)	1 (4.0)	
Body mass index	20.9 (13.3–35.9)	21.1 (13.3–38.9)	20.6 (14.5–30.9)	0.234
Symptoms and signs				
Cough	40 (60.6)	49 (58.3)	17 (68.0)	0.385
Fever	93 (85.3)	71 (84.5)	22 (88.0)	0.666
Dyspnea	103 (94.5)	80 (95.2)	23 (92.0)	0.533
Hypoxia	100 (91.7)	80 (95.2)	20 (80.0)	0.015
Findings of chest computed tomography				
Number of patients evaluated	100	77	23	
Ground glass opacity	96 (96.0)	75 (97.4)	21 (91.3)	0.190
Reticular opacities	26 (26.0)	21 (27.3)	5 (21.7)	0.595
Consolidation	32 (32.0)	27 (35.1)	5 (21.7)	0.229
Nodules	5 (5.0)	5 (6.5)	0 (0.0)	0.210
Pleural effusion	23 (23.0)	22 (28.6)	0 (0.0)	0.003
Laboratory findings				
White blood cell count (cells/μL)				0.002
<2000	16 (14.7)	16 (19.0)	0 (0.0)	
2000–3999	16 (14.7)	13 (15.5)	3 (12.0)	
4000–9999	52 (47.7)	32 (38.1)	20 (80.0)	
≥10,000	25 (22.9)	23 (27.4)	2 (8.0)	
Lymphocyte count (cells/μL)				0.023
<800	69 (63.3)	58 (69.0)	11 (44.0)	
≥800	40 (36.7)	26 (31.0)	14 (56.0)	
Hemoglobin (g/dL)	10.5 (7.2–18.3)	9.9 (7.2–18.3)	12.5 (9.1–15.8)	<0.001
Platelet count (cells $\times 10^3$/μL)				0.024
<50	18 (16.5)	17 (20.2)	1 (4.0)	
50–99	13 (11.9)	12 (14.3)	1 (4.0)	
100–149	15 (13.8)	13 (15.5)	2 (8.0)	
≥150	63 (57.8)	42 (50.0)	21 (84.0)	
C-reactive protein (mg/dL)	9.0 (0.1–40.0)	11.9 (0.1–40.0)	5.74 (0.2–23.0)	<0.001
Ct value of qPCR				0.002
First tertile (≤24.8)	45 (41.3)	28 (33.3)	17 (68.0)	
Second-third tertile (>24.8)	64 (58.7)	56 (66.7)	8 (32.0)	
Hospital-associated infection	39 (35.8)	39 (46.4)	0 (0.0)	<0.001
Treatment				
Symptom onset until treatment (range, days)	11 (1–90)	7 (1–61)	30 (3–90)	<0.001
Radiographic findings until treatment (range, days)	3 (0–25)	4 (0–25)	0 (0–5)	<0.001

(*Continued*)

Table 1 (Continued).

Variables	Number (%) of patients			*P*-value
	All (N=109)	Non-HIV-infected (N=84)	HIV-infected (N=25)	
Adjunctive steroid	87 (79.8)	69 (82.4)	18 (72.0)	0.267
Antifungal agent				0.505
Trimethoprim/sulfamethoxazole	91 (84.3)	71 (85.5)	20 (80.0)	
Echinocandin	17 (15.7)	12 (14.5)	5 (20.0)	
Pneumothorax	10 (9.2)	8 (9.5)	2 (8.0)	0.817
Outcomes				
Respiratory failure	64 (58.7)	57 (67.9)	7 (28.0)	<0.001
Mortality	43 (39.4)	39 (46.4)	4 (16.0)	0.006

Notes: Data are presented as number (%) or median (range).
Abbreviation: Ct, cycle threshold.

