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Original Article

Pneumocystis jirovecii pneumonia in patients with and without human immunodeficiency virus infection

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Background and Purpose: *Pneumocystis jirovecii* pneumonia is an opportunistic infection capable of causing life-threatening pneumonia in immunocompromised patients. To elucidate the clinical presentation and outcome of this disease in Taiwan, we analyzed the patients with *P. jirovecii* pneumonia during a 34-month period.

Methods: We collected data retrospectively from patients with *P. jirovecii* pneumonia at a medical center in northern Taiwan between January 2004 and October 2006. The diagnosis was made by nested polymerase chain reaction (PCR) analysis of expectorated sputum. Demographics, clinical characteristics, laboratory findings, and outcomes were compared between patients with and without human immunodeficiency virus (HIV) infection.

Results: Forty nine patients were included in this study. The most common underlying diseases were HIV and malignancies. The mean (± standard deviation) age of the 49 patients was 54 ± 20.2 years (range, 5 to 96 years). The mean CD4+ T-lymphocyte count was 110 cells/μL (range, 0-670 cells/μL). Although the mean CD4+ T-lymphocyte count of the non-HIV group was higher than that of the HIV group (165 ± 78 cells/μL vs 57.5 ± 97 cells/μL), statistical significance was not obtained (p=0.087). Arterial oxygenation (ratio of arterial oxygenation to fraction of inspired oxygen) was less than 200 mm Hg in 28 patients. Lactate dehydrogenase levels were higher than the normal range in 15 patients. A significantly higher proportion of patients died in the group without HIV compared with the HIV-infected patients (17/34 [50.0%] vs 1/15 [6.7%]; p=0.004).

Conclusion: *P. jirovecii* pneumonia remains a significant problem for immunocompromised patients. The mortality rate for patients without HIV infection was high (50%). Greater alertness with regard to early detection of *P. jirovecii* in HIV-negative immunosuppressed patients with the application of nested PCR may improve the clinical management and outcome.

Key words: AIDS-related opportunistic infections; Lymphopenia; Neoplasms; *Pneumocystis jirovecii*; Pneumonia; Treatment outcome

Introduction

Pneumocystis jirovecii, formerly known as *Pneumocystis carinii*, is a fungal pathogen that causes pneumonia, a common and serious opportunistic infection in immunocompromised patients. Apart from acquired immunodeficiency syndrome (AIDS), the condition has been associated with premature and malnourished children and with patients receiving chemotherapy or organ transplantation. *P. jirovecii* cannot yet be successfully cultured from human specimens. Diagnosis of *P. jirovecii* pneumonia in the laboratory was, until a few years ago, dependent on visualization of *Pneumocystis* organisms in stained preparations of appropriate respiratory specimens using the Giemsa and Gomori-Grocott techniques.

The polymerase chain reaction (PCR) technique has become a promising tool for diagnosis of *P. jirovecii* in recent years, offering sensitivity and negative predictive

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values of 84.62% and 98.41%, respectively, for sputum specimens [1]. An increase of *P. jirovecii* pneumonia at the Tri-Service General Hospital, Taipei, Taiwan, was noted since the establishment of the nested PCR technique for diagnosis in 1997. In this study, the demographics, clinical features, laboratory findings, and outcomes of patients with and without human immunodeficiency virus (HIV) infection were compared.

Methods

The diagnosis of *P. jirovecii* pneumonia was based on the presence of consistent clinical symptoms (i.e., fever, pulmonary symptoms) and/or chest radiograph abnormalities along with the positive finding of nested PCR to identify the fungus from expectorated sputum specimens. From January 2004 to October 2006, 274 sets of sputum from 220 patients were sent for nested PCR for *P. jirovecii*. Positive findings were noted in 52 patients. Three patients were excluded as they discharged themselves against medical advice at diagnosis. Only 2 patients received bronchoalveolar lavage (BAL) and 1 patient received open lung biopsy. We reviewed the charts and clinical findings of the patients with a positive nested PCR test. Clinical data abstracted included the following: clinical features, laboratory analysis, radiological study, including both plain chest radiographs and computed tomography, antibiotic therapy, underlying immunosuppressive condition, and clinical outcome. Tachycardia was defined as a heart rate >100 beats per min. Tachypnea was defined as a respiratory rate >25 breaths/min. Cytomegalovirus (CMV) viremia was defined as detection of CMV DNA in the serum sample by PCR. Lymphopenia was defined as T-lymphocyte counts of <1000 cells/μL. AIDS was defined as previously described [2].

