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ORIGINAL ARTICLE

Pneumocystis jiroveci pneumonia in immunocompromised patients: Delayed diagnosis and poor outcomes in non-HIV-infected individuals

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KEYWORDS
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Background: Pneumocystis jiroveci pneumonia (PJP) is a life-threatening disease in immunocompromised patients. Improved knowledge about the varied characteristics and management in different populations may guide treatment.
Methods: We evaluated the clinical characteristics, management, and outcomes of patients with PJP diagnosed by nested polymerase chain reaction at a medical center in southern Taiwan from 2008 to 2011. The risk factors of mortality among non-human immunodeficiency virus (HIV)-infected patients were analyzed.
Results: During the study period, there were 43 cases of PJP, and the common underlying diseases were HIV infection (23 patients, median CD4 count: 19/μl) and malignancy. The HIV-infected patients had a younger age (36.9 ± 13.7 vs. 50.2 ± 16.2 years, $p = 0.006$), a lower body mass index (19.9 ± 2.3 vs. 22.0 ± 3.7 kg/m^2, $p = 0.035$), a longer duration of symptoms before admission (24 ± 29 vs. 7 ± 15 days, $p = 0.035$), and a lower pneumonia severity index (56 ± 25 vs. 99 ± 35, $p < 0.001$) than non-HIV-infected patients. A delay between admission and starting antimicrobial therapy for PJP (10 ± 10 days vs. 1 ± 3 days, $p = 0.004$) and a high crude mortality (12/20, 60% vs. 2/23, 9%, $p = 0.001$) were noted in non-HIV-infected patients. In the univariate analysis, the risk factors for mortality were a low lymphocyte count ($p < 0.05$) and shock during hospitalization ($p = 0.004$).
Conclusion: A delay in the initiation of antimicrobial therapy for PJP and severe pneumonia were more common in the non-HIV-infected patients and were most likely related

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to the poor prognosis. The utilization of sensitive diagnostic tools to facilitate early diagnosis and treatment may improve the clinical outcomes of non-HIV-infected patients with PJP.

Introduction

Pneumocystis jiroveci pneumonia (PJP) is a severe and potentially fatal opportunistic infection in immunosuppressed patients.[1,2] Several published studies had revealed that that clinical characteristics of PJP vary between patients with and without human immunodeficiency virus (HIV) infection.[2–4] It is difficult to diagnose PJP due to the lack of a reliable culture system for *P. jiroveci*. However, the development of a polymerase chain reaction (PCR) technique has allowed substantial progress in diagnosis and enhanced the sensitivity and specificity of *P. jiroveci* detection.[5]

The incidence of PJP in HIV-infected patients declined 21.5% per year between 1996 and 1998.[6] This decline has been attributed to the availability of highly active antiretroviral therapy.[7] However, an increasing number of sporadic cases of PJP among the individuals without HIV infection has been noted in recent years.[4,8] In addition, the number of susceptible people is increasing because PJP may develop after the clinical application of monoclonal antibody therapy for the treatment of autoimmune diseases.[9,10] In addition, several clusters of *P. jiroveci* infection among kidney transplant recipients have been reported.[11,12] Most importantly, the mortality rate of PJP among the patients without HIV infection was as high as 59%, generally worse than that in the patients with acquired immunodeficiency syndrome.[4]

Thus, we intended to analyze the clinical characteristics, treatment, and outcomes of non-HIV-infected patients with PJP to identify the factors associated with mortality. HIV-infected patients with PJP were used as the control population.

Materials and methods

From October 2008 to September 2011, the patients admitted to the National Cheng Kung University Hospital, a medical center in southern Taiwan, who were diagnosed with PJP were included in this study. Four inclusion criteria had to be met by each patient included in this study: (1) the presence of relevant pulmonary symptoms, i.e., cough or dyspnea, (2) pulmonary infiltration observed by chest radiography or computed tomography, (3) detection of *P. jiroveci* DNA fragments in the samples of sputum, bronchoalveolar lavage fluid, or lung tissue by nested PCR, as described in published reports,[2,3,13] and (4) the receipt of antimicrobial therapy for PJP during hospitalization.