Table 2 Clinical characteristics, management, and outcomes of *Pneumocystis* pneumonia in non-HIV-infected patients according to 60-day mortality

Variables	Number (%) of patients		*P*-value
	Survivors (N=45)	Non-survivors (N=39)	
Age			0.600
<50	13 (28.9)	9 (23.1)	
50–59	9 (20.0)	9 (23.1)	
60–69	13 (28.9)	8 (20.5)	
≥70	10 (22.2)	13 (33.3)	
Sex			0.937
Male	25 (55.6)	22 (56.4)	
Female	20 (44.4)	17 (43.6)	
Body mass index	21.0 (16.8–35.9)	21.2 (13.3–33.5)	0.872
Underlying disease			0.728
Hematologic disorder	2 (4.4)	2 (5.1)	
Hematologic malignancy	17 (37.8)	12 (30.8)	
Solid cancer	8 (17.8)	9 (23.1)	
Solid organ transplantation	5 (11.1)	2 (5.1)	
Autoimmune disease	9 (20.0)	12 (30.8)	
Chronic kidney disease	4 (8.9)	2 (5.1)	
Previous immunosuppressant			
Steroid	27 (60.0)	28 (71.8)	0.259
Dosage (Prednisolone equivalent)			0.227
<10 mg	6 (29.6)	4 (14.3)	
10–19 mg	2 (7.4)	9 (32.1)	
20–39 mg	14 (51.9)	10 (35.7)	
≥40 mg	5 (18.5)	5 (17.9)	
Chemotherapy	12 (26.7)	7 (17.9)	0.343
Immunomodulation drugs	20 (44.4)	21 (53.8)	0.390
mTOR inhibitor	3 (6.7)	1 (2.6)	0.379
Anti-proliferative agents	17 (37.8)	13 (33.3)	0.672

(*Continued*)

Table 2 (Continued).

Variables	Number (%) of patients		
	Survivors (N=45)	**Non-survivors (N=39)**	***P*-value**
Monoclonal Ab	5 (11.1)	6 (15.4)	0.563
Calcineurin inhibitor	6 (13.3)	6 (15.4)	0.789
Symptoms			
Cough	26 (57.8)	23 (59.0)	0.912
Fever	38 (84.4)	33 (84.6)	0.983
Dyspnea	41 (91.1)	39 (100.0)	0.999
Radiographic severity			0.062
Mild–moderate	23 (51.1)	12 (30.8)	
Severe	22 (48.9)	27 (69.2)	
Findings of chest computed tomography			
Number	40	37	
Ground glass opacity	39 (97.5)	36 (97.3)	0.955
Reticular opacities	13 (32.5)	8 (21.6)	0.287
Consolidation	12 (30.0)	15 (40.5)	0.334
Nodules	4 (10.0)	1 (2.7)	0.225
Pleural effusion	12 (30.0)	10 (27.0)	0.875
Laboratory findings			
White blood cell count (cells/μL)			0.642
<2000	7 (15.6)	9 (23.1)	
2000–3999	8 (17.8)	5 (12.8)	
4000–9999	19 (42.2)	13 (33.3)	
≥10,000	11 (24.4)	12 (30.8)	
Lymphocyte count (cells/μL)			0.058
<800	45 (60.0)	39 (79.5)	
≥800	18 (40.0)	8 (20.5)	
Hemoglobin (g/dL)	9.7 (7.2–15.7)	10.1 (7.7–18.3)	0.990
Platelet count (cells $\times 10^3$/μL)			0.611
<50	9 (20.0)	8 (20.5)	
50–99	6 (13.3)	6 (15.4)	
100–149	5 (11.1)	8 (20.5)	
≥150	25 (55.6)	17 (43.6)	
C-reactive protein (mg/dL)	12.0 (0.1–33.8)	11.9 (1.7–40.0)	0.961
Ct value of qPCR			0.039
First tertile (≤24.8)	10 (22.2)	18 (46.2)	
Secondary–third tertile (>24.8)	35 (77.8)	21 (53.8)	
Hospital-associated infection	23 (51.1)	16 (41.0)	0.355
Treatment			
Symptom onset until treatment, days	7 (1–37)	7 (1–61)	0.808
Radiographic change until treatment, days	4 (0–23)	4.5 (0–25)	0.815
Adjunctive steroid use	32 (71.1)	37 (94.9)	0.011
High dose	20 (44.4)	23 (59.0)	
Low dose	12 (26.7)	14 (35.9)	
Antifungal agent			0.319
Trimethoprim/sulfamethoxazole	36 (80.0)	35 (92.1)	
Echinocandin	9 (20.0)	3 (7.9)	
Pneumothorax	1 (2.2)	7 (17.9)	0.038

Notes: Data are presented as number (%) or median (range).
Abbreviations: mTOR, mammalian target of rapamycin; Ab, antibody; Ct, cycle threshold.

