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ORIGINAL ARTICLE

Asymptomatic carriage of *Pneumocystis jiroveci* in elderly patients with rheumatoid arthritis in Japan: a possible association between colonization and development of *Pneumocystis jiroveci* pneumonia during low-dose MTX therapy

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Abstract Low-dose methotrexate (MTX) has been used effectively for rheumatoid arthritis (RA) because of its favorable risk-benefit ratio. One of the recent concerns arising from this therapy is a possible increase in the rate of opportunistic infections, particularly *Pneumocystis jiroveci* pneumonia (PCP). In this study, we report two cases of PCP occurring during low-dose methotrexate therapy for RA and review 13 additional cases from the literature on Japanese patients with RA. The average age of these patients was 67.7 years, and most were over the age of 60. MTX-associated PCP appears to occur more frequently in elderly individuals in Japan. To identify individuals with a high risk of PCP, we performed a polymerase chain reaction on specimens from induced sputum or bronchoalveolar lavage fluids from 55 patients with RA. At that point in time, they showed no evidence of PCP development. We found six patients (10.9%) having asymptomatic carriage of *P. jiroveci*. The mean age of the *P. jiroveci*-positive patients was 74.7 years, which was significantly older than the *P. jiroveci*-negative patients (mean age 63.6 years). Of the RA patients over the age of 65, 18.8% (6 cases out of 32) were carriers of *P. jiroveci*. There were no significant differences in RA duration or counts of white blood cells or lymphocytes between the positive and negative groups. Notably, we encountered a case of PCP occurring in an asymptomatic carrier of *P. jiroveci* during low-dose MTX therapy for RA. This case appeared to be a reactivation of latent infection. By careful follow-up on the carriers of *P. jiroveci*, we succeeded in promptly diagnosing PCP, and we employed the appropriate therapeutic strategies for this possibly life-threatening complication.

Keywords Colonization · Methotrexate · *Pneumocystis jiroveci* · *Pneumocystis jiroveci* pneumonia · Polymerase chain reaction · Rheumatoid arthritis

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Introduction

Methotrexate (MTX) is commonly used as the first-line disease-modifying antirheumatic drug (DMARD) having proven efficacy on radiographic progression in patients with rheumatoid arthritis (RA) [1]. Most recently, the European League Against Rheumatism (EULAR) recommended that patients at risk of developing persistent and/or erosive rheumatic diseases be started with DMARDs, especially MTX, as early as possible, even if they do not fulfill the established classification criteria for RA yet [2]. EULAR also stated that among DMARDs, MTX is considered an anchor drug in RA management since it can be used in combination with anti-tumor necrosis factor α (anti-TNFα) agents if necessary. In addition to its greater effectiveness, when used in low doses, MTX has a better toxicity profile than other DMARDs, and therapy can be continued for a significantly long period [3, 4].

Accompanying the popularity and increased use of MTX, rheumatologists have encountered its toxicity and

side effects, including marrow suppression, hepatic impairment, gastric toxicity, and pneumonitis. Furthermore, patients taking MTX seem to be at risk of opportunistic infections, such as cytomegalovirus pneumonia, disseminated herpes zoster infection, cryptococcosis, widespread nocardiosis, and *Pneumocystis jiroveci* pneumonia (PCP) [5, 6]. However, the exact role of MTX in inducing infections is unclear since patients with RA have a predisposition to pulmonary infections [7], and MTX is sometimes used concomitantly with corticosteroids, a major predisposing factor for PCP development in RA patients [8]. Whether the use of low-dose MTX may confer susceptibility to infectious agents is still debatable since there are conflicting results of in vivo and in vitro tests on immune variables [9].

PCP is recognized as a rare, but serious opportunistic infection exclusively occurring in immunocompromised hosts, such as those with malignancy or acquired immunodeficiency syndrome (AIDS). However, accumulated reports have shown an occurrence of PCP in patients treated with cytotoxic or immunosuppressive drugs for underlying diseases [10–12]. A recent postmarketing surveillance (PMS) report by the Japan College of Rheumatology (JCR) indicated a high incidence of PCP in RA patients receiving infliximab, an anti-TNFα agent [13]. It has been long discussed whether PCP may develop through a reactivation of latent infection or through person-to-person spread [14]. In this study, we show 2 cases of PCP development during the course of low-dose MTX monotherapy for RA, and review 13 Japanese patients in the literature. We also report a case of PCP occurring in an RA patient who has been colonized by *P. jiroveci*. To determine the prevalence of latent infection of *P. jiroveci* in Japanese patients with RA, polymerase chain reaction (PCR) was performed for 55 patients using specimens from induced sputum or bronchoalveolar lavage (BAL) fluids. Based on these findings, we will discuss a risk factor for PCP development in MTX-treated RA patients in Japan.