The clinical samples used for nested PCR can be expectorated or induced sputum, bronchoalveolar lavage fluid, or lung tissue. DNA extraction and DNA amplification were performed as previously described.[14] The first round of PCR was performed with the oligonucleotide primers pAZ102-H and pAZ102-E, which were designed to amplify the gene encoding the mitochondrial large subunit rRNA of *P. jiroveci*. The second round of PCR was performed with two sets of primer pairs, each of which had an EcoRI restriction endonuclease site incorporated at the 5′ end: pAZ102-X/R1 (5′-GGGAATTCGTGAAATACAAATCGGACTAGG-3′) with pAZ102-Y/R1 (5′-GGGAATTCTCACTT AATATTAATTGGGGAGC-3′) and pAZ102-X/R1 with pAZ102-L1R/R1 (5′-GGGAATTCTCTCGACTCCTCACCT TAT-3′).

Clinically, hypoxemia was defined as either an oxyhemoglobin saturation of less than 95% measured by pulse oximetry under room air or the need for oxygen. Hospital-onset PJP referred to the patients who developed relevant symptoms more than 48 hours after admission. In this study, cardiovascular disease refers to stroke and coronary artery disease. Prior pulmonary diseases were defined as a history of physician-diagnosed nonmalignant lung disease, such as pulmonary tuberculosis, chronic bronchitis, emphysema, interstitial lung disease, or autoimmune disease with lung involvement.

When calculating the dose of co-trimoxazole (or trimethoprim/sulfamethoxazole) for the treatment of PJP, we excluded the patients with impaired renal function because the dosage of co-trimoxazole should be lower for such patients; therefore, averaging the doses used in patients with and without impaired renal function will result in an underestimate of the dosage. The clinical data retrieved from the medical records included demographic characteristics, comorbid conditions, clinical features at presentation, laboratory test results, radiologic images, use of antimicrobial therapy for PJP, and clinical outcome. The pneumonia severity index was used to grade the severity of pneumonia.[15]

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows (version 17.0; SPSS Inc., Chicago, IL, USA). Continuous variables were analyzed using the independent *t* test, and categorical variables were compared by the Chi-square or Fisher's exact test. A p value of <0.05 was considered statistically significant.

Results

Demographic data and underlying disease

Forty-three patients were diagnosed as having PJP during the study period. There were 23 HIV-infected patients and 20 patients who were immunocompromised due to medications or illness other than HIV infection (Table 1). Most clinical respiratory samples with positive results in the nested PCR were expectorated or induced sputum (22/23 for the HIV-infected patients and 15/20 for the non-HIV-infected patients). *P. jiroveci* DNA fragments were also

Table 1 Characteristics and outcomes of patients with PJP with and without HIV infection

Variables	Patients, *N* (%)		*p*
	HIV, $n = 23$	Non-HIV, $n = 20$	
Age, y (mean ± SD)	36.9 ± 13.7	50.2 ± 16.2	0.006
Male	23 (100)	9 (45)	<0.001
Body mass index, kg/m^2 (mean ± SD)	19.9 ± 2.3	22.0 ± 3.7	0.04
Underlying diseases			
Hematologic malignancy	0	5 (25)	0.02
Solid organ malignancy	0	3 (15)	0.09
Autoimmune disease	0	8 (40)	0.001
Recipient of a kidney transplant	0	2 (10)	0.21
Chronic kidney disease	0	3 (15)	0.09
Cardiovascular disease	2 (9)	3 (15)	0.65
Prior pulmonary diseases	3 (13)	4 (20)	0.69
Prior PJP chemoprophylaxis	1 (4)	0	1.00
Symptom onset before admission, d (mean ± SD)[a]	24 ± 29	7 ± 15	0.04
Severity markers at presentation			
Hypoxemia[b]	14 (61)	14 (70)	0.53
Pneumonia severity index (mean ± SD)	56 ± 25	99 ± 35	<0.001
Hypotension	1 (4)	4 (20)	0.17
Concurrent respiratory tract infection	8 (35)	2 (10)	0.08
Laboratory findings (mean ± SD)			
White blood cell count, cells/μl	6839 ± 2678	9070 ± 4934	0.07
Lymphocytes, cells/μl	782 ± 609	915 ± 903	0.57
Hemoglobin, g/dl	10.9 ± 1.9	11.2 ± 2.3	0.60
Platelets, cells × 10^3/μl	261 ± 109	205 ± 129	0.13
Treatment of PJP			
Initiation after admission, d (mean ± SD)[a]	1 ± 3	10 ± 10	0.004
Dose of co-trimoxazole, mg/kg/d (mean ± SD)[c]	14.3 ± 2.2	13.5 ± 3.6	0.44
Adjuvant steroid use	16 (70)	20 (100)	0.01
Outcome			
Pneumothorax	1 (4)	2 (10)	0.59
Shock during hospitalization	2 (9)	13 (65)	<0.001
Admission to ICU	2 (9)	18 (90)	<0.001
Total hospital stay, d (mean ± SD)[a]	20 ± 11	31 ± 13	0.007
ICU stay, d (mean ± SD)	18 ± 3	16 ± 3	0.87
Respiratory failure	2 (9)	18 (90)	<0.001
Crude mortality	2 (9)	12 (60)	<0.001

