PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY

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ABSTRACT

Progressive multifocal leukoencephalopathy is a demyelinating disease of the central nervous system caused by the reactivation of latent JC virus. Since its initial description, there have been changes in its epidemiology, clinical presentation, evolution and prognosis. Some decades ago, it was considered a rare disease, but now it is a major complication of AIDS and represents an obstacle to the use of promising treatments for autoimmune disorders. Although the survival rate has increased in the last years, still no specific treatment for JC virus is available and further research is needed for a more effective management of the disease.
Key words: progressive multifocal leukoencephalopathy, JC virus, immunodeficiency.

HISTORY

In 1958, Aström et al. (1) described progressive multifocal leukoencephalopathy (PML) as a disease based on the clinical and histopathological features observed in two patients with chronic lymphocytic leukemia and one patient with Hodgkin's disease. They presented multiple demyelinating lesions of the central nervous system (CNS) and a rapid, fatal progression. In 1961, Richardson (2) reviewing other cases, found that PML was associated with malignant lymphoma or leukemia, sarcoidosis, carcinomatosis, military tuberculosis and advanced age, the patients having in common a relative immunological unresponsiveness. The detection of inclusion bodies in the nuclei of damaged oligodendrocytes indicated a possible viral cause of the disease (3), this hypothesis being confirmed in 1965 when the presence of small virus-like particles with resemblance of papovavirions in PML lesions through electron microscopy were described (4, 5). The virus was isolated in 1971 from the brain of a patient with Hodgkin's disease who died of PML (6), being named JC virus (JCV) after the patient's initials.

MICROBIOLOGY

JCV is a member of the polyomavirus family, being one of the smallest human DNA-viruses, with **a** diameter of 38-43 nm. It has a icosahedral structure and double-stranded, supercoiled 5,1 kb genome (7) that codes for several nonstructural but multifunctional proteins (T, t, T 135, T136, T165), 3 capsid proteins (VP1, VP2, VP3) and a protein known as agnoprotein. Large T mRNA is produced prior to viral DNA replication and from the complementary strand of the capsid and agnoprotein genes which are transcribed after DNA replication. Small t and T RNA transcripts result from the cellular splicing of the large T (8). Several types of JCV with same basic genome configuration have been isolated from PML affected brain tissue. Isolating and cultivating JCV is difficult because it multiplies very slowly (9).

EPIDEMIOLOGY

Seroepidemiologic studies have demonstrated that usually JCV is a harmless virus, at the age of five 10% of children having antibodies against JCV

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and by the middle adulthood 80-90% of the population presenting IgG antibodies against JCV (10). PML is typically observed in patients with immunodeficiency. The first reports were in individuals with chronic lymphocytic leukemia, Hodgkin's disease, carcinomas, granulomatous and inflammatory diseases such as tuberculosis and sarcoidosis, PML being considered a rare disease. Miller et al. (11) reported the first description of PML complicating the acquired immunodeficiency syndrome (AIDS) in 1982, one year later after the initial description of AIDS. Soon afterwards, PML was recognized as a major opportunistic infection of AIDS, occurring in up to 5% of the patients (12). In 2005, two cases of PML were reported in patients with multiple sclerosis (MS) treated with natalizumab – a humanized monoclonal antibody against alpha 4-integrin (13, 14). followed by A retrospective diagnosis of PML in a patient with Crohn's disease also treated with natalizumab, who died two years earlier from what initially was thought to be an astrocytoma followed (15). The review of all other exposed patients revealed no further cases of PML, the estimated risk being assumed 1:1000 with a mean exposure of 18 months of therapy (16). In 2008, two more cases of PML were reported in patients with MS receiving natalizumab monotherapy for longer than a year. Another immunosuppressive drug that was associated with PML is rituximab, indicated for the treatment of non-Hodgkin lymphoma and rheumatoid arthritis.

Epidemiological studies revealed that about 80% of PML patients had AIDS (17) and among HIV-negative cases, 55% had hematological malignancies, 15% had chronic inflammatory disease, 9% had sarcoidosis, 7% were transplant recipients, 7% had other conditions (cirrhosis, pulmonary fibrosis) and 7% had no detectable predisposing illness except for age between 66 and 80 years (18).

