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Detectable Cerebrospinal Fluid JCV DNA in Late-presenting HIV-positive Patients: beyond Progressive Multifocal Leukoencephalopathy?

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Abstract (150 words, unstructured)

In the absence of effective prophylaxis and treatment, therapeutic options in HIV-positive patients with progressive multifocal leukoencephalopathy (PML) are limited to antiretroviral therapy: nevertheless outcome is poor. We conducted a retrospective study (2009-2015) describing the outcome of 25 HIV-positive patients with detectable cerebrospinal fluid JC virus DNA: 14 had a probable PML while the others had evidence of other inflammatory central nervous system (CNS) affecting disorders. In the former group six-month mortality was 45.5% vs. 21.4 in the latter one : survival was higher than previously described but none predictor of poor outcome was identified. Two patients treated with 5HT2-inhibitors survived. The contributing role of JCV replication in other CNS-affecting disorders needs to be assessed as well as the benefits of 5HT2-inhibitors in HIV-positive patients with proven PML.

Background

Progressive multifocal leukoencephalopathy (PML) is a central nervous system (CNS) demyelinating disease caused by reactivation of JC virus (JCV) that occurs primarily in immunosuppressed individuals, most of whom have advanced HIV infection with low CD4+ cell count (<200/µl). In the absence of HIV infection, PML is most likely to be found in hematological and solid organ malignancies, sarcoidosis, autoimmune disorders, congenital immune deficiencies and following the introduction of biological agents [1,2,3].During immunosuppression, JCV can reactivate and, through the blood-brain barrier, infect oligodendrocytes and astrocytes: typical white matter demyelinating lesions are usually observed on magnetic resonance imaging (MRI).[4]Viral DNA detected in patients with PML shows rearrangement of RR region(duplication, tandem repeats and deletions) compared with archetype[5]thus suggesting a “transformation” of JCV within its host rather than acquired transmission[6].

Prognosis of HIV-positive patients with PML is poor and 1-year survival rates was approximately 9% in pre- highly active antiretroviral therapy (HAART) era[7]. Despite HAART introduction and

several potential adjunctive treatments,2-year mortality is still 50-60%[8,9]. Some *in vitro* studies demonstrated that the entrance of JCV within brain is, at least, partially mediated by attachment to host cell serotoninergic receptor 5HT-2A: the blockade of serotoninergic receptors, preventing JCV infection of glial cells, may represent a possible therapeutic target.[10, 11] Recent case reports have shown beneficial effects of these drugs in HIV-positive and negative patients with PML. [11, 12, ,13,14] Aim of this study was to describe the outcome of patients with detectable cerebrospinal fluid (CSF) JCV DNA.

Material and Methods

A retrospective study was conducted in HIV-positive patients admitted with neurological symptoms at a single institution from 2009 to 2014. The study was approved by the local Ethics Committee and patients signed a written informed consent.

Inclusion criteria were confirmed HIV-positivity and detectability of JCV DNA on CSF by real-time PCR. Demographic, radiologic (MRI), clinical and therapeutic data were collected and survivals compared at six months. PML diagnosis was performed according to the following criteria: presence of CSF JCV by PCR with clinical and radiologic features of PML. CPE scores were calculated according to Hammond et al.[15]. Data are expressed as medians(interquartile ranges) and compared through non-parametric tests; survival analysis was performed by Kaplan-Meier curves and log-rank analysis.

Results

We identified 25 patients with detectable CSF JCV DNA of whose 14 presented clinical and radiological features of PML: baseline data are summarized in Table1.

Tabella 1: Clinical, radiological and immunovirological features at baseline. "PML", progressive multifocal leukoencephalopathy.

