CHEST

Primary Prophylaxis for *Pneumocystis jirovecii* Pneumonia in Patients Receiving Rituximab

Jun Won Park, MD; Jeffrey R. Curtis, MD; Kang Il Jun, MD; Tae Min Kim, MD; Dae Seog Heo, MD; Jongwon Ha, MD; Kyung-Suk Suh, MD; Kwang-Woong Lee, MD; Hajeong Lee, MD; Jaeseok Yang, MD; Min Jung Kim, MS; Yunhee Choi, PhD; and Eun Bong Lee, MD

BACKGROUND: Although previous studies suggested that rituximab increases the risk of *Pneumocystis jirovecii* pneumonia (PJP), it is uncertain whether its primary prophylaxis for PJP is justified.

RESEARCH QUESTION: Does the benefit of primary prophylaxis for PJP in patients receiving rituximab treatment outweigh the potential risk of the prophylaxis?

STUDY DESIGN AND METHODS: This retrospective study included 3,524 patients (hematologic diseases, 2,500; rheumatic diseases, 559; pre/post-solid organ transplantation, 465) first exposed to rituximab between 2002 and 2018 in a tertiary referral center in South Korea. Patients were classified into a control group (n = 2,523) and a prophylaxis group (n = 1,001) according to the administration of prophylactic trimethoprim-sulfamethoxazole (TMP-SMX) during the first 28 days after the start of rituximab (intention-to-treat analysis). In addition, exposure to TMP-SMX was examined as a time-varying variable (time-varying analysis). The primary outcome was the prophylactic effect of TMP-SMX on the 1-year incidence of PJP. Inverse probability of treatment weights was applied to minimize the baseline imbalance. The secondary outcome included the incidence of adverse drug reactions (ADRs) related to TMP-SMX.

RESULTS: Over 2,759.9 person-years, 92 PJP infections occurred, with a mortality rate of 27.2%. The prophylaxis group showed a significantly lower incidence of PJP (adjusted subdistribution hazard ratio, 0.20 [95% CI, 0.10-0.42]) than the control group. This result was consistent with the results of time-varying analysis, in which only one PJP infection occurred during TMP-SMX administration (adjusted subdistribution hazard ratio, 0.01 [0.003-0.16]). The incidence of ADRs related to TMP-SMX was 18.1 (14.6-22.2)/100 person-years, and most were of mild to moderate severity. On the basis of 10 severe ADRs, the number needed to harm was 101 (61.9-261.1), whereas the number needed to prevent one PJP infection was 32 (24.8-39.4).

INTERPRETATION: TMP-SMX prophylaxis significantly reduces PJP incidence with a tolerable safety profile in patients receiving rituximab treatment. CHEST 2022; 161(5):1201-1210

KEY WORDS: *Pneumocystis jirovecii*; prophylaxis; rituximab; trimethoprim-sulfamethoxazole

ABBREVIATIONS: ADR = adverse drug reaction; AE = adverse event; IPTW = inverse probability of treatment weights; NNH = number needed to harm; NNT = number needed to treat; PJP = *Pneumocystis jirovecii* pneumonia; SHR = subdistribution hazard ratio; TMP-SMX = trimethoprim-sulfamethoxazole; TPL = solid-organ transplantation

AFFILIATIONS: From the Division of Rheumatology, Department of Internal Medicine (J. W. Park and E. B. Lee), Seoul National University College of Medicine, Seoul, Republic of Korea; the Division of Clinical Immunology and Rheumatology (J. R. Curtis), University of Alabama at Birmingham, Birmingham, AL; the Division of Infectious Disease,

Take-home Points

Study Question: Is primary prophylaxis for *Pneumocystis jirovecii* pneumonia (PJP) necessary for patients receiving rituximab treatment?

Results: Primary prophylaxis for PJP using trimethoprim-sulfamethoxazole was associated with significantly lower PJP incidence (adjusted subdistribution hazard ratio, 0.20; number needed to treat, 32) with a favorable safety profile (number needed to harm, 101).

Interpretation: The potential benefit from the primary prophylaxis for PJP outweighs its potential risk in patients receiving rituximab treatment.

Pneumocystis jirovecii pneumonia (PJP) is a potentially life-threatening infection that occurs mainly in immunocompromised patients.[1] Effective treatment and an established prophylactic strategy in patients with HIV infection have led to a marked fall in occurrence.[2] However, the incidence of non-HIV PJP is increasing, with widespread use of immunosuppressive agents for the treatment of hematologic malignancies, rheumatic diseases, and solid organ transplantation.[3-6] Moreover, non-HIV PJP usually has more severe manifestations and carries a higher mortality rate than that in patients with HIV infection.[7-10]

Department of Internal Medicine (K. I. Jun), Ewha Womans University Seoul Hospital, Seoul, Republic of Korea; the Division of Hematology and Medical Oncology, Department of Internal Medicine (T. M. Kim and D. S. Heo), Seoul National University College of Medicine, Seoul, Republic of Korea; the Cancer Research Institute (T. M. Kim and D. S. Heo), Seoul National University, Seoul, Republic of Korea; the Department of Surgery (J. Ha, K.-S. Suh, and K.-W. Lee), Seoul National University College of Medicine, Seoul, Republic of Korea; the Transplantation Research Institute (J. Ha), Medical Research Center, Seoul National University College of Medicine, Seoul, Republic of Korea; the Division of Nephrology, Department of Internal Medicine (H. Lee), Seoul National University College of Medicine, Seoul, Republic of Korea; the Department of Internal Medicine (J. Yang), Yonsei University College of Medicine, Severance Hospital, Seoul, Republic of Korea; the Division of Medical Statistics (M. J. Kim and Y. Choi), Medical Research Collaborating Center, Seoul National University College of Medicine, Seoul, Republic of Korea; and the Department of Molecular Medicine and Biopharmaceutical Sciences (E. B. Lee), Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Republic of Korea.

FUNDING/SUPPORT: This study was supported by the Seoul National University Hospital Research Fund (grant 0320200170) and by a National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (grant 2021R1A2C2004874).