Patients and methods

Patients

To investigate the presence of latent *P. jiroveci* infection, we collected induced sputum from 47 patients with RA who had no complaints of respiratory symptoms and voluntarily participated in this study. We also used BAL fluids from eight patients with RA who required diagnostic bronchoscopy for their pulmonary symptoms or abnormalities on high-resolution computed tomography (HRCT) and whose symptoms were ultimately not diagnosed as PCP. The specimens were collected from March 2005 to July 2007. No patient was known to be HIV-positive. The ethics committee of our hospital approved the protocol for the study of *P. jiroveci* carriage in the RA patients, and written consent was obtained from all patients.

PCR for *P. jiroveci*

Specimens from 55 patients with RA were subjected to a PCR test for *P. jiroveci.* Template DNAs were extracted from induced sputum and BAL samples by means of proteinase K digestion and phenol/chloroform extraction and subsequently subjected to PCR as templates. The PCR analysis for *P. jiroveci* was performed in 50 μl of amplification reaction mixtures, with denaturation at 94°C for 90 s, annealing at 50°C for 90 s, and extension at 72°C for 2 min (40 cycles). The following oligonucleotide primers were used at 100 pmol: 5′-GAT GGC TGT TTC CAA GCC CA-3′ and 5′-GTG TAC GTT GCA AAG TAC TC-3′. DNA products with lengths of 376 bp were amplified from template DNAs. This analysis was done at SRL Inc. (Tachikawa, Japan). The details of this method and its usefulness for detection of *P. jiroveci* were described by Wakefield et al. [15–17].

Statistical analysis

The characteristics of patients with and without detectable *P. jiroveci* were compared using the Mann-Whitney U test. This method was used to compare quantitative data not distributed normally. Probability values (p values) of <0.05 were considered to be statistically significant.

Results

Cases of PCP during MTX therapy for RA in Japan

We recently encountered two patients with PCP who had been treated with low-dose MTX as therapy for active RA. Case 1 was a 76-year-old woman with a 50-year history of seropositive RA who had been treated for 3 months with 6 mg/week of MTX and 5 mg/day of prednisolone. The patient showed a low level of oxygen saturation at rest (91%) and complained of slight general fatigue. A large amount of β-D-glucan, a composition of fungal cell wall, was detected in serum (210.9 pg/ml), and PCR for *P. jiroveci* was positive with BAL fluids. Case 2 was a 75-year-old man whose symptoms had been diagnosed as RA 8 months earlier. The patient received MTX monotherapy at 8 mg/week for 3 months. The patient complained of general fatigue, high fever, and dyspnea, and oxygen saturation was 92%. Serum β-D-glucan was high (229.3 pg/ml), and PCR for *P. jiroveci* was positive with BAL fluids. In both cases, trimethoprim-

sulfamethoxazole (TMP-SMX) was effective. To examine the characteristics of Japanese patients who developed PCP during MTX therapy, we searched the literature that had been reported from Japan up until 2006, and listed 13 cases [18–27]. Cases with uncertainty regarding the MTX dosage were discarded. Patients taking anti-TNFα agents or more than 30 mg/day of prednisolone in combination with MTX were also excluded since these drugs per se are prone to opportunistic infections [8, 28]. We summarized the clinical features including age, dose and duration of MTX use, dose of prednisolone, and RA duration in Table 1. Our cases were also included in Table 1. We noted that MTX-associated PCP is likely to occur in elderly patients with RA. The average age of these patients was 67.7 years, and all patients except two were above the age of 60. In most cases, PCP occurred within 1 year from the initiation of MTX therapy or increase in MTX dosage, and in the shortest case, PCP developed 1 month after increasing the MTX dosage. In seven cases, this interval was within 3 months. We found four patients who had not received prednisolone therapy, but had developed PCP. In most other cases, the amount of prednisolone used was less than 10 mg/day.

