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ORIGINAL ARTICLE

Point prevalence of *Pneumocystis* pneumonia in patients with non-Hodgkin lymphoma according to the number of cycles of R-CHOP chemotherapy

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Abstract R-CHOP chemotherapy composed of rituximab, cyclophosphamide, adriamycin, vincristine, and prednisolone which might increase the risk of *Pneumocystis* pneumonia in patients with non-Hodgkin lymphoma. We estimated the point prevalence of *Pneumocystis* pneumonia in non-Hodgkin lymphoma patients according to the number of R-CHOP cycles and investigated whether cytoreduction by chemotherapy is associated with *Pneumocystis* pneumonia development. We retrospectively established a cohort of patients who received R-CHOP for non-Hodgkin lymphoma in our institution. Using this cohort, we estimated the incidence rate and point prevalence of definite and probable *Pneumocystis* pneumonia. To assess factors associated with *Pneumocystis* pneumonia development several clinical variables, including absolute neutrophil and lymphocyte count at the time of non-Hodgkin lymphoma diagnosis and when the last R-CHOP cycle was administered, were compared between patients with and without *Pneumocystis* pneumonia. Of 713 patients in the cohort, 14 and 18 patients were diagnosed with definite and probable *Pneumocystis* pneumonia, respectively. The overall incidence of definite and definite plus probable PCP in NHL patients receiving R-CHOP were 2.0 % (14/713; 95 % CI, 1.1–3.3 %) and 4.5 % (32/713; 95 % CI, 3.2–6.4 %), respectively. This corresponded to 3.8 (95 % CI, 2.2–6.4) and 8.4 (95 % CI, 5.9–11.9) per 1000 persons. Many cases of *Pneumocystis* pneumonia (22/32, 68.7 %) developed after administration of the fourth R-CHOP cycle. However, there was no statistical difference in *Pneumocystis* pneumonia prevalence between patients receiving four or more cycles of R-CHOP and fewer than. Higher absolute neutrophil count (4,742/mm^3 vs. 2,627/mm^3; $p<0.01$) was associated with *Pneumocystis* pneumonia development at the last R-CHOP cycle, while absolute lymphocyte count at the time of NHL diagnosis was not. Contrary to expectations, *Pneumocystis* pneumonia is not a frequent complication of R-CHOP treatment for non-Hodgkin lymphoma. Cytoreduction of R-CHOP might not be a risk factor of *Pneumocystis* pneumonia development. Universal prophylaxis against *Pneumocystis* pneumonia during R-CHOP treatment could not be strongly recommended.

Keywords *Pneumocystis* pneumonia · R-CHOP · Non-Hodgkin lymphoma · Prevalence

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Introduction

Pneumocystis jirovecii pneumonia (PCP) is one of the major infectious complications of chemotherapy in patients without human immune-deficiency virus (HIV) infection [1–4]. Systemic corticosteroid usage has been identified as a predisposing factor of PCP in previous studies [4, 5]. It has also known that rituximab usage might be a risk factor of PCP development [6]. In addition, some case series of patients with solid tumor showed that cytoreduction itself after chemotherapy without corticosteroid could be sufficient for being a predisposing condition of PCP [4, 7].

R-CHOP, the regimens of therapy for non-Hodgkin lymphoma (NHL), consists of rituximab, cyclophosphamide, adriamycin, vincristine, and prednisolone [8]. As you see, two kinds of regimens which could increase the risk of PCP development are included: rituximab and prednisolone. In addition, this risk could be more increased by the administration of several cycles of R-CHOP regimens, because cytoreduction might progress and the duration of corticosteroid usage will be lengthened in consequence of repeated chemotherapy. Indeed, a previous report showed that antilymphocyte count (ALC) decreased according to the number of cycles of R-CHOP therapy [9, 10]. Based on these rationales, it can be readily inferred that PCP might develop often in patients with NHL who are treated with R-CHOP and incidence of PCP could increase according to repeated cycles of R-CHOP.

