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Inflammatory Infratentorial Progressive Multifocal Leukoencephalopathy in a Patient with Rheumatoid Arthritis

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Abstract

84–year-old man with rheumatoid arthritis (RA) treated with methotrexate, developed progressive confusion and cerebellar symptoms, and died approximately two months later. Neuropathological examination revealed progressive multifocal leukoencephalopathy (PML) involving the cerebellum and brainstem. The affected tissues displayed intense infiltrations by CD8+ T-cells and microglia. JC virus was localized in oligodendroglia and cerebellar granule cells. This case illustrates unusual localization of inflammatory PML in a patient with RA treated with methotrexate.

Keywords

Progressive Multifocal Leukoencephalopathy; Demyelination; Cerebellar syndrome; Methotrexate; Rheumatoid Arthritis

Introduction

Progressive multifocal leukoencephalopathy (PML) is a demyelinating, usually non-inflammatory disorder of the central nervous system caused by reactivation of a latent JC virus (JCV), in the setting of immunosuppression[1–4]. The most frequent underlying conditions are HIV/AIDS, myelo- and lymphoproliferative disorders, autoimmune and chronic granulomatous diseases, as well as the use of immunomodulatory medications[1–4]. Among autoimmune disorders, the most common is systemic lupus erythematosus[5–7]. PML as a complication of rheumatoid arthritis (RA) treated with immunosuppressive medication is rare[8–19]. We present a patient with rheumatoid arthritis treated with methotrexate who

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developed an uncommon form of inflammatory PML limited to the infratentorial compartment.

Clinical History

An 84-year-old man with approximately one year of subtle symptoms of cognitive decline was admitted to the hospital with worsening confusion, hallucinations and progressive incoordination with frequent falls. He had been taking methotrexate (20mg/week) for rheumatoid arthritis for one year, and continued until his demise. The patient had a past history of myocardial infarction, spontaneous deep vein thrombosis and pulmonary embolus. Examination revealed an afebrile, alert, cachectic man oriented to time and person but not to place. The patient displayed moderate paratonia, mild reduction of vibration sense in big toes, drifting of the left arm up and down when eyes were closed, dysdiadochokinesis and striking bilateral dysmetria in the arms and legs, left worse than right. He had an ataxic gait with marked truncal instability and inconsistent stimulus-sensitive myoclonus.

Laboratory investigations were negative for ANNA-1, ANNA-2 and Purkinje cell antibodies, as well as for Lyme disease and HIV. Levels of serum gamma globulins were normal. Cerebrospinal fluid (CSF) glucose, WBC and protein level were within normal limits. The CSF was negative for JC and BK viruses but was positive for 14-3-3 protein, raising the suspicion of Creutzfeldt-Jacob disease (CJD). Brain magnetic resonance imaging (MRI) revealed non-enhancing white matter hyperintensities in the left cerebellar hemisphere. A repeat MRI scan twelve days later revealed “progressive vasogenic edema” suggestive of an acute progressive demyelinating disease. A CT of the chest, abdomen, and pelvis was noncontributory. Due to his advanced age and the possibility of CJD, no further aggressive diagnostic procedure or treatment was undertaken. He continued to deteriorate and died at home two months after presentation.

