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# Atypical radiological presentation of progressive multifocal leukoencephalopathy following liver transplantation

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**Progressive multifocal leukoencephalopathy (PML), a demyelinating disease of the brain caused by JC virus (JCV), occurs following transplantation and other conditions associated with immunosupression. On magnetic resonance imaging (MRI), PML lesions typically appear as hyperintense signal on T2-weighted and FLAIR images located in the subcortical white matter, which are devoid of contrast enhancement or mass effect. The prognosis is poor, but unusual inflammatory forms of PML characterized by contrast enhancement have been associated with a cellular immune response against JCV and a better prognosis. The authors report an atypical presentation of PML with contrast-enhancing lesions and mass effect on the MRI in a liver transplant recipient, who had a progressive course and fatal outcome.** *Journal of NeuroVirology* (2005) **11,** 46–50.

**Keywords:** demyelinating diseases; polyoma virus; transplantation

## Introduction

Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the brain caused by JC virus (JCV), a polyoma virus. Asymptomatic infection by JCV usually takes place during childhood and antibodies for this virus are found in more than 80% of the healthy adult population (Weber *et al*, 1997). After primary infection, JCV remains latent in the kidney and lymphoid organs but can reactivate and spread to the central nervous system in the setting of cellular immunodeficiency, resulting in PML. Prior to acquired immunodeficiency syndrome (AIDS), PML was a rare disease occurring in approximately 0.07% of patients with hematological malignancies, but it now affects up to 5% of the patients with AIDS and is recognized as a major cause of neurological complication in this group (Power *et al*, 2000).

PML may occur following organ transplantation (Kwak *et al*, 2002) and is usually included in the differential diagnosis of central nervous system (CNS) white matter lesions in these patients. However, transplant recipients present an additional challenge to clinicians, because others causes of leukoencephalopathy, such as other opportunistic infections, toxic effect of drugs, and metabolic disturbances, commonly occur in this setting. Furthermore, more than one condition can coexist in these immunossupressed individuals.

There is no specific treatment available for PML, but cytosine arabinoside (ARA-C) has been shown to have *in vitro* activity against JCV (Hou and

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Major, 1998) and has been used in human immunodeficiency virus (HIV)-negative patients with PML (Aksamit, 2001). We have shown that the detection of JCV-specific cytotoxic T-cell lymphocytes (CTLs), using tetramer staining and functional lysis assays, is associated with prolonged survival in PML patients and could be used as a prognostic marker of disease evolution (Koralnik, 2002; Koralnik *et al*, 2002). We report an atypical clinical and radiological presentation of PML in a liver transplant recipient who had a fatal outcome despite treatment with ARA-C in which the cellular immune response against the JCV was examined using tetramer-staining assay.

## Case report

A 39-year-old woman was admitted to the hospital because of right-sided weakness and progressive confusion.

She underwent living donor right lobe liver transplant in March 2003 because of cirrhosis secondary to hepatitis C virus (HCV). Her past medical history also included diabetes mellitus, hypertension, and a previous admission in October 2003 for small bowel obstruction, treated surgically. Postoperatively she received mycophenolate mofetil (MMF), steroids, cyclosporine, and two doses of anti–interleukin-2 receptor (IL2R) antibody (Simulect) for immunosuppression. Two months later, she was transferred to a chronic rehabilitation facility, on total parenteral nutrition and taking an immunosuppressive regimen that included tacrolimus 2 mg twice a day and prednisone 5 mg once a day.

One week prior to the current admission in December 2003, the patient presented with right hand weakness. On the following days, she started using words inappropriately and became progressively confused and agitated. Three days before admission, she developed right-sided hemiparesis and hypertension (188/117 mm Hg). On the admission day, she became unresponsive and was transferred to our hospital. She was intubated for airway protection and transferred to the intensive care unit. A head computed tomography (CT) scan showed cerebral edema and diffuse hypodense lesions in the posterior white matter bilaterally.

