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BRIEF REPORT

# Atypical *Pneumocystis jiroveci* pneumonia with multiple nodular granulomas after rituximab for refractory nephrotic syndrome

**Mai Sato · Shuichi Ito · Masao Ogura · Koichi Kamei · Isao Miyairi · Ippei Miyata · Masataka Higuchi · Kentaro Matsuoka**

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**Abstract**
*Background* Rituximab, an anti-CD20 antibody that targets B cells, is a promising agent against steroid-dependent and steroid-resistant nephrotic syndrome in children.
*Case-Diagnosis/Treatment* We report a 3-year-old boy who presented with atypical *Pneumocystis jiroveci* pneumonia (PCP) following administration of rituximab for refractory nephrotic syndrome. He had received cyclosporine and daily prednisolone for over 1 year. Following rituximab therapy, a hazy shadow was observed on his chest X-ray. Chest-computed tomography revealed multiple nodular lesions in bilateral lungs, although his clinical symptoms were subtle. PCR analysis demonstrated the presence of *Pneumocystis* DNA in his bronchoalveolar lavage. Lung wedge resection of the nodular lesion exhibited granulomas containing a few cysts of *P. jiroveci* that primarily consisted of T cells and histiocytes and lacked B cells. A deficiency of B cells following rituximab treatment suggests a dramatic effect on the immune response and, therefore, could result in granulomatous PCP. Nodular granulomatous lesions of PCP comprise an emerging concept previously reported in adults with hematological disease, bone marrow transplant, or treatment with rituximab. We report the first pediatric case of nodular PCP. Granulomatous PCP can be life-threatening. Moreover, bronchoalveolar lavage often fails to demonstrate the presence of *P. jiroveci* DNA. Wedge biopsy is warranted for definitive diagnosis. Our patient fully recovered with sulfamethoxazole/trimethoprim treatment because of early detection.
*Conclusions* The indication of rituximab for refractory nephrotic syndrome has increased recently. Therefore, recognition of the risk of atypical PCP is important. Our findings suggest that PCP prophylaxis should be considered following rituximab therapy.

**Keywords** Nephrotic syndrome · Rituximab · *Pneumocystis jiroveci* · Granulomatous · Granulomatous *Pneumocystis jiroveci* pneumonia

**Abbreviations**

| | |
|---|---|
| PCP | *Pneumocystis jiroveci* pneumonia |
| RTX | Rituximab |
| NS | Nephrotic syndrome |
| FRNS | Frequently relapsing nephrotic syndrome |
| SDNS | Steroid-dependent nephrotic syndrome |
| SRNS | Steroid-resistant nephrotic syndrome |

## Introduction

Treatment of refractory childhood nephrotic syndromes, such as frequently relapsing nephrotic syndrome/steroid-dependent (FRNS/SDNS) and steroid-resistant nephrotic syndrome (SRNS), remains a challenge. Although various

M. Sato · S. Ito (✉) · M. Ogura · K. Kamei
Division of Nephrology and Rheumatology,
National Center for Child Health and Development,
2-10-1 Okura,
Setagaya-ku, Tokyo 157-8535, Japan
e-mail: ito-shu@ncchd.go.jp

I. Miyairi · I. Miyata
Division of Infectious Diseases,
National Center for Child Health and Development,
2-10-1 Okura,
Setagaya-ku, Tokyo 157-8535, Japan

M. Higuchi
Division of Pulmonology,
National Center for Child Health and Development,
2-10-1 Okura,
Setagaya-ku, Tokyo 157-8535, Japan