Despite this background, there has not been a well-described study showing the incidence of PCP in patients receiving R-CHOP used for NHL treatment. The development of PCP has not been notable in previous studies addressing the efficacy of R-CHOP in NHL [8, 11, 12]. A few case series of PCP in patients with NHL were reported and some studies have considered the issue of PCP development in patients receiving R-CHOP for NHL [9, 13–18]. However, these studies included only a few patients with PCP confirmed by biopsy or direct immunofluoresecence assay. In addition, they did not show the trend of point prevalence of PCP development according to the number of cycles of R-CHOP therapy.

Therefore, we estimated the point prevalence of PCP in patients with NHL according to the number of cycles of R-CHOP and investigated whether low ALC is associated with PCP development to verify our hypothesis: (1) PCP will frequently develop in NHL patients treated with R-CHOP. (2) Point prevalence of PCP will increase according to the number of cycles. (3) ALC as a marker of cytoreduction resulted from repeated chemotherapy will be associated with PCP development.

Patients and methods

Patient population and study design

This study was performed at the Asan Medical Center, a 2700-bed tertiary care teaching hospital in Seoul, Korea. By searching the electronic medical records for patients who received the R-CHOP regimen for the treatment of NHL, we retrospectively established a cohort of NHL patients who were administered the R-CHOP regimen between January 2007 and December 2011. Patients who were less than 16 years old were excluded. All patients in the cohort were followed for at least 3 months. The R-CHOP regimen of our center consists of 375 mg/m^2 rituximab, 750 mg/m^2 cyclophosphamide, 50 mg/m^2 doxorubicin, and 2 mg vincristine on day 1, and 100 mg prednisone on days 1–5, administered every 3 weeks for 6–8 cycles based on NHL status and the clinician's judgment.

Data collection

Data on demographic (age and gender) and clinical (Ann Arbor stage and pathology of NHL) variables were retrospectively collected. Information on R-CHOP therapy, such as the number and interval of cycles, was recorded. We also noted whether a patient received prior anti-pneumocystis prophylaxis. Laboratory results such as absolute neutrophil count (ANC), ALC, and lactate dehydrogenase (LDH) level at the time of NHL diagnosis and administration of the last R-CHOP were also recorded.

PCP patients

At first, we searched for patients with a positive test result for an immunofluorescence assay for PCP using bronchoalveolar lavage (BAL) samples within the cohort. Among them, those who had respiratory symptoms and radiological findings compatible with PCP were diagnosed with definite PCP. Broncholalveolar lavage was performed through a fiberoptic bronchoscope using standard techniques [19]. A direct immunofluorescence assay to detect *P. jirovecii* was performed using a commercially available murine monoclonal antibody labeled with fluorescein isothiocyanate that reacts with human and rodent *Pneumocystis* cysts and trophozoites in accordance with the manufacturer's instructions (Light Diagnostics TM *Pneumocystis carinii* DFA Kit, Millipore, Billerica, MA, USA). Secondly, we searched for patients who received anti-pneumocystis regimens at therapeutic dose. Among them, those who had following conditions were considered as having probable PCP : 1) symptoms such as fever, cough, or dyspnea, 2) radiologic findings compatible to PCP, 3) no other definite cause of

pneumonia. Events that happened after other kinds of salvage chemotherapy were excluded because the purpose of our study was to estimate the point prevalence of PCP in patients received R-CHOP regimens.

Clinical characteristics such as time of onset of PCP from the first and last R-CHOP cycles, the severity of PCP, treatment duration, adjunctive steroid usage, intensive care unit admission, mechanical ventilation, and 30-day and 90-day mortality were investigated. The classification of the severity of PCP was based on the PaO_2 while breathing room air, or on the alveolar–arterial oxygen difference ($AaDO_2$), prior to the first bronchoscopy (mild PCP: PaO_2 >70 mmHg or $AaDO_2$ <35; moderate PCP: $PaO_2 \leq 70$ mmHg or $AaDO_2 \geq 35$; severe PCP: PaO_2 <60 mmHg or $AaDO_2 \geq 45$) [20]. Laboratory results such as BAL white blood count and C-reactive protein were also collected.

