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【 ORIGINAL ARTICLE 】

Factors Associated with *Pneumocystis jirovecii* Pneumonia in Patients with Rheumatoid Arthritis Receiving Methotrexate: A Single-center Retrospective Study

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Abstract:

Objective To investigate the risk factors for the development of *Pneumocystis jirovecii* pneumonia (PCP) in patients with rheumatoid arthritis (RA) undergoing methotrexate (MTX) therapy.

Methods This single-center retrospective cohort study included consecutive patients with RA who received MTX for at least one year. The study population was divided into PCP and non-PCP groups, depending on the development of PCP, and their characteristics were compared. We excluded patients who received biologic disease-modifying anti-rheumatic drugs (DMARDs), Janus kinase inhibitors, and anti-PCP drugs for prophylaxis.

Results Thirteen patients developed PCP, and 333 did not develop PCP. At the initiation of MTX therapy, the PCP group had lower serum albumin levels, a higher frequency of pulmonary disease and administration of DMARDs, and received a higher dosage of prednisolone (PSL) than the non-PCP group. A multivariate Cox regression analysis revealed that the concomitant use of PSL [hazard ratio (HR) 5.50, p=0.003], other DMARDs (HR 5.98, p=0.002), and serum albumin <3.5 mg/dL (HR 4.30, p=0.01) were risk factors for the development of PCP during MTX therapy. Patients with these risk factors had a significantly higher cumulative probability of developing PCP than patients who lacked these risk factors.

Conclusion Clinicians should pay close attention to patients with RA who possess risk factors for the development of PCP during MTX therapy.

Key words: disease-modifying anti-rheumatic drugs, methotrexate, pneumocystis pneumonia, rheumatoid arthritis

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Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by synovitis and structural damage to multiple joints. The treatment modalities for RA have improved dramatically since the advent of biological agents. Methotrexate (MTX) is considered a first-line therapy for the treatment of active RA, and the European League Against Rheumatism (EULAR) established that MTX is an anchor drug for RA management (1). Rheumatologists should consider administering biologic agents in combination with MTX to patients who do not exhibit a good response to MTX therapy.

However, MTX can induce several adverse drug reactions, including bone marrow suppressions, hepatic toxicity, renal toxicity, gastric toxicity, MTX-induced pneumonia, MTX-associated lymphoproliferative disorder, and opportunistic infections with cytomegalovirus infection, herpes zoster, and *Pneumocystis jirovecii* pneumonia (PCP) (2-10). PCP is not an uncommon opportunistic infection in Japan among patients receiving glucocorticoids, immunosuppressants, and biologics for the treatment of RA (9-14). In Japan, mandatory post-marketing surveillance programs reported that 0.18-0.4% of patients with RA who were treated with bi-

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ologics developed PCP (9-11).

One study reported that an age of at least 65 years old, a daily dose of prednisolone (PSL) of at least 6 mg, and the presence of coexisting pulmonary disease were risk factors for the development of PCP in patients with RA who received infliximab (11). Another study reported that concomitant MTX therapy was an independent risk factor for the development of PCP in patients receiving etanercept (14). While some physicians have reported the incidence of PCP during MTX therapy (10, 15), the clinical characteristics and risk factors for PCP in patients with RA treated with MTX have not been elucidated yet.

In the current study, we investigated the clinical characteristics and prognosis of patients with RA who developed PCP during MTX therapy and identified the risk factors for the development of PCP during MTX therapy.

Materials and Methods

Patients

The medical records of consecutive patients who were diagnosed with RA based on the 1987 American College of Rheumatology (ACR) or 2010 ACR/EULAR criteria for RA (16, 17) and underwent treatment at the Department of Rheumatology, Seirei Hamamatsu General Hospital, from January 2004 through October 2017 were reviewed. The medical records included the patients' demographic data, clinical characteristics, comorbidities, immunosuppressive therapy, laboratory data, radiographic data, treatments for PCP, and outcomes of PCP. The demographic data, clinical characteristics, comorbidities, immunosuppressive therapy including disease-modifying anti-rheumatic drugs (DMARDs), laboratory data, and radiographic data were obtained at the initiation of MTX therapy. We excluded patients who received biologic DMARDs, Janus kinase inhibitor, sulfamethoxazole/trimethoprim (SMX/TMP), inhaled pentamidine, or atovaquone for PCP prophylaxis. Patients with a positive human immunodeficiency virus (HIV) test or malignancy were also excluded.