K. Matsuoka
Division of Pathology,
National Center for Child Health and Development,
2-10-1 Okura,
Setagaya-ku, Tokyo 157-8535, Japan

immunosuppressive agents are effective, a substantial number of children are intractable. Recently, rituximab (RTX), a monoclonal antibody that targets the B cell specific antigen CD20, has been demonstrated to be effective for FRNS/SDNS and SRNS in children [1–3]. RTX is relatively well tolerated; however, occasionally severe or life-threatening adverse events occur, including progressive multifocal leukoencephalopathy [4], interstitial pneumonia [5], and ulcerative colitis [6]. Moreover, a decreased number of B cells potentially induces opportunistic infection and deterioration of infection. *Pneumocystis jiroveci*, formerly known as *Pneumocystis carinii*, pneumonia (PCP) is a rare but serious cause of mortality in patients with acquired immunodeficiency syndrome (AIDS), as well as in immunocompromised hosts. RTX increases susceptibility to PCP. Previous cases of PCP following rituximab treatment have been reported for various indications, such as B cell lymphoma [7–9], rheumatoid arthritis [10], Wegener's granulomatosis [11], autoimmune hemolytic anemia [12, 13], pure red cell aplasia [14], pemphigus [15], and acute rejection of kidney transplants [16, 17]. PCP usually presents as a bilaterally diffuse and fairly symmetric interstitial pattern on chest X-ray and as patchy ground-glass opacities on high-resolution computed tomography (hrCT). However, immunocompromised hosts who exhibit hematological malignancy with or without bone marrow transplants, chemotherapy including RTX, and AIDS infrequently present with atypical PCP with multiple nodular granulomatous lesions [18–20]. We report the first pediatric case of nodular granulomatous PCP in which the patient was treated with a single dose of 375 mg/m$^2$ of RTX against SDNS. RTX potentially increases the risk of granulomatous PCP. Written informed consent for publication of this information was obtained from the child's family.

## Case report

The patient is a 3-year-old boy with SDNS. He had been healthy before he presented with NS at 2 years of age. At primary onset, he was initially treated with 25 mg/day (60 mg/m$^2$/day) of daily prednisolone (PSL) for 4 weeks. NS was in remission 10 days after initiation of PSL. PSL was then reduced to 16 mg/day (40 mg/m$^2$) on alternate days, but NS had relapsed soon after PSL reduction. He was treated with 25 mg of daily PSL again. Proteinuria resolved in 7 days. However, as PSL was reduced to 20 mg/day on alternate days, he experienced a second relapse. Thereafter, we stopped reducing PSL by administration on alternate days. Nevertheless, his NS repeatedly relapsed whenever daily PLS was reduced to less than 15 mg. Cyclosporine, losartan and mizoribine were added after 5, 8, and 9 months after diagnosis, respectively. Renal biopsy was performed 5 months after the onset of NS.

One year after onset, at his fifth relapse, the patient was treated with daily PSL 18 mg/day (32 mg/kg/m2), cyclosporine 50 mg/day (3.3 mg/kg/day), and losartan 15 mg/day (1 mg/kg/day). He was then treated with daily PSL 25 mg/day (1.8 mg/kg/day) for 4 weeks. The dose of PSL was slightly less than that used previously, because he was intolerant to the previous full dose of PSL owing to a mood disorder. After 4 weeks, his urinary protein/creatinine ratio was still 6, and serum albumin level was 2.5 g/dl. Additionally, he suffered from serious steroid-related toxicities, including growth retardation, obesity, and bilateral glaucoma. He was transferred to our institute because of SRNS.

After admission, he was treated with one course of methylprednisolone pulse therapy (30 mg/kg/day, three consecutive days). Four days after this therapy, his NS completely remitted. Thereafter, we administered 375 mg/m$^2$ of RTX 2 days after the remission, and we added two more courses of methylprednisolone pulse therapy for reduction of steroids and prevention of further relapses. The patient did not have a reaction to RTX infusion, and was finally discharged. After RTX infusion, daily PSL 1 mg/kg/day was continued for 1 month, and it was reduced to 0.25 and 0.5 mg/kg/day every other day. Cyclosporine was continued, and its 2-h blood concentration was maintained between 400 and 600 ng/dl. However, his NS relapsed 50 days after RTX infusion, and he was admitted to our clinic again. Upon admission, he experienced no symptoms. His temperature was 36.3 °C, heart rate was 110/min and blood pressure was 100/50 mmHg. However, his respiratory rate was slightly fast (30/min). Oxygen saturation remained above 95 % while the patient was awake, but dropped to 92 % during sleep. Other physical examinations did not exhibit any remarkable findings. However, a chest radiograph exhibited patchy and solid infiltrates spread over both lungs (Fig. 1a). Chest helical computed tomography (CT) revealed multiple solid opacities in bilateral lobes.