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows (Version 13; SPSS, Chicago, IL, USA). Univariate analysis was performed by use of Student's *t* test for continuous variables and chi-squared test for categorical variables. A value of $p<0.05$ was considered statistically significant in all analyses.

PCR technique

DNA was extracted from patients, control specimens, and external controls using a NucliSens kit (Organon Teknika, Durham, NC, USA), according to manufacturers' instructions. Specimens were stored at −70°C in NucliSens lysis buffer. A 50-μL volume of lower respiratory tract specimen was extracted directly. The pellet was digested with proteinase K (0.1 mg/mL) and DNA was extracted with phenol chloroform solution. External primers were used as previously described [3], namely pAZ 102-E (5'-GATGGCTGTTTCCAAGCCCA-3') and pAZ 102-H (5'-GTGTACGTTGCAAAGTACTC-3'). These primers are complementary to sequences of the mitochondrial small subunit rRNA gene of *P. jirovecii*. To increase sensitivity, a set of nested primers, pLE1 (-5'-TCGGACTAGGATATAGCTGG-3') and pLE2 (-5'-CCCTTTCGACTATCTACC-3') was constructed to yield a final product of 193 bp. The PCR reaction was carried out in a 50-μL volume containing PCR buffer (10 mM Tris-HCl [pH, 9.0], 50 mM KCl, and 1.5 mM $MgCl_2$), 200 μM deoxynucleotide triphosphates, 1 μM of each primer, 1.5 U of Taq DNA polymerase and 5 μL of DNA solution. PCR amplification was initially denatured at 95°C for 10 min, followed by amplification for 30 cycles. Each cycle consisted of denaturation at 95°C for 15 sec, followed by annealing at 60°C for 30 sec and extension at 72°C for 30 sec. One microliter of the first PCR product was used for the second round of PCR (35 cycles with the following parameters: 15 sec at 95°C, 30 sec at 60°C and 30 sec at 72°C; final extension for 7 min at 72°C). Amplified products were determined by 2% agarose gel electrophoresis and visualized by ultraviolet light. Positive (*P. jirovecii* DNA) and negative (autoclaved water) controls were tested simultaneously in each round.

Results

Demographic data

Forty nine patients were diagnosed with *P. jirovecii* pneumonia were diagnosed during the study period. There were 36 men and 13 women (Table 1). The mean age (± SD) at the time of diagnosis was 54 ± 20 years (range, 5 to 96 years). The mean age of female patients was 59.8 years and that of male patients was 51.6 years. There were 15 HIV-positive individuals and 34 patients with other immunocompromised states. Although men represented the majority of patients for both groups, women accounted for 35.3% of non-HIV *P. jirovecii* pneumonia patients.

Underlying diseases

The underlying diseases among the 49 patients with *P. jirovecii* pneumonia are shown in Table 2. All patients

Table 1. Characteristics and outcome of patients with *Pneumocystis jirovecii* pneumonia with and without human immunodeficiency virus (HIV) infection

Variable	HIV (n = 15)	Non-HIV (n = 34)	*p*
Age (years; mean ± SD)	38.2 ± 9.5	60.7 ± 19.8	NS
Female sex (no. [%])	1 (6.7)	12 (35)	NS
Symptoms (%)			
Fever	100	100	NS
Tachycardia (>100/min)	80	79.4	NS
Tachypnea (>25/min)	73.3	67.6	NS
Chest radiography			
Bilateral	80	100	NS
Interstitial infiltration only	40	47	NS
Laboratory finding			
Total lymphocytes ($\times 10^9$/L)	0.778 ± 0.699	0.858 ± 1.063	NS
Lymphopenia (<1000 cells/μL) [%]	86.67	32.3	
CD4+ (cells/μL; mean ± SD)	57.5 ± 97	165 ± 178	0.087
CRP (μg/L; mean ± SD)	83 ± 63	100 ± 76	NS
LDH (U/L; mean ± SD)	731 ± 554	581 ± 301	NS
LDH >460 U/L (%)	60	52.9	
Albumin (g/L; mean ± SD)	26.5 ± 5.0	29.7 ± 6.0	0.09
pO_2/FiO_2 <200 (%)	61.5	58	NS
CMV viremia (no. [%])	5/12 (41.6)	6/23 (26)	NS
Treatment with TMP-SMZ (%)	100	76.4	
Mortality (%)	6.7	50.0	0.004