CORRESPONDENCE TO: Eun Bong Lee, MD; email: leb7616@snu.ac.kr

DOI: https://doi.org/10.1016/j.chest.2021.11.007

Many previous studies of the pathophysiology of PJP in patients with HIV infection focus on T cells and show that cell-mediated immunity plays an important role in the clearance of microorganisms.[11,12] However, accumulating evidence suggests that B cells play a critical role in T-cell-mediated immunity, and that abnormalities in B-cell numbers or function predispose to opportunistic infections such as PJP.[13,14] Rituximab, a chimeric anti-CD20 monoclonal antibody, is used widely to treat patients with hematologic malignancies, autoimmune diseases, ABO-incompatible transplantation, and antibody-mediated rejection. It exerts a therapeutic effect by complement and antibody-mediated B-cell depletion; however, this increases the risk of infectious complications.[15,16] However, although the prescription information for rituximab published by the US Food and Drug Administration recommends primary PJP prophylaxis for some indications, the incidence of PJP in patients receiving rituximab is unclear; indeed, few studies have investigated the efficacy of primary PJP prophylaxis in such patients.[17] Therefore, it is uncertain whether PJP prophylaxis is indicated for patients starting rituximab.[18,19]

To address this question, we investigated the incidence of PJP in patients treated with rituximab at a large national tertiary referral center over a 16-year period. The aim was to evaluate the effectiveness and safety of PJP prophylaxis and to quantify the precise risk-benefit profile.

Study Design and Methods

This retrospective study included patients treated with rituximab for the first time between 2002 and 2018 at Seoul National University Hospital. According to the underlying disorder requiring rituximab treatment, all patients were classified into one of the three disease groups: hematologic disease, rheumatic disease, or pre/post-solid organ transplantation (TPL). Further information regarding patient selection, inclusion and exclusion criteria, the collection of clinical data, and diagnostic evaluation of patients with suspicious PJP is given in the online article (e-Appendix 1 and e-Fig 1).

This study was carried out in accordance with the Declaration of Helsinki and was approved by the Seoul National University Hospital Institutional Review Board (Approval No. 1905-173-1036). The need for patient consent was waived due to the retrospective nature of the study.

Exposure to PJP Prophylaxis

Because there have been no established guidelines on primary PJP prophylaxis in patients receiving rituximab treatment, the selection of patients who would receive prophylaxis and the duration of treatment were mainly at the discretion of the treating physician. In our institution, most physicians have prescribed trimethoprim-sulfamethoxazole (TMP-SMX) for primary PJP prophylaxis at a dose

of one single-strength tablet per day, or one double-strength tablet three times per week. The dose of TMP-SMX was adjusted according to each patient's renal function. A review of all prescription data in the study population showed that no patient received second-line prophylactic agents such as dapsone and pentamidine during the observation period.

Considering the heterogeneity regarding the starting point and duration of PJP prophylaxis among the study population, efficacy outcomes were assessed using two different time schemes (e-Fig 2). First, an intention-to-treat design (ie, "first exposure carried forward") was used, in which administration of TMP-SMX during the period between baseline (day 0, the day of the first rituximab administration) and day 28 (lead-in period) was necessary to determine whether a patient was to be included in the prophylaxis or unexposed (control) group. Using this scheme, 1,001 patients who received prophylactic TMP-SMX during the lead-in period were classified into the prophylaxis group. The unexposed group comprised 2,269 patients who were never exposed to TMP-SMX during follow-up and 254 patients in whom the start of TMP-SMX treatment was delayed (> 4 weeks). In the latter case, follow-up was censored if patients subsequently received TMP-SMX. Second, a time-varying analysis, in which prophylactic TMP-SMX use was modeled as a time-dependent variable, was performed. In this analysis, follow-up of all patients began at baseline, and each subsequent person-day of observation was classified according to whether the patient received prophylactic TMP-SMX. In the time-varying analysis, patients could be assigned to the exposed and unexposed categories without restriction. Finally, 5,265 episodes (4,006 without TMP-SMX and 1,259 with TMP-SMX) were analyzed (e-Table 1).

PJP Detection

To detect all PJP cases without misclassification, a two-step algorithm was designed (e-Fig 3). First, because a definite diagnosis of PJP requires identification of the organism, we first captured the 219 cases with positive results from direct immunofluorescence staining and/or PCR assays of induced sputum and BAL during the observation period.

Next, two expert investigators (J. W. P. and K. I. J.) independently reviewed all medical records, laboratory data, and therapeutic antibiotic use in the selected cases to confirm PJP. To minimize bias, the investigators evaluated each case without information regarding whether the patient had received the prophylactic TMP-SMX previously. PJP was confirmed on the basis of (1) the presence of clinical and radiographic features suggestive of PJP, (2) the results of microbiologic tests for the identification of organisms other than *Pneumocystis jirovecii*, and (3) treatment responses to various antimicrobials. The final PJP cases were determined when both assessors agreed that the cases were consistent with PJP. The level of agreement between two investigators was excellent, with a κ value of 0.963 (95% CI, 0.927-0.998).

Outcomes

The primary outcome was the prophylactic effect of TMP-SMX on the 1-year incidence of PJP. Secondary outcomes included the effect of prophylaxis on 1-year PJP-related death (defined as death caused by a progression to ARDS or respiratory failure due to uncontrolled PJP) and the incidence of adverse drug reactions (ADRs) related to prophylactic TMP-SMX. All patients were observed from day 29 (intention-to-treat analysis) or baseline (time-varying analysis), and were monitored up until PJP infection, death, loss to follow-up (defined as no subsequent visit for > 6 months from the last visit), or 52 weeks from the start of observation, whichever came first.

The safety of prophylactic TMP-SMX was evaluated in two stages: first, all adverse events (AEs) that occurred during the period of prophylactic TMP-SMX administration were captured from the electronic medical database. Next, the probability of causation of each AE was estimated by one author (J. W. P.), based on timing, known AE profile, and improvement of AE after cessation of the agent. AEs showing probable/likely or certain causality were regarded as ADRs related to TMP-SMX.[20] The type of ADR and its severity were assessed according to the Common Terminology Criteria for Adverse Events, version 5.0.[21] A severe ADR was defined as grade 3 or higher. For risk-benefit assessment, the number needed to treat (NNT) to prevent one case of PJP with prophylaxis, and the number needed to harm (NNH) for one severe ADR, were compared.