Colonization by *P. jiroveci* among RA patients in Japan

PCR has proven to be the most sensitive technique for detecting *P. jiroveci* in pulmonary specimens [15]. Using this method, very low copies of *P. jiroveci*, which are undetectable by routine histochemical staining, have been detected in the lungs of only mildly immunocompromised or even immunocompetent individuals without PCP development [14]. These cases are considered to have colonization by *P. jiroveci.* To determine the prevalence of colonization by *P. jiroveci* in RA patients in Japan, we performed PCR using induced sputum or BAL fluid obtained from 55 patients with RA. Enrolled patients were predominantly female (15 males and 40 females), with a mean age of 64.8 years and a mean RA duration of 6.9 years. Percentages of rheumatoid factor (RF)-positive and anti-cyclic citrullinated peptide antibody (anti-CCP Ab)-positive patients were 83.6 and 87.3%, respectively. The induced sputum was collected from 47 volunteers with RA who had no respiratory symptoms. The BAL fluids were obtained from eight patients with RA who were ultimately diagnosed with MTX pneumonia (three cases), bacterial pneumonia (two cases), nonspecific interstitial pneumonia (NSIP, one case), organizing pneumonia (OP, one case), or pulmonary hemorrhage (one case). We found six cases (10.9%) of asymptomatic carriage of *P. jiroveci*. The clinical characteristics of patients with and without *P. jiroveci* are shown in Table 2. The mean age of the positive patients was 74.7 years, which was significantly older than the negative patients (mean age 63.6 years). Of the RA patients over the age of 65, 18.8% (6 cases out of 32) were carriers of *P. jiroveci*. There were no significant

Table 1 Characteristics of patients with rheumatoid arthritis who developed *P. jiroveci* pneumonia during low-dose methotrexate therapy in Japan

Case	Age/sex	RA duration (years)	MTX (mg/week)	MTX duration (months)	PSL (mg/day)	Ref.
1	47/F	27	2.5	?	5	[17]
2	71/F	9	6	9	7.5	[18]
3	64/M	3	6	8	None	[19]
4	72/F	19	6	3	None	[20]
5	68/F	7	10	2 years	2.5	[20]
			12	8		
6	74/F	9	4	1	7–10	[21]
			6	3		
7	55/F	11	?	69	7	[22]
			6	5		
8	68/F	17	5	147	6	[23]
9	73/F	14	7.5	13	16	[23]
10	61/F	26	15	?	5	[25]
11	79/M	10	4	4	None	[25]
			8	1		
12	62/M	5	5	1	5–10	[26]
			7.5	1.5		
13	70/F	13	7.5	2.5	7.5–15	[24]
14	76/F	50	6	3	5	this study
15	75/M	8 months	8	3	None	this study

RA rheumatoid arthritis, *MTX* methotrexate, *PSL* prednisolone

Table 2 Comparison of carriers and non-carriers of *P. jiroveci* with rheumatoid arthritis

	PCR-positive patients ($n = 6$)	PCR-negative patients ($n = 49$)	P*
Age (years, mean ± SE)	74.7 ± 2.2	63.6 ± 2.0	0.01
Sex (male/female)	3/3	12/37	
Disease duration (years)	5.8 ± 20	7.0 ± 7.7	0.49
WBC (/μl, mean ± SE)	7,160 ± 778	6,466 ± 324	0.35
Lymphocytes (/μl, mean ± SE)	729 ± 180	981 ± 83	0.54
No. of positive anti-CCP Abs	5 (83%)	43 (88%)	
No. of positive RF	4 (67%)	42 (86%)	
No. of patients taking			
Methotrexate	6 (100%)	37 (76%)	
Prednisolone	3 (50%)	28 (57%)	
Leflunomide	0	3 (6%)	
Infliximab	0	3 (6%)	
Etanercept	0	6 (12%)	
Tacrolimus	3 (50%)	9 (18%)	
Sulfasalazine	0	2 (4%)	

