

## Short Communication

### Effects of Sulfamethoxazole-Trimethoprim on Airway Colonization with *Pneumocystis jirovecii*

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**SUMMARY:** Reactivation of latent infection is considered to be the main mechanism underlying the development of *Pneumocystis* pneumonia in immunosuppressed patients. We retrospectively assessed the effects of prophylactic administration of sulfamethoxazole-trimethoprim on the development of *P. pneumonia* and airway colonization with *P. jirovecii* in patients undergoing examinations to diagnose or rule out *P. pneumonia*. Polymerase chain reaction was performed to detect *P. jirovecii* in bronchoalveolar lavage fluid or sputum of 60 consecutive patients between 2004 and 2012. No patients who received the prophylactic administration of sulfamethoxazole-trimethoprim ( $n = 10$ ) developed *P. pneumonia* or demonstrated airway colonization with *P. jirovecii*, and none of the patients who developed *P. pneumonia* ( $n = 11$ ) or showed colonization ( $n = 9$ ) had received prophylactic treatment. Furthermore, 20 (40%) of 50 patients without prophylactic treatment showed positive results on the *P. jirovecii* DNA polymerase chain reaction, but all 10 patients who had prophylactic treatment showed negative results (Fisher's exact test,  $P = 0.02$ ). Therefore, the prophylactic administration of sulfamethoxazole-trimethoprim has potential to be effective in preventing *P. pneumonia* as well as eliminating airway colonization with *P. jirovecii*. Further studies targeting large cohorts of patients with a variety of underlying diseases are required to develop recommendations regarding the prophylactic administration of sulfamethoxazole-trimethoprim.

*Pneumocystis jirovecii* normally exists in the lungs of humans and other mammals. Reactivation of latent infection is considered to be the main mechanism underlying the development of *P. pneumonia* (PCP) in immunosuppressed patients, and evidence from human and animal studies suggests that mammalian hosts act as a reservoir for *P. jirovecii* (1). The mortality rate associated with PCP is high without an early diagnosis and prompt therapy. In addition, a post-marketing surveillance report issued by the Japan College of Rheumatology indicated a high incidence of PCP in patients treated with infliximab, a monoclonal antibody against tumor necrosis factor (TNF), in combination with methotrexate (MTX) (2), and the incidence of PCP is predicted to increase in association with the popularization of biological agents, including infliximab. Therefore, obtaining an early diagnosis and providing prompt therapy, as well as preventing PCP, is important. In this study, we investigated the contribution of prophylaxis with sulfamethoxazole-trimethoprim (SMX-TMP) in reducing the development of PCP and airway colonization with *P. jirovecii*. Airway colonization with *P. jirovecii* means that the patient exhibits *P. jirovecii*

DNA-positive airway samples without findings suggestive of PCP.

This study was a retrospective analysis of 60 patients examined to diagnose or rule out the presence of PCP between January 2004 and August 2012 at Oita University Hospital, Japan. The ethics review boards of the institutions that contributed cases to this study did not require approval for the retrospective review of the patients' records. The subjects had a variety of underlying diseases and showed radiologically diffuse abnormal shadows in both lungs considered to be indicative of PCP as a differential diagnosis. After obtaining informed consent, we performed polymerase chain reaction (PCR) to detect *P. jirovecii* in bronchoalveolar lavage fluid (BALF) or sputum. BAL was performed using flexible bronchoscopy after the administration of local anesthesia to the upper airway with 4% lidocaine. The bronchoscope was wedged for saline solution lavage into one of the subsegmental bronchi displaying peripheral opacity on chest computed tomography. The samples were analyzed for bacteriological and fungal culture as well as PCR detection of *P. jirovecii*. After crushing yeast cell walls by blending with 0.5-mm glass beads that were pretreated by washing in concentrated nitric acid, fungal DNA was extracted directly from the BALF or sputum using the QiaAmp DNA Mini kit (QIAGEN K.K., Tokyo, Japan). The PCR detection method employed in this study was based on the report by Wakefield et al., with oligonucleotide primers pAZ102-E and pAZ102-H designed for the gene encoding the mitochondrial large subunit rRNA of *Pneumocystis* (3). Negative controls were included in each ex-

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periment for both DNA extraction and amplification. *P. jirovecii* DNA obtained from a patient with positive findings on a microscopic examination was used as a positive control. The subjects were considered to be *P. jirovecii*-positive when the expected *P. jirovecii* DNA-specific 346-bp band was visualized. Other diagnostic examinations, including measurements of the serum  $\beta$ -D-glucan and sialylated carbohydrate antigen KL-6 levels, were routinely conducted. The  $\beta$ -D-glucan levels were measured using an alkaline treatment chromogenic automated kinetic assay (called the MK assay).

