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ORIGINAL ARTICLE

**Noriko Iikuni · Mariko Kitahama · Shuji Ohta
Hiroshi Okamoto · Naoyuki Kamatani
Makoto Nishinarita**

Evaluation of *Pneumocystis* pneumonia infection risk factors in patients with connective tissue disease

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Abstract We conducted a retrospective, clinical evaluation of connective tissue disease (CTD) patients who were tested for either sputum or bronchoalveolar lavage fluid *Pneumocystis* polymerase chain reaction (*PC*-PCR) and analyzed the risk factors that cause *Pneumocystis* pneumonia (PCP) susceptibility and fatality. *PC*-PCR was performed on 66 CTD patients who presented with symptoms, data, or radiological findings strongly suggesting respiratory infection. Patients with higher oral corticosteroid doses, use of oral methotrexate (MTX), bilateral lung findings, positive β-D-glucan, and no prophylaxis use were more susceptible to PCP. They had significantly low immunoglobulin G and significantly high β-D-glucan and lactate dehydrogenase. Survivors and nonsurvivors of PCP were also evaluated. Poor prognoses were observed with older age, elevated β-D-glucan, rheumatoid arthritis (RA) patients using MTX, hypoxemia, bilateral lung findings, and mechanical ventilation use. Nonsurvivors had significantly lower lymphocytes, oxygen saturation, and significantly higher β-D-glucan. In RA, poor prognoses were seen with those taking MTX. Disease duration, underlying pulmonary complications, and oral corticosteroid doses did not lead to poor prognoses in RA. Because PCP in CTD leads to abrupt onset of symptoms with poor survival rates, early diagnosis and initiation of treatment are critical, and it is essential for clinicians to recognize risk factors that predispose patients to PCP and its mortality.

Key words Connective tissue disease (CTD) · Opportunistic infection · *Pneumocystis* infection · Rheumatic disease

N. Iikuni (✉) · M. Kitahama · H. Okamoto · N. Kamatani
Institute of Rheumatology, Tokyo Women's Medical University, 10-22 Kawada-cho, Shinjuku-ku, Tokyo 162-0054, Japan
Tel. +81-3-5269-1725; Fax +81-3-5269-1726
e-mail: iikuni@ior.twmu.ac.jp

S. Ohta · M. Nishinarita
Department of Rheumatology, Taga General Hospital, Hitachi, Japan

Introduction

Pneumocystis pneumonia (PCP) remains the dominant opportunistic infection that causes morbidity and mortality in patients with impaired immune system; and in patients with connective tissue disease (CTD), PCP is more rapid in onset and is usually more severe, often leading to death. Clinical manifestations of PCP often mimic those of pulmonary complications caused by rheumatologic diseases, as well as drug-induced lung injuries, further causing a dilemma in the diagnosis of the cause of the event. Therefore, early diagnosis and treatment become crucial to the prognosis of this disease, and understanding the risk factors of infection may allow for initiation of prophylaxis, earlier suspicion, as well as commencement of treatment.

In this study we analyzed patients at our institution who presented with a suspicion of respiratory infection and who underwent sputum or bronchoalveolar fluid (BALF) *Pneumocystis* polymerase chain reaction (*PC*-PCR) tests. These patients were further analyzed according to *PC*-PCR results, factors controlling susceptibility, prognosis, and fatality of *Pneumocystis* infection. We also investigated *PC*-PCR positive rheumatoid arthritis (RA) patients, for they constituted the largest group of PCR-positive patients.

Patients and methods

We evaluated clinical backgrounds, disease course, and immunosuppressive conditions of both outpatients and hospitalized patients that had sputum or BALF tested for *Pneumocystis* infection using *PC*-PCR from October 2000 to February 2004 in the Department of Rheumatology, Taga General Hospital, Hitachi, Japan. At the time of testing, all patients had respiratory symptoms, radiographic readings, and/or laboratory data indicating respiratory infection and a suspected diagnosis of *Pneumocystis* pneumonia. A total of 66 (22 men, 44 women, mean age 65 years) patients were tested for sputum or BALF *PC*-PCR.