Statistical Analysis

There were no missing values with respect to clinical characteristics or laboratory findings; thus no imputation was performed. Propensity scores were developed to model the likelihood that a patient would receive TMP-SMX prophylaxis. Then, inverse probability of treatment weights (IPTW) was applied to balance the baseline characteristics between the two groups. This approach creates a pseudo-population in which exposure to TMP-SMX was independent of measured covariates.[22] Baseline imbalances before and after applying IPTW were estimated, using the standardized mean difference. For time-varying analysis, in which exposure of an individual patient to TMP-SMX can be changed over time, time-varying inverse probability weights were estimated.[23] The bootstrap method was used to calculate the 95% confidence rate of NNT (NNH).

The efficacy of prophylactic TMP-SMX on outcome was assessed using Fine-Gray models, applying IPTW weights to control for confounders. A competing risk in the analysis of PJP incidence and related mortality was non-PJP-related death.[24] After applying IPTW, the model was adjusted further using prespecified covariates (age, sex, baseline azotemia [glomerular filtration rate < 60 mL/min], baseline lymphopenia [lymphocyte count < 800/μL], and concomitant high-dose steroid [mean daily dose of steroid ≥ 30 mg/d prednisone or equivalent during the lead-in period according to previous studies])[25-27] to generate effect estimates robust to misspecification of the model based on IPTW.[28] A robust sandwich-type variance estimator was used to examine within-subject correlation.[29]

Several sensitivity analyses were performed. First, the efficacy of prophylactic TMP-SMX was estimated after excluding patients with concomitant high-dose steroid treatment (n = 947). Second, multivariable analysis was repeated without IPTW. Third, the main analysis was performed after applying 1:1 propensity-score matching alternatively (n = 1,272; e-Table 2). Fourth, the efficacy of prophylactic TMP-SMX was estimated in the subgroup of patients who were not treated with any concomitant immunosuppressive and/or antineoplastic agents other than rituximab and glucocorticoids (n = 528). Finally, the potential effect of unmeasured confounding was estimated using the E-value, defined as the minimum strength of the association that an unmeasured confounder would need to have with both treatment and outcome to fully explain a specific treatment-outcome association.[30]

All statistical analyses were performed with R version 3.6.1 (R Foundation for Statistical Computing) and SAS version 9.4 (SAS Institute), and a P value < .05 was considered statistically significant.

Results

A total of 3,524 patients treated with rituximab were analyzed. The most common disease group was hematologic disease (n = 2,500; 71.0%), followed by rheumatologic disease (n = 559; 15.9%) and pre/post-TPL (n = 465; 13.2%). The baseline characteristics of the prophylaxis and control groups are presented in Table 1. After applying IPTW, all measured covariates were well balanced (standardized mean difference < 0.1). In the prophylaxis group, the mean (SD) duration of TMP-SMX administration was 153.8 (107.6) days.

PJP Incidence and Associated Factors

Overall, during 2,759.9 person-years, 92 cases of PJP (10 in the rheumatic disease group, 64 in the hematologic disease group, and 18 in the pre/post-TPL disease group) occurred, with a crude incidence rate (per 100 person-years) of 3.33 (95% CI, 2.69-4.09). The cumulative incidence of PJP in the control group was 4.11 (3.26-5.12) with 2.96 (1.19-6.09) for rheumatic disease, 4.50 (3.44-5.78) for hematologic disease, and 7.01 (3.63-12.25) for pre/post-TPL (e-Fig 4). The median (interquartile range) time interval between baseline and PJP infection was 86.0 (80.0) days. PJP-related mortality was 27.2% (25 of 92). The clinical features of these 92 cases at the time of PJP diagnosis were summarized in e-Table 3. A total of 356 non-PJP-related deaths occurred. The prevalence of non-PJP-related death was comparable between the control and the prophylaxis groups (10.3% vs 9.5%; $P = .448$) (e-Table 4).

Univariable analysis identified 1-year incidence of PJP as being significantly associated with age at baseline, disease group, baseline lymphopenia, azotemia, and concomitant treatment with high-dose steroid (e-Table 5). By contrast, concomitant treatment with lower dose of prednisone (20-30 mg/d) was not significantly associated with increased risk of PJP (subdistribution hazard ratio, 1.32 [95% CI, 0.81-2.14]).

Multivariable analysis identified azotemia (adjusted subdistribution hazard ratio [SHR], 2.38 [1.75-3.23]) and concomitant treatment with high-dose steroid (adjusted SHR, 3.09 [2.22-4.30]) as the two most important factors that increase the risk of PJP.

Efficacy of Prophylactic TMP-SMX

Intention-to-treat analysis revealed that 80 and 12 cases of PJP occurred in the control and prophylaxis groups, respectively. Prophylaxis significantly reduced the 1-year incidence of PJP (adjusted SHR, 0.20 [0.10-0.42]) and related mortality (adjusted SHR, 0.21 [0.05-0.84]) (Fig 1, Table 2), and its effect was consistent in all disease groups (Table 3, e-Fig 5).

Time-varying analysis showed that the prophylactic effect of TMP-SMX on the 1-year incidence of PJP was greater (adjusted SHR, 0.01 [0.002-0.09]) than that shown by intention-to-treat analysis (Table 2). Interestingly, among the 16 PJP cases that occurred in the 1,259 patients exposed to TMP-SMX during follow-up, only one case occurred during concomitant administration of TMP-SMX (e-Fig 6). Even after the population was restricted to those ever exposed to TMP-SMX, follow-up after discontinuation of TMP-SMX showed a significant increase in the risk for PJP compared with prophylaxis (adjusted SHR, 10.78 [1.68-69.28]) (e-Fig 7).

The prophylactic effect of TMP-SMX appeared to be duration-dependent. When the prophylaxis group was stratified according to duration of prophylaxis (based on 20 weeks [median value]), patents with a longer period of prophylaxis showed a greater prophylactic effect (Fig 2). When the analysis was performed only in the prophylaxis group, this subgroup also showed a lower incidence of PJP than those with shorter duration of prophylaxis (adjusted SHR, 0.17 [0.04-0.75]).

The prophylactic effect of TMP-SMX on the 1-year incidence of PJP was consistent with the results of sensitivity analyses, including those obtained after excluding patients treated concomitantly with high-dose steroid (adjusted SHR, 0.14 [0.04-0.52]) (e-Table 6), those obtained without applying IPTW (adjusted SHR, 0.26 [0.14-0.47]) (e-Table 7), those obtained in the 1:1 propensity score-matched population (adjusted SHR, 0.35 [0.16-0.79]) (e-Table 8), and those obtained in the subgroup of patients without other immunosuppressive and/or antineoplastic agents (e-Table 9). Finally, the E-value for the primary analysis of the 1-year incidence of PJP was 9.47, with a low 95% CI limit of 4.19, suggesting that an unmeasured confounder associated with both exposure to TMP-SMX and PJP by an SHR of 4.19-fold each would explain the observed prophylactic effect of TMP-SMX. The low 95% CI limit of the E-value for primary analysis in each disease group was 4.70 for rheumatic diseases, 2.90 for hematologic diseases, and 1.43 for the TPL disease group.

