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# Progressive Multifocal Leukoencephalopathy in a HIV-Negative Patient with Small Lymphocytic Leukemia following Treatment with Rituximab

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**Key Words**
Progressive multifocal leukoencephalopathy · Rituximab · Small lymphocytic leukemia · JC virus

**Abstract**
We describe a case of progressive multifocal leukoencephalopathy (PML) caused by infection with the human polyomavirus JC virus in a patient with B-cell small lymphocytic leukemia who was treated with rituximab. The first symptoms of PML appeared immediately following the last of five cycles of rituximab, cyclophosphamide and pentostatin. Magnetic resonance imaging revealed changes consistent with PML, although JC virus DNA was not detected by polymerase chain reaction assay of the cerebrospinal fluid. A stereotactic biopsy of the brain showed histological changes consistent with PML, while electron microscopy revealed JC virus particles attached to the nuclei of astrocytes. The patient was treated supportively but died 53 days after the initial onset of symptoms.

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## Background

Rituximab is a chimeric human/mouse IgG1-κ monoclonal antibody that targets the CD20 antigen expressed on the surface of both normal and malignant B lymphocytes. The CD20 antigen, while being expressed in nearly 90% of B-cell non-Hodgkin lymphomas (NHLs) and a smaller proportion of B-cell chronic lymphocytic leukemia (CLL) cells, is not expressed by hematopoietic stem cells. As a result, treatment with rituximab is associated with an initial decrease in lymphocyte count which recovers 9–12 months following completion of the therapy [1]. Rituximab is currently approved for the treatment of CD20-positive hematologic malignancies (NHLs and CLL) and as maintenance therapy for follicular lymphoma and other indolent B-cell malignancies. Rituximab has also been employed for the treatment of non-malignant autoimmune disorders, chiefly rheumatoid arthritis and systemic lupus erythematosus (SLE) [2].

Progressive multifocal leukoencephalopathy (PML) is a lethal, progressive demyelinating disorder of the central nervous system (CNS) characterized by the destruction of oligodendrocytes due to the reactivation of a type of human polyoma virus called the John Cunningham (JC) virus [3]. Interestingly, while 50–86% of healthy adults are seropositive for JC virus, reactivation of the virus occurs almost exclusively in patients with an impaired cellular immune response (nearly 85% cases of PML occur in human immunodeficiency virus (HIV)-positive patients) [4]. Although the virus ultimately lodges in and damages the CNS, its journey begins by binding to and entering the B-lymphocytes by a clathrin-dependent pathway. Most of the virions however do not enter the B-cells to establish infection, remaining attached instead to their surface. The infected B-lymphocytes enter the CNS through the blood flow, carrying the virus with them. Once in the CNS, the virus infects astrocytes and oligodendrocytes. Infection of the latter causes a massive demyelination in the CNS leading to focal neurological deficits, the hallmark of PML. The disease has a rapid clinical course with an extremely poor clinical outcome, the overall median survival without treatment being a mere 3.5 months [3].

In 2006, following the report of two patients with SLE who developed PML following rituximab treatment, the Food and Drug Administration issued an alert concerning the use of rituximab in SLE [2]. A review of literature published recently [5] revealed that 57 cases of rituximab-associated PML were reported to date, most in patients who had lymphoproliferative disorders. We describe the development of PML in a patient with B-cell CLL who had received rituximab as part of a combination therapy with pentostatin and cyclophosphamide.

## Case Report

A 70-year-old man with stage IV B-cell small lymphocytic leukemia (SLL) presented to the hospital with complaints of confusion, memory loss and loss of vision. He had recently (2 weeks prior to onset of symptoms) completed five cycles of chemotherapy with rituximab, pentostatin and cyclophosphamide for progressive SLL (achieved nearly complete response as assessed by approximately 90% reduction in cervical, submandibular and axillary adenopathy). The patient had been in good health during his chemotherapy except for mild treatment-associated anemia and an episode of herpes zoster (on the left shoulder) after the fourth cycle which resolved completely with valacyclovir. His wife reported that two weeks back she had noticed that he appeared confused and took longer than usual to respond to her questions or suggestions. Around this time, the patient had also noticed a subjective decrease in vision (in both eyes) which had become progressively worse over the last two weeks. There was no history of trauma, seizures or stroke.