For PCR, the primers were 5S sense (5′-AGTTACGGC CATACCTCAGA-3′) and 5S antisense (5′-AAAGCTA CAGCACGTCGTAT-3′), generating a 120-bp product. Denaturation was at 94°C for 1.5 min with annealing at 55°C for 2.5 min and extension at 72°C for 1.9 min; amplification was done by 25 or 40 cycles, as described by Kitada et al.[1]

Each patient was evaluated for duration of disease, coexisting pulmonary conditions, prednisolone dose at the time of *PC*-PCR testing, total amount of prednisolone dose, previous glucocorticoid pulse therapy, intravenous cyclophosphamide therapy, other immunosuppressive agents, symptoms and radiological findings at the time of *PC*-PCR testing, and prophylaxis for *Pneumocystis* infection. For RA patients, the use of methotrexate (MTX) and other disease-modifying antirheumatic drugs (DMARDs) were noted.

Statistical analysis

Data are expressed as mean ± standard deviation. Differences between pairs of groups were analyzed for statistical significance with the Mann–Whitney *U*-test. A *P* value of less than 0.05 denoted a statistically significant difference.

Results

Of the 66 patients tested for *PC*-PCR, 21 had positive (7 men, 14 women; mean age 64.8 years) and 45 negative *PC*-PCR (15 men, 30 women, mean age 65.2 years) (Table 1). Because *PC*-PCR is a reliable diagnostic tool of PCP in connective tissue disease patients,[2] the presence of respiratory symptoms and/or radiological findings along with a positive *PC*-PCR were considered PCP. In both positive and negative groups, RA was the most abundant CTD (57% and 62%, respectively), followed by systemic lupus erythematosus. For RA patients, there was a higher risk of PCP with the use of low-dose MTX, but the use of other DMARDs had no effect on *Pneumocystis* infection. Of the 21 PCP patients, 16 (76.2%) had symptoms such as dyspnea, fever, cough, and sputum. Fifteen (71.4%) of the PCP patients had radiographic indications of respiratory infection, and all had abnormal readings on bilateral lung fields. A significant difference in the average β-D-glucan reading for PCP was observed ($P < 0.005$). Positive β-D-glucan was observed in 77.8% of PCP vs 23.0% in the negative PCR group. Significant differences were observed in serum immunoglobulin G (IgG) ($P < 0.01$), lactate dehydrogenase (LDH) ($P < 0.005$), and β-D-glucan ($P < 0.005$). No significant differences between PCP patients and negative PCR patients were seen with lymphocyte counts, CD4+ lymphocyte counts, KL-6, serum albumin, and pulse oximetry. None of the PCP patients had ever taken any prophylaxis against *Pneumocystis* infection, while 24.4% of negative *PC*-PCR patients were taking prophylaxis.

Survivors and nonsurvivors of PCP were compared (Table 2). Nonsurvivors were older, although this difference was not significant. RA made up for most of the cases for both survivors and nonsurvivors. Six out of the seven fatal cases occurred with RA patients (one was primary Sjögren's syndrome). Duration of disease, existing pulmonary complications, average oral prednisolone, total cumulative average prednisolone, past corticosteroid pulse therapy, past cyclophosphamide i.v. therapy, and use of other immunosuppressants or DMARDs were either no different in the two groups or were higher in the surviving group, and were not factors of PCP fatality. Nonsteroidal anti-inflammatory drugs (NSAIDs) were used in all nonsurviving cases. The number of patients using MTX was higher in the nonsurvivors (83.3% vs 50.0%). However, average MTX dose was lower in the nonsurviving group. Symptoms and radiological findings in bilateral lungs were seen in all fatal cases. Six of the seven fatal cases were intubated and under mechanical respiratory support, while none of the survivors were intubated. Significant differences were observed between survivors and nonsurvivors for lymphocytes ($P < 0.005$), β-D-glucan ($P < 0.005$), and pulse oximetry ($P < 0.05$). No significant differences were observed between the two groups for CD4+ lymphocytes, IgG, KL-6, LDH, and serum albumin.

