Abstract. Background: Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous system which affects the white matter and is caused by reactivation of the JC polyomavirus. Case Report: We report the case of a 63-year-old man with chronic lymphocytic leukemia who was treated with fludarabine; rituximab and fludarabine; fludarabine, cyclophosphamide and rituximab; and lenalidomide. While he underwent chemotherapy, the patient was diagnosed with PML. After stabilization of PML, the patient underwent non-myeloablative allogeneic bone marrow transplantation as a treatment for chronic lymphocytic leukemia. Unfortunately, after several opportunistic infections, the patient died. Discussion: The patient underwent allogeneic bone marrow transplantation with the expectation that donor-derived competent immunological cells would migrate into the cerebral lesions, maintaining immunological response. The effect of bone marrow transplantation in patients with PML requires investigation in larger patient series.

Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous system (CNS) which affects the white matter and is characterized by lytic infection of oligodendrocytes and astrocytes (1, 2). PML is caused by re-activation of the JC polyomavirus (JCV). JCV has been described almost exclusively in immunosuppressed individuals, with the first description made more than 50 years ago in patients with chronic lymphocytic leukemia (CLL) and Hodgkin’s disease (3, 4). Today, JCV represents a major opportunistic infection in HIV-infected patients and PML is most frequently associated with AIDS (5). Apart from HIV, with a much lower but increasingly dreadful incidence, PML is associated with hematological malignancies, solid organ transplantation and chronic inflammatory diseases (6). A high mortality rate is associated with PML and no specific treatment has been established to date (4). There is evidence that a variety of targeted-therapeutics, such as the anti-CD20 monoclonal antibody rituximab can increase the risk of PML by reactivation of dormant JCV (1).

Pathological findings in PML. PML lesions mostly occur in the subcortical white matter with both hemispheres being affected, the parieto-occipital region being the most common location (5). Usually, the de-myelinated areas are diffusely spread throughout the subcortical white matter (7). Lesions also occur in the cerebellum, brain stem, thalamus and in the spinal cord (8, 9). Smaller foci at the junction of cortex and white matter are frequently present, and more widespread lesions in the white matter that are located rather centrally can occur (10). Radiologically, hyperintense signal abnormalities of the white matter on T2-weighted magnetic resonance imaging (MRI) are typical for PML (6).

The histological analysis of PML reveals rather large areas of de-myelination that are often surrounded by smaller foci (7). The lesions contain lipid-laden macrophages and perivascular lymphocytes. Reactive astrocytes and abnormally huge glial cells with large, pleomorphic nuclei that contain basophilic granules are frequently found (11). In the center of the lesions, areas of necrosis occur (12). Oligodendrocytes with enlarged nuclei, containing viral inclusion bodies, can be visualized by electronic microscopy (5, 10). The viral particles measure approximately 30 to 45 nm in diameter and appear in two forms: filamentous and spheric (6). Papovavirus antigens may be detectable in frozen sections by immunohistochemical staining with an antibody directed
against the JCV (6, 10). Oligodendrocytes that react to
demyelination can be stained with carbonic anhydrase
isoenzyme II and cyclic nucleotide phosphodiesterase (10).

Although the JCV normally affects the white matter of the
CNS, a case has been reported in which the virus-affected
granule cells of the cerebellar inner granule cell layer.
Interestingly, the Purkinje cells were not affected and there
were no classic manifestations of PML in the white matter.
The authors named this new manifestation of JC virus
infection ‘JCV granule cell neuropathy’ (8, 13). Further
investigations of cerebellar involvement of JCV infection
revealed that over 90% of the investigated patients with PML
with demyelinated areas in the white matter also had JCV-
infected cells in the cerebellar granule cell layer.

**JCV infection.** JCV can occur via the respiratory or oral
route. Asymptomatic primary infection with JCV occurs in
childhood and antibodies can be found in more than 80% of
healthy adults (6). After primary infection, the virus remains
latent in tubular epithelial cells of the kidneys and lymphoid
organs, but in the context of profound cellular immune
deficiency, it may reactivate and spread to the brain where it
infests and destroys oligodendrocytes, leading to multifocal
areas of demyelination and associated neurological
dysfunction (6). The virus practically never results in disease
in healthy individuals (14).

