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Topoisomerase I Improve JC virus DNA detection

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Abstract

Introduction: Progressive Multifocal Leukoencephalopathy (PML) is a severe and often fatal CNS disease caused by JC human polyomavirus infection. Diagnosis of PML is based upon suggestive clinical symptoms and brain images, supported by the presence of JC virus genome in CSF samples.

Objective: The main objective of our study was the search for an alternative method for JC virus DNA detection in CSF samples, with sensitivity and specificity characteristics close to that of standard techniques, but feasible at any clinical laboratory.

Methods: In order to evaluate the effect of topoisomerase I treatment in the detection of JC virus genome, and its feasibility in laboratory diagnosis of PML, 129 CSF samples were examined for the presence of JC virus DNA by a nested-PCR protocol, with and without previous treatment with topoisomerase I. All CSF samples were also evaluated through a real-time PCR protocol.

Results: Eleven CSF samples presented detectable JC virus DNA with all used protocols. On 9 CSF samples, JC virus DNA was only detectable with topoisomerase I modified nested-PCR and real-time PCR protocols. Real-time PCR was the only protocol able to detect JC virus genome in 4 CSF samples. One CSF sample revealed the presence of the expected amplified fragment only when tested with topoisomerase I modified nested-PCR protocol.

Conclusion: The results of the present study point towards the benefit of using topoisomerase I DNA treatment before amplification reactions in JC virus DNA detection on CSF samples, and confirm that topoisomerase I modified nested-PCR protocol represents a good alternative method to detect JC virus DNA in CSF samples from patients with clinical signs and brain images suggestive of PML.

Keywords: JC Virus; Polymerase chain reaction; Topoisomerase I; Progressive multifocal leukoencephalopathy

Introduction

The human JC virus belongs to the *Polyomavirus* genus within the *Polyomaviridae* family. This non-enveloped virus has an icosahedral capsid and a closed, double-stranded, supercoiled DNA genome of about 5130 bp. JC virus is known to infect a large proportion of the human population worldwide, usually in the absence of clinical symptoms. After the initial infection, JC virus persists in kidney and B lymphocytes also in an asymptomatic way. Under immunossupression conditions this virus may reactivate from latency and disseminate into the CNS, where it could lytically infect oligodendrocytes, resulting in the development of Progressive Multifocal Leukoencephalopathy (PML) [1,2]. PML is a severe demyelinating disease that occurs primarily in AIDS patients. Three to five per cent of these patients develop PML, with a poor prognosis, and, invariably, a fatal evolution, with death occurring 4 to 6 months after diagnosis of the disease [3,4]. Although there is no effective treatment for PML [3], the introduction of Highly Active Antiretroviral Therapy (HAART) for the treatment of HIV infection has been accomplished by a decrease in the incidence of HIV-related PML and by prolonged survival of patients with this disease [3,5,6]. The early diagnosis of PML is crucial for patient prognosis. The definitive diagnosis of PML was intially based on clinical presentation and neuriomaging picture of the brain, along with the concomitant presence of characteristic histopathological and virological features in brain tissue. Despite the high sensitivity and specificity of brain tissue analysis, it implied the realization of a brain biopsy, an invasive method with well recognized risks and high costs [7].

Actually, the presence of JC virus genome in cerebrospinal fluid (CSF) of a suspected PML patient, presenting typical symptoms and characteristic brain images contributes to the diagnosis of these neurological disease [8]. The method of choice for the detection of JC virus genome has long been the PCR amplification. However, the low concentration of JC virus genome in CSF samples of some PML patients, represented a limitation to the use of the conventional protocols using agarose gel electrophoresis to evaluate amplified products. Initially, in order to overcome this limitation, an additional step of southern blot hybridization to detect and identify the amplified products was used [8]. Although highly specific and sensitive, this methodology is not feasible in many clinical laboratories, due to the need of use radio-labeled specific probes, and the equipment and license associated with these compounds. To overcome these difficulties, some of these laboratories have used nested-PCR instead of simple PCR protocols, in order to achieve increased sensitivity and specificity of amplification reaction [9,10].