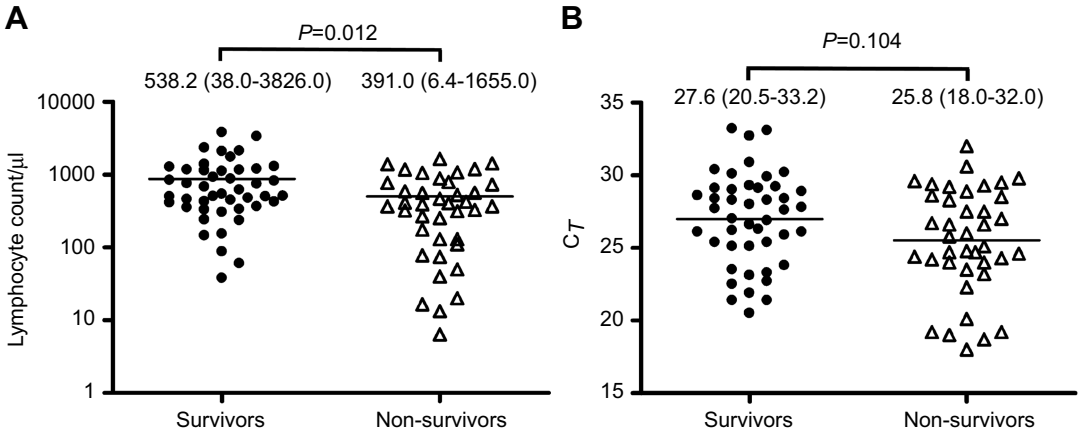

Figure 1 Distribution of (**A**) lymphocyte count and (**B**) *Ct* values according to survivors and non-survivors among non-HIV-infected patients with *Pneumocystis* pneumonia.
Abbreviations: PCP, *Pneumocystis* pneumonia; Ct, cycle threshold.

Table 3 Prognostic factors for 60-day mortality in non-HIV-infected PCP patients

	Univariate			Multivariable		
	Odds ratio	**95% Confidence interval**	***P*-value**	**Odds ratio**	**95% Confidence interval**	***P*-value**
Low Ct of qPCR (≤24.8, 1st tertile)	2.70	1.05–6.96	0.039	1.98	0.68–5.77	0.209
Lymphopenia (<800 cells/μL)	2.58	0.97–6.88	0.058	3.24	1.07–9.79	0.037
Adjunctive steroid	13.68	1.58–35.84	0.011	6.23	1.17–33.14	0.032
Severe radiographic grade	2.35	0.96–5.77	0.062	2.30	0.80–6.60	0.122
Pneumothorax	9.63	1.13–82.2	0.038	10.68	1.00–113.93	0.050

Abbreviation: Ct, cycle threshold.

agents (trimethoprim/sulfamethoxazole or echinocandin), treatment delay (symptom onset and radiographic changes until treatment), or hospital-associated PCP between survivors and non-survivors. Blood lymphocyte count and qPCR *Ct* values of survivors and non-survivors among non-HIV-infected patients with PCP are presented in Figure 1. Compared with survivors, non-survivors had a significantly lower lymphocyte count (391.0 vs 538.2, $P=0.012$) and tended to have lower qPCR *Ct* values (25.8 vs 27.6, $P=0.104$), which indicated a higher fungal burden.

Prognostic factors of 60-day mortality were explored with logistic regression (Table 3). In the univariate analysis, compared with survivors, non-survivors had a higher fungal burden (first tertile of qPCR *Ct* value, 46.2% vs 22.2%, $P=0.039$); adjunctive steroids (94.9% vs 71.1%, $P=0.011$) during PCP treatment; and a higher rate of pneumothorax (17.9% vs 2.2%, $P=0.038$). In the multiple logistic regression, lymphopenia (odds ratio [OR] =3.24, 95% confidence interval [CI] =1.07–9.79, $P=0.037$); adjunctive steroid use (OR =6.23, 95% CI =1.17–33.14, $P=0.032$); and pneumothorax (OR =10.68, 95% CI =1.00–113.93, $P=0.050$) were significantly associated with 60-day mortality.