Abbreviations: SD = standard deviation; CRP = C-reactive protein; LDH = lactate dehydrogenase; pO_2 = partial pressure of oxygen; FiO_2 = fraction of inspired oxygen; CMV = cytomegalovirus; TMP-SMZ = trimethoprim-sulfamethoxazole; NS = not significant

were immunosuppressed prior to the development of *P. jirovecii* pneumonia. The most common diseases were malignancies and HIV infection. Fifteen patients had solid organ malignancies. Thirteen patients had hematological malignancies. Fifteen patients were HIV positive. One child had hypogammaglobulinemia.

Clinical and laboratory data

All of the patients had a body temperature greater than 38°C. Most of the patients had tachypnea (70%) and tachycardia (80%) [Table 1]. At the time of diagnosis, lymphocyte counts were available for all of the 49 patients. Lymphopenia was common (49%). The mean absolute lymphocyte counts were $0.778 \pm 0.699 \times 10^9$/L in HIV-positive patients and $0.858 \pm 1.063 \times 10^9$/L in non-HIV patients. Data on CD4+ T-lymphocyte counts were available for 20 patients, including all of the AIDS patients and 6 of the non-HIV patients. The mean CD4+ T-lymphocyte count was 110 cells/μL (range, 0-670 cells/μL). The mean CD4+ T-cell count was 57.5 ± 97.0 cells/μL (range, 0-280 cells/μL) for AIDS patients and 165.0 ± 178.0 cells/μL (range, 16-366 cells/μL) for non-HIV patients. The mean CD4+ T-lymphocyte count was higher in non-HIV patients, but statistical significance was not obtained (p=0.087). Data for arterial oxygenation (ratio of arterial oxygen to fraction of inspired oxygen) were available for 48 patients; for 28 patients this variable was less than 200 mm Hg. HIV-infected patients had a higher serum lactate dehydrogenase (LDH) level compared with those without HIV infection (731 ± 554 U/L vs 581 ± 301 U/L), but statistical significance was not obtained. Chest radiographs revealed pulmonary consolidation in 27 patients, and alveolar and interstitial infiltrations in 22, with 46 being bilateral and 3 unilateral. High-resolution computed tomography scan of the chest performed for 12 patients showed evidence of consolidation in 2 of 4 HIV-infected patients and in 5 of 8 non-HIV patients. For 11 patients, the diagnosis of CMV viremia was confirmed during episodes of *P. jirovecii* pneumonia.

Therapy and clinical outcome

Eighty four percent of patients (41/49) were treated with trimethoprim-sulfamethoxazole (TMP-SMZ). The most common dose used was trimethoprim 15-20 mg/kg/day in divided doses. One patient discontinued treatment after 14 days due to acute renal failure. The remaining patients chose hospice care and did not take TMP-SMZ. The patients who received TMP-SMZ treatment were also treated with adjunctive corticos-

Table 2. Underlying diseases of patients with *Pneumocystis jirovecii* pneumonia

Underlying disease	No. of patients
HIV infection	15
Solid organ malignancies	15
Adenocarcinoma of lung	6
Squamous cell carcinoma of lung	2
Breast cancer	3
Pancreatic cancer	1
Angiocarcinoma of spleen	1
Prostate cancer	1
Rectal cancer	1
Hematologic malignancies	13
Acute myelogenous leukemia	4
Acute lymphocytic leukemia	1
Non-Hodgkin's lymphoma	4
Hodgkin's lymphoma	2
Myelodysplastic syndrome	1
Multiple myeloma	1
Others	6
Dermatomyositis	1
COPD with steroid use	1
Type 2 diabetes	1
End-stage renal disease with regular hemodialysis	1
Hypogammaglobulinemia	1
Nephrotic syndrome	1

Abbreviations: HIV = human immunodeficiency virus; COPD = chronic obstructive pulmonary disease

teroids. Of the 49 patients, 18 (36.7%) died within 30 days after *P. jirovecii* pneumonia was diagnosed. Among the patients without HIV, 17 of 34 (50%) died, compared with 1 of 15 (6.7%) who died among the HIV-positive patients (p=0.004).