Statistical analysis was performed with the Mann-Whitney *U* test. * Probability values (*P* values) of <0.05 were considered to be statistically significant. Laboratory data were obtained on the same day that PCR specimens were collected. *WBC* white blood cells, *SE* standard error, *RF* rheumatoid factor, *anti-CCP Abs* anti-cyclic citrullinated peptide antibodies, *PCR* polymerase chain reaction

differences in the RA duration between those with and without detectable *P. jiroveci*. The rate of anti-CCP Ab-positive or RF-positive patients was also similar between these groups. The white blood cell or lymphocyte count is unlikely to be a statistically significant predictor of an increased risk for colonization with *P. jiroveci*, although qualitative alterations in the cellular immune system are unknown. The clinical features of six patients with asymptomatic carriage of *P. jiroveci* are shown in Table 3. Two patients were diagnosed as having NSIP (case 5) and pulmonary hemorrhage (case 6), respectively. MTX was used for all positive cases. Although not significant, the *P. jiroveci* carriers more often received tacrolimus compared with the *P. jiroveci*-negative patients. Fifty percent of the carriers received 5 mg/day of prednisolone. Values of serum β-D-glucan in three cases were available (case 1, 4.4 pg/ml; case 4, 5.4 pg/ml; and case 6, 20.0 pg/ml). When compared with the patients developing PCP [29] the values of serum β-D-glucan were lower in the patients having the asymptomatic colonization by *P. jiroveci*.

PCP development in an RA patient with asymptomatic carriage of *P. jiroveci*

One month after the detection of *P. jiroveci* in induced sputum, a 66-year-old man (case 1 in Table 3) developed PCP. One year earlier, the patient had been diagnosed with RA, and he had been treated with 8 mg/week of MTX and 5 mg/day of prednisolone. Titers of RF and anti-CCP Ab were high at the onset of RA, but 3 months after starting MTX therapy, the disease activity score (DAS28) decreased to 3.2. His disease activity was controlled effectively by MTX and prednisolone for 1 year. When colonization analysis for *P. jiroveci* was performed, values of C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR)

Table 3 Characteristics of patients with rheumatoid arthritis who were colonized by *P. jiroveci*

Case	Age/sex	RA duration (years)	DAS28	Treatment	Pulmonary disease
1	66/M	1	2.9	MTX (8 mg/week) PSL (5 mg/day)	No
2	70/F	1	5.1	MTX (8 mg/week)	No
3	76/F	7	4.1	MTX (8 mg/week) FK506 (1 mg/day)	No
4	78/M	2	3.5	MTX (10 mg/week) FK506 (2 mg/day)	No
5	80/M	3	6.7	MTX (8 mg/week) PSL (5 mg/day)	NSIP
6	80/F	8	3.4	MTX (6 mg/week) FK506 (1 mg/day) PSL (5 mg/day)	Pulmonary hemorrhage

MTX methotrexate, *PSL* prednisolone, *FK506* tacrolimus, *DAS* disease activity score, *NSIP* nonspecific interstitial pneumonia

were 0.89 mg/dl and 17 mm/h, respectively, and DAS28 was 3.4. One month later, the CRP value markedly increased to 11.5 mg/dl without the presentation of articular symptoms. Oxygen saturation was 96% at rest, but it dropped to 91% after motion. The patient did not complain of dyspnea, dry cough, or fever. HRCT showed extensive interstitial opacities, while chest radiographs showed no evidence of interstitial pneumonia. The white blood cell count was 9,100/μl, and the lymphocyte rate was 8% (728/μl). Serum β-D-glucan was 134.2 pg/ml. Since PCP development was suspected, we started oral administration of TMP-SMX (5,760 mg/day) and methylprednisolone by intravenous drip (40 mg daily for 5 days). MTX was withdrawn. One week later, the abnormalities on HRCT and the CRP value both improved.