The diagnosis of PCP was modified according to the previously proposed diagnostic criteria for PCP (18-20). Definite PCP was defined as the presence of *Pneumocystis jirovecii* microorganisms in the patients' respiratory samples on microscopic examination or positive results on the *P. jirovecii* DNA polymerase chain reaction (PCR) with respiratory samples and elevated serum 1, 3-β-D-glucan levels above the cut-off value. Presumptive PCP was defined as the presence of clinical manifestations compatible with PCP from either positive results on the *P. jirovecii* DNA PCR test with respiratory samples or serum β-D glucan levels above the cut-off value and a positive response to standard treatment for PCP with TMP/SMX, pentamidine isethionate, or atovaquone. The serum β-D-glucan levels were measured using the FUNGITEC™ G test MK II (Nissui Pharmaceutical, Tokyo, Japan). In the current study, we established 20 pg/mL as the cut-off value to determine abnormal elevations in plasma β-D glucan levels. Chest radiography and/or computed tomography findings were retrospectively evaluated by an expert radiologist (T.M.) who was blinded to the clinical information.

This retrospective study was approved by the institutional review board and ethics committee at Seirei Hamamatsu General Hospital and conducted in accordance with the Declaration of Helsinki and the 2017 Ethical Guidelines for Medical and Health Research Involving Human Subjects in Japan. Written informed consent was waived because of the retrospective design of this study, and information on the right to opt out of the study was presented.

Statistical analyses

We used Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables to perform comparisons between the two groups. The Cox proportional-hazards regression model was used to identify the risk factors for PCP. The cumulative probability for developing PCP with respect to the number of risk factors was calculated using the Kaplan-Meier method, and comparisons between the groups were performed using the log-rank test with Bonferroni correction.

All statistical analyses were performed using the EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) (21).

Results

Patient demographics

During the study period, 802 patients who were diagnosed with RA received MTX, and 346 of these patients were included in this study. Their demographic data are presented in Fig. 1. We identified the presence of PCP in 13 patients with RA during MTX therapy and compared these patients to 333 without PCP, all of whom were recruited from amongst consecutive patients with RA who received MTX for at least 1 year.

The diagnosis and clinical characteristics in PCP patients with RA receiving MTX

The demographics and treatment at the onset of PCP in patients with RA receiving MTX therapy are presented in Table 1. The median age in the PCP group was 69 years old, and 76.9% of patients were women. The median duration of RA was 7.3 years, and the median duration of MTX therapy was 60 (range 8-562) weeks. The median dosages of MTX and PSL were 10 (range 6-14) mg/week and 4 (range 0-30) mg/day, respectively. Two patients received iguratimod, and one patient received salazosulfapyridine in combination with MTX. Six patients had pulmonary comorbidities, including interstitial pneumonia (n=4), and chronic ob-