The Kaplan–Meier survival curve for non-HIV-infected patients with PCP showed that 60-day survival was significantly worse in patients with a lower PJ qPCR *Ct* value ($P=0.025$) and adjunctive steroid use ($P=0.011$) (Figure 2A and B). No significant differences ($P=0.270$) were noted between hospital- or community-associated PCP patients with 60-day survival (Figure 2C). Furthermore, conditional effect plots of lymphocyte counts (Figure 3A), based on the final logistic regression model, revealed that a lower lymphocyte count was associated with an increased risk of mortality, regardless of whether adjunctive steroids were used or not (Figure 3B).

Discussion

This study investigating the predictors of poor outcome for patients with PCP had three major findings. First, in recent years, the number of PCP cases among non-HIV-infected

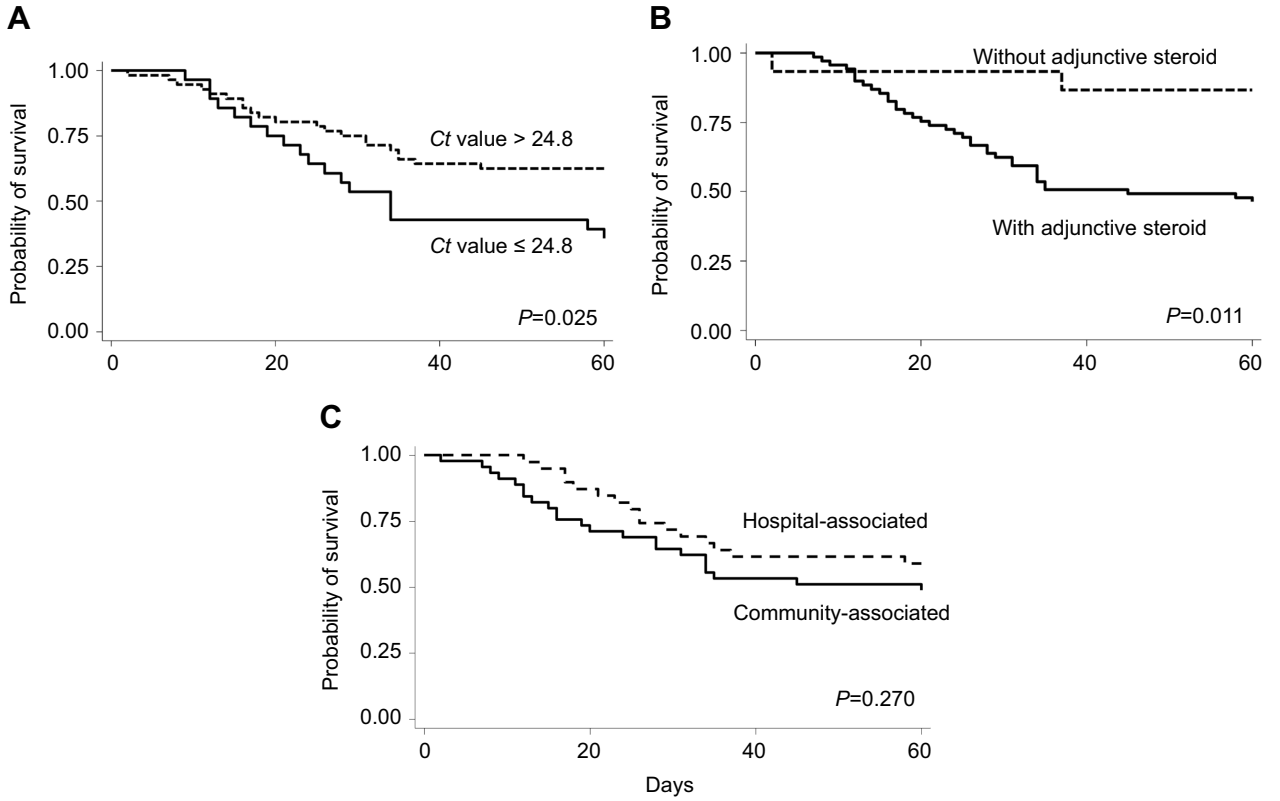

Figure 2 Kaplan–Meier survival curves within 60 days for non-HIV-infected patients with *Pneumocystis* pneumonia stratified by (**A**) *Ct* values, (**B**) adjunctive steroid use, and (**C**) hospital or community-associated infection.
Abbreviations: PCP, *Pneumocystis* pneumonia; Ct, cycle threshold.