Methods

Standard set of neuropathology sections from all brain areas as well as samples of grossly described abnormalities were removed for microscopic examination. The sections were processed to paraffin embedding and stained with hematoxylin and eosin, and in luxol fast blue with PAS methods. Selected sections were routinely immunostained for the following tissue antigens with commercially available primary antibodies (all from DAKO, Carpenteria, CA, USA): glial fibrillary acidic protein (GFAP, polyclonal, 1:3000 dilution), ferritin (polyclonal 1:500), P53 (clone DO-7, 1:50) and neurofilament (NF, monoclonal, 1:4000, clone 2F11). Monoclonal antibodies against SV-40 T antigen (Calbiochem, 1:400) were used for initial detection of the virus. For the identification of inflammatory cells, monoclonal antibodies against CD3, CD4, CD8, CD45 and CD68 (Novocastra, Newcastle-upon-Tyne, 1:50) were also applied. The Streptovidin/biotin detection system (Invitrogen, “Histostatin Plus”) was used for visualization of the immune reactions and followed by a light hematoxylin counter stain. Immunohistochemistry was performed using LabVision autostainer. Cellular localization of the virus was performed by double immunostains combining antibodies against JCV protein Agno 48–71[20], T Ag or VP1 (Santa Cruz Biotechnology, Santa Cruz, CA) with cellular markers: CNPase C5922, 11-5B (oligodendroglia and myelin, Sigma), MAP-2 (neurons), and GFAP(astrocytes), as previously reported[21].

Results

Due to the clinical suspicion of CJD, the autopsy was limited to the brain. The fresh brain weighed 1376 grams and was cut after two weeks of fixation (CJD was excluded after

preliminary examination of multiple brain samples). The cerebral hemispheres showed only mild ventricular dilatation. The cerebellum displayed minimal atrophy of the superior vermis and large geographic areas of poorly demarcated, greyish discoloration of the white matter, more in the left hemisphere.

Microscopic examination revealed extensive loss of myelin involving the white matter of both cerebellar hemispheres, slightly more on the left side (Fig 1). Demyelination was accompanied by a significant dropout of axons, numerous axonal retraction balls, accumulation of ferritin-positive microglia and CD68+ foamy macrophages, and a moderate to severe degree of astrocytosis. These changes were most expressed in the centers of the lesions and gradually blended with relatively normal white matter with numerous small satellite foci of early myelin loss. The periphery of the demyelinated areas displayed many oligodendroglial cells with enlarged nuclei filled by homogeneous, intensely purple intranuclear viral inclusions that were weakly immunoreactive for P53 and strongly positive for JCV antigens. Scattered vessels at the edge of the lesions were surrounded by mild CD8+ inflammatory infiltrations, with few CD3+ and CD4+ T-cells, and no CD20+ B-cells. The population of Purkinje cells and granule cells, as well as neurons in the dentate nucleus appeared normal. Cerebellar cortex contained scattered axonal torpedoes of Purkinje cells. The overall pathological changes were consisted with chronic PML lesions.

The brainstem showed multiple small patches of demyelination with centrifugal distribution of oligodendroglial intranuclear inclusions (Fig 2A and B) and numerous foci of perivascular infiltrations by CD8+ T-cells, and less abundant CD3+ and CD4+ T-cells (Fig 3A and 3B). CD20+ B-cells were entirely absent. The perivascular myelin was not affected. Clusters of normal-appearing neurons outside of areas of demyelination were surrounded by CD8+ T-cells and microglia (Fig 4A and 4B). In addition, the parenchyma of the pons was sprinkled with small collections or individual CD8+ cells without relation to the vessels or neurons. Very careful screening of sections of the brainstem revealed no direct contact of CD8+ T-cells with the oligodendroglial cells containing intranuclear inclusions. CD68+ macrophages and ferritin-positive microglia were massively increased in foci of demyelination and, to a lesser extend, diffusely throughout the entire brainstem. Scattered, well-formed microglial nodules were present as well. Double immunostains of the sections removed from the pons and cerebellum demonstrated viral infections limited to the oligodendroglial cells and few cerebellar granule cells. Very thorough screening of multiple slides revealed only two microscopic foci of early demyelination present in the midbrain and in the deep white matter of the frontal lobe. The meninges showed mild lymphocytic infiltrates slightly more prominent at the base of the brain.