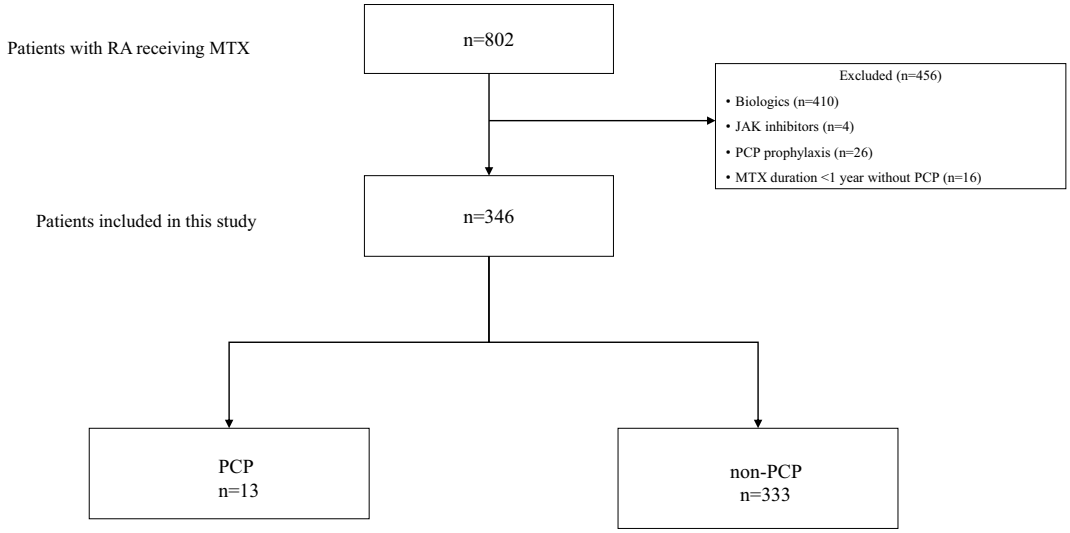

Figure 1. **Patient Demographics. JAK: Janus kinase, MTX: methotrexate, PCP: *Pneumocystis jirovecii* pneumonia, RA: rheumatoid arthritis**

Table 1. **Demographics and Treatment at the Onset of PCP in Patients with RA Receiving MTX Therapy.**

Patient	Age/ Sex	Disease duration (year)	MTX duration (week)	PSL (mg/day)	Other DMARDs	Pulmonary disease	DM	WBC ($\times10^3/\mu L$)	Lymphocyte counts ($\times10^3/\mu L$)
1	81/F	44.3	15.1	0	IGU	COPD	-	11,190	1,488
2	69/F	49.9	19.3	5	-	-	-	12,090	2,310
3	72/F	13.5	103	4	SASP	-	-	19,360	1,181
4	69/F	6.8	246	4	-	-	-	6,330	1,070
5	36/F	0.3	15.9	8	-	-	-	6,510	0,660
6	66/F	7.3	284	2	-	-	-	4,850	0,330
7	72/F	0.4	19.4	3	-	IP	-	4,590	0,670
8	63/F	10.3	318	0	-	-	-	8,640	1,450
9	77/M	0.2	8.0	10	-	IP	-	11,480	1,460
10	78/F	2.6	60.0	0	-	-	-	7,920	0,768
11	59/F	11.0	562	30	IGU	COPD	-	10,480	1,370
12	65/M	1.3	24.6	0	-	IP	+	13,760	1,700
13	79/M	13.5	155	5	-	IP	-	6,830	0,847

COPD: chronic obstructive pulmonary disease, DM: diabetes mellitus, DMARDs: disease modifying anti-rheumatic drugs, F: female, IGU: iguratimod, IP: interstitial pneumonia, M: male, MTX: methotrexate, PCP: *Pneumocystis jirovecii* pneumonia, PSL: prednisolone, RA: rheumatoid arthritis, SASP: salazosulfapyridine, WBC: white blood cell

structive pulmonary disease (n=2). One patient had diabetes mellitus. The serum lymphocyte counts during MTX therapy are depicted in Table 2. The median serum lymphocyte counts at the last observation before the onset of PCP and those at the onset of PCP were lower than those at baseline.

Laboratory data of the PCP group

The laboratory data at the onset of PCP are summarized in Table 3. Five of the 13 patients had definitive PCP, and the other 8 had presumptive PCP. The median serum C-reactive protein (CRP) and lactate dehydrogenase (LDH) levels were 10.5 mg/dL and 323 U/L, respectively. Twelve patients tested positive for serum β-D glucan, and the median β-D glucan level was 73.3 pg/mL. The PCR test for *P. jirovecii* was performed in 11 patients, 5 of whom were positive. Although two patients underwent bronchoscopy, *P. jirovecii* microorganisms in their respiratory samples were negative (Patients 3 and 12).

Treatment and clinical course of the PCP group

The treatment and clinical course of the PCP group are summarized in Table 3. All patients with PCP who were included in this study underwent hospitalization. Eleven patients required oxygen supplementation, one of whom developed respiratory failure and required ventilatory support. The median maximum PSL dose was 80 mg, and 3 patients received methyl-PSL pulse therapy. All patients received SMX/TMP treatment and responded to the treatment; however, it was only continued in two patients. Ten patients required a switch to other PCP drugs, and one required a re-

Table 2. Changing the Serum Lymphocyte Counts during MTX Therapy in Patients with RA who Developed PCP.