Safety of Prophylactic TMP-SMX

During the 509.1-person-year period of TMP-SMX prophylaxis, there were 2,113 AEs in 824 patients

TABLE 1] Baseline Characteristics of Study Population, Grouped According to Intention-to-Treat Analysis

Characteristic	Control Group (n = 2,523)	Prophylaxis Group (n = 1,001)	SMD Before IPTW	SMD After IPTW
Age, mean (SD), y	56.2 (15.1)	56.9 (14.5)	0.046	0.032
Male, No. (%)	1,345 (53.3)	523 (52.2)	0.021	0.005
Underlying diseases, No. (%)				
Rheumatoid arthritis	80 (3.2)	10 (1.0)	0.152	0.029
ANCA-associated vasculitis	22 (0.9)	77 (7.7)	0.342	0.018
Systemic lupus erythematosus	30 (1.2)	17 (1.7)	0.043	0.040
Systemic sclerosis	9 (0.4)	1 (0.1)	0.054	0.074
Sjögren syndrome	7 (0.3)	6 (0.6)	0.049	0.003
Inflammatory myositis	26 (1.0)	20 (2.0)	0.079	0.031
IgG4-related disease	10 (0.4)	10 (1.0)	0.072	0.022
Pemphigus	55 (2.2)	132 (13.2)	0.422	0.004
Other rheumatic diseases[a]	42 (1.7)	5 (0.5)	0.113	0.013
Diffuse large B-cell lymphoma	1,447 (57.4)	232 (23.2)	0.743	0.013
Follicular lymphoma	161 (6.4)	31 (3.1)	0.155	0.011
Mantle cell lymphoma	79 (3.1)	16 (1.6)	0.101	0.014
Primary CNS lymphoma	36 (1.4)	124 (12.4)	0.443	0.025
Chronic lymphoid leukemia	64 (2.5)	6 (0.6)	0.156	0.003
Burkitt lymphoma	51 (2.0)	20 (2.0)	0.002	0.002
Marginal zone B-cell lymphoma	74 (2.9)	13 (1.3)	0.114	0.013
Acute lymphoblastic leukemia	10 (0.4)	10 (1.0)	0.072	0.008
MALToma	40 (1.6)	2 (0.7)	0.083	0.004
Allo-SCT	2 (0.1)	3 (0.3)	0.051	< 0.001
GVHD	3 (0.1)	14 (1.4)	0.148	0.012
Other hematologic diseases[b]	48 (1.9)	8 (0.8)	0.096	0.006
Liver transplantation	9 (0.4)	107 (10.7)	0.464	0.055
Kidney transplantation	203 (8.0)	117 (11.7)	0.122	0.002
Other transplantation	15 (0.6)	14 (1.4)	0.081	0.006
Previous history of chemotherapy, No. (%)	129 (5.1)	53 (5.3)	0.008	0.051
Azotemia,[c] No. (%)	360 (14.3)	226 (22.6)	0.216	0.009
Baseline lymphopenia,[d] No. (%)	443 (17.6)	269 (26.9)	0.225	0.004
Cumulative steroid use (based on prednisone) during past 6 mo, mean (SD), mg	445.6 (2,608.1)	731.7 (1,929.3)	0.125	0.014
Concomitant high-dose steroid,[e] No. (%)	519 (20.6)	428 (42.8)	0.491	0.001

ANCA = anti-neutrophil cytoplasmic antibody; GVHD = graft-vs-host disease; IPTW = inverse probability of treatment weights; MALT = mucosa-associated lymphoid tissue; SCT = stem cell transplantation; SMD = standardized mean difference.
[a]Includes polyarteritis nodosa, anti-phospholipid antibody syndrome, and cryoglobulinemic vasculitis.
[b]Includes immune thrombocytopenic purpura, thrombotic thrombocytopenic purpura, intravascular B-cell lymphoma, and Waldenström macroglobulinemia.
[c]Defined as glomerular filtration rate < 60 mL/min.
[d]Defined as lymphocyte count < 800/μL.
[e]Defined as mean dose of steroid ≥ 30 mg/d of prednisone or equivalent during the lead-in period.

(e-Table 10). Ninety-two of these were ADRs, with an incidence of 18.1 (14.6-22.2) per 100 person-years (e-Table 11). An increased aspartate transaminase and/or alanine transaminase level was the most common ADR (n = 25), followed by azotemia (n = 10), hyponatremia (n = 9), and leukopenia (WBC < 4,000/mm^3) (n = 9). Eighty-two ADRs (89.1%) showed mild-to-moderate severity, and most did not require any intervention. Seventy patients (7.0%) discontinued TMP-SMX due to adverse events, of which 23 cases were ADRs.