Statistical analysis

All statistical analyses were performed using SPSS version 12.0 (SPSS, Chicago, IL, USA). Data are presented as the median score with interquartile range (IQR) in parentheses. The incidence rates of PCP were estimated by dividing the number of PCP cases in the cohort by the number of person-cycles of R-CHOP at-risk in the overall cohort. For point prevalence, we estimated the proportion of patients with PCP at each cycle of R-CHOP therapy. The 95 % confidence intervals (CI) of the incidence rates and prevalence were estimated based on a Poisson distribution. Categorical variables were compared using Fisher's exact test and continuous variables were analyzed by the Mann-Whitney *U* test because the data were not normally distributed. In all patients of our cohort, regardless of PCP development, ANC, ALC, LDH value at the time of NHL diagnosis and at the last R-CHOP cycle was compared by paired *t* test. Analysis of covariance was used to compare the extent of ALC decrease between patients with and without PCP. All tests were two-tailed and differences were considered significant at $p < 0.05$.

Results

Study population

During the study period, 713 patients received R-CHOP for the treatment of NHL. Diffuse large B cell lymphoma (DLBL) was the most common pathologic type (n=672, 94.2 %). Other pathologic types were mantle cell lymphoma (MCL; n=27, 3.8 %), follicular lymphoma (n=8, 1.1 %), marginal zone lymphoma (n=4, 0.6 %), mucosa-associated lymphoid tissue (n=1, 0.1 %), and lymphomatoid granulomatosis (n=1, 0.1 %). A total of 456 (63.7 %) patients were treated with six or more cycles of R-CHOP before definite PCP happened: 371 (52.0 %) received six cycles, 11 (1.5 %) received seven cycles, and 74 (10.4 %) received eight cycles. In addition, 37 (5.2 %), 85 (11.9 %), 71 (10.0 %), 21 (3.0 %), and 43 (6.0 %) patients received 5, 4, 3, 2, and 1 cycle of R-CHOP, respectively. On the other hand, a total of 453 (63.5 %) patients were treated with six or more cycles of R-CHOP before definite or probable PCP happened: 368 (51.6 %) received six cycles, 11 (1.5 %) received seven cycles, and 74 (10.4 %) received eight cycles. In addition, 37 (5.2 %), 85 (11.9 %), 71 (10.0 %), 24 (3.4 %), and 43 (6.0 %) patients received 5, 4, 3, 2, and 1 cycle of R-CHOP, respectively.

Point prevalence of PCP based on the number of R-CHOP cycles

Among the patients enrolled in the cohort, 14 and 18 patients were diagnosed with definite and probable PCP, respectively. Probable patients were searched through following process: we retrospectively found 40 patients who received anti-pneumocystis regimens in the cohort. Of these 40 patients, 7 patients received less than 5 days of anti-pneumocystis regimens, because physicians judged that their events were not likely *P. jirovecii* infection. 2 patients had no signs and symptoms of PCP. A patient had no compatible radiologic finding. 4 patients received other kinds of salvage chemotherapy before event happened. 5 patients had other definite cause of interstitial pneumonia such as CMV (3), influenza (1), and coronavirus (1) which were diagnosed by culture from BAL specimens. All these 5 patients had negative DFA results. A patient received TMP/SMZ for the treatment of S. maltophilia. Two patients empirically received TMP/SMX because fever was not subsided nevertheless of broad-spectrum antibiotics usage. Consequently, 18 patients were suspected to have PCP and received TMP/SMX to treat PCP. Of these 18 patients, BAL was done in 7 patients and negative results of DFA were documented and the results of biopsy and BAL fluid analysis favored the diagnosis of drug-induced interstitial lung disease.