Most PCP cases were RA patients and therefore, they were evaluated independently and survivors and nonsurvivors compared (Table 3). The age of nonsurvivors was slightly higher, but not significant. The length of disease duration, the existence of pulmonary complications, average oral prednisolone dose, total cumulative average prednisolone dose, past corticosteroid pulse therapy, past cyclophosphamide i.v. therapy, use of other immunosuppressants and other DMARDs all did not have any effect on the fatality of RA patients. NSAIDs were used equally in both groups. Fatal cases consisted of more RA patients using MTX; however, average MTX dose per week was higher in the surviving group. All nonsurvivors had radiological findings of PCP in bilateral lung fields. Lymphocytes and CD4+ counts were significantly reduced in nonsurviving cases and β-D-glucan was significantly elevated. No significant differences were observed between the two groups in IgG, KL-6, and pulse oximetry readings.

The trends in laboratory data for RA patients were evaluated at half a year before onset of the respiratory episode, just prior to onset (approximately 2–4 weeks), and at the time of onset to see if these variables had any predicative values (Fig. 1). Fatal cases had decreases in lymphocytes, IgG, and serum albumin across time, suggesting a possibility that these may allow for early recognition of fatality. Sharp increases were observed for KL-6 and LDH at the time of onset and therefore, these were not useful as predicative values. Not all values for KL-6 were available. CD4+ lymphocyte counts at half a year before the event and just prior to the event were unavailable for most of the cases because CD4+ counts are not frequently measured.

Table 1. Positive *PC*-PCR vs. Negative *PC*-PCR

	Positive (n = 21) *PC*-PCR	Negative (n = 45) *PC*-PCR	
Sex	7 males, 14 females	15 males, 30 females	
Age (years)	64.8 ± 12.4 (31–87)	65.2 ± 9.3 (47–85)	
Disease	RA 12, SLE 2, MCTD 1, SSc 1, DM 1, MRA 1, PSjs 1, BD 1, RPC 1, CSS 1	RA 28, SLE 6, MCTD 2, SSc 2, DM 1, MRA 1, SSjs 7, BD 2, MPA 2, CD 1	
Duration of disease (months)	93.1 + 84.1 (4–312)	129.8 + 140.7(1–504)	
Pulmonary complications	61.9% (13/21)	62.2% (28/45)	
Average oral prednisolone dose (mg/day)	19.6 ± 11.1	15.2 ± 22.0	
Total average prednisolone (mg)	15177.1 ± 16872.6	20886.9 ± 34522.0	
Past corticosteroid pulse therapy	14.3% (3/21)	17.8% (8/45)	
Past cyclophosphamide i.v. therapy	19.0% (4/21)	13.3% (6/45)	
Other immunosuppressants			
Mizoribine	4.8% (1/21)	13.3% (6/45)	
Oral cyclophosphamide	0	2.2% (1/45)	
Cyclosporine A	0	2.2% (1/45)	
NSAIDs	71.4% (15/21)	68.2% (30/44)	
No. of RA patients taking MTX	66.7% (8/12)	10.7% (3/28)	
Average MTX (mg/wk)	6.4	4	
RA patients taking other DMARDs	75.0% (9/12)	82.1% (23/28)	
Symptoms at the time of onset (dyspnea, fever, cough, sputum)	76.2% (16/21)	80.0% (36/45)	
Radiological findings at time of onset			
Lateral lung field	0	42.2% (19/45)	
Bilateral lung fields	71.4% (15/21)	20.0% (9/45)	
No. of positive β-D-glucan	77.8% (14/18)	23.1% (6/26)	
No. of cases using prophylaxis for	0	24.4% (11/45)	
Pneumocystis infection			
Laboratory data			
WBC (/mm^3)	7934.8 ± 2514.6	8281.1 ± 4418.6	NS
Lymphocytes (/mm^3)	1004.7 ± 791.9	1167.6 ± 636.6	NS
CD4+ count (/mm^3)	435.1 ± 379.2	421.2 ± 310.3	NS
IgG (mg/dl)	818.7 ± 249.0	1267.5 ± 695.3	$P < 0.01$
β-D-glucan (pg/ml)	87.9 ± 100.3	32.3 ± 80.4	$P < 0.005$
KL-6 (IU/ml)	754.6 ± 582.0	791.6 ± 976.2	NS
LDH (IU/l)	369.5 ± 151.3	269.8 ± 172.1	$P < 0.005$
Serum albumin (g/dl)	3.2 ± 0.5	3.2 ± 0.7	NS
SaO_2 (%)	89.1 ± 8.2	92.1 ± 4.8	NS

RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; MCTD, mixed connective tissue disease; SSc, systemic sclerosis; DM, dermatomyositis; MRA, malignant RA; Sjs, Sjögren's syndrome; BD, Behcet's disease; MPA, microscopic polyangitis; RPC, relapsing polychondritis; CSS, Churg–Strauss syndrome; CD, Castleman's disease; NSAIDs, nonsteroidal anti-inflammatory drugs; MTX, methotrexate; DMARDs, disease-modifying antirheumatic drugs; WBC, white blood cells; IgG, immunoglobulin G; LDH, lactate dehydrogenase; NS, not significant
Pulmonary complications: interstitial pneumonia, organizing pneumonia, diffuse panbronchiolitis, pleural effusion, emphysema, bronchial asthma, lung tuberculosis, lung cancer

Table 2. Patient characteristics of *Pneumocystis* pneumonia survivors vs. nonsurvivors

	Survivors (n = 14)	Nonsurvivors (n = 7)	
Sex	6 males, 8 females	1 male, 6 females	
Age (years)	62.4 ± 12.5 (31–84)	69.6 ± 10.6 (51–87)	
Disease	RA 6, SLE 2, MCTD 1, SSc 1, DM 1, MPA 1, BD 1, RPC 1, CSS 1	RA 6, PSjs 1	
Duration of disease (months)	92.9 ± 78.0 (5–264)	93.1 ± 95.2 (4–312)	
Pulmonary complications	64.3% (9/14)	57.1% (4/7)	
Average oral prednisolone dose (mg/day)	21.6 ± 11.6	15.6 ± 9.0	
Total average prednisolone (mg)	19212.2 ± 18786.8	7106.9 ± 7104	
Past corticosteroid i.v. therapy	14.2% (2/14)	14.3% (1/7)	
Past i.v. CY therapy	28.6% (4/14)	0	
Other drug use			
Mizoribine	7.1% (1/14)	0	
NSAIDs	57.1% (8/14)	100% (7/7)	
RA patients taking	50.0% (3/6)	83.3% (5/6)	
MTX			
Average MTX (mg/week)	7.3	5.8	
RA patients on other	100% (6/6)	50.0% (3/6)	
DMARDs			
Respiratory symptoms	64.3% (9/14)	100% (7/7)	
Chest X-ray			
Bilateral lung fields	57.1% (8/14)	100% (7/7)	
Positive β-D-glucan	63.6% (7/11)	100% (7/7)	
Patients mechanically ventilated	0	85.7% (6/7)	
Laboratory data			
WBC (/mm^3)	7723.6 ± 1788.2	8357.1 ± 3507.9	NS
Lymphocytes (/mm^3)	1303.6 ± 786.2	406.7 ± 329.8	$P < 0.005$
CD4+ count (/mm^3)	542.7 ± 402.5	198.4 ± 149.1	NS
IgG (mg/dl)	862.7 ± 267.0	736.9 ± 185.7	NS
β-D-glucan (pg/ml)	33.4 ± 41.9	173.7 ± 105.2	$P < 0.005$
KL-6 (IU/ml)	609.7 ± 387.7	1044.5 ± 767.8	NS
LDH (IU/l)	354.3 ± 151.1	400.0 ± 146.9	NS
Serum albumin (g/dl)	3.4 ± 0.4	3.0 ± 0.5	NS
SaO_2 (%)	91.7 ± 7.5	84.0 ± 6.7	$P < 0.05$

RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; MCTD, mixed connective tissue disease; SSc, systemic sclerosis; DM, dermatomyositis; MRA, malignant RA; Sjs, Sjögren's syndrome; BD, Behcet's disease; MPA, microscopic polyangitis; RPC, relapsing polychondritis; CSS, Churg–Strauss syndrome; CY, cyclophosphamide; NSAIDs, nonsteroidal anti-inflammatory drugs; MTX, methotrexate; DMARDs, disease-modifying antirheumatic drugs; WBC, white blood cells; IgG, immunoglobulin G; LDH, lactate dehydrogenase; NS, not significant
Pulmonary complications: interstitial pneumonia, organizing pneumonia, diffuse panbronchiolitis, pleural effusion, emphysema, bronchial asthma, lung tuberculosis, lung cancer

Discussion

Pulmonary complications are a frequent cause of morbidity and mortality in immunocompromised patients, and *Pneumocystis* pneumonia is one of the major life-threatening opportunistic infections. Unlike the subacute presentation of PCP during AIDS, PCP in other non-AIDS patients entails a fulminate onset that may correlate either with the tapering of immunosuppressants or an increase in the dosage of immunosuppressants. Despite the availability of effective medications, the mortality of PCP for the non-HIV

Table 3. Rheumatoid arthritis (RA) patients with *Pneumocystis* pneumonia

	Survivors ($n = 6$)	Nonsurvivors ($n = 6$)	
Sex	3 males, 3 females	1 male, 5 females	
Age (years)	64.7 ± 8.8 (48–74)	66.7 ± 8.5 (51–76)	
RA duration (months)	122.0 ± 78.8 (39–264)	96.7 ± 102.4 (4–312)	
Pulmonary complications	83.3% (5/6)	50.0% (3/6)	
Average oral prednisolone dose (mg/day)	18.5 ± 14.4	13.3 ± 7.4	
Total average prednisolone (mg)	15405.5 ± 12862.7	8074.3 ± 7234.1	
Corticosteroid i.v. therapy	16.7% (1/6)	0	
Cyclophosphamide i.v. therapy	33.3% (2/6)	0	
Other drug use			
Mizoribine	16.7% (1/6)	0	
NSAIDs	100% (6/6)	100% (6/6)	
RA patients taking MTX	50.0% (3/6)	66.7% (4/6)	
Average MTX (mg/week)	7.3	5.8	
RA patients on other DMARDs	100% (6/6)	50.0% (3/6)	
Chest X-ray			
Lateral lung field	33.3% (2/6)	0	
Bilateral lung fields	33.3% (2/6)	100% (6/6)	
Lab data at the time of PCP onset			
Lymphocytes (/mm^3)	1407.3 ± 873.0	438.9 ± 345.9	$P < 0.05$
CD4+ count (/mm^3)	593.0 ± 291.4	198.4 ± 149.1	$P < 0.05$
IgG (mg/dl)	830.2 ± 277.9	768.3 ± 182.5	NS
β-D-glucan (pg/ml)	13.8 ± 14.2	161.6 ± 109.0	$P < 0.005$
KL-6 (IU/ml)	627.4 ± 305.5	1044.5 ± 767.8	NS
SaO_2 (%)	91.2 ± 9.5	85.3 ± 6.6	NS

NSAIDs, nonsteroidal anti-inflammatory drugs; MTX, methotrexate; DMARDs, disease-modifying antirheumatic drugs; IgG, immunoglobulin G; NS, not significant

population remains 34%–58%,[3–9] and the outcome of PCP in non-AIDS patients is generally worse than in AIDS patients.