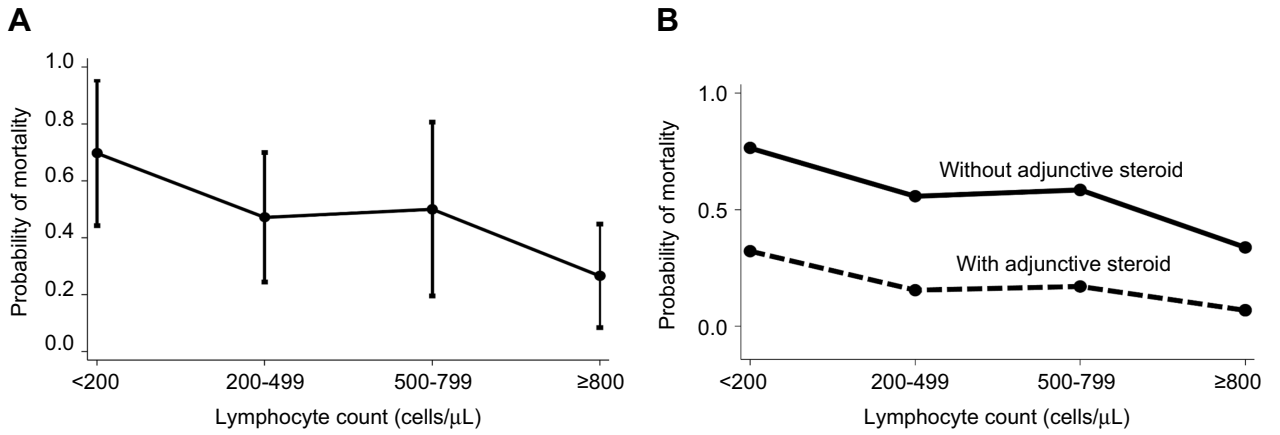

Figure 3 (**A**) Adjusted probabilities of mortality plotted against blood lymphocyte count, based on a multivariable logistic regression model with the rest of the predictors being set to their mean values. (**B**) Adjusted probabilities of mortality against blood lymphocyte count stratified by adjunctive steroid use.

patients have exceeded those of HIV-infected patients. Second, non-HIV-infected patients with PCP had higher rates of respiratory failure (67.9%) and mortality (46.4%). Lastly, lymphopenia, adjunctive steroid therapy, and pneumothorax were independent risk factors for mortality within 60 days among non-HIV-infected patients with PCP.

Corroborating the study conducted by Li et al[13] our current analysis revealed that lymphopenia is associated with a poor prognosis among non-HIV-infected patients with PCP. As shown in Figure 3, the lymphocyte count seems to play an important role as a prognostic factor. In previous studies, a clear relationship between low CD4 T lymphocyte count and PJ infection has also been evident.[20]

Furthermore, passive transfer of immune CD8+ effector T lymphocytes was found to facilitate protection against PCP in mice.[21] These findings support the theory that T-cell immune defects predominate in individuals with a PJ infection. Since lymphopenia can be a surrogate marker for poor T-cell quantification, it could be associated with poor outcomes.

Studies from the 1990s have revealed that adjunctive treatment with high-dose steroids is associated with a considerable reduction in mortality among hypoxic HIV-infected patients with PCP.[22] However, the effects of adjunctive steroid use is still controversial in non-HIV -infected patients with PCP.[23,24] Delclaux et al[23] found no significant differences in mortality between 59 non-HIV-infected patients who received high-dose adjunctive steroids and 29 who did not. Furthermore, Lemiale et al[24] showed that high-dose adjunctive steroids are associated with increased mortality in non-HIV-infected patients with PCP. In 2016, a guideline from the 6th European Conference on Infections in Leukaemia (ECIL)[25] suggested that routine adjunctive steroid use is not advised for PCP treatment in non-HIV-infected hematology patients with PCP and respiratory failure. Our study demonstrated that adjunctive steroid use during treatment was an independent factor for poor prognosis. However, one concern with this practice is that adjunctive steroid use may indicate poor baseline oxygenation and greater disease severity. To eliminate this concern, we included baseline radiographic severity scores and fungal burden (PJ qPCR *Ct* value) as surrogates for disease severity in the multivariable analysis. After adjustment for disease severity, adjunctive steroid use during treatment still presented as a poor prognostic factor. Moreover, as shown in Figure 2B, the survival curves of non-HIV-infected patients with PCP, both with and without adjunctive steroid use, crossed each other at around 15 days after PCP onset. The crossing of the two survival curves might imply that adjunctive steroid use during treatment confers the initial benefit of suppressing the inflammatory process. The beneficial effects, however, are soon counteracted by subsequent adverse effects, such as immunosuppression, hyperglycemia, and new infections.[26] As such, adjunctive steroid use turned out to be a poor prognostic factor. Based on recent studies, as well as the findings from our study, the decision to use steroids as an adjunctive treatment should be made on an individual basis. Further clinical trials are needed to determine the role of adjunctive steroids, including the appropriate dosage and treatment duration, in hypoxic non-HIV-infected patients with PCP.