The disease usually manifests with subacute neurological
deficits such as motor weakness (hemiparesis or
monoparesis), altered mental status, appendicular ataxia (i.e.,
disturbance in carrying out voluntary, planned movements of
the extremities) and visual symptoms (hemianopsia,
diplopia). White matter lesions that undercut relevant cortical
areas may sometimes lead to symptoms indicative of a
cortical disorder (for example, aphasia) (6). Furthermore,
seizures, which are a manifestation of gray matter cortical
dysfunction, can be present in up to 18% of patients with
PML as described by Lima and colleagues (6).

Stereotactic brain biopsy represents the gold standard for
the diagnosis of PML, with a reported sensitivity in patients
with AIDS of 64 to 96% and a specificity of 100%.

Here we report a patient with chronic lymphocytic
leukemia (CLL), who suffered from progressive PML despite
a variety of experimental drug and immunomodulatory
interventions.

**Case Report**

**Case history.** A male patient, aged 50 years at presentation,
was noted to have lymphocytosis on a routine complete blood
cell count in 1996 and thus diagnosis of CLL was made. By
1999, he required treatment and received fludarabine for five
treatment cycles, followed by fludarabine-rituximab for three
treatment cycles. His complete blood cell count normalized
following therapy. However, by December 2001, he required
treatment again because of a rising white cell count,
splenomegaly and lymphadenopathy. At that time, he received
fludarabine, cyclophosphamide and rituximab (FCR) for four
treatment cycles, complicated by autoimmune hemolytic
anemia. His CLL responded to therapy but by January 2006,
he required therapy again and was treated with oral
fludarabine, cyclophosphamide and rituximab for three
treatment cycles without response and then with intravenous
FCR for three treatment cycles, ending in July 2006 and
resulting in a nodular partial response. In 2007, CLL
progressed and he received a two-week course of lenalidomide
that was interrupted when he developed weakness of the right
upper extremity evolving to paralysis, attributed to PML based
on clinical and MRI findings; cerebrospinal fluid viral studies
were initially non-diagnostic. Later in 2007, JCV was detected
in the cerebrospinal fluid, assuring the diagnosis of PML. The
MRI showed demyelinated foci (Figures 1 and 2). In
September 2007, he received a five-day course of cytarabine
for the treatment of PML. In October-November 2007, he
developed refractory focal motor seizures and was treated with
high-dose cytarabine, valproic acid, levetiracetam and
clonazepam. In December 2007, he received investigational
natural killer cell therapy. In February 2008, he was treated
with infusions of CD3/CD28 ex vivo carried out-stimulated
autologous T-cells on a compassionate use protocol at the
University of Pennsylvania in an attempt to reverse his
therapy-related immunodeficiency and to enhance antiviral
immunity. He also received intravenous immunoglobulin. In
May and June 2008, he received investigational JCV peptide-
pulsed dendritic cell vaccinations with granulocyte–monocyte
colonies stimulating factor (GM-CSF). Although neurologically
stable at this point, he developed severe anemia with brisk
hemolysis and symptomatic massive splenomegaly and
underwent splenectomy in July 2008. Other treatment
modalities for PML include mirtazapine and cyproheptadine,
5-hydroxytryptamine 2A (5HT2A) serotonin blockers. There
is evidence suggesting that treatment with 5HT2A blockers
might slow down progression of PML, since the 5HT2A
receptor is a receptor for JCV permitting the infection of glial
cells (15, 16).

In February 2009, the patient underwent human leukocyte
antigen-matched sibling allogeneic stem cell transplantation
following a nonmyeloablative preparative regimen that
included fludarabine, melphalan and total body irradiation
(200 cGy). The donor of bone marrow was the patient’s sister.
Shortly after transplantation, the patient suffered from
intercurrent sepsis caused by *Pseudomonas sp.*, then
contracted viral pneumonia and, subsequently, gastrointestinal
Clostridial infection.

About three months post-transplantation, following
reduction of immunosuppression and a donor lymphocyte
infusion, the patient developed gastrointestinal involvement
by graft-versus-host disease, confirmed by biopsy of large intestine. Graft-versus-host disease later became manifest in the patient’s skin.