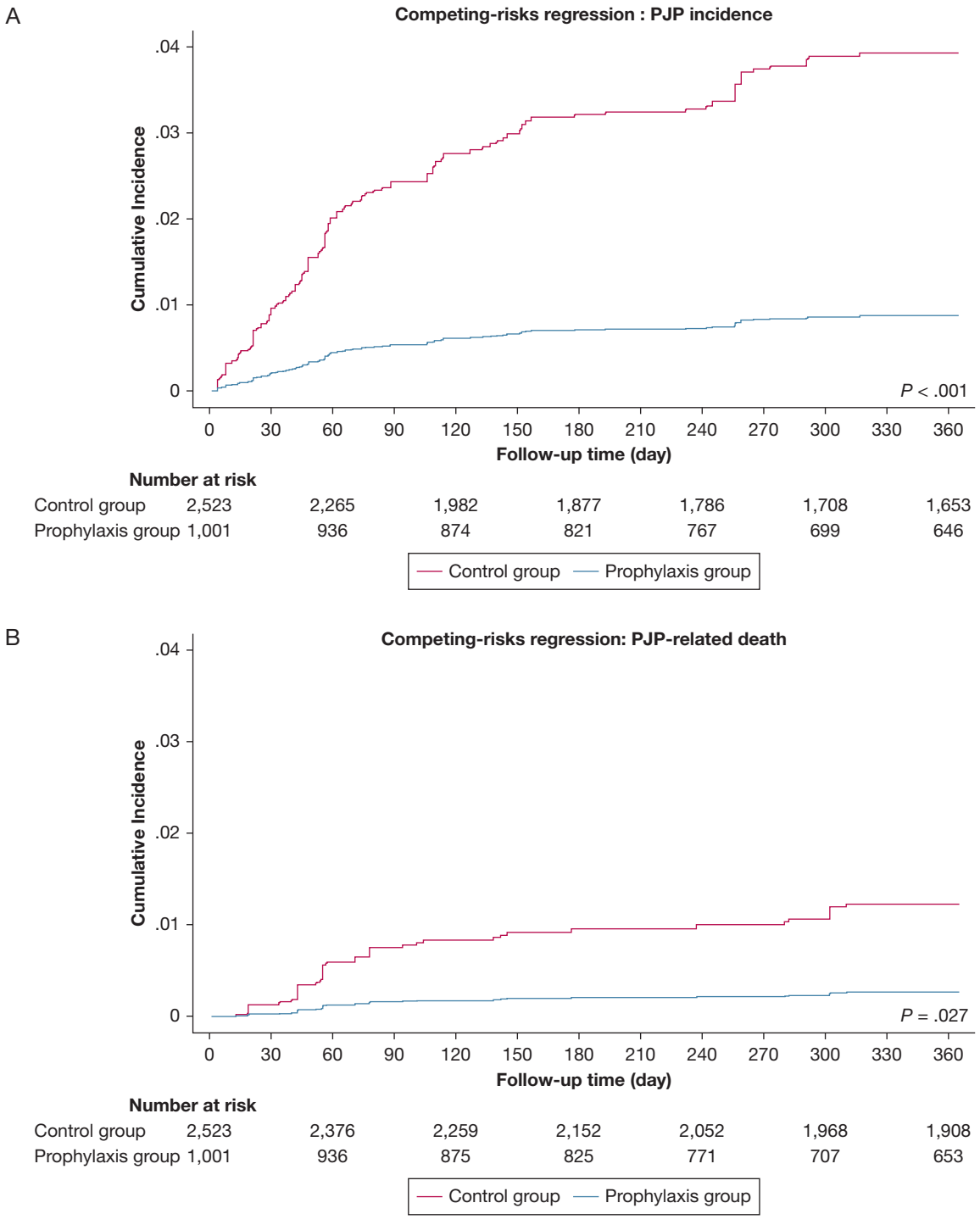

Figure 1 – *A and B, Cumulative incidence of PJP (A) and related mortality (B) in the control and prophylaxis groups. In the intention-to-treat analysis, the prophylaxis group showed significantly lower cumulative PJP incidence and related mortality than the control group. The* P *value was calculated using the Fine-Gray test, accounting for the competing risk of non-PJP-related death by applying inverse probability of treatment weighting. PJP =* Pneumocystis jirovecii *pneumonia.*

Ten severe ADRs occurred in 10 patients; pancytopenia was most common (n = 6) and only one case of Stevens-Johnson syndrome occurred during the observation period. All severe ADRs resolved after discontinuation of TMP-SMX.

Risk-Benefit Analysis for Prophylactic TMP-SMX

On the basis of the intention-to-treat analysis, the NNT to prevent one case of PJP was 32 (24.8-39.4). By contrast, the NNH due to any serious ADR was 101 (61.9-261.1). After stratification according to disease

TABLE 2] Effect of TMP-SMX Prophylaxis on the 1-Year Incidence of PJP and Related Mortality, Based on Intention-to-Treat and Time-Varying Analyses

Parameter	Intention-to-Treat Analysis (No. of Patients = 3,524)	
	Control Group	Prophylaxis Group
No. of PJP cases/follow-up period	80/1,939.6 person-y	12/815.3 person-y
Cumulative incidence of PJP[a] (95% CI)	4.13 (3.27-5.13)	1.47 (0.76-2.57)
No. of PJP-related death cases/follow-up period	21/2,166.5 person-y	4/818.9 person-y
Cumulative incidence of PJP-related death[a] (95% CI)	0.97 (0.60-1.48)	0.49 (0.13-1.25)
Unadjusted SHR for PJP (95% CI)	Reference	0.22 (0.11-0.46)
Adjusted SHR for PJP (95% CI)[b]	Reference	0.20 (0.10-0.42)
Unadjusted SHR for PJP-related death (95% CI)	Reference	0.22 (0.06-0.84)
Adjusted SHR for PJP-related death (95% CI)[b]	Reference	0.21 (0.05-0.84)
Parameter	Time-Varying Analysis (No. of Episodes = 5,265)	
	Episodes Without TMP-SMX	Episodes With TMP-SMX
No. of PJP cases/follow-up period	95/2,472.3 person-y	1/509.1 person-y
Cumulative incidence of PJP[a] (95% CI)	3.84 (3.11-4.70)	0.20 (0.005-1.09)
No. of PJP-related death cases/follow-up period	24/2,518.3 person-y	1/509.1 person-y
Cumulative incidence of PJP-related death[a] (95% CI)	1.17 (0.67-2.05)	0.20 (0.005-1.09)
Unadjusted SHR for PJP (95% CI)	Reference	0.01 (0.003-0.16)
Adjusted SHR for PJP (95% CI)[b]	Reference	0.01 (0.003-0.16)
Unadjusted SHR for PJP-related death (95% CI)	Reference	0.11 (0.01-0.79)
Adjusted SHR for PJP-related death (95% CI)[b]	Reference	0.09 (0.01-0.66)

PJP = *Pneumocystis jirovecii* pneumonia; SHR = subdistribution hazard ratio; TMP-SMX = trimethoprim-sulfamethoxazole.
[a]Per 100 person-years.
[b]Adjusted by age, sex, baseline azotemia, baseline lymphopenia, and concomitant high-dose steroid treatment.

group, the calculated NNT was 23 (16.6-36.4) for the rheumatic disease group, 36 (27.7-46.6) for the hematologic disease group, and 27 (13.6-101.1) for the TPL group, all of which were smaller than the NNH for any serious ADR in each disease group (e-Table 12). Of note, the NNT in the subgroup of patents treated concomitantly with high-dose steroid was lower than that for the other patients (17 [12.1-25.7] vs 46 [34.3-64.6]).