Discussion

The present case is remarkable for the association of PML with RA, intense inflammation in the progressing lesions in the brainstem, and selective involvement of subtentorial compartments. There have only been a few case reports of PML in patients with RA. Amend et al[22] did not find a single case of RA with PML in studies of 138,469 patients with autoimmune disease. However, in a review of 57 HIV-negative PML patients from the Mayo Clinic, Aksamit reported approximately 5% with RA, without details about the topography of lesions, pathology, or specific treatment[23]. Until 2008, only seven patients with PML associated with RA were described, all with the typical clinical and pathological presentation[8–14]. Subsequently, eight additional PML cases were found in the group of RA patients treated with humanized monoclonal antibodies, including five patients taking methotrexate[15–19]. All the RA patients developed typical cerebral lesions and only two (treated with Rituximab), displayed inflammatory changes with the presence of T- and B-cells[15,18].

Classical PML lesions in immunocompromised patients show minimal or no inflammation[1–3]. However, intense inflammation develops in PML cases with immune reconstitution inflammatory syndrome (IRIS), following initiation of HAART in the setting of HIV/AIDS, as well as in HIV-negative patients treated with monoclonal antibodies[24–26]. Clinically, focal inflammation has been reported in about 15% of PML cases using gadolinium-enhanced MRI[2,27]. Although PML is often defined as a non-inflammatory demyelinating disease, some studies suggest that the frequency of inflammation in non-AIDS patients is probably underestimated[28], and it appears to be more common in the individuals with minimal immunosuppression or without immunodeficiency. Several reports indicate that inflammatory PML is associated with better prognosis[14,28–31].

In the inflammatory form of PML, virus specific CD8+ T-cells concentrate in largest number at the borders of progressing demyelination, known to harbor the greatest load of the virus[30]. Furthermore, CD8+ T-cells can be localized in direct contact with the inclusion bearing oligodendroglia[30]. Although the inflammatory cells were concentrated at the progressive edge of the glial infection, direct contact of T-cells and oligodendroglia could not be demonstrated in this patient. This phenomenon could be explained by immune response mounted against the viral antigen released from disintegrated oligodendroglial cells, rather than against intact virus- bearing oligodendroglia. Double immunostains of cerebellum and brainstem for JCV-specific antigens and cell markers (MAP-2, oligodendroglial CNPase or GFAP), revealed viral presence only in the oligodendroglia. With the exception of a few granule cells, there was no sign of invasion of other neurons or astrocytes. Massive neuronal infection has been demonstrated in several entities associated with JCV, such as granule cell neuronopathy[21,32,33] and fulminant encephalopathy with productive infection of cortical pyramidal neurons[34]. The striking CD8 and microglial perineuronal infiltrates in the pons, may suggest greater sensitivity of the hosts immunological system to recognize early JCV neuronal invasion, than the ability of the immunohistochemical methods to detect the virus at the light microscopic level. PML tends to involve subcortical white matter, mostly in the frontal and parieto-occipital areas[1–3]. Predominantly infratentorial localization of PML in non-AIDS patients is approximately ten times less common than the cerebral form[35]. Since 1958, when PML was first described[36], close to 30 case reports of infratentorial PML have been listed in Medline, however, none in RA patients.

In view of the increasing array of new and powerful immunomodulators in the treatment of autoimmune diseases, this case highlights the importance of considering PML in the differential diagnosis for acute or subacute onset of cerebellar or brainstem symptoms in patients with RA on immunosuppressant therapy. Although the frequency of PML with methotrexate use is very low, given the almost uniformly fatal consequences of this infection, patients should be warned of the risk of this complication.

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Abbreviations used

CJD	Creutzfeld-Jacob Disease
JCV	JC Virus

GFAP	Glial Fibrillary Acidic Protein
MAP-2	Microtubule Associated Protein
IRIS	Immune Reconstitution Inflammatory Syndrome
HAART	Highly Active Antiretroviral Therapy