About five months post-transplantation, after an episode of enterococcal cystitis treated with Augmentin®, JCV disease progressed rapidly. This decline happened two years after the first diagnosis of JCV infection. The patient developed tetraparesis, rapidly lost vigilance, and died from respiratory and septic complications due to aspiration pneumonia.

Macroscopic analysis. In the macroscopic autopsy analysis of the brain, no pathological changes were evident at first sight. The brain weighed 1,230 g, and had a smooth surface with normal meninges. The hemispheres were symmetric. Frontotemporally, 3×3×3 cm areas of necrosis were noted bilaterally. In the right thalamus, a macerated area of 0.6 cm that was clearly circumscribed and in the basal ganglia several focal necroses (about 0.4 cm each) were noted. The medulla oblongata was rather pale, and also featured signs of necrosis.

Histology. In the frontal cortex, the cytoarchitecture was widely normal, but within the white matter, focal gliosis encircling demyelinated areas was found. Demyelination was confirmed also by luxol fast blue–periodic acid Schiff (LFB-PAS) staining, which is a routine procedure to assess demyelination (17). In the demyelinated foci, many macrophages were seen. Oligodendroglial JCV enclosure was visible and immunohistochemical staining with JCV antibodies displayed positivity. In the parietal lobes, the areas of demyelination were predominantly found near the cortical surface. CD68-positive macrophages loaded with virus

Figure 1. In the magnetic resonance imaging (MRI) scan, typical progressive multifocal leukoencephalopathy-associated lesions are seen in the precentral gyrus (right and left hemisphere) and in the postcentral gyrus (left hemisphere).
particles, commonly seen in PML, were detected in this patient (18). Few lymphocytes and plasma cells were found. Most of the immune cells were CD68-positive macrophages and siderin-laden macrophages, which were found in the lesions and in perivascular areas. Most of the lymphocytes were CD3-positive, and only a few displayed positivity for CD20. In the occipital lobe, prominent perivascular inflammation and small areas of demyelination were observed. There were numerous viral inclusions in oligodendrocytes that were also immunohistochemically positive for the JCV antibody. The basal ganglia had been extensively destroyed by necrosis. Most of the necrotic areas were inactive and circumscribed by gliosis. Figure 3 shows tissue stained with hematoxylin and eosin, as well as with glial fibrillary acidic protein (Figure 3).

Some areas displayed distinct signs of active inflammation, especially in perivascular regions, where infiltration by CD68-positive macrophages and CD3-positive lymphocytes was evident. In the periphery of the demyelinated areas, there were many eosinophilic oligodendrogial inclusions which stained positively with the JCV antibody. There was diffuse lymphocytic infiltration of the white matter in the thalamus that was positive on LFB-PAS staining. In the pons, there were abundant JCV-positive inclusions, confirmed by immunohistochemistry. Furthermore, the cerebellar tissue displayed signs of demyelination in the white matter, distinct gliosis and JCV inclusions.

In summary, the autopsy findings of the brain demonstrated active JCV infection, with distinct demyelination corresponding to classic PML. It is noteworthy in this case that the cortical affection was located mainly in the frontoparietal cortex.

Since the patient underwent allogeneic bone marrow transplantation with his sister’s bone marrow, we assumed that the inflammatory cells in and around the cerebral PML lesions were derived from the transplanted bone marrow. To test this assumption, we tried to show that the inflammatory cells found in the histological slices post-mortem were female, using fluorescence in-situ hybridization (FISH). Unfortunately, it was not possible to verify the cells’ female origin.

Characterization of the JCV antibody. We used antibody to JCV from Calbiochem (m-a-Polyomavirus JC, monoclonal, clone PAb416; Oncogene Research Products Boston, Massachusetts, US), diluted 1:150 and pretreated in the microwave with Tris Urea 9.5 for 40 minutes.