[a] Four non-HIV-infected patients with hospital-onset PJP were excluded.
[b] Oxyhemoglobin saturation <95% (pulse oximetry) in room air or the need for oxygen.
[c] A total of five patients, including those with impaired renal function, a history of co-trimoxazole allergy, and those whose doses were changed due to adverse events, were excluded for the dose evaluation. The dose refers to that of trimethoprim.
Note: Prior pulmonary disease denotes a history of physician-diagnosed nonmalignant pulmonary disorder; cardiovascular disease: coronary artery disease or stroke.
HIV = human immunodeficiency virus; ICU = intensive care unit; PJP = *Pneumocystis jiroveci* pneumonia; SD = standard deviation.

detected in bronchoalveolar lavage fluid (one HIV-infected patient and three non-HIV-infected patients) and lung tissue (two non-HIV-infected patients).

Clinical characteristics and laboratory findings

Among the HIV-infected patients, the CD4 counts were often less than 100/μl, and the majority of these patients (91%) were naïve to antiretroviral therapy at the time of presentation. Male sex (23, 100%), a younger age (36.9 ± 13.7 vs. 50.2 ± 16.2 years, $p = 0.006$), a lower body mass index (19.9 ± 2.3 vs. 22.0 ± 3.7 kg/m^2, $p = 0.035$), and a longer duration of prodromal symptoms (24 ± 29 vs. 7 ± 15 days; $p = 0.035$) were more common among the HIV-infected patients than among the non-HIV-infected patients (Table 1). However, the severity of illness at presentation, represented by the pneumonia severity index, was lower in the HIV-infected patients (mean 56 vs. 99, $p < 0.001$), although the numbers of patients presenting with hypoxemia or hypotension did not differ between the two groups (Table 1). The white blood cell, lymphocyte, and platelet counts and the hemoglobin levels were similar in the two groups. Regarding concurrent respiratory tract infections, in the HIV group, there were three cases of nontuberculous *Mycobacterium* infection, two cases of pulmonary cryptococcosis, one case of cytomegalovirus infection, one case of influenza virus A infection, and one case of bacterial

infection. In the non-HIV-infected group, one patient had a concurrent cytomegalovirus infection and another was infected with influenza virus A.

Treatment and outcomes

There were four cases of hospital-onset PJP in the non-HIV-infected group and no such cases in the HIV-infected group. The delay in the initialization of specific antimicrobial therapy for PJP, i.e., co-trimoxazole, after admission was longer in the non-HIV-infected group (10 vs. 1 days, $p = 0.004$), after excluding the four patients with hospital-onset PJP (Table 1). Among the 43 patients with PJP, most (33, 73%) received concurrent β-lactam therapy. Furthermore, there were 14 (33%) patients who received a macrolide, 11 (26%) who received fluoroquinolone, four (9%) who received ganciclovir, and three (7%) who received an antifungal agent when they were treated for PJP.

The average dosages of co-trimoxazole for PJP were similar between the two groups (based on trimethoprim: 14.3 mg/kg/day in HIV-infected patients vs. 13.5 mg/kg/day in non-HIV-infected patients, $p = 0.4$). Due to the presence of a history of allergy (one case) or to adverse reactions (18 cases) related to co-trimoxazole, there were 10 (43%), and nine (45%) patients that received a combination of primaquine and clindamycin in the HIV-infected and non-HIV-infected groups, respectively ($p = 0.9$). The intervals between the initiation of co-trimoxazole and the switch to primaquine plus clindamycin were 11.7 ± 3.8 and 11.1 ± 7.4 days in the HIV and non-HIV-infected groups, respectively. With regard to the reasons for the switch to primaquine use, two non-HIV-infected patients had presumed renal toxicity, six (five HIV-infected and one non-HIV-infected) had skin rashes, and eight (three HIV-infected and five non-HIV-infected) had hematologic toxicity. In the HIV-infected group, the hospital stay was shorter (20 vs. 31 days, $p = 0.007$), and there were fewer admissions to an intensive care unit (ICU) due to respiratory failure (2/23, 9% vs. 18/20, 90%; $p < 0.010$). There were more cases of shock during hospitalization (13/20, 65% vs. 2/23, 9%; $p < 0.001$) and more deaths in the non-HIV-infected group (12/20, 60% vs. 2/20, 9%; $p < 0.001$).

