Management and outcome of CSF-JC virus PCR-negative PML in a natalizumab-treated patient with MS

J. Kuhle, MD
R. Gosert, PhD
R. Bühler, MD
T. Derfuss, MD
R. Sutter, MD
Ö. Yaldizli, MD
E.-W. Radue, MD
C. Ryschkewitsch, MT
E.O. Major, PhD
L. Kappos, MD
S. Frank, MD
H.H. Hirsch, MD, MS

Address correspondence and reprint requests to Dr. Ludwig Kappos, Clinical Neuroimmunology, Departments of Biomedicine and Neurology, University Hospital Basel, Petersgraben 4, CH-4031 Basel, Switzerland
kapposl@uhbs.ch

ABSTRACT

Objective: To describe the diagnosis and management of a 49-year-old woman with multiple sclerosis (MS) developing a progressive hemiparesis and expanding MRI lesion suspicious of progressive multifocal leukoencephalopathy (PML) 19 months after starting natalizumab.

Results: Polyomavirus JC (JCV)-specific qPCR in CSF was repeatedly negative, but JCV-specific antibodies indicated intrathecal production. Brain biopsy tissue taken 17 weeks after natalizumab discontinuation and plasmapheresis was positive for JCV DNA with characteristic rearrangements of the noncoding control region, but histology and immunohistochemistry were not informative except for pathologic features compatible with immune reconstitution inflammatory syndrome. A total of 22 months later, the clinical status had returned close to baseline level paralleled by marked improvement of neuroradiologic abnormalities.

Conclusions: This case illustrates diagnostic challenges in the context of incomplete suppression of immune surveillance and the potential of recovery of PML associated with efficient immune function restitution. *Neurology*® 2011;77:2010-2016

GLOSSARY

AI = antibody index; **EDSS** = Expanded Disability Status Scale; **IgG** = immunoglobulin G; **IRIS** = immune reconstitution inflammatory syndrome; **IVIg** = IV immunoglobulin; **JCV** = polyomavirus JC; **MS** = multiple sclerosis; **NTZ** = natalizumab; **PML** = progressive multifocal leukoencephalopathy; **rr-NCCR** = rearranged noncoding control region; **RRMS** = relapsing-remitting multiple sclerosis.

Progressive multifocal leukoencephalopathy (PML) is an infectious demyelinating disease of the brain, caused by the polyomavirus JC (JCV). Typically the disease has been closely associated with profound immunodeficiency, e.g., in the setting of HIV infection and very low CD4 cell counts.^{1,2} Virologic and immunologic studies suggest that activation of JCV replication and the lack of specific cellular immunity are critical in the development of PML.³⁻⁶

Natalizumab (NTZ) has demonstrated high efficacy in 2 phase III trials in relapsing-remitting multiple sclerosis (RRMS).^{7,8} PML has never been reported in multiple sclerosis (MS) prior to introduction of NTZ treatment. As of May 4, 2011, more than 83,300 patients have received NTZ with a total of 124 reported PML cases (Biogen-Idec, data on file, May 4, 2011). The overall incidence of 1.44 in the postmarketing experience (as of May 4, 2011) is similar to that, estimated after the pivotal trials in MS.⁹ In the absence of surrogate markers of the risk of PML, clinical vigilance and a low threshold of intervention is currently recommended for managing patients with MS treated with NTZ and possible PML.¹⁰

CASE REPORT A 48-year-old woman was diagnosed with RRMS in 1995 and temporarily received interferon β -1b (Betaferon) and glatiramer acetate (Copaxone) (table). She reported having approximately 2

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Supplemental Data

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From the Department of Neurology (J.K., T.D., R.S., Ö.Y., L.K.), Clinical Neuroimmunology, Department of Biomedicine (J.K., T.D., L.K.), Intensive Care Unit (R.S.), Medical Image Analysis Center (E.-W.R.), Division of Neuropathology, Institute of Pathology (S.F.), and Division of Infectious Diseases & Hospital Epidemiology (H.H.H.), University Hospital Basel, Basel; Clinical & Transplantation Virology, Institute for Medical Microbiology, Department of Biomedicine (R.G., H.H.H.), University of Basel, Basel, Switzerland; Department of Neurology (R.B.), Bürgerspital, Solothurn, Switzerland; and Laboratory of Molecular Medicine and Neuroscience (C.R., E.O.M.), National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD.

