IDENTIFICATION OF POLYOMA VIRUS JC GENOME SEQUENCES IN TWO HIV-ASSOCIATED PML CASES IN BULGARIA

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ABSTRACT
Active replication of the Polyomavirus hominis 2 (JCV) in glial cells leads to the fatal, demyelinating disease of the central nervous system, called progressive multifocal leukoencephalopathy (PML). Although more than 80% of the population is infected a significant impairment of the immune system is needed for reactivation of the latent virus. Such reactivation can be verified by a Polymerase Chain Reaction (PCR) test for detection of JCV DNA in brain tissue or cerebrospinal fluid (CSF). Here we report and discuss two cases of Bulgarian HIV-infected patients where identification of JCV supported and confirmed the diagnosis of HIV-associated PML.

Keywords: JC polyomavirus, progressive multifocal leukoencephalopathy PML, HIV/AIDS

Introduction
Polyomavirus hominis 2 (JCV) is a small, non-enveloped, DNA virus, that is part of the growing viral family Polyomaviridae (7). JCV has a tropism towards the brain tissue and in particular the glial cells. More than 80% of the population is infected during childhood, but in most cases no clinical manifestation is present (6, 8). The latent condition of the virus remains for years and the impaired immune system acts as a predisposing factor for viral reactivation (1).

JCV has been established as the etiologic agent for the fatal, demyelinating disease of the central nervous system, called progressive multifocal encephalopathy, PML (9, 11). The pandemic level of HIV/AIDS infection/disease worldwide leads to an increased incidence of PML. Not less than 5% of HIV infected patients develop the so called HIV-associated PML, sometimes being the first presentation of the disease (2). Consequently the significance of accurate and prompt diagnosis increases.

An important role in the diagnostic algorithm of PML plays the JC virus DNA identification from different clinical materials and especially from CSF and brain biopsy and a leading method for polyomavirus detection is the Polymerase Chain Reaction, PCR (5).

In this communication we report and discuss JCV detection in two cases of HIV-associated PML. The documented JC virus active replication in brain tissue was a significant criterion for a subsequently diagnosed PML.

Materials and Methods
Descriptive cases:
(a) A 33-year-old HIV infected man (Case 1), showing clinical and laboratory data for progression to AIDS was tested. The HIV viral load was greater than 500 000 copies/ml. The CD4+ count was 32/mm³ and the CD8+ cells were 733/mm³, with a CD4/CD8 ratio of 0,04 respectively. The extensive work up included a magnetic resonance imaging of the brain (MRI) showing multiple lesions in the white matter. Additionally neurological symptoms were present supporting a PML probable case (4). After a brain biopsy was obtained the histological examination indentified astroglial nuclei with a basophilic ground-glass appearance (bizarre astrocytes, which are a typical PML histological finding), lipid-laden macrophages and marked reactive gliosis. No signs of a lymphoproliferative process were present and a virological test for JCV DNA detection was suggested.
(b) A 25-year-old HIV positive woman’s CSF was tested for presence of JCV DNA sequences (Case 2). Apart from high HIV viral load levels flow cytometry determined the number of CD4+ cells as 44/mm³ and the CD8+ cells were 780/mm³, with a CD4/CD8 ratio of 0,06. The patient has been treated according to a Highly active antiretroviral treatment (HAART) protocol for a year during which subacute and remittent neurological symptoms (headache, somnolence and vertigo) were documented.

Samples, DNA preparation and PCR amplification
Clinical specimens of urine, brain biopsy tissue and CSF were collected and tested immediately on laboratory acceptance. A standard Proteinase K digestion followed by phenol/chloroform extraction method was used for DNA isolation from urine and biopsy tissue specimens. CSF initial preparation included incubation of 10 µl aliquots of native liquor for 10 min at 95°C, followed by direct use of the obtained product in the PCR reaction as a template.

For the detection of JCV specific DNA, we used 0.5-1.5 µg in 2-5 µl of whole target DNA. A JTP primer system (10) was employed for amplification of a sequence encoding part...
of the JCV T antigen. The amplification protocol consisted of 40 cycles at 95°C for 60 sec, 55°C for 60 sec and 72°C for 60 sec. The protocol also included an initial denaturation for 5 min and a final extension step for 10 min. All reactions were run in a total volume of 50µl on PTC-200 DNA engine Cycler (MJ Research, Incorporated). A 30 pmol concentration of each primer, 1.25U of recombinant Taq-polymerase (Invitrogen, USA), 200 μM of each dNTP (USB, USA) and a standard reaction buffer were used in the presence of 1.5 mM MgCl₂ (Abgene, USA). Adequate positive and blank controls were used in every run. An aliquot of 20 µl from the PCR amplification products (amplicons) with expected size of 141-bp were run on a 2% agarose gel stained with ethidium bromide together with a 100-bp molecular marker for weight identification (Invitrogen, USA).

Additionally to the conventional PCR technique, we used a TaqMan® probe-based detection protocol (Applied Biosystems, Cat No. 4304437) to test CSF for presence of JCV DNA sequences according to the manufacturer’s instructions.

Results and Discussion
Using a conventional PCR employing the JTP primer system we detect a specific JCV genome sequences in brain tissue and urine (case 1) and in CSF in case 2. Most probably these findings indicate JCV active replication into the brain cells, resulting in certain clinical manifestations, accompanied by an increased viral shedding in CSF and urine (Fig. 1).

![Figure 1](image-url)

Fig. 1. PCR results showing detection of JCV genomic sequences, derived from brain, urine and CSF of HIV positive patients. (a) agarose gel electrophoresis with 100-bp molecular weight marker on lane 1, urine and brain tissue amplicons on lane 2 and 3 respectively; (b) Data graph showing increase of specific fluorescence in 1: JC virus positive control DNA (Ct=33) and 2: JC virus DNA positive CSF sample (Ct=38); no such increase was observed in the negative control sample 3.

Conclusions
Our results suggest that this complex approach including virus detection by molecular techniques (PCR) can be a valuable tool for both diagnosis and monitoring the course of PML. Furthermore, in the presence of viral quantity standards the assay can be used as a real-time quantitative PCR, which will allow viral loads to be determined. All that might result in better understanding the role of JCV for the development and clinical outcome of PML and to increase the survival chances of those patients.

REFERENCES

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