Pneumocystis pneumonia occurs in 1%–2% of all patients with connective tissue disorders,[10] but the exact mechanism of different host responses is still unknown. Owing to the difference in neutrophil numbers of BALF obtained from HIV and non-HIV PCP patients, some suggest that non-HIV patients have higher rates of complication and death, paradoxically, because they have a superior capacity for inflammation.[8,11] Even with its rash and severe course in non-HIV patients, there are no established guidelines for the prevention of *Pneumocystis* infection in these patients. By evaluating connective tissue disease patients that were suspected of PCP, we were able to define some of the risk factors that predispose patients to infection and lead to fatal outcomes. In this study, we utilized *PC*-PCR which is a useful, non-invasive diagnostic tool in patients with connective tissue diseases suspected of having PCP based on the presence of progressive arterial hypoxemia and radiological findings.[2]

Well-cited predisposing factors of PCP are long courses of corticosteroid use, high doses of corticosteroid use, and tapering of corticosteroids.[3,6,9–11,12–14] Our data did not concur with these previous reports, and the total cumulative average corticosteroid dose was not a contributing factor to PCP. Because of the large variability in duration and cumulative dose of immunosuppressive agents, predisposition to PCP cannot be predicted by the total amount of previous immunosuppressive treatment alone.[15] Patients with connective tissue disease are known to have impaired humoral and cellular immunity,[16] and therefore, *Pneumocystis* infection may occur even when patients are not receiving immunosuppressive therapy. Our study supports the view that corticosteroids and immunosuppressants are not necessarily required for causing PCP.

The agent that revealed a noteworthy difference in patient outcome was MTX. Methotrexate is known to impair

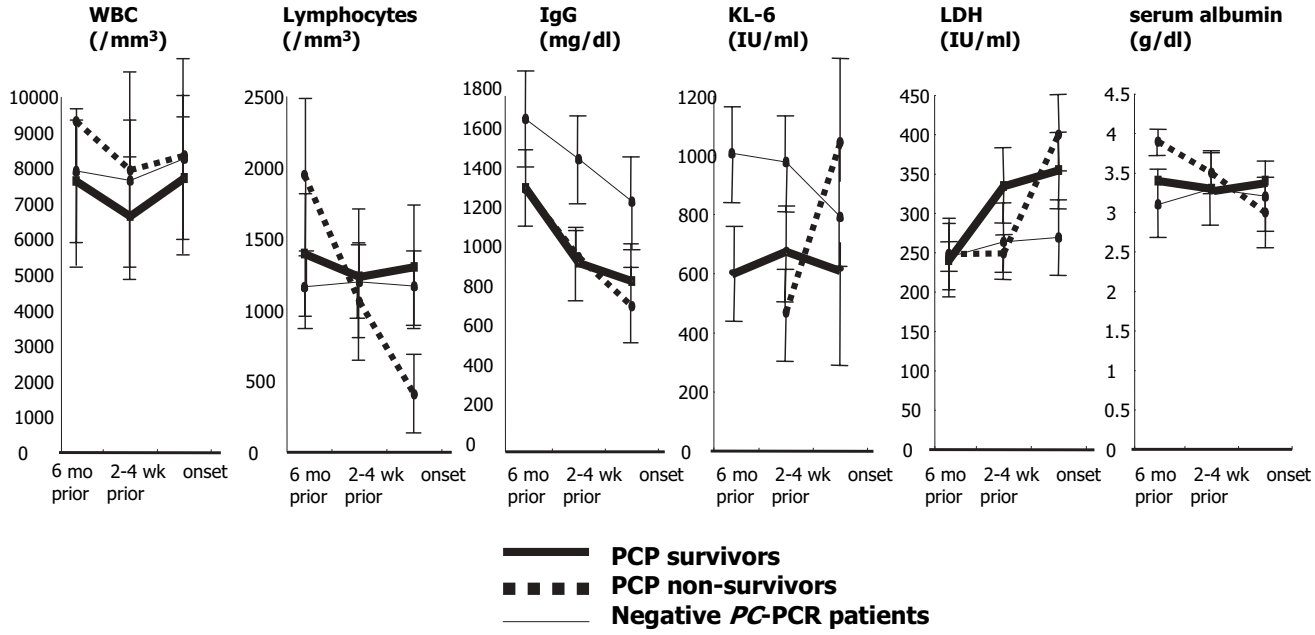

Fig. 1. Changes across time in *Pneumocystis* pneumonia (PCP) survivors, PCP nonsurvivors, and negative *Pneumocystis* polymerase chain reaction (*PC*-PCR) patients. Data for white blood cells (*WBC*), lymphocytes, immunoglobulin G (*IgG*), KL-6, lactate dehydrogenase (*LDH*), and serum albumin at 6 months prior to the respiratory event, 2–4 weeks prior to the event, and at the time of onset are shown for the three groups. *Bars* denote standard deviation