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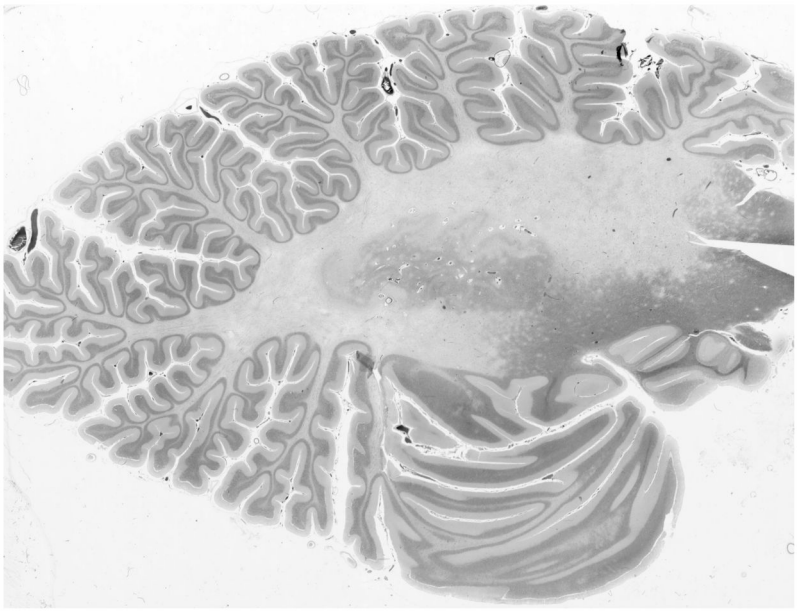


Fig. 1.
Section of cerebellar hemisphere displaying marked demyelination. Luxol Fast Blue combined with PAS.