Despite the use of such amplification reactions, some authors reported poor reproducible results of detection of JC virus DNA in several clinical samples. As consequence, the hypothesis that the closed and supercoiled topology of JC virus DNA molecule could limit the efficiency of its in vitro amplification and, therefore account for the poor PCR reproducibility has been raised [11-14], further suggesting that pre-treatment with topoisomerase I could enhance the efficiency of DNA amplification, and, consequently, the PCR sensitivity. Topoisomerase I is an enzyme able to relax the supercoiled topology of DNA molecules, and, therefore, expose their nucleotide sequences, that, as a consequence, will be more available for primers annealing [11].

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Real-time PCR instrumentation represents one of the latest advances in nucleic acid amplification and detection technology. Some real-time PCR protocols enable the specific detection of as low as 10 viral copies of JC virus genome in a given sample, a similar sensitivity to that obtained with standards PCR-Southern blot hybridization protocols [8,15-17]. Alongside with high sensitivity and specificity, this new methodology presents the advantage of not requiring the use of radionucleotides, along with a more rapid and simple proceeding. Despite these features, real-time PCR performance requires specific and quite expensive equipment, which hamper its realization in some clinical laboratories.

The main objective of the present study was to evaluate the hypothesis of an alternative method for JC virus DNA detection in CSF samples. Our attention pointed to a method with a sensitivity as close as possible to those obtained with standard methods, such as real-time PCR or PCR-Southern blot protocols, but achievable at those laboratories that do not have the equipment necessary to perform this type of methodologies, nor the permit to use radio-labeled compounds. By this manner, we intended to analyze the effect of DNA treatment with Topoisomerase I before PCR amplification, in the detection of JC virus genome on CSF samples, and evaluate an alternative method for JC virus DNA detection on CSF samples from patients with clinical signs and brain images suggestive of PML, and, therefore, confirm the diagnosis of such disease.

For such purpose, CSF samples were tested simultaneously through 3 different PCR protocols: conventional qualitative nested-PCR protocol; topoisomerase I modified nested-PCR protocol, and real-time qualitative PCR methodology using fluorescence resonance energy transfer (FRET) technology, in a hybridization probe format.

Materials and Methods

Clinical samples

CSF samples, stored at Laboratory of Virology from the Infectious Diseases Department of Coimbra University Hospitals between March 1997 and December 2007, were included in the study. A total of 129 CSF samples obtained from 114 patients with suspected viral CNS infection were evaluated. The study group included 37 women and 77 men, with ages comprised between 15 and 94 years old (mean age 43). Seventy two per cent of the patients were HIV seropositive. After collection, CSF samples were frozen at -70ºC until analysis.

Nucleic acid extraction

Nucleic acid extraction was performed in each CSF sample using the QIAampR DNA Blood Mini Kit (QiagenR - Izasa, Portugal), according to the manufacturer's instructions. A starting volume of 200 µl of each CSF specimen was extracted and eluted to a final volume of 100 µl. All eluates were assayed by the three protocols using 10 µl aliquots as template for PCR.

Nested-PCR protocol

The conventional nested-PCR was performed with two sets of primers, specific from the conserved T-antigen coding region of the JC virus genome, described previously [18] and presented in Table 1. PCR reactions, containing 10 µl of DNA, were performed in a total volume of 100 µl with 2.5 U of *Taq* polymerase, 200 µM of each dNTP, and 2 mM $MgCl_2$ as instructed by the manufacturer (PCR Core System I – PromegaR, VWR Portugal). After an initial denaturation period of 5 min at 95ºC, reaction products were amplified for 30 cycles, using the following conditions: 1 min at 95ºC, 1 min at 55ºC, and 2 min at 72ºC. Amplification was completed with a 7 min extension period at 72ºC. Nested PCR was performed with 10 µl of the reaction product from the first amplification.

A total of 20 µl of each PCR reaction was run on a ethidium bromide-containing 1.2% agarose gel for 1 hour at 120 V and photographed under UV illumination. A positive JC virus reaction required the presence of the appropriate 577 bp band.

Topoisomerase I modified nested-PCR protocol

Ten microliters of each DNA template were treated with 0.5 units of topoisomerase I (Amersham Pharmacia BiotechR, GE Health Care, Portugal) at 37ºC for 45 min before undergoing the same nested-PCR amplification and electrophoresis protocols as described previously.