A peripheral blood examination showed the following: hemoglobin, 13.0 g/dl; platelets, $34.6\times10^4$/μl; and white blood cells, $6.1\times10^4$/μl with 72.9 % neutrophils, 18.2 % lymphocytes, 5.2 % monocytes, and 1.5 % eosinophils. The biochemistry profile indicated normal liver and renal function. Lactate dehydrogenase was elevated to 500 IU/l and the isoenzyme patterns suggested that the origin was the lung. The CRP level was 1.0 mg/dl, and erythrocyte sedimentation rate was 44.0 mm/1 h. Serum creatinine and blood urea nitrogen were 0.15 and 11.6 mg/dl, respectively, and serum total protein and albumin were 5.1 and 3.5 g/dl, respectively. Urinary protein was 208.5 mg/dl, and the urinary protein/creatinine ratio was 2.1. The amount of CD19- and CD20-positive B cells in peripheral blood was zero (before RTX infusion, they were 552 and 604/μl, respectively). β-d-Glucan was elevated to 322 pg/ml (normal: <20 pg/ml). Cytomegalovirus antigenemia, Epstein–Barr virus genome, and QuantiFERON (test for tuberculosis)

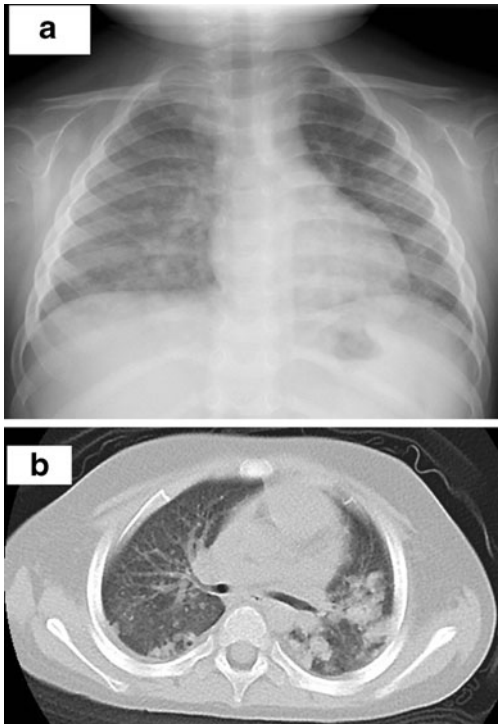


**Fig. 1** Chest X-ray and high-resolution computed tomography (CT) scan of the lung. **a** Chest X-ray shows patchy and solid infiltrates spread over both lungs on admission. **b** A high-resolution CT scan of the chest 3 weeks after admission shows exacerbation of solid opacities in both lobes

results were all negative. Urinalysis exhibited mild albuminuria (urinary protein: 208 mg/dl, urinary creatinine: 99 mg/dl). Because of marked elevation of β-d-glucan, infection with fungus or *P. jiroveci* was suspected. We performed bronchoalveolar lavage (BAL) under general anesthesia on day 3. After bronchoscopy, the patient was treated with oral TMP-SMX for *Pneumocystis* at a dose of TMP 5 mg/kg every 6 h and voriconazole for fungus, including *Aspergillus* 15 mg/kg/day. PSL was maintained at 10 mg/day to prevent a withdrawal reaction, and cyclosporine was halted. Microscopy was negative but polymerase chain reaction analysis revealed *Pneumocystis* DNA. No other pathogens were detected in his blood culture and BAL. We stopped voriconazole and continued a full dose of TMP-SMX for 3 weeks followed by a prophylactic dose of TMP (5 mg/kg/day). Although CD19- and CD20-positive cells in peripheral blood recovered 8 months after RTX, he was treated with prophylactic TMP for 10 months because he was still being treated with MMF.