Discussion

To the best of our knowledge, this is the largest study to examine the efficacy and safety of primary PJP prophylaxis in patients receiving rituximab. Furthermore, the study comprised patients with most of the diseases for which rituximab is the primary treatment option, which increases the generalizability of the results markedly.

We found that the 1-year incidence of PJP without prophylaxis was 4.11/100 person-years; this value was highest in the TPL group, followed by hematologic disease and rheumatic disease groups. Although few studies have investigated the incidence of PJP during rituximab treatment, the incidence reported herein is higher than those previously reported.[17,31,32] From an immunologic perspective, accumulating evidence suggests that B cells play key roles in immunity against *Pneumocystis*.[13,14] Data from a murine model of PJP show that B cells modulate T-cell expansion and differentiation by acting as an antigen-presenting cell to $CD4^+$ T cells.[33] In addition, one study shows that B cells also play a role in lymphocyte reconstitution in the bone marrow following *Pneumocystis* lung infection.[34] These coordinated actions between B and T cells against *Pneumocystis* infection may explain our finding that concomitant treatment with high-dose steroid is the most important risk factor for PJP.

Considering the high incidence of PJP and related mortality in our study population, establishing universal guidelines regarding primary PJP prophylaxis is essential to ensure better outcomes after rituximab treatment. In this context, we demonstrated that prophylactic TMP-SMX is highly effective at reducing the 1-year incidence of PJP and related mortality. This beneficial effect was not accompanied by significant safety issues. This

TABLE 3] Prophylactic Effect of TMP-SMX on Cumulative 1-Year PJP Incidence and Related Mortality in Each Disease Group, Using Intention-To-Treat Analysis

	1-Year PJP Incidence		1-Year PJP-Related Mortality	
	SHR (95% CI)		SHR (95% CI)	
Disease Group	Univariable Analysis	Multivariable Analysis[a]	Univariable Analysis	Multivariable Analysis[a]
Rheumatic disease	0.12 (0.03-0.50)	0.10 (0.02-0.38)	0.13 (0.02-0.75)	0.06 (0.003-0.99)
Hematologic disease	0.16 (0.04-0.59)	0.16 (0.04-0.57)	0.22 (0.03-1.81)	0.21 (0.03-1.75)
Pre/post-TPL	0.46 (0.23-0.92)	0.46 (0.23-0.91)	0.95 (0.11-8.31)	1.01 (0.13-7.64)

PJP = *Pneumocystis jirovecii* pneumonia; SHR = subdistribution hazard ratio; TMP-SMX = trimethoprim-sulfamethoxazole; TPL = transplantation.
[a]Adjusted by age, sex, baseline azotemia, baseline lymphopenia, and concomitant high-dose steroid treatment.

strongly suggests that any potential benefit from TMP-SMX prophylaxis outweighs any likely harm. Furthermore, this result was consistent irrespective of the grouped disease indications for rituximab, suggesting that the prophylactic strategy can be applied to all patients receiving rituximab.

It is noteworthy that most PJP cases (15 of 16) in patients exposed to prophylactic TMP-SMX occurred a few months after discontinuation. The B-cell-depleting effect of rituximab persists for 6 to 12 months, so a longer duration of TMP-SMX treatment may be necessary for better PJP prophylaxis.[35,36] We found that the prophylactic efficacy of TMP-SMX was more prominent in the time-varying analysis, which supports this hypothesis. Our intention-to-treat analysis also showed that the 1-year incidence of PJP was significantly lower in the subgroup of patients that received TMP-SMX prophylaxis for > 20 weeks. This result is consistent with that from another cohort of patients who underwent kidney transplantation, which showed that nearly 80% of PJP cases in patients receiving rituximab occurred within 6 months after discontinuation of a 6-month course of prophylaxis.[37] A case series of non-HIV

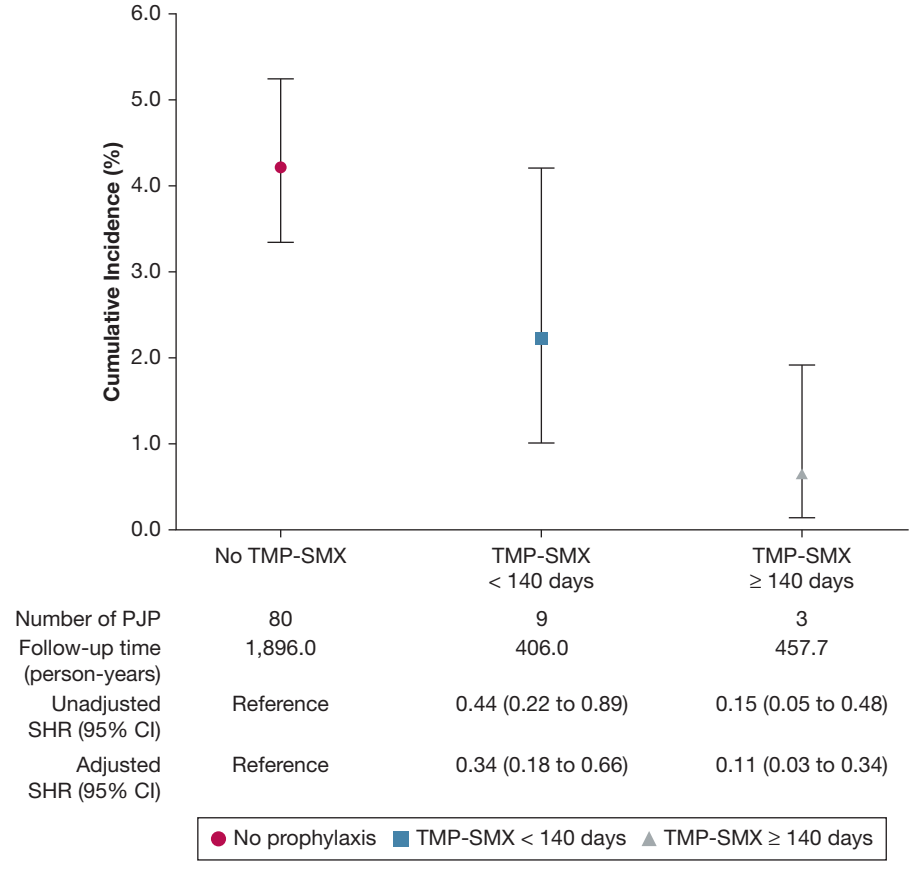

Figure 2 – *Effect of duration of prophylactic TMP-SMX treatment on the 1-year incidence of PJP. PJP* = Pneumocystis jirovecii *pneumonia; SHR = subdistribution hazard ratio; TMP-SMX = trimethoprim-sulfamethoxazole.*

PJP also showed that in PJP cases associated with rituximab treatment, most patients received the treatment in the year before infection.[38] Taken together, the data suggest that TMP-SMX should be continued to ensure a better outcome. However, although we showed the benefit of prolonged prophylaxis, the optimal duration remains to be determined, and it may differ according to the underlying disease or concomitant use of immunosuppressants. Therefore, further studies are required.