The general examination was unremarkable except for elevated blood pressure (220/126 mm Hg). On neurological examination, she was comatose and did not open her eyes to voice or painful stimuli. Fundoscopic examination showed bilateral papilledema. No obvious cranial nerve defects were noticed. She moved all four extremities spontaneously but with an apparent right hemiparesis. The deep tendon reflexes were depressed on the right, and the plantar responses were extensor bilaterally. No meningeal signs were present. The laboratory tests revealed thrombocytopenia (97,000/$mm^3$), hyperglicemia (277 mg/dl), and hyponatremia (119 mEq/L). The aminotransaminases were mildly elevated (alanine aminotransaminase 128 IU/L and aspartate aminotransaminase 85 IU/L), the alkaline phosphatase was 1540 IU/L and total billirrubin was 1.9 mg/dl. Tacrolimus plasma level was 5.4 ng/ml (therapeutic range 5 to 20 ng/ml). On the next day, a cranial magnetic resonance imaging (MRI) study showed extensive areas of abnormal T2-weighted and FLAIR signal intensity in the white matter of the parietal lobes, the posterior left frontal and temporal lobe, and the superior occipital lobes. There was mass effect with compression of the left lateral ventricle and slight midline shift. Following contrast administration, irregular and ring-enhancing lesions in multiple locations were observed. A second MRI done 2 days later showed a worsening of the swelling and a more evident contrast enhancement associated with the lesions (Figure 1A to C). A chest/abdomen/pelvis CT scan was negative for malignancy.

Tacrolimus was discontinued due to the possibility of a drug induced posterior leukoencephalopathy (Wijdicks, 2001). The patient was treated with mannitol 25 mg intravenously every 6 h and dexamethasone 8 mg every 6 h to decrease the brain edema and prevent uncal herniation. The sodium serum concentration increased progressively toward normal range and the systemic blood pressure was controlled with intravenous labetalol 0.5 to 2 mg/min and nitroprusside sodium 0.1 $\mu$g/kg/min on the following days. These measures resulted in a partial improvement of the neurological condition leading to extubation. At that time, the patient was found to have an expressive aphasia characterized by repetitive use of monosyllables and decreased fluency, cortical blindness and right side hemiparesis. Sirolimus 2 mg daily was started for transplant related immunosupression.

Five days after admission, a lumbar puncture was performed to rule out infectious etiologies. The cerebrospinal fluid (CSF) was clear and colorless. Cell count demonstrated two red cells and one white cell per cubic millimeter. The protein content was mildly elevated (56 mg/dl) but glucose was within normal limits. Gram and acid-fast staining were negative as was India ink for encapsulated yeast. Cultures for bacteria, fungi and virus remained sterile. Polymerase chain reaction (PCR) studies for cytomegalovirus (CMV), Epstein-Barr virus (EBV), and *Mycobacterium tuberculosis* were negative in the CSF; however, JCV PCR was positive in the CSF and a diagnosis of PML was established. It is known that JCV can be detected in peripheral blood from immunossupressed patients who do not have any neurological disease and, thus, it can be a potential source of CSF contamination. In order to exclude this possibility, the CSF and plasma JCV DNA load were assessed using a real time quantified PCR protocol. The CSF JCV viral load was 166 copies/ml in the CSF and undetectable in the plasma.

A third MRI done 2 weeks after the first one did not show any improvement in the mass effect associated with the lesions. There was some improvement