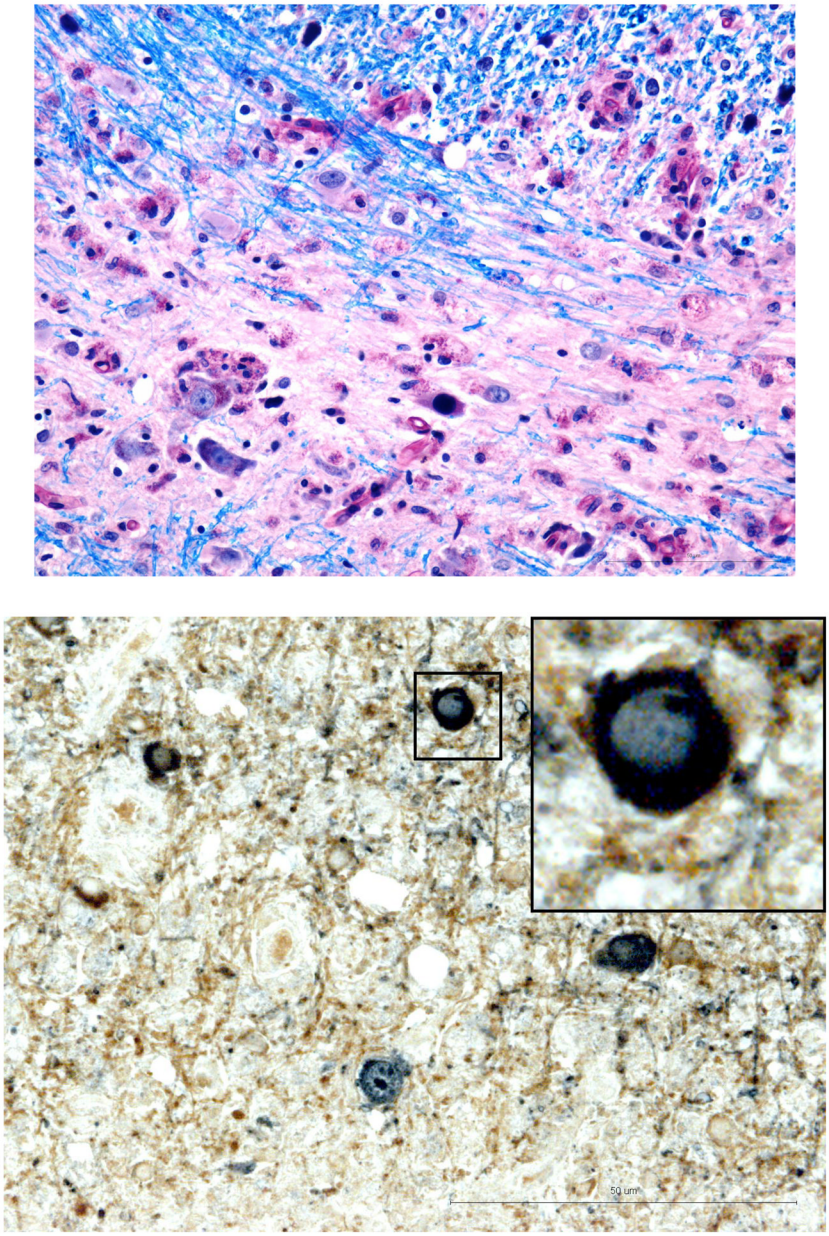
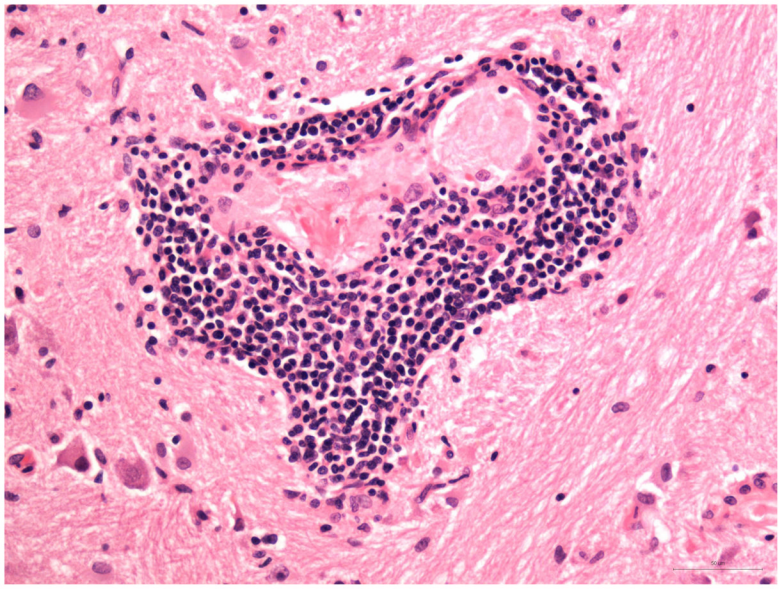


Fig. 2. Pons

A) Intranuclear inclusion in oligodendroglial cells in a small focus of demyelination. Luxol Fast Blue combined with PAS. Additional inclusion at the edge of demyelination in upper part of the photograph. Bar 50 μm.

B) Double immunostaining for CNPase (Brown) and JCV Agno protein (blue). JCV antigen co-localizes with reaction for CNPase indicates infection of oligodendrocytes. Bar 50μ. Window – details of JCV infected oligodendroglial cell.