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Table Clinical status and treatments			
Date	Symptoms and CSF examinations	Treatment	Cranial MRI
1991 and 1995	Transient numbness right face in 1991, sensorimotor paraparesis in 1995: diagnosis of RRMS	MP IV: 1995	
October 1996–January 1999	Approximately 2 relapses per year (last in July 2007)	IFN β -1b	
		MP IV (for relapses)	
June–September 1999		GA	
		MP IV (for relapses)	
January 2008	Mild left spastic atactic hemiparesis, walking distance 3–4 km, EDSS 3.0	Initiation of NTZ: January 29	
January 2009	Left-sided hypoesthesia and dysesthesia, disturbance of equilibrium and weakness of left leg; spontaneous recovery within 4 weeks		See figure 1
May–September 2009	Progressive ataxia and weakness of the left leg, unsteady gait	Last treatment with NTZ: August 11	See figure 1
		MP IV: August 24–28	
	CSF 1: September 2, 2009	Plasma exchange: September 4 and 7	
October–November 2009	Progression of left-sided atactic hemiparesis: increased muscle tone in left leg, positive Babinski sign, unable to lift extended left leg against gravity; sensitivity moderately impaired below left knee with disturbed position sense of left foot; walking distance 500–600 m, EDSS 4.0	MP IV: October 19–23	See figure 1
	Diagnosis of IRIS		
	CSF 2: October 15, 2009		
	CSF 3: November 27, 2009		
December 2009	Stereotactic brain biopsy; clinical deterioration	IVlg: December 17–21, Trazodone	
January 2010–January 2011	Improvement of hypesthesia, strength of left leg, and walking ability on January 5, 2010; further improvement on January 28, 2010: slight pronator drift but no sinking of left arm, paresis of left leg M4/5, able to lift stretched leg by 45° for 5 seconds, walking distance improved to 1.5 km; EDSS 3.5; further improvement (walking distance 1.5–2 km) in June 2010 and January 2011; focal motor seizures of left leg on October 10, 2010, and January 7, 2011	IVlg: February 10–14 and March 17–21	See figure 1

Abbreviations: EDSS = Expanded Disability Status Scale; GA = glatiramer acetate (Copaxone), 20 mg, SC, QD; IFN β -1b = interferon β -1b (Betaferon), 8 MIU, SC, EOD; IRIS = immune reconstitution inflammatory syndrome; IVlg = IV immunoglobulin, 0.4 g/Kg body weight, 21 g; MP IV = methylprednisolone, 1,000 mg, IV, once daily; NTZ = natalizumab (Tysabri), 300 mg, IV, 4 weekly; RRMS = relapsing-remitting multiple sclerosis.

relapses per year, the last relapse with a sensorimotor paresis of the left arm occurring in July 2007. In December 2007 the Expanded Disability Status Scale (EDSS) was 3.0 (scale ranging from 0 to 10, with higher scores indicating greater disability).

NTZ therapy was initiated on January 29, 2008. The third administration was delayed due to unspecified rhinosinusitis for 16 weeks. In January 2009, she presented with subacute left-sided hypoesthesia and dysesthesia, disturbance of equilibrium, and slight weakness of the left leg. Cranial and spinal MRI showed no enhancing or new T2 lesions (figure 1) and symptoms resolved spontaneously within 4 weeks.

On June 2, 2009 (14th NTZ administration), she reported a new weakness of the left leg and an unsteady gait lasting since mid May 2009. The 15th NTZ infusion was delayed by 10 weeks until August 11, 2009, due to a right-sided zoster ophthalmicus

and a respiratory tract infection. Symptoms persisted and were treated with high-dose corticosteroids for suspected relapse in August 2009. MRI on August 29, 2009, showed 2 new lesions (figure 1), and CSF on September 2, 2009, 1.0 white cells/mm³, normal albumin CSF/serum ratio (qAlb 3.0×10^{-3}), and presence of oligoclonal immunoglobulin G (IgG) bands. The qPCR for JCV performed in 3 laboratories was undetected (H.H. Hirsch, Basel; M. Gorgievski, Berne; and E.O. Major, Bethesda, MD), and plasma exchange (September 4 and 7, 2009; 1.5 plasma-volume exchange each) was stopped.^{11–13}

A follow-up MRI on September 28, 2009 (figure 1) showed a slight progression of the new T2 hyperintense and enhancing lesion.