Real-time PCR protocol

Real-time qualitative PCR was performed with hybridization probes and FRET chemistry in the Light Cycler 1.5 instrument (RocheR, Roche Diagnostica, Portugal), using a set of primers and probes specific for major capsid protein gene of JC virus (Table 1). The primers and probes used showed no cross reactivity with human genomic DNA or with DNA from HSV-1, HSV-2, VZV, EBV, BKV, SV40, CMV, HHV6A, HHV6B, or Adenovirus (data not shown). A LightCycler-FastStart DNA Master Plus Hybridization Probes (RocheR, Roche Diagnostica, Portugal) containing FastStart Taq DNA polymerase, reaction buffer, deoxynucleoside triphosphates, and $MgCl_2$, was used as the basis for the reaction mixture in the real-time PCR protocol. Each reaction was performed in a final volume of 20 µl containing 10 µl of extracted DNA and 10 µl of reaction mixture with 1X LightCycler-FastStart DNA Master Plus Hybridization Probes reaction mixture, primers at concentrations of 1.0 µM, and FRET probes at concentrations of 0.2 µM. For the thermal cycling protocol an initial 8-min incubation at 95°C to activate the DNA polymerase, and melt double-stranded DNA, was followed by 45 cycles of 5 s at 95°C, 10 s at 62°C, and 20 s at 72°C.

Name	Target (Primer or probe)	Amplicon (bp)	Sequence (5'-3')
Nested PCR	T antigen		
D07	External Primer	768	GAATAGGGAGGAATCCATGG
D08	External Primer		GGAATGCATGCAGATCTACAGG
D09	Internal Primer	577	GGTGGGGACGAAGACAAGATG
D10	Internal Primer		GTGTTGGGATCCTGTGTTTTC
Real time PCR	VP1		
JC.For.2	Primer	195	GGA TGT TGC CTT TAC TTT TAG GGT
JC.Rev.2	Primer		TAA AAA GCA TTC TAC CTC TGT AAT TGA
JC.FRET.up	Probe		GGG TCC TTC CTT TCT CCT TTT CTT TT - Fluorescein
JC.FRET.Dn	Probe		Red640 –GTT GGG GCC ATC TTC ATA TGC TTC AAG - phosphate

Table 1: Primers and probes sequences used for JC virus DNA detection in CSF samples

Melting curve analysis was performed following PCR amplification.

Controls

One positive and two negative controls were included in all PCR experiments. One negative control consisted of the reaction mixture containing water instead of DNA template. The other negative control was the eluate obtained from nucleic acid extraction protocol performed on sterilized water instead of CSF sample. Tissue culture supernatant of JC virus Mad-4-infected SVG cells, with a concentration of 128 HAU/50 µl was used as positive control.

Evaluation of Topoisomerase I modified nested-PCR protocol detection limit, relatively to that of real-time PCR protocol

One DNA specimen previously extracted from a CSF sample that tested positive for JC virus DNA by conventional nested-PCR protocol, was diluted in 10-fold serial dilutions ranging from 10^{-1} to 10^{-6} in PCR-grade water. These dilutions were tested with conventional nested-PCR, topoisomerase I modified nested-PCR, and real-time PCR protocols, using the conditions described above. The detection limit of each assay was determined as the lowest dilution returning a positive reaction.

Results

Detection of JC virus genome in CSF samples with different protocols

A total of 129 CSF samples were obtained from 114 patients with suspected viral CNS infection. All CSF were evaluated for the presence of JC virus DNA by three methodologies in parallel: nested-PCR, topoisomerase I modified nested-PCR, and real-time PCR protocols. JC virus genome sequences were detected in 25 out of 129 CSF samples tested, with, at least one of the protocols used. From these positive samples, 11 presented JC virus DNA detectable with all used protocols. On 9 CSF samples, JC virus DNA was only detectable with topoisomerase I modified nested-PCR and realtime PCR protocols. Real-time PCR was the only protocol able to detect JC virus genome in 4 CSF samples. One CSF sample revealed the presence of the expected amplified fragment only when tested with topoisomerase I modified nested-PCR protocol (Table 2).

One hundred four CSF samples did not reveal detectable JC virus DNA with any of the protocols used. These results were repeated and confirmed. Retrospectively, it was tried to obtain the clinical information from those patients with JC virus DNA detectable in CSF, however, hospital records were available only for 20 of those 25 patients.