	Detectable JCV	PML (n=14)	p value

	DNA (n=11)		
Male gender(%)	**8** (72,7)	**8** (57.1)	0.35
Age (years)	**45.1** (43.5-49.5)	**46.6** (41.2-51.5)	0.65
CD4+ T lymphocyte (cell/uL)	**93** (23.5-269.5)	**82.5** (57.3-159.5)	0.85
plasma HIV RNA (Log_{10}copies/mL)	**4.97** (1.6-5.3)	**5.05** (1.9-5.5)	0.66
plasma HIV RNA <50 copies/mL(%)	**3** (27.2)	**4** (28.6)	0.65
CSF HIV RNA (Log_{10}copies/mL)	**3.6** (2.11-5.39)	**3.4** (2.4-3.9)	0.63
CSF JCV DNA (Log_{10} copies/mL)	**<2.00** (<2.00-2.64)	**2.78** (<2.00-3.6)	0.02
Neurological symptoms:			
hemiparesis/hemiplegia	**1** (9%)	**7** (50%)	0.18
mono-ocular blindness	**0** (0%)	**2** (14.3%)	
generalized seizures	**0** (0%)	**1** (7.1%)	
ataxia	**2** (18.2%)	**1** (7.1%)	
confusion	**3** (27.3%)	**3** (21.4%)	
neurocognitive impairment	**4** (36.4%)	**2** (14.3%)	
MRI characteristics			
T2-hyperintenseWMA	**7** (63.6%)	**13**/13(100%)	0.41
mass lesion	**2** (18.2%)	**1**/13 (7.7%)	
contrast enhancement	0 (0%)	**1**/13 (7.7%)	
CNS disease other than PML	**3** (27.3%)	**0**(0%)	0.03
neurotoxoplasmosis	**1** (9%)	**0** (0%)	
Cryptococcal meningitis			
6-month mortality	**5**(45.5%)	**3**(21.4%)	0.20

Among the 11 patients with detectable CSF JCV without features of PML ("JCV+") 7 subjects (63.6%) had a concomitant opportunistic infections, 3 of which (27.3%) affected the CNS (1 cryptococcal meningitis, 2 neurotoxoplasmosis); additionally 7 patients (63.6%) revealed some kind of CNS dysfunction on neurological examination. 7 of these JCV+ patients had white-matter lesions, usually T2-hyperintense lesions. 2 subjects had periventricular and subcortical white matter abnormalities with features of PML; both of them presented subacute neurological deficits but low level CSF JCV DNA (<100cp/ml). While one of the two remained stable over time, the other one died 2 months later for cryptococcal meningitis and Human herpesvirus 6 encephalitis.

Despite HAART introduction (median CPE 7, range 7 to 11) 6-month mortality was 21.4%in PML patients group and 45.5% (5 patients) in JCV+ individuals (p=0.383); overall survival was 23.5 months (6.5-44) vs. 18 months (0-36) (p=0.98) and overall mortality was 50% vs. 45.5% (p=0.82), respectively. The 5 patients who died in the JCV+ group had lower baseline CD4+ T cell (19 vs 82.5 cell/uL), higher serum and CSF HIV viral load (respectively 216216 vs 90749 and 211.56 vs 2.451cp/mL) respect to PML patients. Likewise, patients who died in the latter group tended to have more opportunistic infections (100% vs 36.36%); 3 had CNS-affecting conditions (2 neurotoxoplasmosis, 1 cryptococcal meningitis). The remaining two revealed disseminated cryptococcosis in one case and cytomegalovirus encephalitis in the other one. In the PML group, 4 patients had opportunistic infections but none involved the CNS. No clinical, radiological or immunovirological variable was found to significantly predict mortality. Two PML patients treated with 5HT2-inhibitors survived (one with mirtazapine and one with risperidone) over a long follow up (above 5 years).

Discussion

Our retrospective study conducted in modern HAART era showed that JCV DNA may be detected in patients with AIDS as well as in those with clinical and radiological features of PML. 6-month mortality was lower than previously described (21.4%)[16,17] but we couldn't identify any predictor of poor outcome (including CD4 cell count, CSF JCV DNA viral load, HAART neuroefficacy, etc.); nevertheless the observation of long-term success with the use of 5HT2-inhibitors may warrant further studies (in addition to effective HAART) in patients with HIV-associated PML.