PATHOPHYSIOLOGY

The initial infection with JCV is prevalent in childhood, after the primary infection the virus remaining latent in the kidneys and the lymphoid organs. It has been detected in the urine samples of 30% (19) and in the tonsils of 39% (20) of subjects regardless of their immune status. It also has been found in bone marrow samples from PML patients (21), leukemia patients (22) and bone marrow transplant recipients (23). Some authors also reported the presence of JCV in normal digestive system (24) and brain (25).

The reactivation of JCV and the development of PML are still under debate, although immunosuppresion is likely a major component. The loss of specific immune cells may allow for active viral replication and infection, but the fact that the immune suppression creates suitable conditions for changes in the JCV regulatory region has also been proposed. The fact that some immunocompromised patients develop PML while others with similar JCV burdens do not suggest that beside the immune status, there are also other factors that determine the spread of JCV and the development of PML (8).

The JCV reactivation is associated with viremia, and the evidences of multiple tissue involvement indicate that the spread of JCV to the CNS occurs via the hematogenous route. The blood cells that are able to carry JCV are B lymphocytes (26), T lymphocytes, monocytes, and polymorphonucleares (27).

It is assumed that JCV uses the lymphocytes as a Trojan horse to penetrate the blood-brain barrier (BBB), the polymorphonucleares being found in many PML brain tissue samples (28). The virions that are released from lymphocytes attack the glial cells and are internalized rapidly via an endocytic mechanism (29), the DNA binding proteins of infected cells regulating and promoting JCV transcription and DNA replication.

The infection of oligodendrocytes determines demyelination and abortive infection in some astrocytes, with a cytolytic effect on oligodendrocytes, the most commonly involved areas being the subcortical white matter, the deep layers of the cortex and the corticomedulary transitional zone (9).

CLINICAL PRESENTATION

Classic PML symptoms consist in various subacute neurological deficits because the lesions may occur anywhere in the CNS white matter, although they appear to spare the optic nerve.

For example, Stone et al. reported that among the patients with verified PML the following range of symptoms was encountered: mental impairments (e.g. memory disturbances, confusion, personality changes, dementia) 44%, visual impairments (e.g. homonymous hemianopia, diplopia, visual blurring) 41%, motor deficits (mono or hemiparesis) 31%, incoordination (ataxia, cerebellar dysartria) 20%, speech disturbances (dysartria, aphasia) 19%, sensory impairments (numbness, hypo/anesthesia)

3%, miscellaneous (e.g. headache, vertigo, seizures) 8% (30). The symptoms that mimic a cortical disorder are considered to be due to the areas of demyelination immediately adjacent to the cortex. (e.g. lesions underlying the language centers determine aphasia, lesions in the occipital white matter can cause cortical blindness).

In the majority of cases, the disease course is progressive, most of the patients dying within a few months. Because remyelination does not occur in affected areas, many PML survivors remain with neurological sequels.

PML associated with immune reconstitution inflammatory syndrome (IRIS) is a novel presentation of PML consisting in new onset or clinical worsening of symptoms shortly after initiation of HAART therapy. This fulminant inflammatory leukoencephalopathy is a paradoxical deterioration in clinical status that occurs in the setting of a recovery of the immune system marked by an increase of CD4 cells and a decrease in HIV plasma viral load (17).

JCV infection of cerebellar granule cell neuronopathy is a chronic disease, isolated or associated with PML. Recently it was reported that JCV can cause a productive and lytic infection of cerebellar granule cell neurons associated with a cerebellar syndrome in HIV-infected patients with (31, 32) or without PML (33).

PARACLINICAL FINDINGS

The *peripheral blood* studies do not detect any results that can support the diagnostic; leucocytosis might be rarely encountered.

Upon PML suspicion , an extensive immunological examination including determination of types and functions of lymphocytes is necessary for demonstrating the changes in lymphocyte subpopulations (especially low CD4 cell count). Moreover testing for HIV infection is mandatory.

The *cerebrospinal fluid* (CSF) examination usually reveals normal pressure and composition, and serves to exclude other etiologies. A slightly elevated protein content and the presence of myelin basic protein is present sometimes. Oligoclonal bands have been detected in single cases. There can be a slight mononuclear pleiocytosis (9). Hypoglycorrachia was observed in some patients (34), but this abnormality was also reported to occur with HIV infection alone (35, 36).