Whenever clinical notes were available, detailed information was collected regarding the clinical episode associated with CSF sample collection, as well as the results of CNS imaging [obtained by computed tomography (CT) and/or magnetic resonance imaging (MRI)] if it was performed. Both clinical signs and brain images were recorded as suggestive or not suggestive of PML. Of the 20 patients with available clinical records, 17 were seropositive for HIV. Among the HIV negative patients, one presented acute lymphoblastic leukaemia (patient #21), patient #25 presented a monoclonal spike in the gamma region of serum protein electrophoresis, and no immunossupression condition was described for patient #23.

Among the 20 patients with JC virus DNA detectable in CSF and available clinical information, 17 also presented clinical symptoms as well as brain images (obtained by CT or MRI) suggestive of PML. In these patients, the detection of JC virus genome in CSF enabled the confirmation of PML diagnosis. In respect to those 17 patients with confirmed PML diagnosis, real-time PCR was able to detect JC virus DNA in CSF samples from 16 patients (94%), while topoisomerase I modified nested-PCR achieved to detect JC virus genome in 13 (76%), and conventional nested-PCR only achieved to detect JC virus DNA in CSF samples from 9 patients(53%). Three patients with available clinical records, although had JC virus DNA detectable in CSF, brain images were not suggestive of PML at the time of sample collection.

Patient #5 presented clinical signs compatible with PML; however CT brain images were not specific of this disease. Culture of CSF revealed negative results for bacteria and fungi. The patient died 1 month later, and no autopsy has been done.

Patient #7 presented at our hospital with neurological signs and clinical symptoms suggestive of PML. However, MRI did not revealed any alteration suggestive of such disease. Neither bacteria nor fungi were detected by culture of CSF from these two patients. The patient was transferred to another hospital, before a definitive diagnosis was established.

Patient #22, seropositive for HIV, was admitted to our hospital due to clinical signs of CNS infection compatible with a PML situation. However, MRI and CT did not reveal significant alterations. Although CSF culture gave negative results for fungi and bacteria, VDRL test revealed a positive result. JC virus DNA was detected on CSF samples from these 3 patients, by real-time PCR and topoisomerase I modified nested-PCR. Conventional nested-PCR was also able to detect JC virus genome on CSF of patient #22.

Detection limits of JC virus genome with different amplification protocols

Testing of 10-fold serial dilution of DNA template from a positive CSF sample showed that realtime (Figure 1) and topoisomerase I modified nested-PCR protocols (Figure 2A) could detect dilutions equal to or less than 10^{-3}, whereas conventional nested-PCR (Figure 2B) was only able to detect dilutions equal or less than 10^{-2}.

Discussion

The human JC virus is considered to be the causal agent of PML. The incidence of this demyelinating disease of the CNS increased in recent years due to rising in the number of immunocompromised patients, specially those associated with HIV infection [4,15].

Initially, definitive PML diagnosis was dependent upon histophatological and virological evaluation of brain tissue obtained by brain biopsy. The risks associated with such invasive and traumatic procedure triggered the search for a less aggressive diagnostic method for this disease. Some authors assumed the proven value of the detection of JC virus DNA in CSF for the diagnosis of PML; and as consequence, brain biopsies have been gradually replaced in the diagnosis of this CNS

Detection of JC virus genome	**Detectable** (% of total samples)	**Undetectable** (% of total samples)	**Sensitivity** (relatively to clinic PML diagnosis)
Real-Time PCR	24 (18,6%)	105 (81,4%)	94%
Topoisomerase I Nested PCR	21 (16,3%)	108 (83,7%)	76%
Nested PCR	11 (8,5%)	118 (91,5%)	53%

Table 2: Summary of the results for JC virus DNA detection in CSF samples using real-time PCR, topoisomerase I modified nested-PCR and conventional nested-PCR.