Discussion

There is increasing evidence that *P. jiroveci* may colonize the lungs of asymptomatic individuals with mild immunosuppression induced by HIV infection, malignancy, or long-term glucocorticoid therapy, and the lungs of immunocompetent persons with chronic pulmonary diseases [30–34]. Using the PCR technique, we found that in Japan *P. jiroveci* organisms predominantly colonize elderly individuals with RA, irrespective of the disease duration (Tables 2, 3). The mean ages of the carriers and non-carriers of *P. jiroveci* were significantly different (74.7 years vs. 63.9 years). This parasite was detected in 18.8% of the asymptomatic RA patients over the age of 65. Thus, advanced age is apparently a predisposing factor for colonization by *P. jiroveci* in Japan. Furthermore, we reported two patients who developed PCP during MTX therapy for RA, and we reviewed 13 additional cases of PCP that were associated with MTX therapy for Japanese RA patients (Table 1). The average age of these patients was 67.7 years, and most patients were over the age of 60. These findings showed that MTX-associated PCP occurs more frequently in elderly individuals. The PMS report on infliximab by JCR also indicated a high incidence of PCP [13]. The average age of these patients was 64.0 years. Considering that the average age of enrolled patients was 55.1 years, increasing age appears to be one of the predisposing factors for PCP development in RA patients. In a case-control study of PCP in RA patients receiving infliximab, Harigai et al. [35] also reported that Japanese patients with PCP were significantly older (average age, 65 years) than those who did not have PCP (average age, 55 years). The increased incidence of PCP in elderly individuals with RA may be explained by the high frequency of colonization by *P. jiroveci* in this patient population. Recently, Doran et al. showed that patients with RA were at increased risk of developing infectious diseases, as compared to non-RA subjects, and the hazard ratio of infection in RA patients was nearly twice the ratio in matched non-RA controls [7]. Our study is likely to cause further concern about infectious complications in RA patients. The elderly appear to be particularly susceptible to *P. jiroveci* infection. We recommend that rheumatologists be alert to the possibility of PCP development during the course of RA, especially when immunosuppressive drugs are administered to elderly patients.

It is a major point of discussion whether the development of PCP is due to a reactivation of latent infection, *de novo* acquisition by person-to-person transmission, or a combination of both. As shown in Table 1, most patients developed PCP within 1 year from the initiation of low-dose MTX therapy or an increase in MTX dosage, and in seven patients, PCP occurred within 3 months. Furthermore, the data from the PMS report on infliximab in Japan showed that the median interval from the first infusion to the occurrence of PCP was 70.0 days, and PCP developed after a mean of 2.8 infusions of infliximab [13]. We also reported a case of PCP occurring 4 weeks after the first infliximab infusion [36]. These data suggest the possibility that PCP development may result from a reactivation of latent infection rather than from new acquisition by environmental exposure. Of note, we encountered a case of PCP occurring in a *P. jiroveci* carrier who had been receiving low-dose MTX and prednisolone for RA. This case appears to be a reactivation of latent infection. On the contrary, Miller et al. examined the molecular epidemiology of *P. jiroveci* and showed the possibility that person-to-person infection via an airborne route may be involved in the occurrence of PCP [17]. They also showed that immunocompetent health care workers in occupational contact with *P. jiroveci*-infected patients may have been colonized by this parasite, leading to asymptomatic carriage [16]. These data suggest that immunocompetent *P. jiroveci* carriers may be a source of infection to immunocompromised, susceptible hosts. Nevertheless, we should consider the reactivation of latent infection as the cause of PCP in elderly individuals with RA. In healthy, immunocompetent hosts, the immune response may completely clear *P. jiroveci* from the host; namely, colonization by this parasite is a time-limited phenomenon. However, recent laboratory data on RA patients showed contraction of the T cell receptor repertoire and fundamental alterations in T cell dynamics [37, 38]. Age-dependent shrinkage of T cell receptor diversity is well known [39]. Such perturbation of T cell homeostasis results in decreased ability to recognize potential antigens. Under these circumstances, *P. jiroveci* may evade the immune system and successfully parasitize the hosts for relatively long periods. Lifelong latency is not necessary. As immune dysfunction

progresses due to the use of MTX or other immunosuppressive agents, the colonized species propagates, and it may reach a level high enough to cause pneumonia. We should also keep in a mind the possibility that elderly patients with RA might serve as an important reservoir for *P. jiroveci.*