This study has some limitations. First, the baseline characteristics of the control and prophylaxis groups were not fully balanced, which is inevitable in an observational study. We applied the IPTW method to solve the problem, but it could simultaneously lead to substantial bias in estimating SE of the effect and to an increased probability of type I error.[39] Second, the presence of unmeasured confounders such as physician's preference for TMP-SMX cannot be removed by IPTW and could inevitably lead to biased results. However, our sensitivity analysis suggests that extreme unmeasured confounders would be needed to explain an effect estimate. Third, the number of patients with certain specific diseases was rather small, so we could not perform subgroup analyses stratified according to a specific disease. In addition, the effect of concomitant immunosuppressive agents or antineoplastic agents on the risk of PJP was not fully evaluated in our generalized analysis encompassing patients with most of the diseases for which rituximab is the primary treatment option. Therefore, it should be further investigated in future studies. Finally, because this was not a randomized-controlled study, we could not compare the prevalence of AEs between the two groups. Therefore, the NNH was calculated on the basis of ADRs captured from the prophylaxis group. Furthermore, because all safety data were collected retrospectively, it is possible that some ADRs were not identified, and/or the cause of some AEs was misclassified.

Interpretation

In conclusion, we showed that TMP-SMX was associated with a significantly reduced 1-year incidence of PJP in patients receiving rituximab with a favorable safety profile. Although this result should be confirmed in future randomized studies, it may affect practice regarding the use of PJP prophylaxis concomitant with rituximab treatment.

Acknowledgments

Author contributions: E. B. L. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: E. B. L., J. R. C., and J.W. P. Acquisition, analysis, or interpretation of data: J. W. P., J. R. C., K. I. J., T. M. K., D. S. H., J. H., K.-S. S., K.-W. L., H. L., J. Y., M. J. K., Y. C., and E. B. L. Drafting of the manuscript: E. B. L., J. R. C., and J. W. P. Critical revision of the manuscript for important intellectual content: J. W. P., J. R. C., K. I. J., T. M. K., D. S. H., J. H., K.-S. S., K.-W. L., H. L., J. Y., M. J. K., Y. C., and E. B. L. Statistical analysis: E. B. L., J. R. C., M. J. K., Y. C., and J. W. P.

Financial/nonfinancial disclosures: The authors have reported to *CHEST* the following: E. B. L. has worked as a consultant to Pfizer and received research grants from GC Pharma, Handok, and ImmuneMed, Seoul, Republic of Korea. J. R. C. reported grants and personal fees from Abbvie, grants and personal fees from Amgen, grants and personal fees from BMS, grants and personal fees from Corrona, grants and personal fees from Eli Lilly, grants and personal fees from Jannsen, grants and personal fees from Myriad, grants and personal fees from Pfizer, grants and personal fees from Regeneron, grants and personal fees from Roche, and grants and personal fees from UCB, during the conduct of the study. None declared (J. W. P., K. I. J., T. M. K., D. S. H., J. H., K.-S. S., K.-W. L., H. L., J. Y., M. J. K., Y. C.).

Role of sponsors: The funders had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript.

Other contributions: The authors especially thank Myung Nam Yoo, BS, for her commitment to the initial collection of TMP-SMX safety data from the patients' medical database.

Additional information: The e-Appendix, e-Figures, and e-Tables are available online under "Supplementary Data".

References

1. Thomas CF Jr, Limper AH. Pneumocystis pneumonia. *N Engl J Med*. 2004;350(24):2487-2498.
2. Kaplan JE, Hanson D, Dworkin MS, et al. Epidemiology of human immunodeficiency virus-associated opportunistic infections in the United States in the era of highly active antiretroviral therapy. *Clin Infect Dis*. 2000;30(suppl 1):S5-S14.
3. Morris A, Lundgren JD, Masur H, et al. Current epidemiology of *Pneumocystis* pneumonia. *Emerg Infect Dis*. 2004;10(10):1713-1720.
4. Maini R, Henderson KL, Sheridan EA, et al. Increasing *Pneumocystis* pneumonia, England, UK, 2000-2010. *Emerg Infect Dis*. 2013;19(3):386-392.
5. Fillatre P, Decaux O, Jouneau S, et al. Incidence of *Pneumocystis jiroveci* pneumonia among groups at risk in HIV-negative patients. *Am J Med*. 2014;127(12):1242.e11-1242.e17.
6. Martin SI, Fishman JA; AST Infectious Diseases Community of Practice. *Pneumocystis* pneumonia in solid organ transplantation. *Am J Transplant*. 2013;13(suppl 4):272-279.
7. Yale SH, Limper AH. *Pneumocystis carinii* pneumonia in patients without acquired immunodeficiency syndrome: associated illness and prior corticosteroid therapy. *Mayo Clin Proc*. 1996;71(1):5-13.
8. Bienvenu AL, Traore K, Plekhanova I, Bouchrik M, Bossard C, Picot S. *Pneumocystis* pneumonia suspected cases in 604 non-HIV and HIV patients. *Int J Infect Dis*. 2016;46:11-17.
9. Cordonnier C, Cesaro S, Maschmeyer G, et al. *Pneumocystis jirovecii* pneumonia: still a concern in patients with haematological malignancies and stem cell transplant recipients. *J Antimicrob Chemother*. 2016;71(9):2379-2385.
10. Monnet X, Vidal-Petiot E, Osman D, et al. Critical care management and outcome of severe *Pneumocystis* pneumonia in

patients with and without HIV infection. *Crit Care*. 2008;12(1):R28.