Although the patient's oxygen saturation improved, and β-d-glucan returned to a normal range, lactate dehydrogenase levels remained elevated. Another chest hrCT scan 3 weeks from the initiation of TMP-SMX demonstrated a further increase in the number and size of the pulmonary nodules (Fig. 1b). To rule out any other causes that were overlooked, the patient underwent open thoracic wedge resection of the nodule lesion. A biopsy sample showed necrotic granulomas containing a few cysts of *P. jiroveci* and Ziehl-Neelsen staining was negative (Fig. 2a/e). Immunohistochemistry revealed that the granulomas primarily consisted of CD3-positive T cells and CD68-positive histiocytes; however, there was an absence of B cells (Fig. 2b, c, d). His respiratory status remained stable, and lactate dehydrogenase soon returned to the normal range. A chest hrCT on the 52nd day exhibited an apparent decrease in nodular lesions. The patient's NS also spontaneously remitted along with the recovery of pulmonary findings.

## Discussion

This is the first childhood case of granulomatous PCP. Additionally, this is the first case of PCP following treatment with rituximab against SDNS. PCP is a serious cause of mortality in immunocompromised patients, affecting up to 50 % of patients. Our patient was treated with a high dose of daily PSL, cyclosporine, and mizoribine for an extended period. Additional rituximab potentially strengthens immunosuppression and increases the susceptibility to PCP. However, our patient's clinical and radiological findings substantially differed from typical PCP, and we had to rule out viral or fungal infection, tuberculosis, and rituximab-induced interstitial pneumonia.

Accumulating evidence suggests that, under some circumstances, RTX may significantly increase the risk of PCP. Recently, cyclophosphamide, doxorubicin, vincristine and prednisone with rituximab (R-CHOP) against B-cell lymphoma was shown to increase the prevalence of PCP compared with classical CHOP (identical treatment without RTX). Additionally, PCP after rituximab has been previously reported in patients with rheumatoid arthritis, Wegener's granulomatosis, autoimmune hemolytic anemia, pure red cell aplasia, pemphigus, and acute rejection of kidney transplants. Therefore, B cell depletion by RTX may increase the risk of PCP. They suggested the necessity of PCP prophylaxis after RTX therapy.

The host's defense against *Pneumocystis* is thought to be critically dependent on CD4+ helper T cells. The incidence of PCP in human immunodeficiency virus (HIV) infected patients is elevated when the level of circulating CD4 cells falls below 200/μl. However, B cells potentially play a significant role in protection against *Pneumocystis*. Transgenic B-cell functionally deficient mice, in which B cells do not express major histocompatibility complex class 2 antigens (and thus were unable to act as antigen-presenting cells), are susceptible to *Pneumocystis carinii* (formerly the species *Murina*). These mice fail to clear the *Pneumocystis* infection, most likely because of the inefficient generation of protective CD4+ memory and effector T cells in the lungs [21]. The