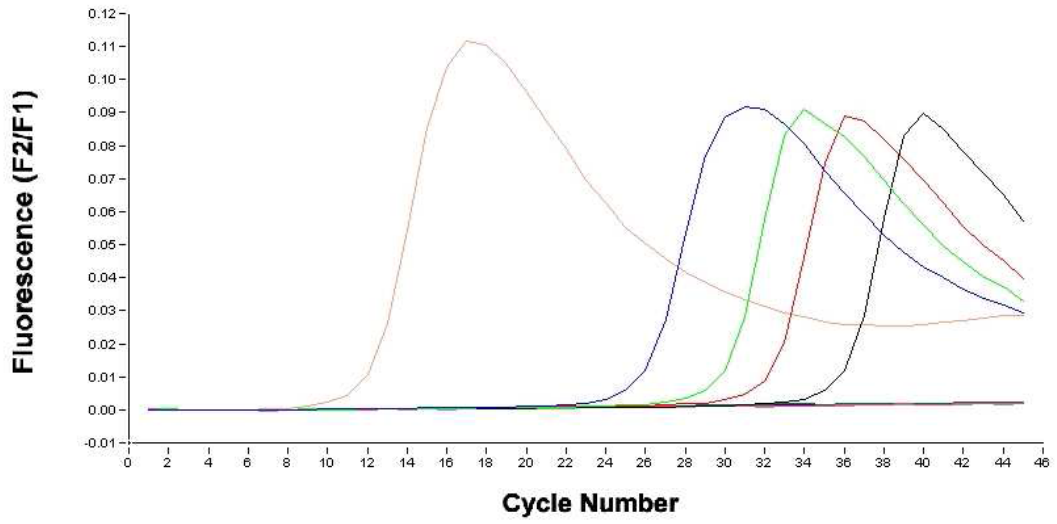

Figure 1: Real-time PCR amplification of 10-fold serial dilutions of a positive CSF sample (0 to 5 – decimal dilutions of positive CSF sample, from 10^0 to 10^{-5}; P – positive control).

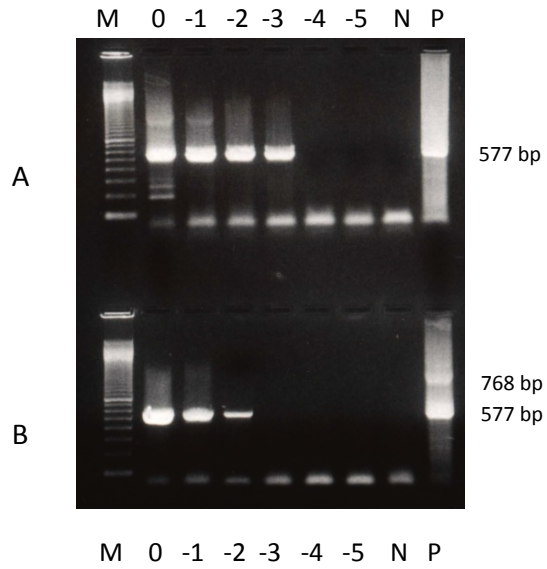

Figure 2: Electrophoresis of products from amplification of 10-fold serial dilutions of a positive CSF sample using topoisomerase I modified nested-PCR (A) and conventional nested-PCR (B) protocols. (M – molecular weight ladder; 0 to 5 – decimal dilutions of positive CSF sample, from 10^0 to 10^{-5}; N – negative control, P– positive control).

disease by molecular approaches, namely PCR amplification of DNA template molecules obtained from CSF samples [8,15].

Different conventional PCR-based strategies have demonstrated 74 to 92% sensitivity and 92 to 100% specificity, with greater sensitivity values correspond, in general, to PCR followed by Southern blot hybridization for detection of amplified products [19]. However, the use of radioactive compounds implicated in these detection methodologies, make it not well suited for use in many clinical laboratories. Real-time PCR technology represented a significant breakthrough in PCR amplification and amplicon detection, enabling the same specificity and sensitivity as the PCR-Southern blot method, with the advantage of using a more rapid and simple protocol, without the need of radionucleotides [8,15-17]. Nevertheless, real-time PCR experiments require specific and quite expensive equipment, which makes this highly specific and sensitive technique, not accessible to all clinical laboratories. Due to the crescent number of PML cases, as well as the need for an early diagnosis, the availability of a diagnostic confirmation method is imperious for such laboratories. In this order, the main objective of our study was the search for an alternative method for JC virus DNA detection in CSF samples, with sensitivity and specificity values close to that of standard techniques, but feasible at any clinical laboratory. Nested-PCR protocols have been used as an alternative method, since it presents better sensitivity and specificity than simple PCR amplification. However, some authors have hypothesized that JC virus supercoiled DNA topology could limit the efficiency of its PCR amplification [12-14], and suggested the treatment of DNA templates with topoisomerase I in order to obtain reproducible and more sensitive results [11].