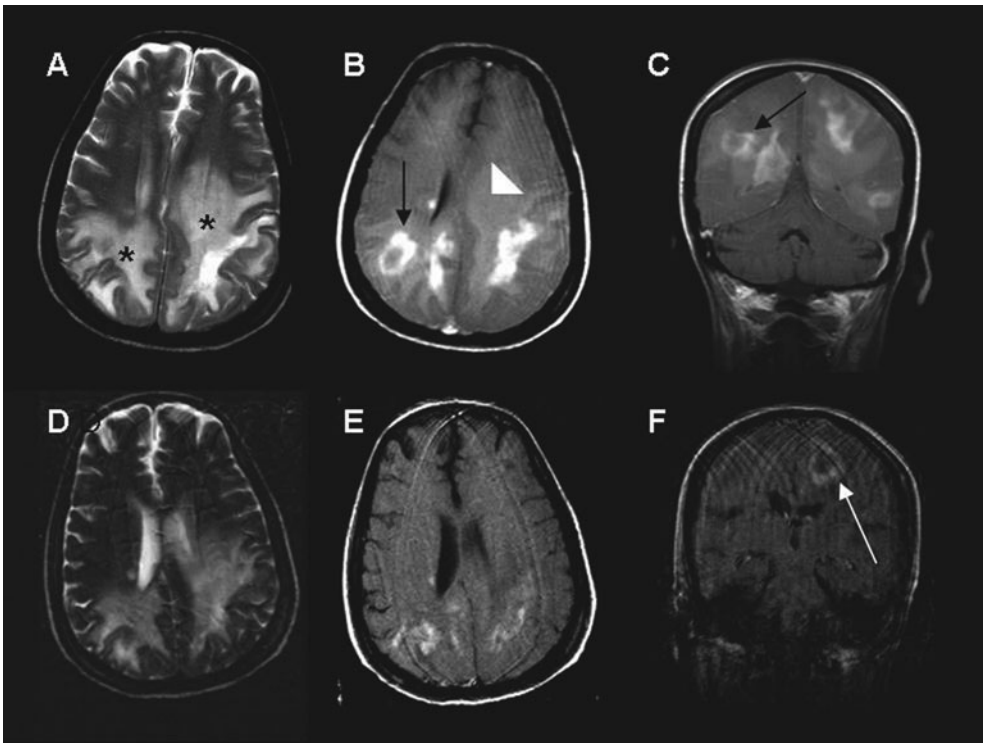


**Figure 1** A T2-weighted image (**A**) shows extensive hyperintense lesions in the white matter of both cerebral hemispheres (*asterisks*). On contrast-enhanced T1-weighted images (**B** and **C**), irregular and ring enhancing lesions are present (*black arrows*). Mass effect and compression of the left lateral ventricle is evident (*white arrow head*, **B**). After treatment with steroids, mannitol and ARA-C, a partial improvement in the mass effect is observed (**D** and **E**), but ring enhancing lesions could still be seen (*white arrow*, **F**).

in the pattern of enhancement, but multiple foci of hyperintense FLAIR signal and T2-weighted signal appeared within the central portions of these white matter areas, suggesting the development of necrosis in these areas. Based on these results, she received a 5-day course of intravenous ARAC 2 mg/kg for treatment of PML (Aksamit, 2001). During this period, she continued to receive dexamethasone and mannitol. A follow-up MRI done at the end of the treatment showed a partial improvement of the brain edema (Figure 1D to F), but this was not followed by apparent neurological improvement.

She concomitantly developed progressive drug associated pancytopenia and liver function abnormalities due to recurrent HCV (viral load >500,000 IU/ml). Her clinical condition worsened without neurological improvement and comfort care measures were initiated. The patient died 6 weeks after admission. An autopsy was not performed.

*Cellular immune response against JCV VP1 protein*

To determine if JCV-specific CTLs could be detected in the peripheral blood, a tetramer-staining assay was performed. This technique permits the identification of CTL that recognize specifically HLA-A*0201–restricted JCV VP1 epitopes in HLA-A*0201–positive individuals (approximately 40% of the population). The major histocompatibility complex (MHC) class I alleles expressed by the patient were determined by using standard tissue typing procedures and she was found to be HLA-A*0201–positive. No JCV-specific CTLs could be detected in her blood (data not shown).

## Discussion

Neurological complications are frequently seen after liver transplantation. In a recent study with 657 patients, 27% of these presented with new neurological events (Lewis and Howdle, 2003). The most common etiologies are anoxia, metabolic derangements, rejection, adverse effects of the immunosuppressive drugs, malignancies, and infections (Bronster *et al*, 2000). PML has been described in solid-organ or bone marrow transplant recipients (Kwak *et al*, 2002). In a series of 463 liver transplant recipients, the incidence of the disease was 0.21% (Bronster *et al*, 2000) and in a postmortem series, PML was found in 1 out 132 individuals (0.8%) (Martinez and Ahdab-Barmada, 1993).

PML is usually a subacute disease with progressive focal neurological deficits related to the white matter lesions. Common signs and symptoms are weakness, gait abnormalities, visual and language complaints, and cognitive dysfunction. Fever or other constitutional symptoms are absent (Berger *et al*, 1998b; Mamidi *et al*, 2002). The typical radiological findings on MRI studies are subcortical high signal images

on T2-weighted and FLAIR and low signal on T1-weighted images located in subcortical white matter (Mader *et al*, 2003; Thurnher *et al*, 2001). Although seldom reported in association with PML in HIV-infected patients (Hoffmann *et al*, 2003; Thurnher *et al*, 2001), the presence of ring enhancement is more frequently seen in other neurological conditions associated with immunosupression such as toxoplasmosis, fungal abscesses, and CNS lymphoma. In PML, the presence of contrast enhancement has been interpreted as the result of an intense inflammatory reaction against the virus (Thurnher *et al*, 2001) and, recently, has been reported in HIV-positive patients in the setting of the immune reconstitution inflammatory syndrome (IRIS) (Du Pasquier and Koralnik, 2003). These inflammatory forms of PML are usually associated with a better prognosis (Berger *et al*, 1998a; Du Pasquier and Koralnik, 2003). On the contrary, the presence of mass effect has been correlated with a shorter survival in these patients (Post *et al*, 1999).