Serological studies for JCV of the blood are not very useful as antibodies are found in many healthy subjects and PML patients might not have raised titers as they have an underlying disorder that compromises the immune system. Contrarily, polymerase chain reaction (PCR) detection of JCV DNA in CSF helps in confirming the diagnosis of PML in patients with suggestive neurological and radiological findings, with sensitivity between 72-92% and specificity of 92% to 100% before the HAART era (37). In patients receiving HAART therapy, the sensitivity of the PCR has been reported to be of 58% (38). Finding negative CSF JCV PCR in AIDS patients with clinical and imagistic findings indistinguishable from PML it is likely to be due to the recovery of the immune system and the decrease of viral replication induced by HAART. Therefore, a negative PCR test does not exclude the diagnosis of PML; although the effects of HAART treatment are beneficial, this shift in PCR's sensitivity for JCV DNA caused the return to the brain biopsy that might be necessary to ascertain the diagnosis.

The *histological* examination requires a tissue specimen from a lesion area obtained by *biopsy*. The sensitivity of brain biopsy is between 64-96% and the specificity of 100% for PML (19), with a risk for fatal complications in 2,9 % and morbidity in 8,4% in HIV-positive patients (39). Additionally, the lesions may not be accessible for biopsy, or the patients may be too debilitated to withstand the procedure (17).

The histological changes in classic PML consist in demyelination areas containing infected oligodendrocytes with enlarged amphophilic nuclei located at the periphery of the lesions, reactive gliosis with enlarged bizarre astrocytes, lipid-laden macrophages that phagocyte myelin and cellular debris and CD8 cells near JCV infected cells. The presence of JCV-infected cells can be confirmed by immunohistochemistry for polyomavirus proteins or in situ hybridization for JCV DNA (17). In inflammatory PML associated with IRIS, the histological findings are the same as in classic PML, but the inflammatory infiltrates are more conspicuous (17). In JCV granule cell neuronopathy, the biopsy reveals focal cell loss in internal granule cell layer of the cerebellum and immunohistochemistry is positive for JCV in enlarged granule cell neurons (17).

NEUROIMAGING

Computerized tomography (CT) typically shows diffuse hypodense asymmetric lesions in the white matter. The absence of edema, contrast enhancement or mass effect, helps to the differential diagnosis with tumors, granulomas and other

infections (40). Sometimes the lesions can be confluent and rarely do they show contrast enhancement, appearing as homogenous hypodense areas that do not follow vascular territories. However, in the most of the cases on CT scan appear only modest changes that contrast with the pronounced clinical signs (40).

Magnetic resonance imaging (MRI) is more sensitive than CT in determining the size and extent of the lesions. In classic forms of PML, the lesions appear hypointense on T1-weighted images and hyperintense on T2-weighted and fluid-attenuated inversion recovery images (41). Usually, they are well demarcated, non-contrast enhancing and there is no surrounding edema, without mass effect. Most of the patients present bilateral, asymmetric lesions, localized preferentially in the subcortical white matter, sometimes being restricted to the arcuate fibers (U fibers) (42), but there have been reported cases presenting initially with a single lesion which can be mistaken for a stroke or a tumor (17, 43).

The frontal and parieto-occipital lobes are the most commonly affected. The deep nuclear structures are involved in 12 % of the patients (34) and the isolate involvement of basal ganglia was also reported (42). In 48% of all patients, MRI scan shows lesions in brain stem or cerebellum, with 20% of lesions being located solely infratentorially (34).

In PML associated to IRIS, the MRI findings are the same as for classical forms, but the lesions may present rim or speckled enhancement, as well as mild swelling and mass effect (17) which suggest an unusually intense inflammatory reaction.

In JCV infections of cerebellar granule cell neurons, neuroimaging studies detect cerebellar atrophy (17).

DIFFERENTIAL DIAGNOSIS

Differential diagnosis should consider other primary as well as opportunistic infections of CNS (e.g. Toxoplasma, Cryptococus, Herpes Simplex virus, Varicella-Zoster virus, Cytomegalovirus, Epstein Barr virus, Treponema Pallidum, Mycobacterium tuberculosis), other demyelinating diseases (such as multiple sclerosis), vascular lesions (e.g. ischemic stroke, HIV vasculopathy, arterial dissection), neoplastic diseases (e.g. lymphoma, paraneoplasic syndrome) and HIV encephalopathy with secondary changes in white matter.