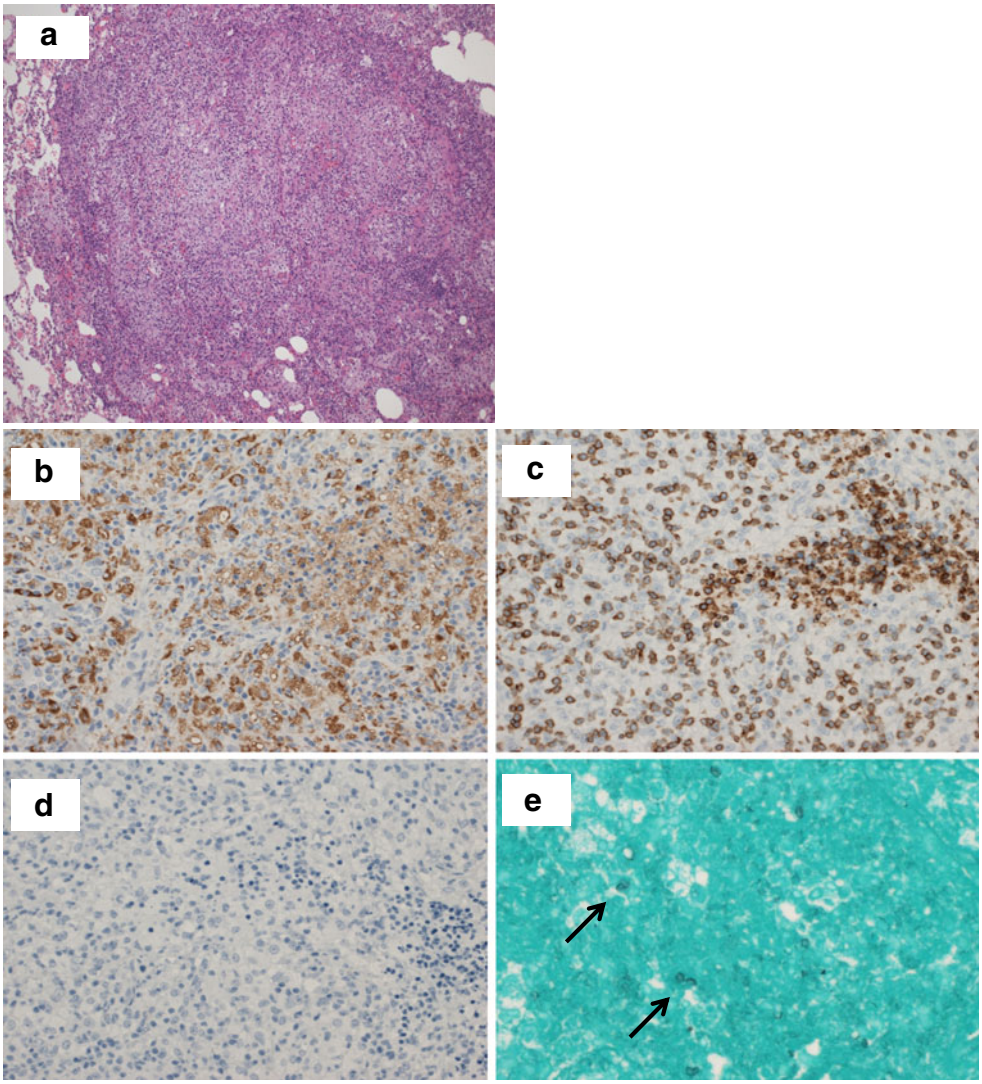


**Fig. 2** Pathological findings of lung biopsy. The biopsy sample shows necrotic granulomas (**a**, hematoxylin-eosin stain, magnification ×100), consistent with CD3-positive T cells (**b**) and CD68-positive histiocytes (**c**) (positive cells were stained brown), but without CD20-positive B cells (**d**). Several cysts of *P. jiroveci* were identified inside of the granulomas by Grocott's stain (**e**, *arrows*)

deficiency of B cells potentially results in attenuated immunoprotection against *P. jiroveci*.

Our patient was likely infected with *P. jiroveci*. However, he developed granulomatous PCP after RTX treatment; therefore, RTX potentially contributed to the development of granulomatous PCP. A defect in B cells induces high susceptibility to *P. jiroveci*, as well as development of granulomatous PCP. Granulomatous PCP is an emerging concept. Granulomatous PCP has been previously reported in up to 4–5 % of PCP patients. A total of 35 adult patients have previously exhibited granulomatous PCP. They suffered from AIDS, hematological neoplasms, and solid malignancy, but none of these patients had NS. Interestingly, three of them were treated with RTX prior to the development of PCP [19, 20]. B cell function appears to be impaired in most of these patients, as in our patient. In our patient, immunohistochemical staining revealed that granulomas primarily consisted of T cells and histiocytes without B cells. B cells may be essential for the clearance of *P. jiroveci*, and macrophages may substitute for the clearance of *P. jiroveci* by forming granulomas. In fact, we found several cysts of *P. jiroveci* inside the granulomas. B cell deficiency following RTX potentially modifies the immune response to *P. jiroveci* and induces a granulomatous reaction. Furthermore, Totet et al. reported that granuloma formation is not related to any specific genotype of *P. jiroveci* [22, 23].