Similar to the findings of previous studies,[24,27] pneumothorax was associated with poor prognosis in our study. To our knowledge, no prior study has demonstrated the relationship between PJ fungal burden and prognosis. Based on our findings, a higher fungal burden (low *Ct* value) was associated with increased mortality in the univariate analysis, but was not an independent risk factor for mortality in the multivariable analysis. The relationship between PJ fungal burden and mortality still requires further investigation.

Nosocomial acquisition and possible person-to-person transmission of PJ infection has occurred in hospitals.[28] However, the clinical characteristics and clinical outcomes of hospital-associated PCP have not yet been evaluated. Our study showed that non-HIV-infected patients with PCP had a significantly higher rate of hospital-associated PCP than HIV-infected patients. However, among non-HIV-infected patients with PCP, no differences were noted between hospital- and community-associated PCP.

Compared with HIV-infected patients with PCP, non-HIV-infected patients with PCP had shorter duration between symptom onset and treatment (7 vs 30 days, $P<0.001$), which may represent the much more fulminant course among non-HIV-infected patients and less patient delay. In contrast, the treatment delay between radiographic findings till initiation of anti-PCP treatment were longer in non-HIV-infected PCP patients than HIV-infected PCP patients (4 vs 0 days, $P<0.001$). This may indicate medical team had less awareness about PCP among non-HIV-infected population, so it took longer time to initiate treatment even the presentation of typical radiographic finding. The awareness of medical team about the incidence of PCP among non-HIV-infected patients should be improved.

Our study had several limitations. First, the number of study participants was relatively small, and recruitment occurred at a single medical center. Besides, this was a retrospective study, without a standardized treatment and follow-up protocol. Second, despite the combined use of clinical symptoms, radiographic findings, and qPCR for PCP diagnosis, the possibility of including patients with PJ colonization could not have been completed eliminated. Third, respiratory specimens collected for determination of qPCR *Ct* values were obtained from sputum, bronchial washing, and bronchoalveolar lavage. These heterogeneous sources, different specimen concentration and timing of the exam may have interfered with the final *Ct* value analysis. Fourth, we only

used a baseline chest X-ray severity score and fungal burden as surrogates of disease severity. Parameters such as the APACHE (Acute Physiology and Chronic Health Evaluation) score and oxygenation ratio were unavailable because there were many missing values. Finally, we did not analyze the influence of co-infection with other bacteria, fungi, or viruses.

Conclusions

In conclusion, PCP in non-HIV-infected patients is associated with a higher risk of respiratory failure and mortality. Lymphopenia, adjunctive steroid use during treatment, and pneumothorax were independent factors of poor prognosis among non-HIV-infected patients with PCP.

Ethical approval and consent

The study was approved by the Institutional Review Board (IRB) of the National Taiwan University Hospital (201802082RIND). Informed consent was waived by IRB due to the retrospective nature and study was performed according to Declaration of Helsinki.

Acknowledgments

The abstract of this paper was presented as an oral presentation at the 2017 annual meeting of the Asian Pacific Society of Respirology (APSR). The poster's abstract (AO077) was published in *Respirology*. This study was supported by funding from the Ministry of Science and Technology, Taiwan (MOST 107-2314-B-002-245-)

Disclosure

The authors report no conflicts of interest in this work.

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