Patient	Lymphocyte counts before MTX therapy ($\times 10^3/\mu L$)	Lymphocyte counts at last observation before the onset of PCP ($\times 10^3/\mu L$)	Lymphocyte counts at the onset of PCP ($\times 10^3/\mu L$)
1	1,470	0,840	1,488
2	2,090	0,990	2,310
3	0,842	1,578	1,181
4	1,553	1,619	1,070
5	0,890	0,590	0,660
6	1,148	0,900	0,330
7	2,200	0,680	0,670
8	1,560	2,110	1,450
9	2,390	1,690	1,460
10	1,879	2,410	0,768
11	1,788	0,470	1,370
12	2,314	2,095	1,700
13	1,730	0,846	0,847
Median (range)	1,730 (0,842-2,390)	0,945 (0,470-2,110)	1,181 (0,330-2,310)

MTX: methotrexate, PCP: *Pneumocystis jirovecii* pneumonia, RA: rheumatoid arthritis

Table 3. Clinical Characteristics at the Onset of PCP and Treatment Outcome in Patients with RA with MTX.

Patient	Clinical symptoms	Criteria for PCP	CRP (mg/dL)	LDH (U/L)	β-D glucan (pg/mL)	PCR test	PaO_2 (Torr) [O_2 (L/min)][a]	Oxygen supplementation	mPSL pulse	Maximal PSL dosage (mg/day)	Ventilation support or ICU admission	Recovery of PCP	MTX Restart	Relapse of PCP Within year
1	Fever/ cough/ dyspnea	P	19.1	362	83.6	-	79.6 [2]	+	-	80	-	+	+	-
2	Fever/ cough/ dyspnea	P	18.9	465	229	-	29.1 [0]	+	+	100	-	+	-	-
3	Fever/ cough	P	8.7	323	23.6	-[b]	55.7 [0]	+	-	80	-	+	+	-
4	Fever	D	5.3	301	209	+	64.0 [0]	+	-	80	-	+	+	-
5	Fever/ cough/ dyspnea	D	4.9	223	31.2	+	70.6 [9]	+	+	80	+	+	-	-
6	Cough/ dyspnea	P	14.7	320	54.3	-	44.4 [0]	+	-	80	-	+	+	-
7	Fever/ cough/ dyspnea	D	13.4	432	288	+	58.2 [0]	+	+	80	-	+	-	-
8	Fever/ cough	P	15.8	462	105	-	60.5 [0]	+	-	80	-	+	+	-
9	Fever/ cough/ dyspnea	P	6.3	247	73.3	NA	57.2 [0]	+	-	80	-	+	+	-
10	Fever/ dyspnea	P	10.5	419	29.5	-	54.3 [0]	+	-	80	-	+	-	-
11	Fever	P	8.2	428	48.6	NA	50.1 [0]	-	-	30	-	+	-	-
12	Fever/ cough/ dyspnea	D	8.8	308	168	+[b]	67.7 [0]	-	-	30	-	+	+	-
13	Fever	D	22.8	277	11.3	+	51.7[0]	+	-	5	-	+	-	-

CRP: C-reactive protein, D: definitive, LDH: lactate dehydrogenase, ICU: intensive care unit, mPSL: methyl-prednisolone, MTX: methotrexate, NA: not assessed, P: presumptive, PaO_2: partial pressure of arterial oxygen, PCP: *Pneumocystis jirovecii* pneumonia, PCR: polymerase chain reaction, PSL: prednisolone, RA: rheumatoid arthritis

[a]Oxygen therapy at the measurement of PaO_2. [b]*Pneumocystis jirovecii* microscopically detected in bronchoalveolar-lavage fluid.

Table 4. Clinical Characteristics at the Initiation of the MTX.