JCV qPCR performed in Basel and Berne on a second CSF sample (October 15, 2009) was again negative. However, the JCV-specific IgG antibody

Figure 1 MRI

Left image: axial fluid-attenuated inversion recovery (FLAIR) sequence, except January 2009: axial T2-weighted sequence; middle image: coronal T2-weighted sequence, except January 2010: coronal FLAIR sequence; right image: contrast-enhanced T1-weighted images. (A) January 14, 2009: few periventricular, nonenhancing lesions compatible with multiple sclerosis (arrowheads). (B) August 29, 2009: initial demonstration of a new, hyperintense, faintly contrast-enhancing, subcortical, ribbon-like lesion in the right central region, showing no mass effect and sparing the cortex (lesion 1, arrow). A second small hyperintense and noncontrast enhancing lesion was seen further rostrally (lesion 2, short

titers in the CSF were positive at a dilution 1:400 and had increased significantly by 8-fold over the first CSF sample (titer 1:50). Immune reconstitution inflammatory syndrome (IRIS) was diagnosed based on clinical progression and the MRI findings, and corticosteroid treatment initiated.

Brain MRI on November 23, 2009, showed a dramatic spread of the lesion in the right central region and enhancement was more prominent (figure 1). At that time the patient had a progressive left-sided hemiparesis. She was unable to walk more than 500–600 meters without help; EDSS score was 4.0.

JCV DNA in a third CSF sample (November 27, 2009) was again negative in all 3 laboratories. At this time, the JCV-specific IgG antibody titer in CSF was found to be 1:1,200, indicating an intrathecal JCV-specific antibody production with an antibody index (AI) of 13 (AI: ratio between the CSF/serum quotients for JCV antibody [Q_{JCV}] and Q_{IgG} : $AI = Q_{JCV}/Q_{IgG}$; reference <1.5).¹⁴

A stereotactic brain biopsy of the right parietal lobe was performed on December 10, 2009 (figure 2). There were no signs of demyelination, bizarre astrocytes, nuclear inclusions, or nuclear p53 immunoreactivity. Immunohistochemistry was negative for the viral capsid protein VP1 (antibody gift of R. Frisque, Huck Institute of Life Sciences, Pennsylvania State University) and the large T-antigen using cross-reacting antibodies raised to SV-40 (Ventana Medical Systems, Inc., AZ). In situ hybridization for JCV was negative, but qPCR from the paraffin-embedded material was positive in an independent laboratory (National Institute of Neurological Disorders and Stroke). Quantification of the JCV brain tissue viral load was positive (93,100 Geq/mL, corresponding to 12,670 JCV-Geq/100,000 cells). Direct sequencing revealed a rearranged noncoding control region (rr-NCCR) JCV variant with partial deletions and duplication as commonly detected in PML (figure 3). Cloning and sequencing confirmed that this rr-NCCR JCV represented the majority species which coexisted side by side with a smaller number of closely related quasispecies, but the archetype at-NCCR JCV was not found (figure e-1 on the *Neurology*[®] Web site at www.neurology.org).

arrow). (C) September 28, 2009: slight progression of lesion 1 and 2 and persisting sparse rim-shape contrast enhancement. (D) November 23, 2009: dramatic enlargement of the meanwhile confluent lesion 1 and 2. The corresponding T1 signal was hypointense, ribbon-band-like, and also speckled pattern contrast enhancement more prominent. (E) January 28, 2010: lesion slightly smaller compared with November 2009. Ribbon-like contrast enhancement persisted, whereas the punctiform enhancement pattern was slightly reversible. (F) March 25, 2010 and (G) October 21, 2010: clear reduction of lesion size, no contrast enhancement. No additional new lesions.

Figure 2 Histology of the brain biopsy material

Intraparenchymal and perivascular chronic lymphocytic inflammation (A: hematoxylin & eosin). T cells (CD5) were clearly dominating over B cells (CD20), and CD4+ T cells (B) were similarly abundant as CD8+ T cells (C). Frequent microglial activation was demonstrated (CD68) (D). There were no viral inclusions, no bizarre astrocytes, or nuclear p53 immunoreactivity. JCV- and SV-40 staining and in situ hybridization for JCV (National Institute of Neurological Disorders and Stroke) were negative (all images at same magnification; scale bar (D) 50 μ m).

Due to progressive clinical deterioration attributed to IRIS, IV immunoglobulins (IVIg, 0.4 g/kg body weight) were administered (December 17 to December 21, 2009). Trazodone was given for treatment of depression.

On January 5, 2010, hypesthesia, left leg weakness, and impaired gait had improved. Left-sided reflexes were still exaggerated and the Babinski sign was positive.