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A neurologic examination revealed that he was oriented to person, generally to place but not to time. Objective ophthalmologic examination revealed significantly decreased vision in all planes bilaterally. Other significant findings included difficulty with upward gaze, reduced vibration sense bilaterally in the lower extremities and a positive Babinski response. An magnetic resonance imaging (MRI) scan with T2-weighting and fluid-attenuated inversion recovery (**fig. 1**) obtained at presentation revealed a hyperintense lesion in the deep white matter of the left temporal lobe and several hyperintense lesions in the subcortical, deep and periventricular white matter of the parieto-occipital region bilaterally. The lesions were mainly confined to the white matter with relative sparing of the cortex. No enhancement was observed following administration of gadolinium. There were no signs of a mass effect or midline shift. The white matter changes were thought to be consistent with either PML or posterior reversible encephalopathy syndrome. Laboratory investigations were significant for leukopenia (total WBC count 2,400/μl, reference range: 4,000–11,000/μl), anemia (hemoglobin 11.6 g/dl, reference range: 13.0–18.0 g/dl), thrombocytopenia (platelet count 105,000/μl, reference range: 150,000–400,000/μl) and decreased serum IgG (591 mg/dl, reference range: 768–1,632 mg/dl). Cerebrospinal fluid analysis revealed presence of erythrocytes (32/μl, reference range: 0/μl), elevated total protein (73 mg/dl, reference range: 15–45 mg/dl) and a decreased IgG to albumin ratio (0.06, reference range: 0.09–0.25). Polymerase chain reaction for cytomegalovirus and JC virus DNA was negative.

Over the next five days, the patient's symptoms progressively worsened with psychomotor slowing, appearance of abnormal eye movements and psychotic episodes together with persistence of his earlier subjective visual impairment. Laboratory studies at this time revealed persistence of the hematological changes with a further drop in the platelet count (97,000/μl). Tests for anti-thyroid peroxidase antibodies and anti-thyroglobulin antibodies and assays for syphilis, HIV-1 and HIV-2, IgG and IgM antibodies to *Borrelia burgdorferi* were negative.

An MRI scan of the brain revealed a slight increase in the size of the lesions in the parieto-occipital regions although no new lesions were noticed (**fig. 2**). A week later (3rd week post-admission), a stereotactic biopsy of the right parietal lobe was performed. Hematoxylin and eosin staining (**fig. 3**) revealed diffuse astrocytosis with many of the astrocytes containing large, irregular nuclei. Occasionally, a smudgy material was observed within their nuclei. Oligodendroglial cells were sparse, enlarged and contained ground glass inclusions. Conventional electron microscopy images of astrocytes in the same sections revealed presence of enlarged and irregular nuclei. The nuclei exhibited focal loss of nuclear envelope with viral particles attached to the nuclear envelope in clusters. Higher magnification revealed numerous icosahedral 35–45 nm viral particles within the astrocyte nucleus consistent with a papovavirus, specifically JC virus. Following diagnosis, the patient was counseled regarding possible treatment options including cidofovir therapy. However, the patient decided against treatment and died 53 days after the initial onset of his symptoms.

## Discussion

PML is a lethal, progressive demyelinating disorder of the CNS characterized by destruction of oligodendrocytes due to the reactivation of a type of human polyoma virus called the JC virus [3]. While 50–86% of healthy adults are seropositive for the virus, reactivation occurs almost exclusively in patients with an impaired cellular immune response (nearly 85% of PML cases occur in HIV-positive patients) [4]. The disease has a rapid clinical course with an extremely poor clinical outcome, the overall median survival without treatment being a mere 3.5 months [3]. Rituximab is a chimeric human/mouse IgG1-κ monoclonal antibody that targets the CD20 antigen expressed on the surface of both normal and malignant B lymphocytes (both in NHLs and CLL). It is currently approved for the treatment of CD20-positive hematologic malignancies (NHLs and CLL) and as maintenance therapy for follicular lymphoma and other indolent B-cell malignancies. Rituximab has also been employed for the treatment of non-malignant autoimmune disorders, chiefly rheumatoid arthritis and SLE [2]. In 2006, following the report of two patients with SLE who developed PML after rituximab treatment, the Food