Clinical characteristics	PCP group (n=13)	non-PCP group (n=333)	p value
Age (years old)	68.0 [36.0, 81.0]	62.0 [24.0, 87.0]	0.11
Age ≥65 (%)	61.5	42.5	0.25
Female (%)	76.9	64.4	0.56
Body weight (kg)	46.4 [32.4, 70.8]	53.0 [30.7, 105.0]	**0.02**
Body weight <40kg (%)	15.4	7.6	0.27
Disease duration (years)	1.84 [0.02, 49.54]	0.66 [0.00, 33.3]	0.30
Pulmonary disease (%)	46.2	20.4	**0.04**
Diabetes (%)	7.7	8.7	1.00
PSL (%)	61.5	16.8	**0.001**
PSL (mg/day)	5.0 [0.0, 10.0]	0.0 [0.0, 15.0]	**<0.001**
Initial MTX dose (mg/week)	6.0 [4.0, 12.0]	8.0 [2.0, 12.0]	**0.04**
Other DMARDs (%)	53.8	12.3	**0.001**
-SASP (%)	38.4	11.1	
-BUC (%)	7.7	0.9	
-MZB (%)	0.0	0.3	
-IGU (%)	7.7	0.0	
White blood cell count ($\times10^3/\mu L$)	7,500 [5,050, 12,210]	6,690 [3,280, 33,320]	**0.04**
Lymphocyte count ($\times10^3/\mu L$)	1,730[0,840, 2,390]	1,555.5 [0,320, 4,380]	0.42
Lymphocyte count <1,000$\times10^3$/L (%)	15.4	11.1	0.65
Serum albumin (mg/dL)	3.80 [2.90, 4.50]	4.00 [2.10, 4.90]	0.13
Serum albumin <3.5mg/dL (%)	38.5	13.3	**0.03**
Serum Creatinine (mg/dL)	0.61 [0.41, 0.99]	0.62 [0.30, 1.50]	0.84
eGFR (mL/min/1.73m^2)	87.0 [42.0, 124.0]	83.0 [34.0, 173.0]	1.00

Laboratory data were obtained at the initiation of the MTX.

Statistical analysis was performed with the Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables.

Probability values (p values) of less than 0.05 were considered to be statistically significant.

BUC: bucillamine, DMARDs: disease modifying anti-rheumatic drugs, eGFR: estimated Glomerular Filtration Rate, IGU: iguratimod, mPSL: methylprednisolone, MTX: methotrexate, MZB: mizoribine, PCP: *Pneumocystis jirovecii* pneumonia, PCR: polymerase chain reaction, PSL: prednisolone, SASP: salazosulfapyridine

Table 5. Risk Factors for the Development of PCP in Patients with RA Receiving MTX.

Variable	Hazard ratio	95% CI	p value
Concomitant use of PSL	5.50	1.80-16.88	**0.003**
Concomitant use of other DMARDs	5.98	1.91-18.74	**0.002**
Serum albumin <3.5 mg/dL	4.30	1.33-13.90	**0.01**

CI: confidence interval, DMARDs: disease modifying anti-rheumatic drugs, MTX: methotrexate, PCP: *Pneumocystis jirovecii* pneumonia, PSL: prednisolone, RA: rheumatoid arthritis

duction in the dose of SMX/TMP because of adverse drug reactions. All patients eventually recovered from PCP. MTX therapy was reinstated in 7 patients after discharge from the hospital, 6 of whom received PCP prophylaxis with SMX/TMP (n=3), inhaled pentamidine (n=2), and atovaquone (n=1). None of the patients with PCP experienced relapse within two years.

Factors associated with PCP during MTX therapy

Table 4 presents the clinical characteristics at the initiation of MTX therapy. The patients in the PCP group had a significantly lower body weight, higher frequency of pulmonary disease, higher frequency of concomitant administration of PSL, higher initial dose of MTX, higher proportion of other DMARDs, higher white blood cell count, and higher proportion of serum hypoalbuminemia than those in the non-PCP group. Based on the results of the univariate analysis, we identified independent risk factors for PCP in patients with RA treated with MTX using Cox proportional hazard models (Table 5). The results showed that the development of PCP was significantly associated with the concomitant use of PSL [hazard ratio (HR) 5.50, 95% confidence interval (CI) 1.80-16.88, p=0.003], concomitant use of other DMARDs (HR 5.98, 95% CI 1.91-18.74, p=