Although our patient exhibited no symptoms and his general condition was well on admission, the mortality rate can be 35–50 % in patients without HIV compared with 10–20 % in those with HIV [24, 25]. The diagnosis of conventional PCP is traditionally performed by BAL. However, in the case of granulomatous PCP, BAL frequently fails to detect *P. jiroveci* [18]. An open lung biopsy is required to generate the correct diagnosis if BAL fails to detect *P. jiroveci*.

Our case study suggests that RTX may modulate T cell immunity and increase susceptibility to PCP. Furthermore, RTX treatment may cause a granulomatous pattern and make the correct diagnosis of PCP difficult. Indication of rituximab

for refractory NS has been recently expanding. Therefore, physicians should be aware of the risk of PCP and its atypical manifestations under combined immunosuppressive therapy, including RTX. Prophylaxis for PCP should be considered after RTX against refractory NS.

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## References


1. Bagga A, Sinha A, Moudgil A (2007) Rituximab in patients with the steroid-resistant nephrotic syndrome. N Engl J Med 356:2751–2752
2. Guigonis V, Dallocchio A, Baudouin V, Dehennault M, Hachon-Le Camus C, Afanetti M, Groothoff J, Llanas E, Nivet H, Raynaud N, Taque S, Ronco P, Bouissou F (2008) Rituximab treatment for severe steroid-or cyclosporine-dependent nephrotic syndrome: a multicentric series of 22 cases. Pediatr Nephrol 23:1269–1279
3. Kamei K, Ito S, Nozu K, Fujinaga S, Nakayama M, Sako M, Saito M, Yoneko M, Iijima K (2009) Single dose of rituximab for refractory steroid-dependent nephrotic syndrome in children. Pediatr Nephrol 24:1321–1328
4. Carson KR, Evens AM, Richey EA, Habermann TM, Focosi D, Seymour JF, Laubach J, Bawn SD, Gordon LI, Winter JN, Furmann RR, Vose JM, Zelenets AD, Mamtani R, Raisch DW, Dorshimer GW, Rosen ST, Muro K, Gottardi-Litell NR, Talley RL, Sartot O, Green D, Major EO, Cl B (2009) Progressive multifocal leukoencephalopathy after rituximab therapy in HIV-negative patients: a report of 57 cases from the research on adverse drug events and reports project. Blood 113:4834–4840
5. Burton C, Kaczmarski R, Jan-Mohamed R (2003) Interstitial pneumonitis related to rituximab therapy. N Engl J Med 348:2690–2691
6. Ardelean DS, Gonska T, Wires S, Cutz E, Griffiths A, Harvey E, Tse SM, Benseler SM (2010) Severe ulcerative colitis after rituximab therapy. Pediatrics 126:e243–e246
7. Brusamolino E, Rusconi C, Montalbetti L, Gargantini L, Uziel L, Pinotti G, Fava S, Rigacci L, Pagnucco G, Pascutto C, Morra E, Lazzarino M (2006) Dose-dense R-CHOP-14 supported by pegfilgrastim in patients with diffuse large B-cell lymphoma: a phase II study of feasibility and toxicity. Haematologica 91:496–502
8. Ennishi D, Terui Y, Yokoyama M, Mishima Y, Takahashi S, Takeuchi K, Ikeda K, Tanimoto M, Hatake K (2008) Increased incidence of interstitial pneumonia by CHOP combined with rituximab. Int J Hematol 87:393–397
9. Katsuya H, Suzumiya J, Sasaki H, Ishitsuka K, Takamatsu Y, Tamura K (2009) Addition of rituximab to cyclophosphamide, doxorubicin, vincristine, and prednisolone therapy has a high risk of developing interstitial pneumonia in patients with non-Hodgkin lymphoma. Leuk Lymphoma 50:1818–1823
