Progressive multifocal leukoencephalopathy: a report of three cases in HIV-negative patients with non-Hodgkin's lymphomas treated with rituximab

Marco Tuccori • Daniele Focosi • Fabrizio Maggi •
Mirco Cosottini • Barbara Meini • Fabio Lena •
Corrado Blandizzi • Mario Del Tacca • Mario Petrini

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Dear Editor,

Progressive multifocal leukoencephalopathy (PML) results from productive infection of glial cells by John Cunningham polyomavirus (JCV), facilitated by immune deficiency [1]. We report three cases of PML in HIV-negative patients who had been treated with rituximab (their main clinical features are summarized in Table 1).

Case 1 A 45-year-old male patient with mantle cell lymphoma was treated with four courses of R-hyperCVAD (October 2000–August 2001), followed by mitoxantrone and melphalan supported by autologous hematopoietic stem cell transplantation (HSCT; October 2001). Complete remission was maintained with four courses of rituximab 375 mg/m² (May–June 2002). On November 2005, a bone relapse required four courses of R-DHAP (ended in February 2006)

and BEAM-conditioned allogeneic HSCT (March 2006). Graft-versus-host disease prophylaxis was carried out with methotrexate 15 mg/m² on day +1 and 10 mg/m² on days +3, +6, and +11, and cyclosporine A titrated to keep serum levels between 200 and 400 pg/ml until October 2006. Five courses of maintenance rituximab (375 mg/m²) were then administered (January 2007–April 2008). On April 2008, while in complete remission, he developed bilateral hemianopsia. Magnetic resonance imaging (MRI) displayed subcortical lesions of the white matter. A stereotactic brain biopsy (June 2008) revealed the presence of JCV DNA by polymerase chain reaction (PCR), and histomorphology confirmed PML. Despite treatments with mirtazapine 30–45 mg/day orally, cidofovir 5 mg/kg intravenously (four courses), and risperidone 4 mg/bid orally, follow-up MRIs (June–September 2008) showed progression of

Marco Tuccori and Daniele Focosi contributed equally to this manuscript.

M. Tuccori (✉) • C. Blandizzi • M. Del Tacca
Division of Pharmacology and Chemotherapy,
Department of Internal Medicine, University of Pisa,
Via Roma 55,
56126 Pisa, Italy
e-mail: m.tuccori@ao-pisa.toscana.it

M. Tuccori • B. Meini • F. Lena • C. Blandizzi • M. Del Tacca
Tuscan Regional Centre for Pharmacovigilance,
Florence, Italy

D. Focosi • M. Petrini
Division of Haematology, Department of Oncology,
Transplantations and New Technologies in Medicine,
University of Pisa,
Pisa, Italy

F. Maggi
Virology Section and Retrovirus Centre,
Department of Experimental Pathology, University of Pisa,
Pisa, Italy

M. Cosottini
Department of Neuroscience, University of Pisa,
Pisa, Italy

B. Meini • F. Lena
Pharmaceutical Unit, Hospital of Livorno,
Livorno, Italy

Table 1 Summary of patients' clinical features

Features	Case 1	Case 2	Case 3
Age/gender	45/male	68/male	66/male
Malignancy	Mantle cell lymphoma	Splenic marginal zone lymphoma	Mantle cell lymphoma
Rituximab exposures (regimens)	R-hyperCVAD R-DHAP Maintenance	R-CVP Maintenance	R-CHOP
Exposure to drugs for which evidence exists of a possible association with PML	Cyclosporine methotrexate Oral glucocorticoids (dexamethasone)	Cladribine Cyclophosphamide Oral glucocorticoids (prednisone)	Cyclophosphamide Oral glucocorticoids (prednisone)
Other drug/regimen exposures	Mitoxantrone Melphalan BEAM	Bendamustine Lenalidomide COMP	P-VMYBEC
Stem cell transplantation	Allogeneic	Not performed	Not performed
Laboratory analysis at PML diagnosis	WBC: 7,660; RBC: 5.18; PLT: 237,000; NEU: 62; LYM: 24; BC: absent; IgG: 520; IgA: 22; IgM: 18	WBC: 34,000; RBC: 3.49; PLT: 102,000; NEU: 5; LYM: 18; BC: 75; IgG: 565; IgA: 109; IgM: 21	WBC: 23,200; RBC: NA; PLT: 299,000; NEU: 17; LYM: 74; BC: absent; IgG: 930; IgA: 198; IgM: 50
PML treatments (ineffective)	Cidofovir Mirtazapine Risperidone	Mirtazapine	None