On January 28, 2010 (table, figure 1), the patient reported further improvement in strength of the left leg and walking ability, confirmed in the clinical examination. The EDSS score was 3.5, and the MRI lesion had slightly decreased in size and contrast enhancement.

Two IVIg courses were administered again in February 2010 and March 2010. Follow-up MRIs clearly demonstrated shrinkage of the lesion and

Figure 3 Rearrangements of the noncoding control region (NCCR)

DNA from frozen brain biopsy was extracted using the QIAamp kit according to the manufacturer's instruction and the respective JCV DNA was amplified and sequenced as described.⁴ Characteristic rearrangements of the NCCR were found as had been reported for JCV strains isolated from the brain or CSF of patients with progressive multifocal leukoencephalopathy.^{4,18,19}

complete resolution of enhancement (figure 1). In June 2010, the patient reported having intermittent headaches responding to paracetamol; walking distance had stabilized (1.5–2 km) without any new neurologic symptoms. Focal motor seizures of her left leg on October 10, 2010, and January 7, 2011, led to the diagnosis of a lesional epilepsy, currently treated with levetiracetam; up to January 27, 2011, she had experienced no more relapses or neurologic worsening.

DISCUSSION The onset of PML is insidious and its diagnosis may represent a formidable challenge despite a high index of suspicion and extensive imaging. This challenge is accentuated in patients with RRMS on NTZ where the differential diagnosis of the underlying disease and the potential consequences of disease-modifying therapies have blurred the boundaries between 2 formerly mutually exclusive diagnoses of PML and MS. However, in face of the dire prognosis, a high level of clinical vigilance and a low threshold for invasive diagnostic measures and intervention are currently recommended for managing patients with MS on NTZ and possible PML.

Detecting JCV DNA in CSF is accepted for the diagnosis of a laboratory-confirmed PML,¹⁵ but has a sensitivity of approximately 80%.^{11,15,16} In our case, the delay of approximately 15 weeks from initial symptoms (May 2009) to first JCV CSF PCR (September 2009) and an efficient reconstitution of CNS immune surveillance have likely contributed to repeatedly negative JCV PCR results.

Similar to previously reported cases of patients with HIV-AIDS with PML and IRIS following initiation of highly active antiretroviral therapy, the histopathology studies of our case did not reveal any of the findings of florid PML.¹⁷ However, qPCR from brain biopsy in December 2009 was clearly positive for JCV and characteristic rearrangements of the NCCR were found as had been reported for JCV strains isolated from the brain or CSF of patients with PML (figure 3).^{4,18,19} Moreover, cloning and sequencing confirmed the presence of a closely related rr-NCCR quasispecies in addition to the one identified as majority species (GenBank submission and figure e-1) as discussed, while JCV archetype NCCR was not detected.

The diagnosis of PML in our patient is further supported by the detection of a 24-fold rise of JCV-specific IgG antibodies in the CSF with clear evidence for a preferential intrathecal production (AI of 13, with values of AI ≥ 1.5 indicating a local specific antibody synthesis in the CNS).¹⁴ An intrathecal humoral immune response to JCV-specific VP1 has been found in 78% of patients with PML but only

3.2% of controls,²⁰ and can be detected in cases in which the JCV load in CSF is below the detection limit of PCR.^{21,22} Recently, the presence and rise of JCV IgG antibody levels in serum were proposed to be a significant indication of virus exposure and active infection. A rise in serum antibody titers was highly indicative of active PML disease. Similarly, in our patient serum JCV IgG antibody titers were 4-fold higher on November 27, 2009, compared with the first sample obtained on September 2, 2009.²³

IRIS occurs due to a restoration of a pathogen-specific immune response and is linked to clinical disease or worsening of disease.²⁴ Unlike classic PML, IRIS lesions may show contrast enhancement in MRI. In our patient, MRI and CSF examination were obtained approximately 15 weeks after initial PML symptoms. At this time point, the brain MRI displayed enhancing lesions, probably already indicating IRIS.²⁵

In contrast to patients with HIV-AIDS and PML, the immune system is not compromised in MS. The reduced frequency of NTZ infusions in our patient together with the 2 plasma exchange sessions probably have contributed to an early reconstitution of immune surveillance of the CNS. Conversely, the reduced frequency of NTZ might have contributed to this “smoldering PML” with its diagnostic challenges that clinical trials examining NTZ drug holidays should be aware of. Restoration of immune control over JCV replication resulted in a highly efficient IRIS and early suppressed JCV replication. As a consequence we could neither detect virus in the CSF nor histologic findings that are usually found in full-blown JCV CNS infection.²⁶ Spontaneous “inflammatory PML,” which has been occasionally observed during a well-controlled HIV infection as compared to the classic noninflammatory variant, has been associated with a better prognosis.^{3,27} Similarly, in patients with HIV higher CD4 lymphocyte counts were associated with significant gains in survival.²⁸