10. Teichmann LL, Woenckhaus M, Vogel C, Salzberger B, Scholmerich J, Fleck M (2008) Fatal *Pneumocystis* pneumonia following rituximab administration for rheumatoid arthritis. Rheumatology 47:1256–1257
11. Hugle B, Solomon M, Harvey E, James A, Wadhwa A, Amin R, Bell-Peter A, Benseler S (2010) *Pneumocystis jiroveci* pneumonia following rituximab treatment in Wegener's granulomatosis. Arthritis Care Res (Hoboken) 62(11):1661–1664
12. Motto DG, Williams JA, Boxer LA (2002) Rituximab for refractory childhood autoimmune hemolytic anemia. Isr Med Assoc J 4:1006–1008
13. Bussone G, Ribeiro E, Dechartres A, Viallard JF, Bonnotte B, Fain O, Godeau B, Michel M (2009) Efficacy and safety of rituximab in adult's warm antibody autoimmune haemolytic anemia: retrospective analysis of 27 cases. Am J Hematol 84:153–157
14. Dungarwalla M, Marsh JC, Tooze JA, Lucas G, Ouwehand W, Pettengell R, Dearden CE, Gordon Smith EC, Elebute MO (2007) Lack of clinical efficacy of rituximab in the treatment of autoimmune neutropenia and pure red cell aplasia: implications for their pathophysiology. Ann Hematol 86:191–197
15. Morrison LH (2004) Therapy of refractory pemphigus vulgaris with monoclonal anti-CD20 antibody (rituximab). J Am Acad Dermatol 51:817–819
16. Kumar D, Gourishankar S, Mueller T, Cockfield S, Weinkauf J, Vethanayagam D, Humar A (2009) *Pneumocystis jirovecii* pneumonia after rituximab therapy for antibody-mediated rejection in a renal transplant recipient. Transpl Infect Dis 11:167–170
17. Shelton E, Yong M, Cohney S (2009) Late onset *Pneumocystis* pneumonia in patients receiving rituximab for humoral renal transplant rejection. Nephrology 14:696–699
18. Hartel PH, Shilo K, Klassen-Fischer M, Neafie RC, Ozbudak IH, Galvin JR, Franks TJ (2010) Granulomatous reaction to *Pneumocystis jirovecii*: clinicopathologic review of 20 cases. Am J Surg Pathol 34:730–734
19. Chang H, Shih LY, Wang CW, Chuang WY, Chen CC (2010) Granulomatous *Pneumocystis jiroveci* pneumonia in a patient with diffuse large B-cell lymphoma: case report and review of the literature. Acta Haematol 123:30–33
20. Kumar N, Bazari F, Rhodes A, Chua F, Tinwell B (2011) Chronic *Pneumocystis jiroveci* presenting as asymptomatic granulomatous pulmonary nodules in lymphoma. J Infect 62:484–486
21. Lund FE, Hollifield M, Schuer K, Lines JL, Randall TD, Garvy BA (2006) B cells are required for generation of protective effector and memory CD4 cells in response to *Pneumocystis* lung infection. J Immunol 176:6147–6154
22. Totet A, Duwat H, Magois E, Jounieaux V, Roux P, Raccurt C, Nevez G (2004) Similar genotypes of *Pneumocystis jirovecii* in different forms of *Pneumocystis* infection. Microbiology 150:1173–1178
23. Totet A, Duwat H, Daste G, Berry A, Escamilla R, Nevez G (2006) *Pneumocystis jirovecii* genotypes and granulomatous pneumocystosis. Med Mal Infect 36:229–231
24. Kovacs JA, Hiemenz JW, Macher AM, Stover D, Murray HW, Shelhamer J, Lane HC, Urmacher C, Honig C, Longo DL (1984) *Pneumocystis carinii* pneumonia: a comparison between patients with the acquired immunodeficiency syndrome and patients with other immunodeficiencies. Ann Intern Med 100:663–671
25. Mansharamani NG, Garland R, Delaney D, Koiziel H (2000) Management and outcome patterns for adult *Pneumocystis carinii* pneumonia, 1985 to 1995: comparison of HIV-associated cases to other immunocompromised state. Chest 118:704–711