Discussion

It is well known that *P. jirovecii* pneumonia is common in patients with AIDS [4]. In contrast, *P. jirovecii* has been considered as an infrequent event among patients with solid tumor or hematological malignancies in the past [5]. However, an increase in the incidence of *P. jirovecii* pneumonia in patients without HIV infection, but with neoplastic disease and connective tissue diseases has been observed in the past decade [6-8]. Explanations for the increase in the diagnosis of *P. jirovecii* pneumonia include improved diagnostic techniques, more types and doses of immunosuppressive agents used for patients with malignancies and connective tissue diseases, and alertness of physicians.

The use of PCR has resulted in increased detection of *P. jirovecii* from BAL fluid, induced sputum, and expectorated sputum [9]. The PCR technique can be used for a large number of samples at one time, and results can be obtained within a day, without any subjective bias, which may occur for microscope examinations. This avoids false-positive results caused by related fungi. DNA amplification on induced sputum will offer a powerful technique for diagnosis and epidemiological study of *P. jirovecii* pneumonia, thereby reducing the need for obtaining BAL by bronchoscopy [1].

CD4+ T-lymphocyte count <200 cells/μL is a well-known risk factor for *P. jirovecii* pneumonia, and initiating chemoprophylaxis helps to decrease its incidence [10]. In this study, 3 patients with solid organ malignancies showed CD4+ T-lymphocyte counts <200 cells/μL (median, 77 cells/μL; range, 17-177 cells/μL). The CD4+ T-lymphocyte counts were significantly lower in patients who died (p=0.018). More studies to establish the relationship between the CD4+ T-lymphocyte count and *P. jirovecii* pneumonia infection in non-HIV patients may help to establish the indications for prophylaxis and reduce the associated morbidity and mortality.

Fever, dyspnea, and tachycardia remain the main clinical symptoms of *P. jirovecii* infection. It is known that the prodrome duration of *P. jirovecii* infection is longer and the symptoms milder in HIV-positive patients, despite a higher fungal load [11]. In contrast, immunocompromised HIV-negative patients with *P. jirovecii* pneumonia may progress rapidly to respiratory failure [12].

Treatment of *P. jirovecii* pneumonia has changed little since the 1970s [13]. In this study, the treatment of choice was TMP-SMZ for both HIV-positive and HIV-negative patients. There were no severe side effects associated with the use of TMP-SMZ in these patients, except for 1 patient who had acute renal failure during the therapy.

P. jirovecii has been reported as a fungal opportunistic pathogen causing serious pneumonia in patients with immunodeficiency. *P. jirovecii* pneumonia was one of the leading causes of morbidity and mortality among HIV-infected patients in the 1980s. Studies in animals and humans favor an airborne route of transmission [14]. Although the diagnostic procedures have improved over the years, the outcome among patients with *P. jirovecii* pneumonia who do not have HIV infection has improved little: only 40% to 70% survive. In the 1970s, Hughes et al [15] reported a 68% survival rate among children with acute lymphoblastic leukemia, and Walzer et al [16] found a 42% survival

rate among all reported patients in the United States. Throughout 3 decades of reports from the Memorial Sloan-Kettering Cancer Center, the survival rate has been around 50% [17]. In our study, the mortality rate of *P. jirovecii* pneumonia in patients with HIV infection was significantly lower than in patients without HIV infection (6.7% vs 50%, p=0.004). According to the attending physicians' judgments, 5 mortality cases of non-HIV patients were associated with *P. jirovecii* pneumonia. However, our study has some limitations due to its retrospective nature and the relatively small number of cases. These limitations hindered analysis of risk factors predicting mortality.

These data suggest that *P. jirovecii* pneumonia remains a serious illness with high mortality among non-HIV-infected patients. In contrast, with *P. jirovecii* pneumonia in HIV patients, the introduction of highly-active antiretroviral therapy and adjunctive corticosteroid therapy has reduced the mortality rate. Since the application of bronchoscopy in patients with severe pneumonia and in a hypoxic state was not practical in Taiwan, PCR of sputum provides a promising non-invasive diagnostic test for *P. jirovecii*. To refine therapeutic strategies and improve the prognosis of *P. jirovecii* pneumonia, attention must be paid to the diagnosis of *P. jirovecii* infection in immunocompromised patients with respiratory symptoms. Alertness with regard to early diagnosis of *P. jirovecii* pneumonia in HIV-negative immunosuppressed patients with the application of nested PCR may improve patient outcomes in this setting.

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