Individual clinical characteristics of these patients are shown in supplement Table 1. Median onset times of definite PCP from first and last R-CHOP cycle were 102 days (IQR, 85–147) and 19 days (IQR, 17–24), while those of probable PCP were 84 days (IQR, 75–113) and 22 days (IQR, 17–40), respectively. Clinical manifestations and outcomes of definite and probable PCP were summarized in Table 1. Most common symptom was fever (29/32, 90.6 %), followed by dyspnea (25/32, 78.1 %), cough (18/32, 56.3 %), and sputum (34.4 %). Initial severity was classified as severe in 83.3 % (25/30) of patients. Diffuse ground glass

Table 1 Clinical manifestations and outcomes of *Pneumocystis* pneumonia in patients receiving R-CHOP regimens for non-Hogkin lymphoma

	Definite PCP (%) N=14	Probable PCP (%) N=18
Fever (%)	13 (92.9)	16 (88.9)
Cough (%)	9 (64.3)	9 (50)
Sputum (%)	5 (35.7)	6 (33.3)
Dyspnea (%)	13 (92.9)	12 (66.7)
Initial severity		
Severe	11 (78.6)	14 (87.5)
Mild-to-Moderate	3 (21.4)	2 (12.5)
Radiographic findings		
Diffuse GGO (%)	14 (100)	18 (100)
Consolidation (%)	5 (35.7)	5 (27.8)
Bilateral pleural effusion (%)	1 (7.1)	3 (16.7)
Pneumothorax (%)	0	1 (5.6)
Treatment duration, median days (IQR)	15 (14–19)	9 (7–14)
Adjunctive corticosteroid usage (%)	12 (85.7)	16 (88.9)
Admission to intensive care unit (%)	5 (35.7)	8 (44.4)
Need for mechanical ventilation (%)	5 (35.7)	7 (38.9)
90-day mortality (%)	1 (7.1)	5 (27.8)

Abbreviations: PCP: *Pneumocystis* pneumonia; GGO: Ground Glass opacity; IQR: interquartile range

opacities were found in all PCP patients, while consolidation, bilateral pleural effusion, and pneumothorax were found in only small proportion of patients. All patients with either definite or probable PCP received trimethoprim/sulfamethoxazole (TMP/SMX) for first-line therapy. The median treatment duration was 13 days (IQR, 8–16). 87.5 % (28/32) patients received adjunctive corticosteroid. 13 (40.6 %) patients with definite PCP were admitted to the intensive care unit and 12 (37.5 %) patients needed mechanical ventilation. Only one (7.1 %) of patients with definite PCP had 90-day mortality, while 5 (27.8 %) patients with probable PCP died at the time of 90-day from PCP development.

Figure 1 and Table 2 show the point prevalence rates of PCP at each cycle of R-CHOP chemotherapy. The overall incidence of definite and definite plus probable PCP in NHL patients receiving R-CHOP were 2.0 % (14/713; 95 % CI, 1.1–3.3 %) and 4.5 % (32/713; 95 % CI, 3.2–6.4 %), respectively. This corresponded to 3.8 (95 % CI, 2.2–6.4) and 8.4 (95 % CI, 5.9–11.9) per 1000 persons. Most cases of definite PCP (12, 85.7 %) occurred after administration of four or more cycles of RCHOP. However, there was no statistical difference in PCP prevalence between patients receiving four or more cycles of R-CHOP and those receiving fewer than four cycles (12/578, 2.1 % vs. 2/135, 1.5 %; p=0.74). When probable PCP was included in the analysis, this ratio was lowered (22, 68.7 %), because only 55.6 %

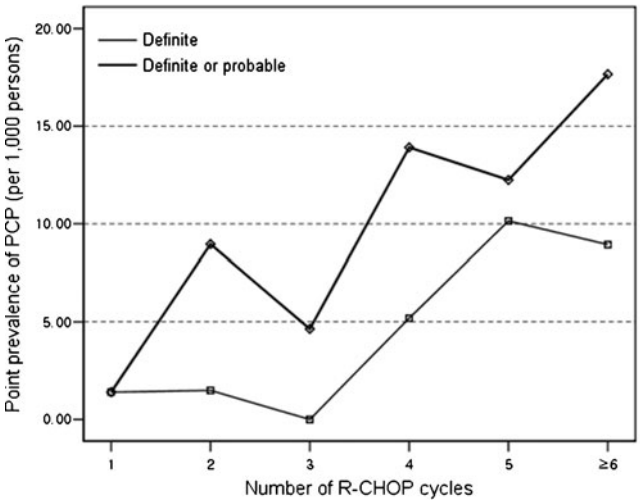

Fig. 1 Point prevalence of definite or probable Pneumocystis pneumonia in patients with non-Hodgkin lymphoma according to the number of cycles of R-CHOP chemotherapy

(10/18) of patients had events of probable PCP after administration of four or more cycles of RCHOP.