Review of Existing Data

Obviously patients undergoing immunomodulatory therapy are at a higher risk for JCV infection. Even if an individual is not severely immune-suppressed, therapy with biological agents may facilitate reactivation of JCV infection and consequently PML. The association between biological drugs and JCV-triggered disease has been found in patients treated with the rituximab antibody to CD20, natalizumab antibody to α4 integrin, and efalizumab antibody to lymphocyte function-associated antigen-1 (LFA-1) (19). A case of an HIV-negative individual with non-Hodgkin’s lymphoma who developed PML after rituximab therapy has also been reported (20).

Natalizumab therapy is a risk factor for JCV infection. Natalizumab is a humanized monoclonal antibody targeting the α4 subunit of α4β1 and α4β7 integrins, which are involved in the migration of T-cells into the CNS, interacting with ligands in the extracellular matrix (21). Natalizumab is used in therapy of multiple sclerosis. It is suggested that patients suffering from multiple sclerosis undergo a JCV antibody test to estimate their risk for PML manifestation upon treatment with natalizumab. Patients who are found to be JCV antibody-negative carry a very small risk for developing PML (21). However, patients with JCV antibody positivity have an increased risk for PML upon natalizumab treatment (22). Prior immunosuppressive therapy is also a risk factor for PML manifestation when patients are treated with natalizumab (21, 23, 24). When the JCV antibody is measured in the patients’ serum, the specific titer can be determined. Recent data suggest that JCV antibody-positive patients should be further stratified into "high positive" and "low positive", because "low positive" patients are clearly at a substantially lower natalizumab-associated PML risk compared to "high positive" individuals (25). Several cases of patients developing PML when treated with natalizumab have been reported. For example, in a case of a patient with multiple sclerosis who had been treated with natalizumab, plasma exchange was performed to accelerate the clearance of natalizumab. The patient had also been treated with corticosteroids (26). Interestingly, some weeks after plasma exchange, JCV DNA was no longer detectable and the patient’s symptoms improved (27). This finding indicates that prompt diagnosis of JCV infection and, in the case of monoclonal antibody therapy, plasma exchange, can stop progression of the disease in some cases (26).

Yet it has not been totally resolved why treatment with biological agents can lead to JCV infection. However, in the case of efalizumab, this effect could be due to LFA-1 inhibition, preventing extravasation of T-cells. T-cells can act against JCV and may even have the potential to inhibit the clinical manifestation of PML. If T-cell extravasation cannot take place, infection by and replication of the JCV is probably facilitated (1).

There is evidence that the use of the purine analog fludarabine increases the risk of developing PML (11). Fludarabine is used as a chemotherapeutic agent for the treatment of hematological malignancies. Several cases of PML after fludarabine treatment have been reported (28). The toxic side-effects of fludarabine include myelosuppression,
immunosuppression and sporadic life-threatening neurotoxicity (29). Very high doses of fludarabine (90-120 mg/m²) increase the risk for PML, whereas standard doses (18-25 mg/m²) are considered safer (28). D’Souza and colleagues have reported three cases of patients with CLL that developed PML. All three patients had received fludarabine and rituximab as chemotherapy (30). In a similar case of a CLL patient that was diagnosed with PML after fludarabine therapy, the neurological deterioration was especially fast (29). Kiewe et al. also reported a case of a patient with CLL who developed PML during fludarabine therapy. This patient received virostatic treatment with cidofovir, but neurological symptoms were progressive and the patient died. The authors suggested that PML might be triggered not only by fludarabine treatment but also by the immunosuppression caused by the lymphoproliferative malignancy itself (31).

According to two case reports of CLL patients that had been treated with fludarabine, white matter lesions occurred in absence of JCV infection (32, 33). The patients developed encephalopathy with distinct neurological symptoms, such as altered sensorium and hemiplegia. The MRI showed multiple white matter lesions mimicking PML, although neither of the two patients was infected with JCV (32, 33). A similar case has been reported of a CLL patient who developed rapidly progressive neurological symptoms with normal cerebrospinal fluid. The authors attributed the neurological symptoms to direct brain infiltration by lymphoma cells, although no biopsy was performed (34).

Farge et al. have described a case of a non-HIV patient who suffered from CLL with PML and concomitant cerebral Epstein-Barr virus (EBV) infection. The brain parenchyma was diffusely infiltrated by leukemia cells in the presence of both JCV and EBV. The authors suggested EBV-transformed B-lymphocytes as possibly favoring JCV penetration and the activation of a previously latent JCV infection (35).