The 60 patients evaluated in this study comprised 38 men and 22 women, with a mean age of 63 years (range: 27–89 years). The underlying diseases were malignant solid tumors (19 patients, 32%), hematopoietic malignancy (13 patients, 22%), interstitial lung disease associated with connective tissue disease (8 patients, 13%), idiopathic interstitial pneumonia (7 patients, 12%), acquired immunodeficiency syndrome (3 patients, 5%), connective tissue disease without interstitial lung disease (3 patients, 5%), and others (7 patients, 12%). Medications included corticosteroids and/or immunosuppressants (24 patients, 40%), anticancer drugs (24 patients, 40%), radiation therapy (17 patients, 28%) and biological the lungs in 1 case.

As shown in Table 1, 20 patients (33%) demonstrated positive results on the *P. jirovecii* DNA PCR assay. We comprehensively judged whether the positive results represented colonization of *P. jirovecii* or PCP based on chest computed tomography findings, subsequent clinical course, and the serum levels of  $\beta$ -D-glucan and KL-6 for reference. We considered that the patients with

*P. jirovecii* PCR-positive results and normal levels of serum  $\beta$ -D-glucan but without typical findings on chest images as having colonization of *P. jirovecii*. We diagnosed 9 patients as being colonized with *P. jirovecii* (cases 1–9 in Table 1) and 11 patients as having developed PCP (cases 10–20). Among the patients with *P. jirovecii* colonization, the serum  $\beta$ -D-glucan levels were within the normal range ( $<20$  pg/mL, mean: 2.8 [range: 0.7–7.8] pg/mL), the mean serum KL-6 level was 1,087 (383–2,340) U/mL, and the subjects were diagnosed with drug-induced pneumonitis and radiation pneumonitis among others. The PCP group included 3 patients with human immunodeficiency virus (HIV) infection and 1 patient with rheumatoid arthritis (RA) treated with infliximab. Among the patients with PCP, the mean serum  $\beta$ -D-glucan and KL-6 levels were 71.1 (3.0–345) pg/mL and 1,379 (312–2,233) U/mL, respectively. None of the patients with airway colonization with *P. jirovecii* or exhibiting PCP had received prophylactic SMX-TMP.

Fifty of the 60 patients had not received prophylactic SMX-TMP and 10 patients had received prophylactic SMX-TMP. Forty of the 60 patients showed negative results on the *P. jirovecii* DNA PCR assays; 30 of the 40 patients with negative results had not received prophylactic SMX-TMP and 10 patients who had received prophylactic SMX-TMP neither developed PCP nor showed airway colonization with *P. jirovecii*. Furthermore, 20 (40%) of 50 patients without prophylactic treatment showed positive results on the *P. jirovecii* DNA PCR. On the other hand, all 10 patients with prophylactic treatment showed negative results on

Table 1. Characteristics of the patients with positive results for *Pneumocystis jirovecii* DNA in respiratory samples

	sample	age	sex	underlying disease	$\beta$ -D-glucan (pg/ml)	KL-6 (U/ml)	medications for underlying disease	diagnosis	SMX-TMP
1	sputum	75	M	lung cancer	0.7	983	anticancer drug	drug-IP	—
2	sputum	75	M	lung cancer	ND	983	anticancer drug	drug-IP	—
3	sputum	58	M	colon cancer	2.8	2,160	anticancer drug	drug-IP	—
4	BALF	72	F	CTD-ILD	0.8	886	steroid, MTX	drug-IP	—
5	BALF	55	M	renal cancer	3	677	—	drug-IP	—
6	sputum	61	M	CTD-ILD	2	2,340	steroid, CPA	IP-AE	—
7	BALF	74	M	lung cancer	7.8	393	anticancer drug	radiation pneumonitis	—
8	BALF	69	M	lung cancer/ gastric cancer	2	383	anticancer drug	radiation pneumonitis	—
9	BALF	79	M	lung cancer	3.3	977	—	radiation pneumonitis	—
10	BALF	77	M	CBD cancer/ CMV pneumonia	79.7	315	anticancer drug, steroid	PCP	—
11	autopsy lung	77	M	CBD cancer/ CMV pneumonia	ND	ND	anticancer drug, steroid	PCP	—
12	BALF	35	M	AIDS	34.5	1,160	—	PCP	—
13	sputum	67	F	ATL	elevated	399	—	PCP	—
14	BALF	63	M	RA	43.14	729	MTX, infliximab	PCP	—
15	sputum	75	F	RA	26.42	323	steroid, MTX	PCP	—
16	sputum	39	M	AIDS	345	788	—	PCP	—
17	sputum	67	M	lung cancer	18.7	615	steroid	PCP	—
18	BALF	68	M	malignant melanoma	11.2	312	anticancer drug	PCP	—
19	sputum	81	F	chronic HP	73.43	2,233	steroid	PCP	—
20	BALF	35	M	AIDS	3.0	ND	—	PCP	—

SMX-TMP, sulfamethoxazole-trimethoprim; CTD-ILD, connective tissue disease-related interstitial lung disease; CBD, common bile duct; CMV, cytomegalovirus; RA, rheumatoid arthritis; AIDS, acquired immunodeficiency syndrome; ATL, adult T-cell leukemia/lymphoma; HP, hypersensitivity pneumonia; drug-IP, drug-induced interstitial pneumonia; AE, acute exacerbation; PCP, *Pneumocystis pneumonia*; ND, not detectable; MTX, methotrexate; CPA, cyclophosphamide.

the DNA PCR. This result indicated a statistically significant difference between the groups (Fisher's exact test,  $P = 0.02$ ). Comparing the characteristics of the 10 *P. jirovecii*-negative patients who had received prophylaxis and 9 *P. jirovecii*-colonized patients who had not received prophylaxis, there were no statistical differences in age, daily dose of prednisolone, or the presence or absence of existing pulmonary lesions ( $\chi^2$  test).