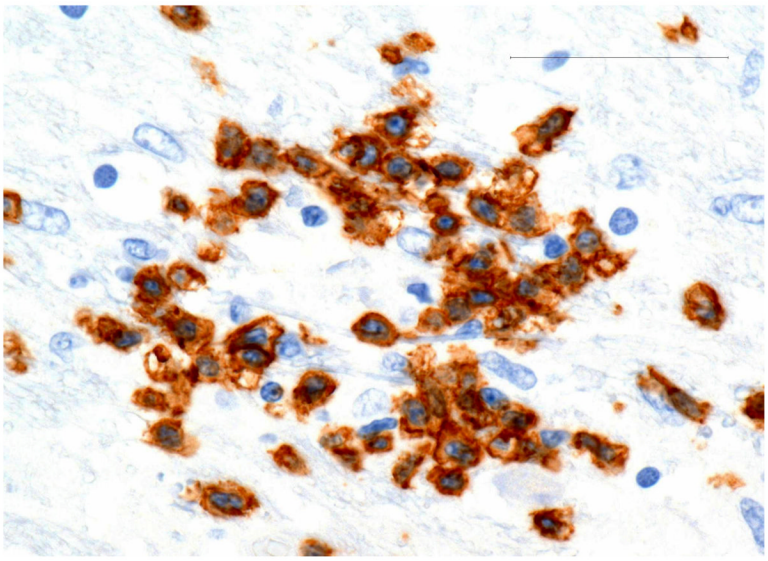


Fig. 3. Pons

A) Perivascular mononuclear inflammatory infiltrate (H&E).

B) Capillary surrounded by CD8+ T-cells. Bar 50μm

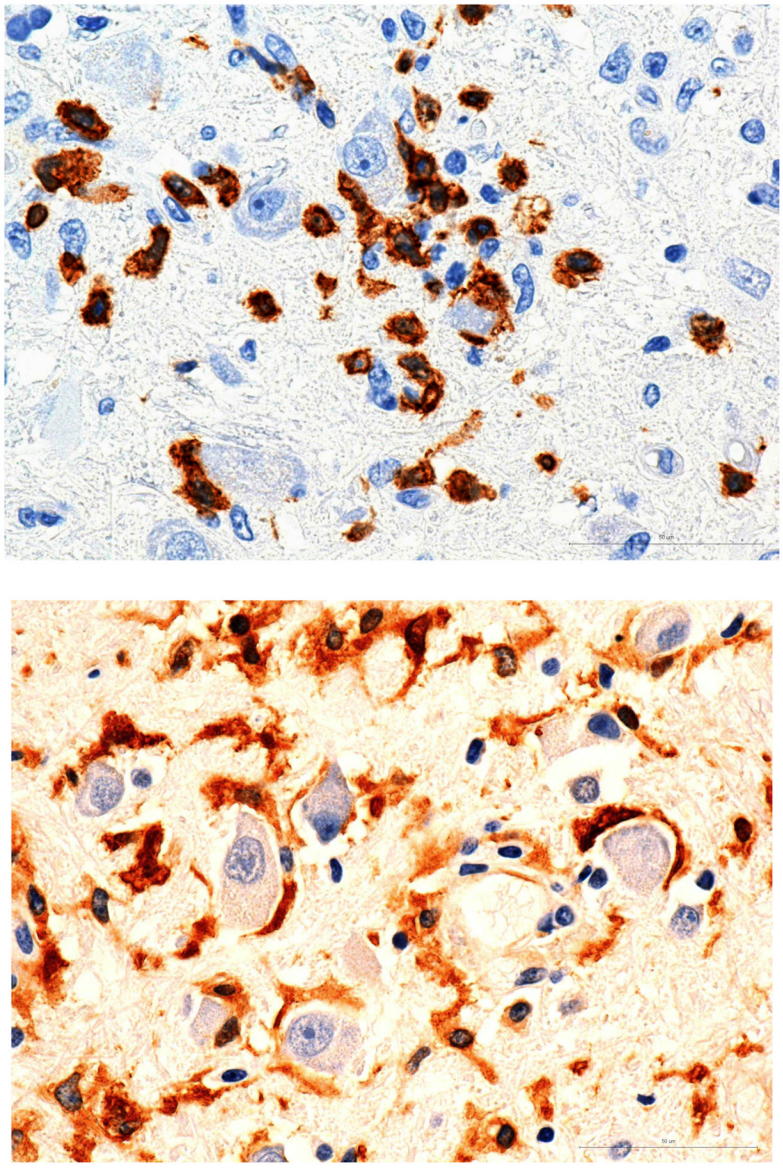


Fig. 4. Pons

A) Group of normal appearing neurons surrounded by CD8+ T-cells. Please note absence of intranuclear inclusions in any cell type in this microscopic field.

B) Morphologically normal neurons surrounded by ferritin positive microglia.