Takayanagi et al. reported different patterns of opportunistic pulmonary infections among MTX, prednisolone and anti-TNFα agents, and a higher incidence of PCP in MTX-treated RA patients in comparison with those treated with the other two drugs [40]. Iikuni et al. also showed that the use of MTX for patients with connective tissue disease is related to susceptibility to PCP [41]. A search of literature from Western countries revealed that *P. jiroveci* is the most common opportunistic infection in RA patients treated with low-dose MTX [5, 6, 42]. As mentioned above, the PMS report on infliximab by JCR indicated a high incidence of PCP in RA patients treated with infliximab (22 cases of PCP out of 5,000 patients) [13]. In contrast, there were no cases of PCP in 2,820 patients with Crohn's disease during infliximab therapy (PMS report by Tanabe Pharmaceutical Company). Considering there was no use of MTX in treatment for Crohn's disease and the younger age of the patients with this syndrome, the use of MTX and older age are factors likely to be associated with increased risk of PCP. Prednisolone is considered to be a major predisposing factor for PCP development in patients who have a variety of underlying conditions [8, 10–12]. Most recently, Harigai et al. showed that 6 mg/day or more of prednisolone is a risk factor for developing PCP [35]. Wolfe et al. [43] also indicated a dose-related association between prednisolone use and pneumonia risk in RA, and this relationship was evident with average daily doses of 5 mg. These data suggest that even low-dose prednisolone is not safe from the increased risk of PCP. To reduce the rate of radiographic progression in RA, low-dose prednisolone is widely used in combination with DMARD treatment [44]. It may contribute to the high incidence of PCP during the MTX therapy for RA.

Recently, the use of TMP-SMX as a prophylactic for PCP has been recommended for collagen disease patients receiving high-dose steroid therapy or whose lymphocyte counts are less than 500/μl [45]. However, the preventive use of TMP-SMX is not viable during MTX therapy, since a combination of these agents may induce pancytopenia. Moreover, the clinical presentation of PCP is sometimes subtle and nonspecific, and the radiographic findings are often difficult to discriminate from those of MTX-induced lung injury (MTX pneumonia) and RA-related interstitial pneumonia. Therefore, it is important to identify patients at high risk of PCP. Using the PCR technique, we indicated the high frequency of colonization by *P. jiroveci* in elderly RA patients. Such carriers may be at risk of a reactivation of *P. jiroveci* or may be the source of hospital-acquired transmission. Low-dose MTX, particular when used for this patient population, becomes a predisposing factor to the development of PCP. We should therefore be vigilant in evaluation of any pulmonary symptom presented by elderly RA patients during MTX therapy. Identification of high-risk patients and close follow-up with a high degree of suspicion will lead to prompt diagnosis and implementation of therapeutic strategies for PCP in RA patients.

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References

1. Pavy S, Constantin A, Pham T, Gossec L, Maillefert JF, Cantagrel A, et al. Methotrexate therapy for rheumatoid arthritis: clinical practice guidelines based on published evidence and expert opinion. Joint Bone Spine. 2006;73:388–95.
2. Combe B, Landewe R, Lukas C, Bolosiu HD, Breedveld F, Dougados M, et al. EULAR recommendations for the management of early arthritis: report of a task force of the European Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT). Ann Rheum Dis. 2007;66:34–45.
3. Buchbinder R, Hall S, Sambrook PN, Champion GD, Harkness A, Lewis D, et al. Methotrexate therapy in rheumatoid arthritis: a life table review of 587 patients treated in community practice. J Rheumatol. 1993;20:639–44.
4. Weinblatt ME, Maier AL, Fraser PA, Coblyn JS. Long-term prospective study of methotrexate in rheumatoid arthritis: conclusion after 132 months of therapy. J Rheumatol. 1998;25:238–42.
5. Kanik KS, Cash JM. Does methotrexate increase the risk of infection or malignancy? Rheum Dis Clin North Am. 1997;23:955–67.
6. Boerbooms AM, Kerstens PJ, van Loenhout JW, Mulder J, van de Putte LB. Infections during low-dose methotrexate treatment in rheumatoid arthritis. Semin Arthritis Rheum. 1995;24:411–21.
7. Doran MF, Crowson CS, Pond GR, O'Fallon WM, Gabriel SE. Frequency of infection in patients with rheumatoid arthritis compared with controls: a population-based study. Arthritis Rheum. 2002;46:2287–93.
8. White DA. Drug-induced pulmonary infection. Clin Chest Med. 2004;25:179–87.
9. Segal R, Yaron M, Tartakovsky B. Methotrexate: mechanism of action in rheumatoid arthritis. Semin Arthritis Rheum. 1990;20:190–200.
10. Roblot F, Godet C, Le Moal G, Garo B, Faouzi Souala M, Dary M, et al. Analysis of underlying diseases and prognosis factors associated with *Pneumocystis carinii* pneumonia in immunocompromised HIV-negative patients. Eur J Clin Microbiol Infect Dis. 2002;21:523–31.
11. Yale SH, Limper AH. *Pneumocystis carinii* pneumonia in patients without acquired immunodeficiency syndrome: associated illness and prior corticosteroid therapy. Mayo Clin Proc. 1996;71:5–13.
12. Sepkowitz KA. *Pneumocystis carinii* pneumonia in patients without AIDS. Clin Infect Dis. 1993;17(Suppl 2):S416–22.
13. Takeuchi T, Tatsuki Y, Nogami Y, Ishiguro N, Tanaka Y, Yamanaka H, et al. Post-marketing surveillance of the safety profile of infliximab in 5,000 Japanese patients with rheumatoid arthritis. Ann Rheum Dis. 2007;67:189–94.
14. Stringer JR, Beard CB, Miller RF, Wakefield AE. A new name (*Pneumocystis jiroveci*) for *Pneumocystis* from humans. Emerg Infect Dis. 2002;8:891–6.

