J Clin Exp Hematopathol
Vol. 50, No. 2, Nov. 2010

Case Study

Two Cases of *Pneumocystis jiroveci* Pneumonia with Non-Hodgkin's Lymphoma after CHOP-Based Chemotherapy Containing Rituximab

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We report two cases of *Pneumocystis jiroveci* pneumonia (PCP) with $CD20^+$ B-cell lymphoma. They were treated by several courses of CHOP-based chemotherapy containing rituximab. We confirmed by flow cytometric analysis that both of them completely lost $CD19^+$ and $CD20^+$ B-cells from their peripheral blood after the first course of chemotherapy. They were successfully treated with Trimethoprim-sulfamethoxazole (TMP-SMX) after the diagnosis of PCP by polymerase chain reaction (PCR). We overviewed $CD20^+$ B-cell lymphoma patients treated with CHOP-based regimens from 1997 until 2005 in our hospital. We treated 114 patients with and 121 patients without rituximab. Five patients in the group with rituximab developed interstitial pneumonia (IP). Two of them were confirmed to have PCP and the other three were suspected cases ; however, no patients with IP were seen in the group without rituximab. We strongly suggest the necessity of PCP prophylaxis with oral TMP-SMX when treating B-cell lymphoma patients with chemotherapy containing rituximab. 〔*J Clin Exp Hematopathol 50(2) : 159-162, 2010*〕

Keywords: rituximab, R-CHOP, B-cell lymphoma, *Pneumocystis jiroveci* pneumonia

INTRODUCTION

Pneumocystis jiroveci (formerly *Pneumocystis carinii*) pneumonia (PCP) remains a serious cause of morbidity and mortality not only in patients with acquired immunodeficiency syndrome (AIDS), but also in immunocompromised hosts.[1] Rituximab, a chimeric human/murine monoclonal anitibody targeting the B-cell-specific antigen CD20, has been effectively used for the treatment of B-cell lymphoma.[2-4] Rituximab has been reported to induce interstitial pneumonia (IP) with B-cell lymphoma patients because of its cytokine release.[5,6] On the other hand, a decreased number of B-cells may induce opportunistic infections after treatment with rituximab. We report here two B-cell lymphoma patients with PCP after several cycles of CHOP-based chemotherapy including rituximab. Their specimens of bronchoalveolar (BAL) fluid and sputum were positive for *Pneumocystis jiroveci* by polymerase chain reaction (PCR) and they were successfully treated with oral Trimethoprim-sulfamethoxazole (TMP-SMX) therapy.

Received : November 3, 2009
Revised : December 21, 2009
Accepted : December 30, 2009

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CASE REPORT

Case 1

A 49-year-old man was admitted to our hospital in June 2005 with a fever having a duration of 3 days. He was diagnosed with follicular lymphoma (Grade 1) and presented with systemic lymphadenopathy, hepatosplenomegaly, and bone marrow involvement in March 2005. The clinical stage according to the Ann Arbor classification was IVB and the Follicular Lymphoma International Prognostic Index[7] showed a high risk. He was treated with rituximab plus CHOP which consists of cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP). He was treated with R-CHOP every four weeks. After 3 cycles of R-CHOP therapy, partial remission was achieved. We confirmed the complete disappearance of $CD19^+$ and $CD20^+$ B-cells from his peripheral blood after the first cycle of R-CHOP by flow cytometric analysis (data not shown). With the use of granulocyte colony-stimulating factor (G-CSF) from day 8 of the fourth cycle of R-CHOP, he had a fever from day 12 and was admitted to our hospital on day 15. The results of physical examination were normal except for a temperature of 40.2°C. A computed tomography (CT) scan of the chest revealed ground-glass opacities in the bilateral lobes (Fig. 1). WBC and the abso-

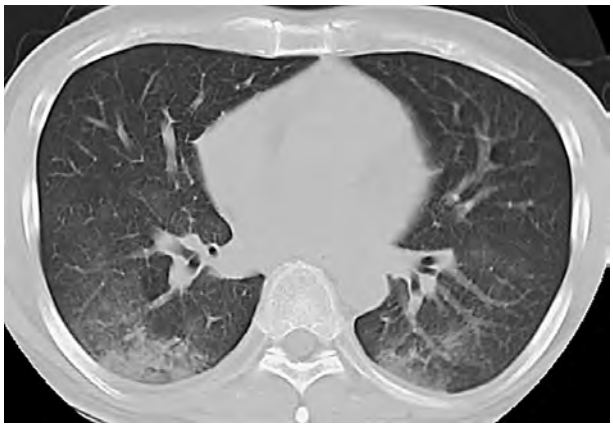

Fig. 1. A computed tomographic scan of the chest revealed ground-glass opacities in the bilateral lower lobes on admission in Case 1.