TREATMENT

There is no specific treatment for PML. The therapeutic strategy includes early diagnosis, discontinuation or at least minimalization of all immunosuppressive drugs and, in HIV-positive patients – optimization of HAART medication.

Administration of HAART was associated with prolonged survival in AIDS patients with PML (44, 45, 46), generally improved survival correlating with higher CD4+ cell counts (47). However, a few studies have reported a failure to obtain benefits from HAART (48). One study reported three patients that developed PML while receiving HAART, with no improvement of PML despite the virologic response (48) and another study reported two patients with clinical symptoms of PML during the first weeks of HAART (49), but follow-up MRI-studies in these patients revealed a clear improvement. The results of another study indicate that a clinical and radiological response to HAART may be seen in some patients with PML, while in others there may be no beneficial response (50). These observations suggest that HAART may not be effective in all PML patients and may not prevent the disease in patients under HAART therapy.

Due to the PML high mortality rate, many treatments have been tried.

Small trials with antineoplasic agents such as cytarabine (Ara C) either intravenously or itrathecally in HIV-positive patients with PML showed either no survival benefit or undesirable side effects (51, 52). Interestingly, in a retrospective study, 36% of HIV-negative patients with PML showed stabilization of the disease under treatment with Ara C (53) and clinical improvement was noted in one MS patient that developed PML under natalizumab (14).

Cidofofir, an antiviral agent against cytomegalovirus was demonstrated to produce no additional benefit over HAART alone in AIDS patients (54) or in HIV-negative patients with PML (55), although in vitro studies showed that it was active against polyomaviruses (56).

The interferon alpha-2b has been reported to improve the survival time in AIDS patients with PML that were not under HAART therapy (57), but a retrospective study which included patients on HAART failed to show any survival benefit (58).

Recently, it was demonstrated that the serotoninergic receptor 5-hydroxytryptamine-2 a serotonin receptor (5HT2AR) could act as the

cellular receptor for JCV on human glial cells (59) and further studies identified possible therapeutic agents for PML such as chlorpromazine, clozapine (atypical antipsychotics), and mirtazapine (antidepressant) as they are known to be 5HT2AR antagonists (60).

PROGNOSIS

Before AIDS epidemic, the prognosis of PML was almost always rapidly fatal, the most of HIV-negative patients dying within 4-6 months after the diagnosis; only a few cases presented prolonged survival with recovery of neurological and radiological signs (61). The one year survival in HIV-infected patients was 10 % in pre-HAART era (34) and 50% with HAART therapy (38).

The effects of HAART on neurologic function are an issue of controversy, some studies reporting clinical improvement in an important number of patients (44, 46, 62), whereas others found no clinical improvement among survivors (63).

Some studies have demonstrated an inverse correlation between JCV load values in the CSF and the clinical outcome of patients with PML (19, 64). Also, an inverse correlation was found between the JCV load in CSF and survival time, there being significant differences in survival between the patients with a JCV load of > 4,68 log compared with that found for patients with a load of < 4,68 log (65). A low JCV burden in the CSF (50-100 copies / µl) was found to be predictive of longer survival when compared with patients with high JCV burden (62). Furthermore, in HIV-positive patients that survived after PML, it was observed a progressive decline in JCV DNA levels during HAART treatment, but not in those who dyed (66).

Another predictive factor for prolonged survival is the presence of contrast enhancement on radiological investigations (50, 67, 68). The contrast enhancement is suggestive of development of an inflammatory response against JCV mediated by CD8 cells and facilitated by CD4 cells, leading to a breakdown of the BBB; once JCV replication is under control, the viral load decreases to undetectable levels in the CSF (67).

CONCLUSIONS

Since its initial description, there have been changes in progressive multifocal encephalopathy's epidemiology, clinical presentation, evolution and prognosis. If some decades ago it was considered a rare disease, now it is a major complication of AIDS and represents an obstacle to the use of promising treatments for autoimmune disorders. Although the survival rate has increased in the last years, there is still no specific treatment for JC virus, and further research is required for a more effective management of the disease.

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