Comparison of clinical variables between patients with and without PCP

Table 3 shows the comparison of clinical variables between patients with and without PCP. Comparing patients with and without definite PCP, none of the variables showed a statistically significant difference. However, comparing patients with and without either definite or probable PCP, higher ANC (4,742/mm^3 vs. 2,627/mm^3; p<0.01) was found with statistically significance in patients with definite or probable PCP than those without it. Median ANC, ALC, and LDH at the time of last R-CHOP administration were 4884/mm^3 (IQR, 4081–7495), 1068/mm^3 (IQR, 739–1680), and 305 IU/L (IQR, 226–374) in patients with probable PCP, respectively.

Against our expectations, ALC at the time of NHL diagnosis and at the last cycle of R-CHOP was not statistically different between patients with and without PCP. The mean ALC at the time of the last R-CHOP cycle was lower than that at the time of NHL diagnosis (1,185/mm^3 vs. 1,757/mm^3; p<0.001 by paired t test in all patients of the cohort), but the extent of ALC decrease was not statistically different between patients with and without definite PCP. No statistical difference was shown regardless of including probable PCP patients in the analysis.

Discussion

One of the aims of this study was to determine the incidence rate of PCP in NHL patients receiving R-CHOP

Table 2 Point prevalence of definite and probable *Pneumocystis* pneumonia according to the number of cycles of R-CHOP chemotherapy

Category	Cycle number of R-CHOP	No. of patients on each cycle of R-CHOP	No. of patients censored before the cycle	Number of patients with PCP	Point prevalence	
					Per 1000 persons	95 % confidence interval
Definite only (N=14)	1	713		1	1.40	0.20–9.96
	2	670	43	1	1.49	0.21–10.60
	3	649	21	0	0	Not applicable
	4	578	71	3	5.19	1.67–16.10
	5	493	85	5	10.14	4.22–23.47
	≥ 6	456*	37	4	8.93	3.35–24.79
Definite & probable (N=32)	1	713		1	1.40	0.20–9.96
	2	670	43	6	8.96	4.02–19.93
	3	646	24	3	4.64	1.50–14.40
	4	575	71	8	13.91	6.96–27.82
	5	490	85	6	12.24	5.50–27.26
	≥ 6	453†	37	8	17.66	8.83–35.31

* Of 456 patients, 371, 11, and 74 patients received 6, 7, and 8 cycles of R-CHOP, respectively. † Of 453 patients, 368, 11, and 74 patients received 6, 7, and 8 cycles of R-CHOP, respectively. Abbreviations: PCP: *Pneumocystis* pneumonia

Table 3 Comparison of clinical variables between non-Hodgkin lymphoma patients with and without *Pneumocystis* pneumonia who received R-CHOP