Despite the florid contrast enhancement of the lesions observed on the MRI, we were not able to detect any significant cellular immune response against JCV in this patient's blood using a tetramer staining assay. This finding is usually associated with a poor prognosis in PML patients (Du Pasquier *et al*, 2003). One possible explanation is the interference of the immunosuppressive drugs on the T cells and, subsequently, on the results of the tetramer-staining assay. Tacrolimus acts via inhibition of calcineurine mediated IL-2 production resulting in blockage of T-cell proliferation and differentiation, whereas corticosteroids use is associated with lymphocyte depletion (Mueller, 2004). Furthermore, the leukopenia induced by ARA-C could have also contributed to the failure in detecting JCV-specific CTLs. However, the breakdown of the blood-brain barrier (BBB) in PML lesions is more likely explained by other known causes of CNS injury at the time of diagnosis, including uncontrolled hypertension, hyponatremia, and tacrolimus use. These conditions could have contributed to additional blood brain barrier disruption resulting in this atypical radiological presentation of PML.

We used a quantified PCR technique for detection of JCV DNA in the plasma and CSF samples of the patient with the purpose of excluding the possibility of as false positive CSF PCR due to blood contamination in a setting of a breakdown of the BBB. The JCV viral load was 166 copies/ml in the CSF and undetectable in the plasma. Therefore, we can conclude that there was active JCV replication in the CSF and no blood contamination.

The prognosis of this condition has changed recently in the HIV-infected population with the use of highly active antiretroviral therapy (HAART). In one study, the 1-year survival was 4% in patients without HAART and 46% in treated ones (De Luca *et al*, 2000). However, the prognosis is still poor when PML is associated with other forms of immunosupression and the survival for most of the cases is usually limited to a few months. Several drugs, including ARA-C, cidofovir, alpha-interferon, topothecan, heparin sulfate, and IL-2, have been evaluated for the treatment of PML with inconsistent and nonreproducible results, precluding any formal recommendation (Kwak *et al*, 2002; Seth *et al*, 2003). In this case, ARA-C was used based on its efficacy in preventing JCV replication *in vitro* (Hou and Major, 1998) and on retrospective study that showed a 36% chance of disease stabilization at 1 year in non-AIDS PML patients (Aksamit, 2001). Because JCV reactivation occurs in association with immunosupression and there is no efficient available treatment at this time, the goal is, whenever possible, the correction of the predisposing condition. In the particular case of solid organ transplant recipients, decreasing the immunosuppressive treatment incurs the risk of organ rejection. However, a cautious reduction of the immunosuppressive drug regimen in these patients could be considered.

This case report illustrates that ring-enhancing lesions and mass effect, although infrequent, can be seen in patients with PML. This disease must, therefore, be included in the differential diagnosis of inflammatory focal brain lesions in organ transplant recipients.

## Materials and methods

DNA extraction from CSF and plasma were performed as previously described (Koralnik *et al*, 1999).

*Qualitative CSF PCR for JCV*

PCR amplification of JCV VP1 was performed using primer pair CJS2474 (5′-CA GGAGACCCAGATATGATGAGATA-3′, nucleotides [nt] 2465 to 2489) and CJR2578 (5′-TGGTTATACTTTATTAAAATGTACTGCATATT-3′nt 2578 to 2547RC), which amplify a ~114-bp fragment. The PCR reaction was performed in PE 9700 thermal cycler using 5 $\mu$l DNA, 25 pmol of each primer, 1.5 mM $MgCl_2$, and 1 U of Ampli Taq Gold DNA Polymerase (Applied Biosystems, Foster City, CA) in a final volume of 50 $\mu$l. The cycles of amplification consisted of 94°C for 10 min followed by 40 cycles of 94°C for 30 s, 56°C for 1 min, 72°C for 1 min, and 15 min of elongation at 72°C.

*Quantified PCR for JCV*

To obtain precise and reproducible measurements of JCV CSF and plasma viral load, a real time quantified PCR protocol was developed using primer JC25: 5′-CTGGTGAATTTATAGAAAGAAGTATTGCA-3′(nt 1343 to 1371) and JC23-5′-GGGCCATCTT CATATGCTTCAA-3′ (nt 1475 to 1454) that amplify a 133-bp fragment of the VP2 gene. The fluorogenic probe labeled with a reporter and quencher dye: 5′-ATCTGCTCCTCAATGGATGTTGCCTTTACTT-3′ (nt 1392

to 1422) was added to the PCR reaction mixture and the reaction was carried out using 40 cycles of amplification in an ABI Prism 7700 Sequence Detection System (PE Applied Biosystems). A linear range of amplification was obtained from $10^1$ to $10^4$ target copy number of control DNA. This method could detect as low as 10 copies of JCV VP2 gene/sample and the primers did not cross-react with simian virus 40 (SV40), BKV, and human genomic DNA.

*Cellular immune response against JCV VP1 protein*
The determination of the immune response against JCV using HLA-A*0201–restricted tetramer was performed as previously described (Du Pasquier *et al*, 2003).

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