According to another case report, an HIV-negative individual presented with CNS symptoms and PML was diagnosed by brain biopsy and by polymerase chain reaction testing of the cerebrospinal fluid for JCV. The patient had never received immunosuppressive therapy. Sarcoïdosis with pulmonary, cardiac and lymph node involvement was discovered at autopsy. This suggests a possible relationship between sarcoïdosis and JCV infection (36).

In a case presented by Balduzzi and colleagues, a 19-year-old patient was diagnosed with PML, after having received allogeneic hematopoietic stem cell transplantation (37). The patient had undergone prolonged immunosuppression for the treatment of severe graft-versus-host disease. PML occurred, most probably, due to immunosuppression. After anti-viral treatment did not lead to an improvement of symptoms, donor-derived JCV antigen-specific T-lymphocytes were generated in vitro after stimulation with 15-mer peptides derived from the polyomavirus capsid protein (VP1) and large T-viral proteins. After the infusion of these T-cells, JCV DNA was cleared in the cerebrospinal fluid and the patient’s symptoms evidently improved. This case suggests that adoptive infusion of JCV-targeted T-lymphocytes may restore the JCV-specific immune competence and improve the outcome (37).

Discussion

Certain diseases and medical therapies impairing cellular immunity can cause JCV reactivation. In a series of 89 patients from Beth Israel Deaconess Medical Center with proven or possible PML, diagnosed between 1995 and 2005, 71% had AIDS, 15.7% hematological malignancies, 5.6% were recipients of bone marrow or solid organ transplantation, 3.4% had prolonged corticosteroid use, 1.1% solid organ malignancy, 1.1% granulomatous disease, 1.1% hepatitis C and 1.1% isolated CD8 T-cell lymphopenia (6). Furthermore, PML was described in three patients (two with multiple sclerosis and one with Crohn’s disease) treated with natalizumab, as well as LFA-1 antibody efalizumab (6, 19). In our case report, it is likely that both the CLL and the immunosuppression caused by the chemotherapy caused PML.

Our patient was also treated with the experimental drug CMX001 (1-O-hexadecyloxypropyl-cidofovir), a drug that inhibits JCV replication in human brain progenitor-derived astrocytes in vitro (38). This treatment modality was meant to improve cellular immunity against JCV during the allogeneic bone marrow transplantation. It is difficult to draw a conclusion from this experimental treatment, since we only used it in a single patient. We consider CMX001 treatment as a reasonable therapeutic option in patients with PML; however, this needs to be investigated in larger patient series.

Cells of the immunological response continue to react against JCV after the onset of PML, even in immune-incompetent patients. Especially in AIDS-related PML,
distinct lymphocytic infiltration of the lesions is common and there is evidence that lymphocytic infiltration is associated with a slightly better prognosis (12). According to previous studies, CD8 positive T-cells have been found in brain biopsy material at perivascular sites and also within and at the border of PML lesions. The number of infected glial cells correlates positively with the number of infiltrating CD8-positive T-cells. Therefore, it has been proposed that activated immune cells have the ability to penetrate the brain and destroy virus-infected glial cells, eventually leading to a

Figure 3. The frontal cortex (A: ×10, B: ×20) and the parietal cortex (C: ×10, D: ×20) feature typical signs of progressive multifocal leukoencephalopathy on hematoxylin and eosin staining. E and F show glial fibrillary acidic protein staining (E: ×10, F: ×20).
healing of PML (39). Our patient underwent bone marrow transplantation, and we assume that this procedure slowed the progression of his disease. We expected that the allogeneic bone marrow transplantation would lead to the migration of competent immunological cells into the cerebral lesions, and improve the patient’s immunological response. Accordingly, we hypothesized that the immune cells that were found in the brain sections would be female, since the bone marrow donor was the patient’s sister. Using FISH, we tried to classify the immune cells within the brain sections as either male or female. Unfortunately, we were not able to determine the cells’ gender in the PML lesions. Nevertheless, bone marrow transplantation could be a potential immunotherapeutic approach for PML, circumventing the period of post-transplant immunosuppression.

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