Because there was a lower mortality rate in the HIV-infected group, further analysis to identify prognostic factors was limited to the non-HIV-infected group. Age, sex, body mass index, underlying disease, the duration of symptoms before admission, the pulmonary severity index and hypoxemia at presentation, hypotension, and the leukocyte, hemoglobin, and platelet counts were not associated with mortality, ICU admission, or respiratory failure during hospitalization (Table 2). However, a greater degree of lymphopenia (593.0 vs. 1398.9 cells/μl, $p < 0.05$) and shock during hospitalization (92% vs. 25%, $p = 0.004$) were associated with mortality.

Discussion

This retrospective study showed that the clinical characteristics, treatment, and outcomes of PJP differ significantly among the patients with and without HIV infection. The mortality rates of HIV-infected and non-HIV-infected patients in the present study were similar to those of published studies.[3,16] Previous studies have noted that PJP can be more fulminant among immunosuppressed non-HIV-infected patients than among HIV-infected patients,[8,17] resulting in a mortality rate as high as 50% in non-HIV-infected patients with PJP.[3,8,18] In our study, the duration between admission and the initialization of PJP treatment was longer in the non-HIV-infected group. Such a delay in treatment is likely to be related to the delay in the diagnosis of PJP, for which the clinical manifestations and radiologic abnormalities are nonspecific to make it difficult to differentiate PJP from other opportunistic pulmonary infections. Most importantly, the diagnostic yield of staining methods for respiratory samples for *P. jiroveci* is limited,[19] leading to an underestimation of the prevalence of PJP in the at-risk population.

The prognosis of HIV-infected individuals with PJP has not changed much despite major advances in caring for HIV-infected patients during the past quarter of a century.[20] By contrast, the high mortality rate of non-HIV-infected patients with PJP should prompt continued efforts to improve their outcomes. The importance of early treatment of non-HIV-infected patients with PJP has not be emphasized until recently.[13,21] Our study also demonstrated that a delay in anti-PJP treatment was more common among non-HIV-infected patients. Physicians should be alert for the possibility of PJP in immunocompromised patients with respiratory complaints and radiologic pulmonary infiltration.

In accordance with earlier studies,[3,22] the clinical symptoms of the HIV-infected and non-HIV-infected patients with PJP were similar and nonspecific. In addition, these two populations have been shown to share common findings for chest films or computed tomography,[22,23] although some differences in computed tomography images have been reported.[22] However, the similar clinical presentations of PJP did not lead to early therapy in the non-HIV-infected patients. In general, the HIV-infected patients often received early antimicrobial therapy for PJP and had a favorable outcome. However, the non-HIV-infected patients were usually treated with appropriate antimicrobial therapy starting, on average, 10 days after admission and had complicated courses. One of the possible reasons for this discrepancy may be related to the variation in clinical alertness for PJP among attending physicians. The HIV-infected patients were predominantly cared for by infectious disease specialists, and most of the non-HIV-infected patients were cared for by medical professionals other than infectious disease specialists.

The standard therapy for PJP is co-trimoxazole at a dose of trimethoprim 5–20 mg/kg/day. Nevertheless, only a limited proportion of patients can complete the recommended treatment course, usually 21 days, without experiencing adverse events.[24] We found that the treatment regimen had been changed for almost one-half of patients due to the adverse events of co-trimoxazole such as skin rash or bone marrow suppression.

Owing to the lack of a reliable culture system of *P. jiroveci*, the diagnosis traditionally relied on conventional staining, but this method is limited by a low sensitivity.[19] The development of PCR-based diagnostic assays improved the sensitivity to between 87 and 100%.[19,25] The negative predictive rate can be as high as 100%.[19,25] Thus,

Table 2 Univariate analysis of risk factors associated with crude mortality in 20 non-HIV-infected patients with *Pneumocystis jiroveci* pneumonia