15. Wakefield AE, Pixley FJ, Banerji S, Sinclair K, Miller RF, Moxon ER, et al. Detection of *Pneumocystis carinii* with DNA amplification. Lancet. 1990;336:451–3.
16. Miller RF, Ambrose HE, Wakefield AE. *Pneumocystis carinii* f. sp. hominis DNA in immunocompetent health care workers in contact with patients with *P. carinii* pneumonia. J Clin Microbiol. 2001;39:3877–82.
17. Miller RF, Lindley AR, Copas A, Ambrose HE, Davies RJ, Wakefield AE. Genotypic variation in *Pneumocystis jiroveci* isolates in Britain. Thorax. 2005;60:679–82.
18. Ihata A, Soga T, Takemura S, Fujita H. A case of *Pneumocystis* pneumonia associated with rheumatoid arthritis. Shizuoka Sekijyuji Byoin Kenkyuhou. 2002;22:67–71.
19. Nakashima A, Toufuku Y, Ojima T. Seventy one year-old female patient with rheumatoid arthritis developed *Pneumocystis carinii* pneumonia mimicking methotrexate pneumonitis. J Chubu Rheum Assoc. 2005;36:74–5.
20. Jojima H, Naito M, Shiraishi M, Matsumoto T, Noritomi T, Hayashi H. *Pneumocystis carinii* pneumonia and perforation of the colon in a patient with rheumatoid arthritis receiving low dose methotrexate and glucocorticoid hormone. Kyushu J Rheumatol. 2005;25:30–4.
21. Kawagishi Y, Yamaguchi T, Matsui S, Narukawa M, Fujita T, Oda H, et al. A case of *Pneumocystis* pneumonia misidentified as methotrexate-induced pneumonitis. J Jap Respir Soc. 2005;43:486–90.
22. Kaneko Y, Suwa A, Ikeda Y, Hirakata M. *Pneumocystis jiroveci* pneumonia associated with low-dose methotrexate treatment for rheumatoid arthritis: report of two cases and review of the literature. Mod Rheumatol. 2006;16:36–8.
23. Ohashi H, Izui Y, Kondo M, Saito M, Suzuki D. *Pneumocystis carinii* pneumonia treated with low dose methotrexate pulse therapy for rheumatoid arthritis: four cases report. J Chubu Rheum Assoc. 2006;37:26–7.
24. Kitsuwa S, Matsunaga K, Kawai M, Tsuzi T, Kato K, Tani K, et al. Pancytopenia and *Pneumocystis carinii* pneumonia associated with low dose methotrexate pulse therapy for rheumatoid arthritis–case report and review of literature. Ryumachi. 1996;36:551–8.
25. Okuda Y, Oyama T, Oyama H, Miyamoto T, Takasugi K. *Pneumocystis carinii* pneumonia associated with low dose methotrexate treatment for malignant rheumatoid arthritis. Ryumachi. 1995;35:699–704.
26. Shoda H, Inokuma S, Yajima N, Tanaka Y, Setoguchi K. Two cases of rheumatoid arthritis presenting lung injury during low dose methotrexate pulse therapy. Kanto Ryumachi. 2005;38:78–88.
27. Shimada T, Nishimura Y, Funada Y, Takenaka K, Kobayashi K, Urata Y, et al. A case of *pneumocystis carinii* pneumonia associated with low dose methotrexate treatment for rheumatoid arthritis and trimethoprim-sulfamethoxazole induced pancytopenia. Arerugi. 2004;53:575–81.
28. Saketkoo LA, Espinoza LR. Impact of biologic agents on infectious diseases. Infect Dis Clin North Am. 2006;20:931–61, viii.
29. Yasuoka A, Tachikawa N, Shimada K, Kimura S, Oka S. (1–>3) beta-D-glucan as a quantitative serological marker for *Pneumocystis carinii* pneumonia. Clin Diagn Lab Immunol. 1996;3:197–9.