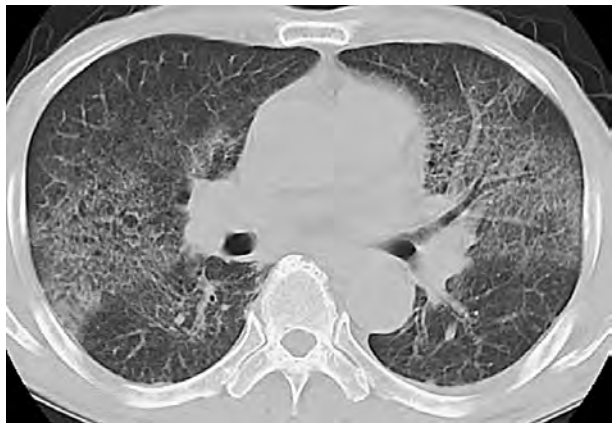

Fig. 2. A computed tomographic scan of the chest showed diffuse pulmonary ground-glass attenuation in the bilateral middle lung field on admission in Case 2.

lute neutrophil count were 2,500/μL and 1,100/μL, respectively. Arterial blood gas measurements in room air revealed : pH, 7.43 ; carbon dioxide partial pressure, 38.0 mmHg ; oxygen partial pressure, 81.2 mmHg ; and saturation, 96.6%. (1→3) β-D glucan (β-glucan) was 182 pg/mL (normal range : below 20 pg/mL). He was diagnosed with IP. After bronchoscopic examination, he was treated with oral TMP-SMX, intravenous panipenem/betamipron, micafungin, and ganciclovir on the assumption of a pulmonary infection. No pathogens were detected in his blood or BAL fluid. After we obtained positive results for *Pneumocystis jiroveci* from BAL fluid by PCR, we continued only TMP-SMX. He had no fever after the seventh day of treatment. A chest CT scan after two weeks of TMP-SMX therapy showed the complete disappearance of pulmonary infiltrates.

Case 2

A 71-year-old man was referred to our hospital in September 2005, because of a 2-day history of fever and productive cough. He was diagnosed with diffuse large B-cell lymphoma of the thyroid in June 2005. The clinical stage was IIA and the International Prognostic Index[8] was low-intermediate. He was treated with a rituximab plus THP (pirarubicin)-COP (cyclophosphamide, vincristine, and prednisolone) (R-THP-COP) regimen. We treat patients aged 70 or older with THP instead of doxorubicin because THP has been reported to have a low cardiac toxicity.[9] He was treated with R-THP-COP every four weeks. After 2 courses of R-THP-COP therapy, complete remission was achieved. Flow cytometric analysis showed the complete absence of $CD19^+$ and $CD20^+$ B-lymphocytes in his peripheral blood after the first course of R-THP-COP (data not shown). After the use of G-CSF from day 10 of the third cycle of R-THP-COP, he had a fever and productive cough from day 14 and was admitted to our hospital on day 16. Temperature was 39.2°C and inspiratory fine crackles were present on bilateral lower lung fields. A CT scan of the chest showed diffuse pulmonary ground-glass attenuation (Fig. 2). WBC and the absolute neutrophil count were 4,800/μL and 3,200/μL, respectively. Pulse oximetry showed oxygen saturation to be 87% in room air. β-glucan was 106 pg/mL. He was diagnosed with IP. After collecting sputum for culture and PCR, he was treated with oral TMP-SMX, intravenous panipenem/betamipron, micafungin, ganciclovir, and methylprednisolone. He had no fever after the second day of these treatments. Sputum and blood culture yielded no bacteria or fungi. After we obtained positive results for *Pneumocystis jiroveci* from sputum by PCR, we continued only TMP-SMX. A chest CT scan after three weeks of TMP-SMX administration revealed significant improvement.

DISCUSSION

Here, we reported two cases of PCP with B-cell lymphoma after CHOP-based chemotherapy containing rituximab. Both of them were successfully treated with TMP-SMX, which acts by inhibiting folic acid synthesis and has been used against *Pneumocystis jiroveci* with a high degree of success.[10] We empirically started treatment with a combination of TMP-SMX, antibiotics, antifungal agents, ganciclovir, and methylprednisolone because we could not identify the pathogen at the time of onset. After the prompt diagnosis of *Pneumocystis jiroveci* infection in both cases by PCR using specimens obtained on the day of admission, they were successfully treated with only TMP-SMX.

We overviewed the patients with B-cell lymphoma treated with CHOP-based regimens from 1997 until 2005 in our hospital. The characteristics of patients are summarized in Table 1. In Japan, we have been able to use rituximab since

2001. We treated 114 patients with and 121 patients without rituximab. We observed five patients with IP in the former group ; however, no patients were observed to have IP in the latter group. The clinical course of five patients who developed IP are summarized in Table 2. We diagnosed two patients reported in this paper with PCP and suspected PCP in three patients due to high levels of serum β-glucan. β-glucan is one of the major components of the cyst wall of *Pneumocystis jiroveci* and is a practical serological marker for the monitoring of PCP.[11] Two patients suspected to have PCP given high levels of β-glucan were also treated successfully with TMP-SMX. One suspected patient died of respiratory insufficiency. On the other hand, there were no patients with PCP among 121 patients treated with CHOP-based regimens lacking rituximab even if corticosteroids, which are the most common immunosuppressive drugs implicated in the development of PCP, were included.