11. Kelly MN, Shellito JE. Current understanding of *Pneumocystis* immunology. *Future Microbiol*. 2010;5(1):43-65.
12. Kelly MN, Zheng M, Ruan S, Kolls J, D'Souza A, Shellito JE. Memory $CD4^+$ T cells are required for optimal NK cell effector functions against the opportunistic fungal pathogen *Pneumocystis murina*. *J Immunol*. 2013;190(1):285-295.
13. Hu Y, Wang D, Zhai K, Tong Z. Transcriptomic analysis reveals significant B lymphocyte suppression in corticosteroid-treated hosts with *Pneumocystis* pneumonia. *Am J Respir Cell Mol Biol*. 2017;56(3):322-331.
14. Rong HM, Li T, Zhang C, et al. IL-10-producing B cells regulate Th1/Th17-cell immune responses in *Pneumocystis* pneumonia. *Am J Physiol Lung Cell Mol Physiol*. 2019;316(1):L291-L301.
15. Gea-Banacloche JC. Rituximab-associated infections. *Semin Hematol*. 2010;47(2):187-198.
16. Aksoy S, Dizdar O, Hayran M, Harputluoglu H. Infectious complications of rituximab in patients with lymphoma during maintenance therapy: a systematic review and meta-analysis. *Leuk Lymphoma*. 2009;50(3):357-365.
17. Wei KC, Sy C, Wu SY, Chuang TJ, Huang WC, Lai PC. *Pneumocystis jirovecii* pneumonia in HIV-uninfected, rituximab treated non-Hodgkin lymphoma patients. *Sci Rep*. 2018;8(1):8321.
18. Martin-Garrido I, Carmona EM, Specks U, Limper AH. *Pneumocystis* pneumonia in patients treated with rituximab. *Chest*. 2013;144(1):258-265.
19. Besada E. Routine *Pneumocystis* pneumonia prophylaxis in patients treated with rituximab? *Chest*. 2013;144(1):359-360.
20. Edwards IR, Aronson JK. Adverse drug reactions: definitions, diagnosis, and management. *Lancet*. 2000;356(9237):1255-1259.
21. US Department of Health and Human Services. *Common Terminology Criteria for Adverse Events (CTCAE)*. Version 5.0. November 27, 2017. Accessed January 27, 2022. https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference_5x7.pdf
22. Austin PC. The performance of different propensity-score methods for estimating differences in proportions (risk differences or absolute risk reductions) in observational studies. *Stat Med*. 2010;29(20):2137-2148.
23. Grafféo N, Latouche A, Geskus RB, Chevret S. Modeling time-varying exposure using inverse probability of treatment weights. *Biom J*. 2018;60(2):323-332.
24. Austin PC, Fine JP. Practical recommendations for reporting Fine-Gray model analyses for competing risk data. *Stat Med*. 2017;36(27):4391-4400.
25. Buttgereit F, da Silva JA, Boers M, et al. Standardised nomenclature for glucocorticoid dosages and glucocorticoid treatment regimens: current questions and tentative answers in rheumatology. *Ann Rheum Dis*. 2002;61(8):718-722.
26. Park JW, Curtis JR, Moon J, Song YW, Kim S, Lee EB. Prophylactic effect of trimethoprim-sulfamethoxazole for pneumocystis pneumonia in patients with rheumatic diseases exposed to prolonged high-dose glucocorticoids. *Ann Rheum Dis*. 2018;77(5):644-649.
27. Park JW, Curtis JR, Kim MJ, Lee H, Song YW, Lee EB. Pneumocystis pneumonia in patients with rheumatic diseases receiving prolonged, non-high-dose steroids: clinical implication of primary prophylaxis using trimethoprim-sulfamethoxazole. *Arthritis Res Ther*. 2019;21(1):207.
28. Funk MJ, Westreich D, Wiesen C, Sturmer T, Brookhart MA, Davidian M. Doubly robust estimation of causal effects. *Am J Epidemiol*. 2011;173(7):761-767.
29. Ellis AR, Brookhart MA. Approaches to inverse-probability-of-treatment-weighted estimation with concurrent treatments. *J Clin Epidemiol*. 2013;66(8 suppl):S51-S56.
30. VanderWeele TJ, Ding P. Sensitivity analysis in observational research: introducing the E-value. *Ann Intern Med*. 2017;167(4):268-274.
31. Jiang X, Mei X, Feng D, Wang X. Prophylaxis and treatment of *Pneumocystis jiroveci* pneumonia in lymphoma patients subjected to rituximab-contained therapy: a systemic review and meta-analysis. *PLoS One*. 2015;10(4):e0122171.
32. Barreto JN, Ice LL, Thompson CA, et al. Low incidence of pneumocystis pneumonia utilizing PCR-based diagnosis in patients with B-cell lymphoma receiving rituximab-containing combination chemotherapy. *Am J Hematol*. 2016;91(11):1113-1117.
33. Elsegeiny W, Eddens T, Chen K, Kolls JK. Anti-CD20 antibody therapy and susceptibility to *Pneumocystis* pneumonia. *Infect Immun*. 2015;83(5):2043-2052.
34. Hoyt TR, Dobrinen E, Kochetkova I, Meissner N. B cells modulate systemic responses to *Pneumocystis murina* lung infection and protect on-demand hematopoiesis via T cell-independent innate mechanisms when type I interferon signaling is absent. *Infect Immun*. 2015;83(2):743-758.
35. Dunleavy K, Hakim F, Kim HK, et al. B-cell recovery following rituximab-based therapy is associated with perturbations in stromal derived factor-1 and granulocyte homeostasis. *Blood*. 2005;106(3):795-802.
36. Colucci M, Carsetti R, Cascioli S, et al. B cell reconstitution after rituximab treatment in idiopathic nephrotic syndrome. *J Am Soc Nephrol*. 2016;27(6):1811-1822.
37. Kim YH, Kim JY, Kim DH, et al. Pneumocystis pneumonia occurrence and prophylaxis duration in kidney transplant recipients according to perioperative treatment with rituximab. *BMC Nephrol*. 2020;21(1):93.
38. Verhaert M, Blockmans D, De Langhe E, Henckaerts L. *Pneumocystis jirovecii* pneumonia in patients treated for systemic autoimmune disorders: a retrospective analysis of patient characteristics and outcome. *Scand J Rheumatol*. 2020;49(5):345-352.
39. Austin PC. Variance estimation when using inverse probability of treatment weighting (IPTW) with survival analysis. *Stat Med*. 2016;35(30):5642-5655.