High-dose corticosteroid therapy has been consistently used to treat IRIS and often resulted in clinical improvement.²⁵ In an attempt to balance the immune reconstitution allowing to control the viral infection but avoiding bystander inflammatory damage, we decided to administer IVIg. Several immunomodulatory rather than immunosuppressive treatments have been proposed in the management of IRIS. Since PML/IRIS is a rare condition, there will probably be no controlled studies in the near future. Hence, for the present, management will be based on expert opinion.²⁴ We speculate that IVIg treatment in our patient—despite overall beneficial survival of PML in MS compared to AIDS—added

to the favorable outcome of PML-IRIS: after a prolonged worsening from May 2009 to January 2010, our patient's clinical condition and the MRI findings clearly improved after one initial and two consecutive courses of IVIg.^{24,29}

Clinicians need to be aware that JCV-PCR in CSF can be negative especially in early PML as well as during IRIS despite typical clinical and radiologic findings. Our case clearly exemplifies that an intrathecal IgG response against JCV may help in guiding further treatment and diagnostic decisions. If a repeat CSF examination is negative, a brain biopsy should be considered if suspicion of PML persists.

AUTHOR CONTRIBUTIONS

Dr. Kuhle, Dr. Bühler, Dr. Sutter, Dr. Yaldizli, Dr. Kappos: patient counseling. Dr. Kuhle, Dr. Gosert, C. Ryschkewitsch, Dr. Major, Dr. Kappos, Dr. Frank, Dr. Hirsch: analysis of patient samples, interpretation of results and writing of the manuscript. Dr. Bühler, Dr. Derfuss, Dr. Sutter, Dr. Yaldizli, Dr. Radue: interpretation of results and revising the manuscript.

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DISCLOSURE

Dr. Kuhle receives research support from the Swiss Multiple Sclerosis Society. Dr. Gosert reports no disclosures. Dr. Bühler has served on a scientific advisory board for Biogen-Dompé AG and has received funding for travel from Merck Serono, sanofi-aventis, UCB, Biogen-Dompé AG, and Janssen. Dr. Derfuss serves on scientific advisory boards for and has received funding for travel and speaker honoraria from Merck Serono, Bayer Schering Pharma, Novartis, and Biogen Idec; his spouse is an employee of Novartis; and he receives research support from Merck Serono, Novartis, Biogen Idec, the German Research Foundation, and the Swiss MS Society. Dr. Sutter has received research support from the Swiss Scientific Society and the Gottfried and Julia Bangerter-Rhyner Foundation and holds stock options from Novartis and Roche. Dr. Yaldizli serves on a scientific advisory board for Merck Serono and has received funding for travel or speaker honoraria from Bayer Schering Pharma and Teva Pharmaceutical Industries Ltd. Dr. Radue has served as a consultant for and received funding for travel and speaker honoraria from Bayer Schering Pharma, Biogen Idec, Novartis, and Merck Serono; and receives research support from Novartis, Biogen Idec, Merck Serono, Actelion Pharmaceuticals Ltd, and Basilea Pharmaceutica Ltd. C. Ryschkewitsch reports no disclosures. Dr. Major receives research support from the NIH/NINDS, Division of Intramural Research. Dr. Kappos has received research support through the University Hospital Basel from Acorda Therapeutics Inc., Actelion Pharmaceuticals Ltd, Advancell, Allozyme, Barofold, Bayer HealthCare Pharmaceuticals, Bayer Schering Pharma, Bayhill, Biogen Idec, BioMarin, Boehringer Ingelheim, CSL Behring, Geneuro, Genmab, GlaxoSmithKline, Glenmark, Merck Serono, MediciNova, Novartis, sanofi-aventis, Santhera Pharmaceuticals, Shire Plc, Roche, Teva, UCB, and Wyeth, and also from the Swiss MS Society, the Swiss National Research Foundation, European Union, Gianni Rubato, and Roche and Novartis Foundations; and serves on the editorial board of *Multiple Sclerosis Journal*, *International Journal of Multiple Sclerosis*, and *Swiss Archives of Neurology and Psychiatry*. Dr. Frank and Dr. Hirsch report no disclosures.

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