In the 1960s, PCP was first recognized as a major complication in immunosuppressed patients receiving steroids or anticancer drugs, particularly for leukemia or lymphoma. Since the 1980s, PCP has most frequently been seen in HIV-positive patients, and T-cell immune defects in HIV-positive patients are recognized to be the primary risk factor. In the present study, 9 of 49 patients (18.4%, excepting 11 patients with PCP) were found to be colonized with *P. jirovecii*, indicating that airway colonization with *P. jirovecii* is relatively common in patients with various HIV-negative underlying diseases treated with steroids, immunosuppressants, and/or anticancer drugs. Mansharamani et al. reported that the peripheral CD4<sup>+</sup> T-lymphocyte count is not a useful biological marker for identifying specific risk factors for PCP in HIV-negative immunosuppressive patients (4). Patients displaying airway colonization with *P. jirovecii* have the potential to develop PCP. Therefore, it may be useful to perform screening examinations for airway colonization with *P. jirovecii* using respiratory samples of HIV-negative immunosuppressive patients, regardless of the presence of a normal peripheral CD4<sup>+</sup> T-lymphocyte count. In the current study, neither the patients exhibiting airway colonization with *P. jirovecii* nor those with PCP had received prophylactic treatment with SMX-TMP. In other words, none of the patients who had undergone prophylactic SMX-TMP therapy demonstrated *P. jirovecii* DNA in their respiratory samples. This finding suggests that the prophylactic administration of SMX-TMP has the potential to both prevent PCP and eliminate airway colonization with *P. jirovecii*. The antibiotic combination SMX-TMP is an inhibitor of folic acid metabolism and may cause bone marrow suppression. Additionally, SMX-TMP is known to decrease the renal excretion of MTX, a commonly used immunosuppressant for RA. Patients receiving low-dose MTX are at risk for developing opportunistic infections, including PCP, despite having a normal leukocyte count (5), although the concomitant use of SMX-TMP and MTX is not usually recommended (6). However, a systematic review and meta-analysis by Green et al. demonstrated that the prophylactic administration of SMX-TMP in immunocompromised non-HIV-infected patients may be warranted at lower rates of PCP (7). It has also been reported that patients with interstitial pneumonia and connective tissue disease receiving steroid therapy benefit from prophylactic treatment against PCP (8,9). Mori et al. reported that 5 of 9 RA patients showing colonization with *P. jirovecii* (asymptomatic carriers) who had received MTX tested negative for PCR within 1 month after the introduction of PCP prophylaxis (10). PCP has been reported to occur in 0.2% to 0.4% of patients with RA treated with biological agents, including infliximab and etanercept (recombinant TNF receptor), and there is a causal association between an in-

creasing incidence of PCP and the use of these drugs (2). As the frequency of PCP is predicted to increase in association with the popularization of biological agents, the ability to prevent PCP is a pressing issue. In patients exhibiting risk factors, including an age of at least 65 years, treatment with a daily dose of prednisolone of at least 6 mg, and the presence of coexisting pulmonary disease, and/or those treated with biological agents, the identification of *P. jirovecii* carriers should encourage the prompt introduction of PCP prophylaxis (11).

PCP is infrequently seen in Africa, particularly in Uganda, where the incidence of AIDS is the highest in the world, suggesting an absence of the organism in that environment (12). In this context, it is important to reduce or eradicate cases of airway colonization with *P. jirovecii* before the patient develops PCP, as PCP is occasionally fatal and *P. jirovecii*-colonized individuals may serve as reservoirs for disease transmission (13). However, few studies have investigated the contribution of SMX-TMP to preventing airway colonization with *P. jirovecii*. In this study, although there was a possibility that all 60 patients showed negative results on the *P. jirovecii* DNA PCR even if they had not received prophylaxis with SMX-TMP, the patients who had received the prophylaxis showed a significantly lower positive rate on the PCR compared with the patients without the prophylaxis. We herein showed that the prophylactic administration of SMX-TMP has the potential to eliminate colonization with *P. jirovecii* in patients receiving steroids, immunosuppressants, or anticancer drugs. Further studies targeting large cohorts of patients with a variety of underlying diseases are required to develop recommendations for the prophylactic administration of SMX-TMP against PCP.

**Conflict of interest** None to declare.

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