Here, we evaluated the feasibility of treating DNA templates from CSF samples with topoisomerase I, before subjecting them to PCR amplification, and compared the results obtained with those of conventional nested-PCR as well as real-time PCR. Treatment of DNA with topoisomerase I was shown to be necessary to detect JC virus DNA in a greater number of CSF samples. In fact, comparing the results obtained by nested-PCR with and without pre-treatment with topoisomerase I, we observed a decrease in detectable JC virus DNA from 21 to 11 CSF samples. This is in accordance with the 10-fold greater relative sensitivity observed for topoisomerase I modified nested-PCR protocol, when compared to the conventional nested-PCR. Regarding the 10 CSF samples in which nested-PCR was unable to detect JC virus DNA, but the viral genome was detected after topoisomerase I treatment, perhaps they have a small number of viral particles, insufficient to generate enough linear DNA molecules to be efficiently amplified. These findings are in accordance with those from Laghi and collaborators [12], suggesting that supercoiled topology of JC virus DNA molecule could limit its efficient amplification, and that lower sensibility accompanied by inconsistent PCR results could result from analysis of intact supercoiled DNA.

The correlation of clinical notes (whenever it was available) with JC virus DNA detection on CSF samples confirms this hypothesis: topoisomerase I modified nested-PCR enabled the confirmation of PML diagnosis on 76% of patients with clinical signs and brain images

suggestive of such disease, while conventional nested-PCR only confirmed PML in 53% of those patients. Therefore, our results point to the requirement of using topoisomerase I treatment before nested-PCR amplification, in order to enhance the efficiency of the technique. All but one DNA template amplified with nested-PCR primers, also amplified with real-time primer set, and hibridized with FRET probes with the expected melting curve characteristic, which confirms the specificity of the amplified products by the nested-PCR and topoisomerase I modified nested-PCR protocols.

Real-time PCR was able to detect JC virus DNA in 24 CSF samples. Of these, topoisomerase I modified nested-PCR also detected JC virus DNA on 20, while the conventional nested-PCR only enabled the detection of JC virus genome on 11 CSF samples. Our results demonstrates that besides topoisomerase I modified nested PCR protocol was not able to detect JC virus DNA in all samples with detectable JC virus by real-time PCR, it is far most sensible than nested-PCR alone. The evaluation of 10-fold dilutions of a positive sample, confirm these results, once it showed that topoisomerase I modified nested-PCR and real-time PCR protocols presents a similar detection limit, which is 10 times lower then the one observed for conventional nested-PCR. The comparison of results generated by topoisomerase I modified nested-PCR and real-time PCR results corroborated the applicability of the first methodology, and proves that it presents PML diagnostic features closer to that of standard methodologies. The correlation of laboratory results with clinical records showed that real-time PCR presents high sensitivity when compared to conventional nested-PCR, which is in accordance with Elfaitouri and collaborators [15,16]. But also, that we could increase the sensitivity values of nested-PCR until values closer to that of real-time PCR, if prior to amplification reaction, we subject DNA template to treatment with topoisomerase I.

Conclusion

The results of the present study point towards the benefit of using topoisomerase I DNA treatment before amplification reactions in JC virus DNA detection on CSF samples, specially in samples presenting low viral loads. Nested-PCR amplification of DNA templates treated with topoisomerase I, and detection of amplified products by agarose gel electrophoresis, is a quite simple protocol, feasible at those clinical laboratories where it is not possible to use radioactive compounds to perform the Southern-blot hybridization reactions advisable for the evaluation of amplified products, nor have the equipment necessary to perform real-time PCR methodologies. In our opinion, topoisomerase I modified nested-PCR protocol represents a good alternative method to detect JC virus DNA in CSF samples from patients with clinical signs and brain images suggestive of PML. Using this methodology, all laboratories will be able to perform the confirmation technique needed for PML diagnosis.