Variables	Definite only			Definite & probable		
	PCP (%) N=14	Non-PCP (%) N=699	*p* value	PCP (%) N=32	Non-PCP (%) N=681	*p* value
Male	9 (64.3)	396 (56.7)	0.57	20 (62.5)	385 (56.5)	0.51
Age (years), median (IQR)	61 (42-65)	58 (47-68)	0.61	63 (53-71)	57 (47-68)	0.06
Ann Arbor stage						
I	3 (21.4)	146 (20.9)	1.00	5 (15.6)	144 (21.1)	0.66
II	4 (28.6)	183 (26.2)	0.77	7 (21.9)	180 (26.4)	0.68
III	0	90 (12.9)	0.24	4 (12.5)	86 (12.6)	1.00
IV	7 (50.0)	280 (40.1)	0.58	16 (50.0)	271 (39.8)	0.27
Type of pathology						
DLBL	12 (85.7)	660 (94.4)	0.19	29 (90.6)	643 (94.4)	0.42
MCL	2 (14.3)	25 (3.6)	0.10	3 (9.4)	24 (3.5)	0.12
Others	0 (1.0)	14 (2.0)	1.00	0	14 (2.1)	1.00
Four or more cycles of R-CHOP	12 (85.7)	566 (81.0)	0.74	22 (68.7)	553 (81.3)	0.11
Laboratory data at the time of initial R-CHOP						
ANC/mm^3, median (IQR)	3274 (2463–5873)	3668 (2701–4964)	0.98	3588 (2599–5803)	3666 (2702–4920)	0.80
ALC/mm^3, median (IQR)	1467 (863–1690)	1589 (1065–2151)	0.27	1476 (1008–1765)	1603 (1059–2152)	0.39
LDH IU/L, median (IQR)	249 (188–415)	243 (188–400)	0.84	290 (199–422)	241 (188–400)	0.36
Laboratory data at the time of last R-CHOP*						
ANC/mm^3, median (IQR)	3664 (2111–5921)	2615 (1858–3993)	0.10	4742 (3039–6772)	2627 (1866–4019)	<0.01
ALC/mm^3, median (IQR)	864 (539–1377)	1076 (786–1443)	0.10	1003 (695–1590)	1076 (783–1454)	0.45
LDH IU/L, median (IQR)	351 (240–414)	264 (217–320)	0.06	309 (235–393)	269 (217–326)	0.07
Decrease in ALC/mm^3, median (IQR)†	346 (-80–996)	475 (-1–1008)	0.25	346 (-349–1018)	269 (217–327)	0.39
Prophylaxis for PCP	0	13 (1.9)	1.00	1 (3.1)	12 (1.8)	0.45

* Patients who received only one cycle of R-CHOP were excluded; † compared by analysis of covariance. Abbreviations: PCP: *pneumocystis* pneumonia; IQR: Interquartile range; DLBL: diffuse large B cell lymphoma; MCL: mantle cell lymphoma; ANC: absolute neutrophil count; ALC: absolute lymphocyte count; LDH: lactate dehydrogenase

chemotherapy. Our study included 14 and 18 patients with definite and probable PCP among 713 patients who received R-CHOP for NHL. To our knowledge, this is the largest study investigating the incidence rate of PCP in NHL treated with R-CHOP.

The overall incidence rate of definite PCP in our study cohort was 2.0 % (14/713), which seems to be lower than that of previous studies (6.6–13 %) [9, 16, 17]. This difference might arise from the difference in the interval between R-CHOP cycles. In previous studies the interval between R-CHOP cycles was 14 days, whereas the patients in our study received a R-CHOP regimen with an interval of more than 21 days between the cycles. In fact, a study with a similar result to ours reported a PCP incidence rate of 2.6 % (1/47) in patients receiving R-CHOP with a cycle interval of 21 days [9]. The low incidence rate of PCP in our study supports a previous observation that PCP developed more frequently in patients receiving R-CHOP with a 14-day interval than in those with a 21-day interval [9].

Another aim of our study was to show the point prevalence of PCP based on the number of R-CHOP cycles. In our study most cases of definite PCP (85.7 %, 12/14) developed after the fourth cycle of R-CHOP, consistent with previous results. When we reviewed the case series of previous studies, we found that 78 % (18/23) of cases of PCP occurred after the patients received four cycles of R-CHOP [9, 16–18]. Including the cases of probable PCP, however, this proportion was lowered (22, 68.7 %). This difference might come from the possibility that some of patients with probable PCP might not actually have PCP. That is to say, viral pneumonia or drug-related lung diseases which resemble clinical manifestations of PCP might be happened at the earlier cycles of R-CHOP.

This result implies that repeated R-CHOP cycles might increase the susceptibility of patients with NHL to PCP. Repeated R-CHOP has been shown to change the host immune status by depleting lymphocytes [10, 15], and a trial based on this finding showed that low ALC was associated with PCP development in patients with NHL [15]. However, this previous study only included cross-sectional comparison of ALC values at the time of NHL diagnosis between patients with and without PCP [15]. This was not sufficient to confirm the validity of ALC for prediction of PCP because the impact of R-CHOP on the value of ALC was not reflected in the analysis. Therefore, we compared the ALC at both the time of NHL diagnosis and at the last R-CHOP treatment.