30. Sing A, Roggenkamp A, Autenrieth IB, Heesemann J. *Pneumocystis carinii* carriage in immunocompetent patients with primary pulmonary disorders as detected by single or nested PCR. J Clin Microbiol. 1999;37:3409–10.
31. Calderon EJ, Regordan C, Medrano FJ, Ollero M, Varela JM. *Pneumocystis carinii* infection in patients with chronic bronchial disease. Lancet. 1996;347:977.
32. Armbruster C, Hassl A, Kriwanek S. *Pneumocystis carinii* colonization in the absence of immunosuppression. Scand J Infect Dis. 1997;29:591–3.
33. Nevez G, Raccurt C, Jounieaux V, Dei-Cas E, Mazars E. Pneumocystosis versus pulmonary *Pneumocystis carinii* colonization in HIV-negative and HIV-positive patients. AIDS. 1999;13:535–6.
34. Maskell NA, Waine DJ, Lindley A, Pepperell JC, Wakefield AE, Miller RF, et al. Asymptomatic carriage of *Pneumocystis jiroveci* in subjects undergoing bronchoscopy: a prospective study. Thorax. 2003;58:594–7.
35. Harigai M, Koike R, Miyasaka N. *Pneumocystis* pneumonia associated with infliximab in Japan. N Engl J Med. 2007;357:1874–6.
36. Mori S, Imamura F, Kiyofuji C, Ito K, Koga Y, Honda I, et al. *Pneumocystis jiroveci* pneumonia in a patient with rheumatoid arthritis as a complication of treatment with infliximab, anti-tumor necrosis factor alpha neutralizing antibody. Mod Rheumatol. 2006;16:58–62.
37. Koetz K, Bryl E, Spickschen K, O'Fallon WM, Goronzy JJ, Weyand CM. T cell homeostasis in patients with rheumatoid arthritis. Proc Natl Acad Sci USA. 2000;97:9203–8.
38. Wagner UG, Koetz K, Weyand CM, Goronzy JJ. Perturbation of the T cell repertoire in rheumatoid arthritis. Proc Natl Acad Sci USA. 1998;95:14447–52.
39. Vallejo AN. Age-dependent alterations of the T cell repertoire and functional diversity of T cells of the aged. Immunol Res. 2006;36:221–8.
40. Takayanagi NTY, Tokunaga D, Miyahara Y, Yamaguchi S, Saito H, Ubukata M, et al. Pulmonary infections in patients with rheumatoid arthritis. J Jap Respir Soc. 2007;45:465–73.
41. Iikuni N, Kitahama M, Ohta S, Okamoto H, Kamatani N, Nishinarita M. Evaluation of *Pneumocystis* pneumonia infection risk factors in patients with connective tissue disease. Mod Rheumatol. 2006;16:282–8.
42. LeMense GP, Sahn SA. Opportunistic infection during treatment with low dose methotrexate. Am J Respir Crit Care Med. 1994;150:258–60.
43. Wolfe F, Caplan L, Michaud K. Treatment for rheumatoid arthritis and the risk of hospitalization for pneumonia: associations with prednisone, disease-modifying antirheumatic drugs, and anti-tumor necrosis factor therapy. Arthritis Rheum. 2006;54:628–34.
44. Kirwan JR, Bijlsma JW, Boers M, Shea BJ. Effects of glucocorticoids on radiological progression in rheumatoid arthritis. Cochrane Database Syst Rev. 2007:CD006356.
45. Hashimoto H. Prophylaxis of *Pneumocystis jiroveci* pneumonia in autoimmune disease (in Japanese). Tokyo: Japanese Ministry of Health, Labor and Welfare Study Group on complication and treatment of immune disease. 2005:14–9.