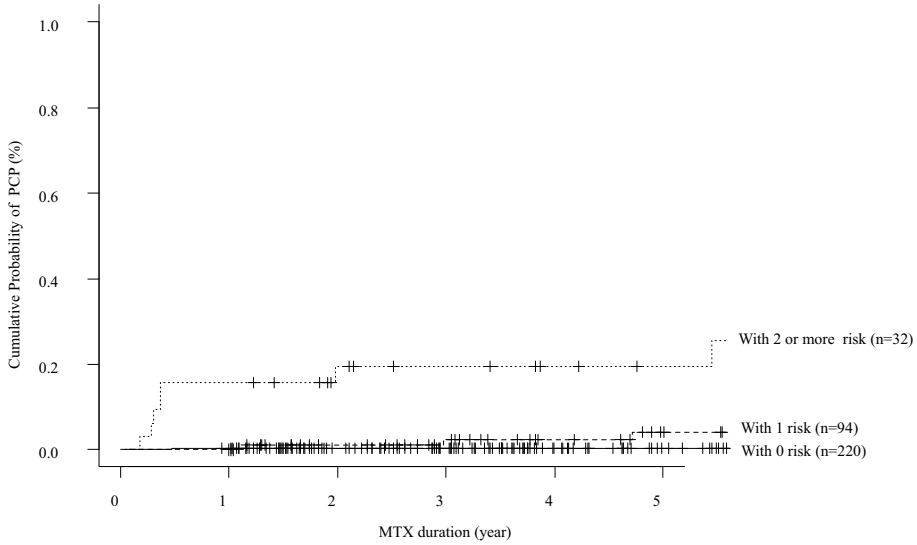

Figure 2. Cumulative probability of PCP in patients with RA associated with MTX therapy, according to the number of risk factors. The patients were stratified by the number of risk factors, including the concomitant use of other DMARDs, concomitant use of PSL, and serum albumin <3.5 mg/dL. The cumulative probability for developing PCP according to the number of risk factors was calculated using the Kaplan-Meier method and the comparison between the groups was performed using the log rank test with Bonferroni's correction. Patients with two or more risk factors had a significantly higher cumulative probability of developing PCP than patients with one or no risk factors (p<0.001), and those with one risk factor had a significantly higher cumulative probability of developing PCP than those without any risk factors (p<0.05). DMARDs: disease-modifying anti-rheumatic drugs, MTX: methotrexate, PCP: *Pneumocystis jirovecii* pneumonia, PSL: prednisolone, RA: rheumatoid arthritis

0.002), and serum albumin <3.5 mg/dL (HR 4.30, 95% CI 1.33-13.90, p=0.01).

The accumulation of risk factors and development of PCP

A total of 346 patients with RA were stratified according to the number of presenting risk factors, including the concomitant use of PSL, concomitant use of the other DMARDs, and serum albumin level <3.5 mg/dL. The cumulative probability of PCP was calculated using the Kaplan-Meier method. Patients with a greater number of risk factors possessed a significantly higher cumulative probability for the development of PCP than other patients (Fig. 2).

Discussion

The results of our retrospective observational study revealed two points. First, all patients with RA who received MTX and developed PCP recovered without relapse. Second, the concomitant use of PSL and other DMARDs as well as serum hypoalbuminemia were risk factors associated with the development of PCP during MTX therapy, and PCP developed more frequently with an increasing number of presenting risk factors.

MTX therapy is associated with numerous adverse effects, such as liver dysfunction, renal dysfunction, and bone marrow suppression (2-10). In Japan, the initial recommended dose of MTX is 6-8 mg/week, and physicians must take care when increasing the dose (22). The most common pulmonary complication during MTX therapy is MTX pneumonia, which was reported in 1.0-7.0% of patients, and most cases developed within 1 year after the initiation of MTX (23, 24). The factors associated with MTX pneumonia include an older age, chronic pulmonary disease, diabetes, hypoalbuminemia, and a history of DMARDs use (25, 26). However, 3.6% of patients with RA receiving MTX developed PCP, and the colonization of *P. jirovecii* in elderly patients was shown to be a risk factor for the development of PCP (10). In the current study, 13 of 346 (3.8%) of patients with RA receiving MTX developed PCP, and the median age at the onset was 69 years old, with 11 of the 13 patients (84.6%) ≥65 years old, which was consistent with the previous study (10).

Several studies have reported that an older age, glucocorticoid use, pulmonary disease, and MTX use were factors associated with the development of PCP in patients with RA receiving tumor necrosis factor (TNF-α) inhibitors (11, 14, 18). In contrast, the current study showed that the concomitant use of PSL and other DMARDs as well as serum hypoalbuminemia were factors associated with the development of PCP. The concomitant use of PSL was a risk factor for the development of PCP in patients with RA with TNF inhibitors (11, 18). One study reported that two or more immunosuppressant medications increase the incidence

Table 6. Characteristics of Patients with RA Developing PCP during MTX.