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and Drug Administration issued an alert concerning the use of rituximab in SLE [2]. A review of literature published recently [5] revealed that 57 cases of rituximab-associated PML were reported to date, most in patients who had lymphoproliferative disorders. A few cases have also been observed in patients treated for other immune-mediated illnesses including SLE, rheumatoid arthritis, idiopathic autoimmune pancytopenia and immune mediated thrombocytopenia. The disease appears to set in relatively early in affected patients following the start of rituximab therapy (median time of onset from the last dose being about 6 months), progresses rapidly (median time to death following diagnosis about 2 months) and is nearly always fatal. Factors predicting a rapid progression of the disease include a CD4+ lymphocyte count <500 cells/μl and diagnosis of PML within 3 months following start of rituximab therapy. Nearly all patients who survive suffer from residual neurologic deficits including hemiparesis, motor aphasia and visual defects. The treatment of PML is mostly supportive and removal of the offending drug (rituximab) is a key part of the therapeutic regimen. Plasma exchange to remove the drug has also been shown to be helpful in treatment and possibly works by restoring the immune function of the CNS [6]. A comparison of our patient with other reported cases revealed several unique characteristics. The most striking feature was the extremely short duration between the last dose of rituximab and the first onset of symptoms (1–2 days). The lowest interval reported so far is 9 days [5]. It has been suggested that a combination of a low CD4+ count and low serum IgG levels is associated with a syndrome of rapid activation of the JC virus following rituximab administration [5, 7]. Although a CD4+ count (at the time of diagnosis) was not available in our case, the patient did have an episode of zoster after the fourth cycle of chemotherapy, suggestive of a decrease in cell-mediated immunity. Further, he had a low serum IgG at presentation. We hypothesize that a combination of decreased cellular and humoral immune response prompted a reactivation of the dormant virus in this patient. Further, the patient had not received prior stem cell transplant, and presented with significant and rapidly progressing loss of vision, a relatively uncommon symptom reported by only 18% of non-transplanted and 13% of post-transplant patients with post-rituximab PML [6]. A second observation was the presence of JC virus particles in astrocytes, a unique finding as the virus typically infects oligodendrocytes although both glial cells and astrocytes express the receptor for JC virus (5-hydroxytryptamine receptor 2A) [8].

The present case represents a rare but deadly complication of rituximab therapy in SLL patients. A deciphering of the molecular mechanisms underlying JC virus infection will potentially permit a better understanding of the pathogenesis of JC virus-associated PML and could possibly lead to the development of novel targeted therapeutic approaches in the future.

## Disclosure Statement

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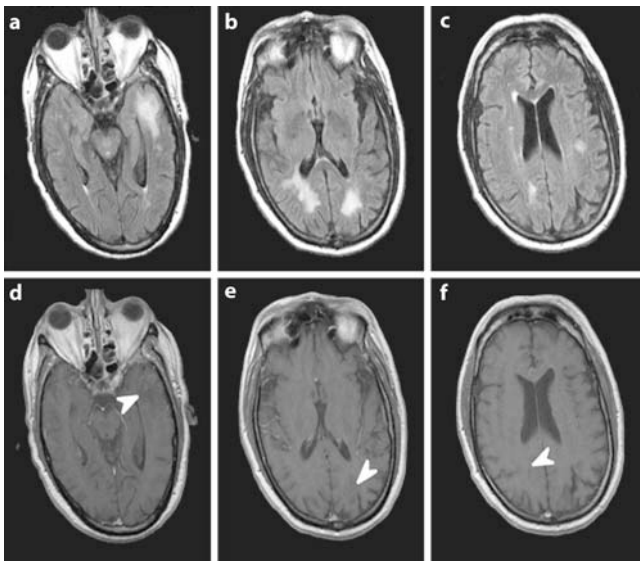


**Fig. 1.** An MRI scan with T2-weighting and with fluid-attenuated inversion recovery (**a–c**) obtained at the time of initial presentation shows a hyperintense lesion in the deep white matter of the left temporal lobe (**a**) and several lesions in the subcortical, deep and periventricular white matter of the parieto-occipital region bilaterally (**b**, **c**). These lesions were mainly confined to the white matter as shown by the relative sparing of the cortex and were not enhanced by the administration of gadolinium on T1-weighted MRI (**d–f**). Arrowheads: hypointense lesions corresponding to the lesions in **a**, **b** and **c**, respectively.

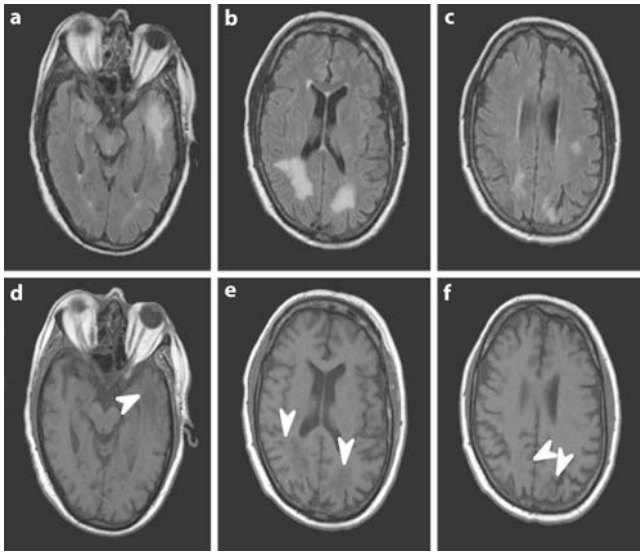


**Fig. 2.** Follow-up MRI scan two weeks after initial presentation revealed a slight increase in size of lesions in the parieto-occipital region (comparison with fig. 1). However, no new lesions were noted anywhere. A gadolinium-enhanced T1-weighted MRI revealed that the non-enhancing lesions were better delineated (**d–f**). Arrowheads: hypointense lesions corresponding to the lesions in **a**, **b** and **c**, respectively.