Variables	Patients, *N* (%)		*p*
	Survived ($n = 8$)	Fatal ($n = 12$)	
Female	5 (63)	6 (50)	0.67
Age, y (mean ± SD)	53.6 ± 15.1	47.9 ± 17.2	0.46
Body mass index, kg/m^2 (mean ± SD)	23.2 ± 3.3	21.3 ± 1.1	0.26
Underlying diseases			
Prior pulmonary disease	1 (13)	3 (25)	0.62
Chronic kidney disease	1 (13)	2 (17)	1.00
Diabetes mellitus	1 (13)	0 (0)	0.40
Hypertension	5 (63)	3 (25)	0.17
Cardiovascular disease	0 (0)	3 (25)	0.24
Hematologic malignancy	1 (13)	4 (33)	0.60
Solid cancer	1 (13)	2 (17)	1.00
Autoimmune disease	4 (50)	4 (33)	0.65
Recipient of a kidney transplant	2 (25)	0 (0)	0.15
Symptom onset before admission, d[a]	3.3 ± 5.2	8.9 ± 18.2	0.48
Severity of illness at presentation			
Pneumonia severity index	95.1 ± 37.9	102.3 ± 33.5	0.66
Hypoxemia[b]	4 (50)	9 (75)	0.36
Hypotension	1 (13)	3 (25)	0.62
Concurrent respiratory tract infection	1 (13)	1 (8)	1.00
Laboratory findings (mean ± SD)			
White blood cell count, cells/μl	11,262.5 ± 5449.8	7608.3 ± 4161.6	0.11
Hemoglobin, g/dl	11.1 ± 2.4	11.3 ± 2.3	0.81
Platelets, cells × 10^3/μl	202.6 ± 119.0	205.8 ± 140.5	0.96
Lymphocytes, cells/μl	1398.9 ± 1026.5	593.0 ± 675.4	<0.05
Treatment and outcomes			
Initiation of treatment after admission, d[a]	8.8 ± 7.2	11.1 ± 12.0	0.68
Admission to intensive care unit	7 (88)	11 (92)	1.00
Respiratory failure	6 (75)	12 (100)	0.15
Shock during hospitalization	2 (25)	11 (92)	0.004
Pneumothorax	1 (13)	1 (8)	1.00

[a] Four non-HIV-infected patients with hospital-onset *Pneumocystis jiroveci* pneumonia were excluded.
[b] Oxyhemoglobin saturation by pulse oximetry <95% in room air or the need for oxygen.
Note: Prior pulmonary disease denotes a history of physician-diagnosed nonmalignant pulmonary disorder; cardiovascular disease: coronary artery disease or stroke.
HIV = human immunodeficiency virus; SD = standard deviation.

a negative PCR result allows the withdrawal of potentially toxic empirical antimicrobial therapy for PJP.[19] Therefore, PCR-based assays are useful tools to guide the clinical management of patients with suspected PJP.

In HIV-infected patients with PJP, a low lymphocyte percentage (≤ 10%) has been identified as a predictor of mortality.[26] In our analysis of non-HIV-infected patients with PJP, lymphopenia and shock during hospitalization were associated with mortality. The association of the lymphocyte count with the mortality rate of PJP in non-HIV-infected individuals is parallel to a previous study.[27]

This study has several limitations. First, the clinical data were collected retrospectively. Some factors that may influence the outcome of PJP may be not available, such as the serum levels of lactate dehydrogenase, C-reactive protein,[18] and albumin.[28] In addition, not all patients in the non-HIV-infected group received an HIV screening test. Second, positive results in the nested PCR were not confirmed by other tests, such as conventional staining. In addition, these positive results can be argued to represent airway colonization rather than true infection with *P. jiroveci*.[29,30] However, all of our patients had clinical symptoms and radiologic abnormalities compatible with true infection. Therefore, the possibility of *P. jiroveci* colonization is minimal. In addition, the non-HIV-infected patients with PJP had fewer fungal cells in the clinical samples than the HIV-infected patients.[31] Thus, the PCR-based studies would be more sensitive to diagnose PJP for the non-HIV-infected patients. Last, the number of patients was relatively small, especially for analyzing the association between clinical characters and mortality. Thus, the clinical implications of our findings cannot be generally applied to other patient populations. Some factors, such the pneumonia severity index or the delayed treatment of PJP, may have reached statistical significance if more patients had been included in the present study.

In conclusion, among non-HIV-infected patients, even under modern medical care, a delay in the initiation of

antimicrobial therapy for PJP was common, and this delay was associated with a worse outcome than that in the HIV-infected population. Rapid and sensitive diagnostic assays to confirm or rule out PJP are urgently needed to improve the clinical outcomes of patients with PJP and to prevent unnecessary exposure to co-trimoxazole in immunocompromised individuals.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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