PML is one of the major opportunistic infection caused by JCV following a DNA rearrangement leading to CNS invasion, that occurs almost exclusively in immunosuppressed individuals, inducing a lytic infection of oligodendrocytes with subsequent demyelinating. PML typically affects HIV+ individuals and less frequently patients with granulomatous, lymphoproliferative or myeloproliferative diseases, solid organ malignancies, organ transplant recipients and relapsing-

remittent multiple sclerosis treated with natalizumab. We collected data from 25 HIV-1 patients with CSF JCV-DNA, 14 of whom had clinical and radiological features compatible with PML. At the time of diagnosis of PML, median CD4 count was 82.5 cc/ul and only 4 patients were HIV-RNA below 50cp/uL. Following introduction of HAART the 6 months mortality in this group was 28.6 % (vs 45.5% in PML negative patients), probably due to selection bias and higher proportion of comorbidities in PML negative patients. This is consistent to previous studies in which near 50% of HAART treated patients still die in 6 months[18]. Immunovirological and clinical features were unfavorable in JCV+ patients with advanced immune depression and multiple concomitant opportunistic infections. In this setting JCV DNA positivity may represent a proxy of profoundly depressed immune system, regardless HIV seropositivity. This observation is supported by the finding that, as previously reported, a large proportion of patients with CNS JCV was affected by hematologic disorders besides HIV infection[19]. Moreover in HIV + it has been suggested that JCV+ tends to be associated with low CD4+ cells and not necessarily with PML development.[20] Similarly several studies found an association between CMV viremia and mortality: however it is unclear if CMV concurs to mortality or if reactivation is a proxy of a deeply compromised immune systems.[21,22]

Two patients with PML-like radiological features had barely detectable CSF JCV DNA. This may suggest a trafficking of JCV-infected cells during HIV-associated CNS diseases (with common blood brain barrier impairment) or the incomplete diagnostic sensitivity of the test[23]. It has been previously evaluated that CSF JCV DNA measured by PCR, presents diagnostic sensitivity ranging from 58% to 92% according to different PCR methods and multiple sample testing in patients with biopsy proven PML[24,25,26]. More recent studies evaluated a real-time PCR targeting a more conserved region of JCV-DNA compared to VP1 nested PCR demonstrating a higher sensibility that may result in less false negative results, making it an effective tool for early diagnosis[27].

PML remains a great concern in individuals for individuals with compromised immune system regardless medical history and prognosis is still poor. There is neither a prophylaxis for PML nor specific anti-JCV treatment and the main approach is restoring the host immune response by HAART in HIV+ patients or discontinuing sources of immunosuppression in other populations. We have found a positive trend for lower mortality in patients treated with 5HT-2 inhibitors and with high CPE. The limitation in our study are due to very low sample size, the retrospective study design, bias for concurrent HAART treatment and the lack of plasma JCV DNA.

In conclusion the presence of JCV in CSF of patients without clinically evident PML suggests that JCV may be present in the central nervous system of HIV positive subjects. One possible explanation is that in patients with a deep and prolonged immusuppression, as though HIV positive patients, JCV may reactivates and spreads out. As a matter of fact, in this specific population, the presence and persistence of JC virus into any body compartment, as bone marrow[28], may play an important role in reactivation and/or CNS neurotropism. The CNS has also been suggested as a potential site for JCV persistence too. Moreover it would be interesting to determine if all HIV positive or by contrast only patients who are initially serum and CNS seronegative for JCV are at risk for developing PML. Nevertheless our study could not distinguish between primary JCV infection and reactivation of CNS JCV latent infection. It should be promptly considered that PcR is a high sensitive tool and because of this, an increase of inflammatory cells, as in the case of CNS infections, could account for a false positive JCV PcR on CSF sample. Effectively, we have found that in the PML negative group, the 3 patients presented with CNS opportunistic infections(2 neurotoxoplasmosis, 1 cryptococcal meningitis) had an higher JCV viral load on CSF respect to the others.

We found that 6 months mortality was higher in detectable CNS JCV positivity respect to PML patients group in controls (21.4% vs. 45.5%). This could be probably related to a major burden of opportunistic infections and comorbidities in the former group or too short follow-up time to come to a significant conclusion because there is a marked variability in the duration and progression of

PML. Effectively all 5 patients died in the first group presented in our hospital with at least an opportunistic infection, 3 of which affected CNS. In addition we observed that patients without PML were more often hospitalized for precipitating factors such as acute infections. In summary we have detected 25 HIV positive patients with positivity of JCV on CSF examination, 11 of them without clinical evidence of PML. We have found that 6 mortality was higher in this latter group. Further investigation is warranted to better define the pathogenesis and factor involved in development of an asymptomatic CNS JCV persistence in some patients to a demyelinating and often fatal disease in others.

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