In contrast to the previous study, we failed to prove that ALC is associated with PCP development; the ALC was not statistically different between patients with and without PCP at either the time of NHL diagnosis or at the last cycle of R-CHOP. Furthermore, although the ALC at the time of the last R-CHOP chemotherapy was lower than that at the time of NHL diagnosis, the extent of decrease was not statistically different between patients with and without PCP. In HIV infected patients, it has been the total CD4 number that has been associated with PCP development [21]. Using ALC as a surrogate indicator of the CD4 count may not be appropriate in this setting, particularly because CD8 cell count is known to recover faster than CD4 count [22]. Thus, the lack of association with the ALC could be explained and ALC might not be a reliable tool for prediction of PCP development.

Unexpectedly, higher ANC was associated with development of definite or probable PCP, although it was not valid in the analysis of only the cases of definite PCP. There has not been such a study showing this kind of association. Higher ANC might come from preceding inflammatory change before clinical manifestation of PCP appeared. In addition, some of probable PCP might be drug-induced interstitial pneumonitis that could make ANC being higher. This finding suggests that cytoreduction by R-CHOP might not be a risk factor of PCP development. This statement is not confirmative, however, because the cases of probable PCP were included in the analysis

It is currently unclear whether anti-pneumocystis prophylaxis is necessary for patients receiving R-CHOP. Based on following reasons, anti-pneumocystis prophylaxis in these patients could be suggested: (1) R-CHOP might increase the risk of PCP development, (2) It might be helpful to differentiate PCP with drug-induced lung interstitial diseases, since breakthrough development of PCP seems unlikely to happen frequently [23]. In other words, we might say that diagnosis of drug-induced lung interstitial diseases seems to be favored when patients who received anti-pneumocystis prophylaxis suffered from interstitial pneumonitis.

Despite of these, there are some obstacles that make clinicians hesitant to start prophylaxis. First, the incidence rate of definite PCP is not considerably high. Although almost all patients in our cohort did not receive anti-pneumocystis prophylaxis, the rate of PCP development was low. Some experts assert that anti-pneumocystis prophylaxis is necessary only for situations where the incidence rate of PCP is greater than 3–5 % [24]. Second, in our study the development of definite PCP was not associated with a bad prognosis; in fact, only one patient died as a result of definite PCP. This suggests that it is not too late to start anti-pneumocystis therapy after the symptoms of PCP manifest, as long as the patients are cautiously observed. Lastly, one of the important side effects of TMP/SMX used for anti-pneumocystis prophylaxis is bone marrow suppression [25]. This crucial side effect can disturb the normal course of NHL therapy. Hence, a study estimating the cost-effectiveness of anti-pneumocystis prophylaxis in patients receiving R-CHOP used for NHL should be performed.

There were some limitations in this study. First, it is possible that the incidence rate of PCP might be underestimated,

because our study was retrospective and clinicians sometimes empirically administered anti-pneumocystis regimens without diagnostic tests to patients who were suspected of having PCP. Additionally, sensitive methods such as PCP and beta-D-glucan were not used for diagnosis of PCP. To avoid an error of lower estimation of PCP incidence, we added the analysis of probable PCP and the overall incidence of definite or probable PCP was less than 5 % of patients receiving R-CHOP regimens. Hence, our statement seems to be still considerable. In our study, however, almost all patients receive R-CHOP every three weeks. Therefore, our statement would not be applicable in the center where R-CHOP is administered every two weeks. Another limitation is that candidate variables were not fully investigated to determine the risk factors of PCP development.

In conclusion, contrary to expectations, PCP is not a frequent complication of R-CHOP treatment for non-Hodgkin lymphoma. Therefore, universal prophylaxis against *P. jirovecii* during R-CHOP treatment could not be strongly recommended. Well-designed studies addressing the cost-effectiveness of anti-pneumocystis prophylaxis in patients with NHL treated with R-CHOP should be followed to justify the usage of anti-pneumocystis regimen for prophylaxis. In addition, we could not conclude the association of cytoreduction with PCP development. Further trials should be carried out to identify the predictive factors of PCP development in these patients.

Conflict of interest The authors declare that they have no conflict of interest.

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