cellular immunity, and reports of PCP associated with RA patients on low-dose MTX is not uncommon.[17–19] One must also keep in mind that patients administered low-dose MTX are equally susceptible to MTX-induced pneumonitis, where symptoms and radiographic findings are often difficult to discriminate, and a number of our patients could have simultaneously suffered from lung injury due to MTX.

Certain data may define the outcome of PCP patients. A helpful laboratory tool was β-D-glucan, which was detected in most of the patients with positive PCR, and levels of β-D-glucan were elevated in these subjects as well. All fatal cases had positive β-D-glucan and serum levels were significantly high. β-D-Glucan may serve as an indication for the severity of PCP as indicated in previous studies,[2] and may aid in predicting the prognosis of PCP patients. KL-6 and LDH were also greatly increased at the onset of PCP, especially in fatal cases. However, these variables are nonspecific and they may only be useful in expressing the severity of lung injury. Moreover, these values increase only once lung injury occurs and therefore do not serve as predictors of patients susceptible to infection or their prognosis, and they also do not serve as markers as to when to start prophylaxis.

Hypoalbuminemia has been reported as a factor of poor prognosis in PCP,[4] although we could not find a direct correlation between serum albumin level and PCP. It is difficult to say whether low albumin was a factor that contributed in causing infection or whether it was a result of PCP. When a severe pulmonary event occurs, such as PCP, there is a possibility that lung alveolar permeability increases, allowing an outflow of serum albumin towards alveolar space of the lungs, resulting in hypoalbuminemia.

Although not significant, poor prognosis was seen with higher age. It has been reported that there is an age-related decline in T-cell function; the memory T cells include a spectrum of T cells with normal function and hypofunctioning T cells, suggesting that old age may be a risk factor for the occurrence of PCP even when CD4+ counts may not be decreased.[20]

There is an emphasis of the importance of lymphocyte depletion predisposing to PCP, in which lymphocytopenia and a reduction of CD4+ lymphocytes prior to PCP are risk factors for infection.[9] Therefore, the trends of patient data across time, rather than the data at the time of infection, are important when considering predisposition of PCP. Past reports suggest CD4+counts <300 cells/mm^3 as the initiation of chemoprophylaxis in non-HIV infection;[7] lymphocyte count <400/mm^3 for lupus;[21] lymphocyte count <400/mm^3 and serum IgG <1000 mg/dl for RA.[2] However, when analyzing changes across time for our patients, lymphocyte counts less than 1500/mm^3 and serum IgG levels less than 1000 mg/dl already posed possible threats of *Pneumocystis* infection (Fig. 1). Rheumatoid arthritis patients are usually not targets for prophylaxis use, and when these patients are subjected to PCP, the outcomes are severe. We propose prophylaxis use at these data points, especially in RA patients on low-dose MTX. Prophylaxis proved to be 100% effective in our study, as well as in other studies.[7] Although the use of trimethoprim–sulfamethoxazole (TMP-SMX) for prophylaxis in patients on MTX must be done cautiously because of toxicity, we experienced very few cases with toxicity due to TMP-SMX, and aerolized pentamidine was equally effective as an alternative prophylactic agent. In addition, prophylaxis use may allow for differentiation between PCP and drug-induced pneumonitis in RA patients.

The number of non-HIV PCP cases has increased, and the need for hospitalization, intensive care unit admission,

intubation, and overall mortality rates remain high. With the availability of newer immunosuppressive drugs for RA, such as biological agents, cases of PCP may further increase in these patients and precautions must be taken.[22–24] Recognition of risk factors, careful monitoring of data, and the appropriate initiation and management of prophylaxis will no doubt lead to effective prevention of PCP in connective tissue diseases.

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