PML Progressive multifocal leukoencephalopathy; *NA* not available; *WBC* white blood count (normal values (n.v.): 5,000–10,000/mm³); *RBC* red blood count (n.v.: 4.4–5.6×10⁶/mm³); *PLT* platelets (n.v.: 150,000–400,000/mm³); *NEU* neutrophils (n.v.:40–75%); *LYM* lymphocytes (n.v.: 20–45); *BC* blast cells (n.v.: 0%); *IgG* G immunoglobulins (n.v.: 800–1,800 mg/dL); *IgA* A immunoglobulins (n.v.: 90–400 mg/dL); *IgM* M immunoglobulins (n.v.: 60–280 mg/dL); *R-hyperCVAD* rituximab 375 mg/m² intravenously (i.v.), cyclophosphamide 300 mg/m² i.v., doxorubicin 50 mg/m² i.v., vincristine 2 mg i.v., dexamethasone 40 mg i.v. or orally, G-CSF 10 µg/kg, and methotrexate 200 mg/m² i.v.; *R-DHAP* rituximab 375 mg/m² i.v. on day 1, cisplatin 100 mg/m² i.v. on day 2, cytosine arabinoside 2 g/m² twice daily i.v. on day 3, and dexamethasone 40 mg i.v. on days 2 through 5; *R-CVP* rituximab 375 mg/m² i.v., cyclophosphamide 400 mg/m² i.v. on days 1 through 5, vincristine 1.4 mg/m² i.v. on day 1, and prednisone 100 mg/m² orally days 1 through 5; *BEAM* carmustine 300 mg/m² i.v. on day –6, etoposide 200 mg/m² i.v., followed by cytarabine 200 mg/m² i.v. twice daily, on days –5 through –2, and melphalan 140 mg/m² i.v. on day –1; *COMP* cyclophosphamide 750 mg/m² i.v. on day 1, doxorubicin 50 mg/m², vincristine 2 mg, and prednisone 100 mg orally on days 1 through 5; *R-CHOP* cyclophosphamide 750 mg/m² i.v. on day 1, doxorubicin 50 mg/m² i.v. on day 1, vincristine 1.4 mg/m² on day 1, and prednisone 100 mg orally on day 1 through 5; *P-VMYBEC* prednisone 50 mg orally, liposomal doxorubicin 30 mg/m² i.v. bolus, etoposide 100 mg/m² i.v. on days 1, 15, 29, and 43, cyclophosphamide 350 mg/m² i.v. on days 1,14, 28, and 42, vincristine 2 mg i.v. bolus, and bleomycin 5 mg/m² i.v. bolus on days 7, 21, and 35

demyelinating lesions. The patient died from respiratory failure on September 2008.

Case 2 A 68-year-old male patient received nine courses of intravenous cladribine for splenic marginal zone lymphoma (May–December 2002), achieving complete remission. Because of relapsing disease, he received six courses of R-CVP (November 2006–April 2007). Since a partial response was achieved, he was treated with ten courses of maintenance rituximab (375 mg/m²; June–October 2007). On November 2007, disease progression prompted a treatment with intravenous bendamustine 90 mg/m² (five courses). On December 2007, the patient complained of dysmnnesia and visual disturbances, and MRI revealed demyelinating lesions suggestive of PML (Fig. 1a–d). Due to lymphoma progression, the patient started lenalidomide 25 mg/day (May–June 2008). Histomorphology and a

positive JCV PCR on a stereotactic brain biopsy confirmed PML (June 2008). Treatment with mirtazapine 30 mg/day was then started. On September 2008, a follow-up MRI showed new areas of demyelination, despite a moderate improvement in the original demyelinated area (Fig. 1e–f). The patient then underwent four courses of COMP chemotherapy to treat lymphoma progression. On December 2008, a new MRI evaluation showed further worsening of the disease (Fig. 1g–h). The patient finally died from leukostasis in March 2009.

Case 3 A 66-year-old male patient with leukemic mantle cell lymphoma received two doses of rituximab (375 mg/m²) within CHOP regimen (January–February 2004). Due to occurrence of infusion reactions (fever, chills, and lumbar pain), the last two doses were not administered. As only a partial response was achieved, the patient received eight

Fig. 1 MRI in case 2. The first MRI examination (December 2007) revealed a left frontal homogeneous hyperintense lesion of the centrum semiovalis on axial fluid-attenuated inversion recovery (FLAIR) images (a) corresponding to an hypointense (c) nonenhancing (d) area on T1-weighted images. Diffusion-weighted imaging image (b) of the same lesion depicted a central core of hypointensity surrounded by a rim of high signal intensity suggesting a demyelinating lesion

probably due to PML. Nine months later, the left frontal lesion showed a slight size reduction with atrophic feature of involved cerebral gyri (e). Nevertheless, similar white matter lesions involving also the subcortical “U” fibers with “scalloped” appearance developed in the left temporal and parietal lobes (f). White matter lesions progressively became multiple and confluent, affecting asymmetrically both cerebral hemispheres as shown in axial FLAIR images obtained 12 months later (g–h)

courses of P-VMYBEC (September–October 2004). On December 2004, the patient developed paraplegia. Brain MRI and computed tomography showed multiple areas of subcortical demyelination. Despite JCV DNA testing negative in cerebrospinal fluid, due to the low sensitivity of this assay in HIV-negative patients [2], he was finally diagnosed as probable PML, according to consensus terminology [3]. Unfortunately, the patient died in December 2004 before a stereotactic brain biopsy could be performed, and no necropsy was carried out.

PML occurs uncommonly in HIV-negative patients with lymphoproliferative disorders, and it has been rarely reported during rituximab therapy [4–6]. The causal involvement of antineoplastic therapies (i.e., purine analogs) in PML is not conclusive [1], and the possible contribution of drugs other than rituximab to PML occurrence in our cases cannot be ruled out. However, case 1 developed PML while in complete remission and free from drug treatments (other than rituximab) since 18 months, making causality highly suspected.

Although immunodeficiency is recognized as a necessary condition for PML development, the mechanism by which JCV crosses the blood–brain barrier (BBB) remains uncertain. Serotonin receptor 2A (5-HT_{2A}R) seems to be involved in JCV internalization into glial cells and some serotonin receptor antagonists (i.e., risperidone, mirtazapine) have been proposed as agents potentially blocking JCV entry [7, 8]. Two of the present cases received mirtazapine and/or risperidone as a tentative salvage therapy, but these drugs did not provide appreciable benefits. Of note, case 2 showed a delay in the progression of the primary lesion, but new focal lesions developed concomitantly. Consistently with recent investigations [9, 10], the unfavorable outcome in our patients suggests that JCV might use alternative gates, besides 5-HT_{2A}R, to cross the BBB.

In conclusion, the present cases support the hypothesis that exposure to rituximab may be an independent risk factor for the development of PML. Moreover, 5-HT_{2A}R antagonists were ineffective in two of our cases, thus pointing out the need for more suitable therapeutic options.

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