Table 1. Summary of the patients with B-cell lymphoma treated with CHOP-based chemotherapy in Toyama Prefectural Central Hospital (1997-2005)

Rituximab	+	−
Number	114	121
Age [mean (range)]	63 (13-88)	60 (27-87)
Sex (Male/Female)	54/60	63/58
Disease		
DLBCL	61	81
FL	46	22
MCL	2	3
MALT-lymphoma	2	6
Others	3	9
Clinical stage		
I	22	28
II	27	30
III	26	17
IV	39	46
Number of courses [mean (range)]	4.5 (1-12)	4.5 (1-12)
IP	5	0

DLBCL, diffuse large B-cell lymphoma ; FL, follicular lymphoma ; MCL, mantle cell lymphoma ; MALT-lymphoma, extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue ; IP, interstitial pneumonia.

There have been several reports which described PCP after R-CHOP therapy. Brusamolino *et al*. reported three out of 50 diffuse large B-cell lymphoma patients treated with R-CHOP-14 developed PCP infections.[12] Ennishi *et al*. also reported that 13 of 90 (14%) patients developed IP during R-CHOP therapy, compared with none of 105 patients treated with CHOP alone and confirmed two PCP patients among R-CHOP-treated group.[13] Katsuya *et al*. analyzed 129 patients treated with R-CHOP regimen and pointed out the low number of lymphocytes both at diagnosis of lymphoma (<1,000/μL) and at the onset of IP (<500/μL) in all three PCP patients.[14] Among the five patients who developed IP in our study, we experienced only one patient whose lymphocyte counts were <1, 000/μL at diagnosis of lymphoma (510/μL in Case 4) and two patients whose lymphocyte counts were <500/μL at onset of IP (72/μL in Case 4, 50/μL in Case 5).

It is understandable that patients treated by chemotherapy with rituximab lack humoral immune responses. In fact, we confirmed that the two patients in this report completely lost all B-cells from their peripheral blood after the first course of the CHOP-based regimen. $CD4^+$ T-cells play a central role in the host defenses against *Pneumocystis jiroveci*.[15] The risk of

Table 2. Summary of five patients with interstitial pneumonia

Case	Age, Sex	Disease (CS)	No. of courses	β-glucan (pg/mL)	PCR for *Pneumocystis Jiroveci*	Treatment	Outcome
1*	49, M	FL (IV)	R-CHOP, ×4	182	+	TMP-SMX	Cured
2*	71, M	DLBCL (II)	R-THP-COP, ×3	106	+	TMP-SMX, mPSL	Cured
3	62, M	DLBCL (III)	R-CHOP, ×2	259	ND	TMP-SMX, mPSL	Cured
4	78, F	FL (II)	R-THP-COP, ×5	> 300	ND	TMP-SMX, mPSL	Cured
5	67, M	DLBCL (II)	R-CHOP, ×4	> 300	ND	TMP-SMX, GCV, mPSL	Dead

CS, clinical stage according to the Ann Arbor classification ; PCR, polymerase chain reaction ; FL, follicular lymphoma ; DLBCL, diffuse large B-cell lymphoma ; TMP-SMX, Trimethoprim-sulfamethoxazole ; GCV, ganciclovir ; mPSL, methylprednisolone ; ND, not done.
*Cases 1 and 2 represent the same patients in the text, respectively.

developing PCP in human immunodeficiency virus (HIV)-infected patients increases greatly when circulating CD4 cells fall below 200/μL.[16] However, it is not clear how the patients treated with rituximab were immunodeficient against *Pneumocystis jiroveci* even though their cellular immune responses were spared. PCP has been reported in patients and mice with B-cell defects.[17] Lund *et al*. reported that B-cells played a vital role in the generation of $CD4^+$ memory T-cells in response to PCP in mice.[18] The interaction between B-cells and T-cells via the CD40-CD40 ligand pathway has been shown to be necessary for the resolution of PCP in mice.[19] We realize that not only cellular but also humoral immune responses play a crucial role against *Pneumocystis jiroveci*. In contrast to HIV-infected patients, there are no national guidelines for *Pneumocystis jiroveci* prophylaxis in other immunocompromised hosts. Halaas *et al*. reported that they observed cases of PCP when PCP prophylaxis was not routinely prescribed, and the subsequent use of PCP prophylaxis avoided any further cases of PCP[20] ; however, the full details of the cases of PCP and the method of prophylaxis are not clear. We have given one prophylactic tablet of TMP-SMX (80 mg TMP and 400 mg SMX) daily to patients with non-Hodgkin's lymphoma treated with CHOP-based regimens containing rituximab since 2006, and no patients have developed PCP so far. No adverse reactions to TMP-SMX have been observed in these patients under the administration of one tablet of TMP-SMX. In conclusion, we strongly suggest that oral TMP-SMX prophylaxis should be given to patients treated with CHOP-based chemotherapy containing rituximab.

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