Case	Age	Sex	RA duration (year)	MTX (mg/week)	MTX duration (month)	PSL (mg/day)	Other DMARDs	WBC ($\times10^3$/μL)	Lymphocyte counts ($\times10^3$/μL)	Ref.
1	68	F	17	5.0	147	6.0	Bucillamine	13,200	0,660	[9]
2	73	F	14	7.5	14	16.0	none	4,400	0,044	[10]
3	56	F	5	7.5	48	2.5	none	3,300	0,099	[29]
4	49	F	4	7.5-15.0	9	0.0	none	3,500	0,595	[29]
5	64	F	15	15.0	30	7.0	none	2,200	0,154	[29]
6	74	F	N.A	15.0	8	5.0	none	8,200	N.A	[29]
7	16	M	N.A	10.0	10	3.0	none	15,100	N.A	[29]
8	66	M	N.A	22.5	6	0.0	none	4,200	N.A	[29]
9	57	M	11	15.0-20.0	N.A	5.0	none	0,580	0,080	[30]
10	76	F	50	6.0	3	5.0	none	N.A	N.A	[10]
11	75	M	8 months	8.0	3	0.0	none	N.A	N.A	[10]
12	66	M	1	8.0	N.A	5.0	none	9,100	0,728	[10]
13	70	F	1	8.0	N.A	0.0	none	N.A	N.A	[10]
14	76	F	7	8.0	N.A	0.0	Tacrolimus	N.A	N.A	[10]
15	78	M	2	10.0	N.A	0.0	Tacrolimus	N.A	N.A	[10]
16	80	M	3	8.0	N.A	5.0	none	N.A	N.A	[10]
17	80	F	8	6.0	N.A	5.0	Tacrolimus	N.A	N.A	[10]
18	62	F	9	15.0	7	10.0	none	N.A	0,700	[31]
19	58	F	1.5	15.0	8	12.5	none	N.A	0,600	[31]
20	74	F	N.A	15.0	8	5.0	none	N.A	N.A	[32]
21	66	M	N.A	22.5	6	0.0	none	N.A	N.A	[33]
22	69	F	N.A	10.0	12	0.0	none	5,700	0,600	[34]
23	44	F	16	15.0	N.A	10.0	Cyclosporine A	6,360	0,630	[35]
24	63	F	14	5.0	10	0.0	none	1,200	1,080	[36]
25	66	F	11	10.0	3	10.0-12.5	none	6,500	N.A	[37]
26	76	F	N.A	15.0	N.A	5.0	none	12,360	0,750	[38]
27	63	M	N.A	5.0-7.5	N.A	0.0	none	2,500	0,450	[39]
28	39	M	10	7.5-15.0	48	0.0	D-penicillamin	4,700	0,564	[39]
29	42	F	18	7.5	4	0.0	none	14,600	0,290	[40]
30	81	F	44.3	8.0	3.5	0.0	Iguratimod	11,190	1,488	this study
31	69	F	49.9	14.0	4.5	5.0	none	12,090	2,310	this study
32	72	F	13.5	8.0	24	4.0	SASP	19,360	1,181	this study
33	69	F	6.8	12.0	57.4	4.0	none	6,330	1,070	this study
34	36	F	0.3	12.0	3.7	8.0	none	6,510	0,660	this study
35	66	F	7.3	8.0	66.3	2.0	none	4,850	0,330	this study
36	72	F	0.4	12.0	4.5	3.0	none	4,590	0,670	this study
37	63	F	10.3	10.0	74.2	0.0	none	8,640	1,450	this study
38	77	M	0.2	8.0	1.9	10.0	none	11,480	1,460	this study
39	78	F	2.6	6.0	14	0.0	none	7,920	0,768	this study
40	59	F	11	10.0	131	30.0	Iguratimod	10,480	1,370	this study
41	65	M	1.3	10.0	5.7	0.0	none	13,760	1,700	this study
42	79	M	13.5	6.0	36.1	5.0	none	6,830	0,847	this study

DMARDs: disease modifying anti-rheumatic drugs, F: female, M: male, MTX: methotrexate, N.A: not available, PCP: *Pneumocystis jirovecii* pneumonia, PSL: prednisolone, RA: rheumatoid arthritis, SASP: salazosulfapyridine, WBC: white blood cell

of PCP (27), while another study reported that serum hypoalbuminemia was a risk factor for pneumonia (28).