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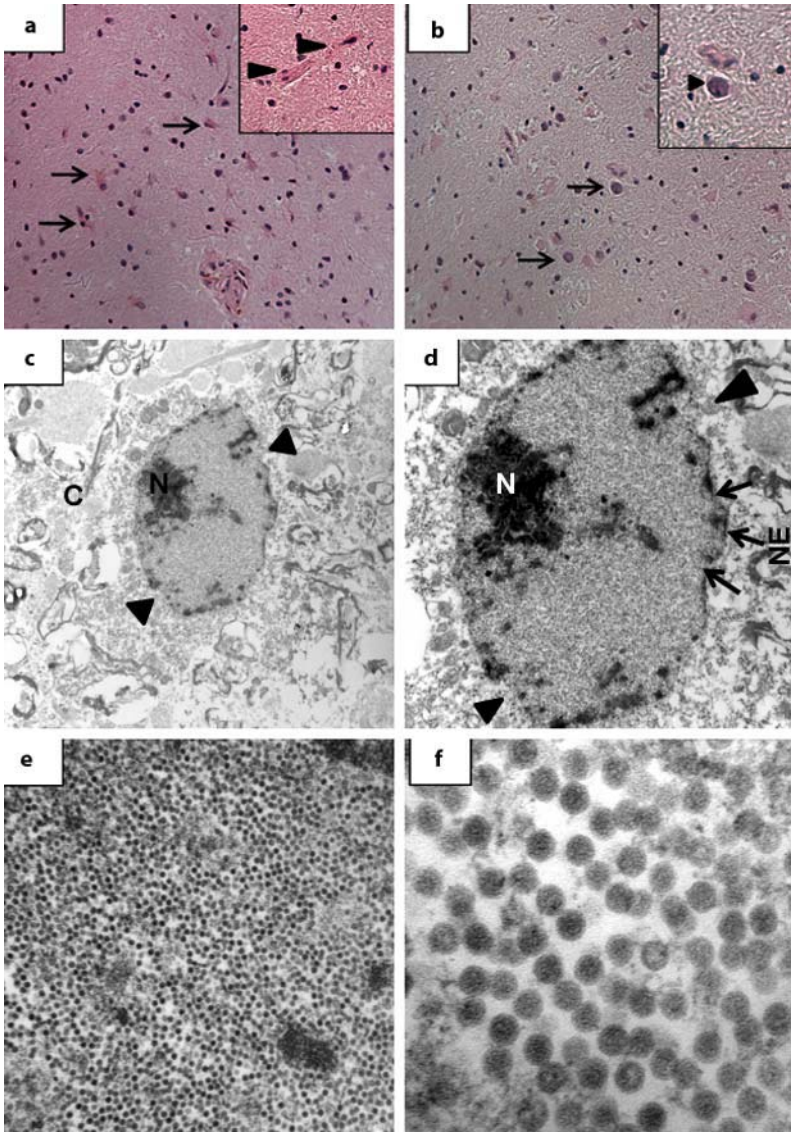


**Fig. 3.** Histologic and electron microscopic findings of PML. Hematoxylin and eosin-stained section of the right parietal lobe (**a**, **b**) showing shows diffuse astrocytosis (**a**, original magnification ×200) with many of the astrocytes containing large, irregular nuclei (arrows). Occasionally, a smudgy type of material was observed within their nuclei (inset shows a magnified view of astrocytes with enlarged nuclei, original magnification ×400). Oligodendroglial cells were sparse and enlarged and contained ground glass inclusions (**b**, arrows, original magnification ×200) (inset shows the magnified image of an oligodendrocyte with viral inclusion body in the nucleus, original magnification ×400). Conventional electron microscopy images of astrocytes from a stereotactic biopsy of the right parietal lobe showing enlarged and irregular nuclei (**c**, original magnification ×8,000, and **d**, original magnification ×15,000). The nuclei exhibit focal loss of nuclear envelope (NE) (arrowheads) while viral particles are observed attached to the nuclear membrane in clusters (**e**, original magnification ×60,000). Higher magnification reveals numerous icosahedral 35–45 nm viral particles within the astrocyte nucleus (**f**, original magnification ×300,000). C = Cytoplasm; N = nucleus; NE = nuclear envelope.

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