In daily clinical practice, there have been no reports regarding patients with RA who developed PCP during MTX therapy. In the current study, we investigated the clinical characteristics and prognosis of patients with RA who developed PCP during MTX therapy and identified the associated risk factors, which is very meaningful.

However, the scale of the present study was insufficient to investigate the clinical characteristics of PCP during MTX therapy. We searched the literature and selected 29 cases (Table 6) to investigate the characteristics of patients with RA who developed PCP during MTX therapy (29-40).

Almost all cases of PCP occurred within one year of the initiation of MTX therapy. Most patients received glucocorticoids, and the median dosage was 4 mg/day. The serum lymphocyte counts were 0.685×10^3/L on average, and the serum lymphocyte counts were $<1.0\times10^3$/μL in 20 cases.

Furthermore, the serum lymphocyte counts were $<0.5\times10^3$/μL in 7 patients. Several studies have reported that PCP developed frequently within half a year after the initiation of TNF-α inhibitor therapy (18, 27). One study also reported that most patients developed PCP within one year of the initiation or increase in the dosage of MTX (10). These results suggest that PCP may represent the reactivation of a latent infection.

Several studies have indicated that low lymphocyte counts were associated with an increased risk for PCP in patients with systemic rheumatic diseases (SRDs) or HIV (41, 42). One study also found that patients with nadir serum lymphocyte counts of $<1.0\times10^3$/μL were at high risk of serious infections, especially patients with counts under 0.5×10^3/μL (43). Further studies are required to investigate whether or not the monitoring of serum lymphocyte counts can predict PCP in patients with SRD.

One study indicated that the administration of corticosteroid doses exceeding 20 mg for more than 4 weeks, the current use of ≥2 DMARDs, including biologic agents, an absolute lymphocyte count $<0.35\times10^3$/μL, and underlying parenchymal lung disease were risk factors for the development of PCP in patients with SRD and also suggested that patients with ≥2 risk factors should receive PCP prophylaxis (44). SMX/TMP was recommended for PCP prophylaxis in patients without HIV (45). SMX/TMP is an antibiotic widely applied for urinary tract infections, diarrhea, and PCP. The recommended prophylactic dose of SMX/TMP for PCP is one tablet per day or two tablets three times per week (46). The prophylactic effectiveness of SMX/TMP was shown to be excellent in patients with SRD (47). However, SMX/TMP causes numerous adverse reactions, such as a fever, rash, abnormal serum electrolytes, renal dysfunction, and liver dysfunction, and several patients discontinued SMX/TMP due to adverse drug reactions (48). Furthermore, MTX and SMX/TMP are dihydrofolate reductase enzyme inhibitors, and the administration of MTX as well as its combination with SMX/TMP may lead to strong inhibition of folate metabolism; thus, this combination can result in several adverse drug reactions (49, 50). Patients on immunosuppressive therapy should thus not be prescribed SMX/TMP for PCP prophylaxis; indeed, experts recommend that the administration of SMX/TMP be considered only if the PCP risk is >3% (48). Secondary prophylaxis is recommended after the development of PCP in patients with HIV (46, 51). However, whether or not secondary prophylaxis for PCP is needed in patients without HIV is unclear (52). In the current study, no patients who restarted MTX experienced relapse of PCP within two years. However, our data were obtained from a very small number of patients, and further investigations are required to investigate secondary PCP prophylaxis in patients with SRD.

This study has several limitations that warrant mention, mainly due to its study design. First, 5 of the 13 patients had definitive PCP, and only 2 underwent bronchoscopy, indicating that almost all patients clinically had PCP. We set the diagnostic criteria for PCP in the current study (akin to several previous studies) based on the clinical symptoms, radiological findings, and serum β-D glucan or PCR test for *P. jirovecii* that supplemented the diagnostic criteria for PCP, independent of the microscopic detection of *P. jirovecii* (18, 19). Second, the results of this study are not generalizable to all patients with RA, as our study included only 13 patients with PCP.

In conclusion, PCP may develop during MTX therapy if the frequency of risk factors is high in patients with RA.

The authors state that they have no Conflict of Interest (COI).

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