References

1. Major EO (2001) Human polyomaviruses. In: Knipe DM, Howley PM, Griffin DE, Lam RA, Martin MA, Roizman B, Straus SE (ed.). Fields Virology. Philapdelphia: Lippincott Williams and Wilkins 2157-2196.
2. Matos A, Duque V, Beato S, da Silva JP, Major E, et al. (2010) Characterization of JC human polyomavirus infection in a Portuguese population. J Med Virol 82: 494-504.
3. Berenguer J, Miralles P, Arrizabalaga J, Ribera E, Dronda F, et al. (2003) Clinical course and prognostic factors of progressive multifocal leukoencephalopathy in patients treated with highly active antiretroviral therapy. Clin Infect Dis 36: 1047-1052.
4. Berger JR (2003) Progressive multifocal leukoencephalopathy in acquired immunodeficiency syndrome: explaining the high incidence and disproportionate frequency of the illness relative to other immunosuppressive conditions. J Neurovirol 9 Suppl 1: 38-41.
5. Giancola ML, Rizzi EB, Lorenzini P, Rovighi L, Baldini F, et al. (2008) Progressive multifocal leukoencephalopathy in HIV-infected patients in the era of HAART: radiological features at diagnosis and follow-up and correlation with clinical variables. AIDS Res Hum Retroviruses 24: 155-162.
6. Kraemer C, Evers S, Nolting T, Arendt G, Husstedt IW (2008) Cidofovir in combination with HAART and survival in AIDS-associated progressive multifocal leukoencephalopathy. J Neurol 255: 526-531.
7. Marzocchetti A, Di Giambenedetto S, Cingolani A, Ammassari A, Cauda R, et al. (2005) Reduced rate of diagnostic positive detection of JC virus DNA in cerebrospinal fluid in cases of suspected progressive multifocal leukoencephalopathy in the era of potent antiretroviral therapy. J Clin Microbiol 43: 4175-4177.
8. Ryschkewitsch C, Jensen P, Hou J, Fahle G, Fischer S, et al. (2004) Comparison of PCR-southern hybridization and quantitative real-time PCR for the detection of JC and BK viral nucleotide sequences in urine and cerebrospinal fluid. J Virol Methods 121: 217-221.
9. Hammarin AL, Bogdanovic G, Svedhem V, Pirskanen R, Morfeldt L, et al. (1996) Analysis of PCR as a tool for detection of JC virus DNA in cerebrospinal fluid for diagnosis of progressive multifocal leukoencephalopathy. J Clin Microbiol 34: 2929-2932.
10. de Viedma DG, Alonso R, Miralles P, Berenguer J, Rodriguez-Creixems M, Bouza E. 1999. Dual qualitative-quantitative nested PCR for detection of JC virus in cerebrospinal fluid: high potential for evaluation and monitoring of progressive multifocal leukoencephalopathy in AIDS patients receiving highly active antiretroviral therapy. J Clin Microbiol 37:724-728.
11. Laghi L, Randolph AE, Malesci A, Boland CR (2004) Constraints imposed by supercoiling on in vitro amplification of polyomavirus DNA. J Gen Virol 85: 3383-3388.
12. Laghi L, Randolph AE, Chauhan DP, Marra G, Major EO, et al. (1999) JC virus DNA is present in the mucosa of the human colon and in colorectal cancers. Proc Natl Acad Sci U S A 96: 7484-7489.
13. Ricciardiello L, Chang DK, Laghi L, Goel A, Chang CL, et al. (2001) Mad-1 is the exclusive JC virus strain present in the human colon, and its transcriptional control region has a deleted 98-base-pair sequence in colon cancer tissues. J Virol 75: 1996-2001.
14. Ricciardiello L, Laghi L, Ramamirtham P, Chang CL, Chang DK, et al. (2000) JC virus DNA sequences are frequently present in the human upper and lower gastrointestinal tract. Gastroenterology 119: 1228-1235.
15. McNees AL, White ZS, Zanwar P, Vilchez RA, Butel JS (2005) Specific and quantitative detection of human polyomaviruses BKV, JCV, and SV40 by real time PCR. J Clin Virol 34: 52-62.
16. Elfaitouri A, Hammarin AL, Blomberg J (2006) Quantitative real-time PCR assay for detection of human polyomavirus infection. J Virol Methods 135: 207-213.
17. Sehbani L, Kabamba-Mukadi B, Vandenbroucke AT, Bodéus M, Goubau P (2006) Specific and quantitative detection of human polyomaviruses BKV and JCV by LightCycler real-time PCR. J Clin Virol 36: 159-162.
18. Monaco MC, Atwood WJ, Gravell M, Tornatore CS, Major EO (1996) JC virus infection of hematopoietic progenitor cells, primary B lymphocytes, and tonsillar stromal cells: implications for viral latency. J Virol 70: 7004-7012.
19. Bossolasco S, Calori G, Moretti F, Boschini A, Bertelli D, et al. (2005) Prognostic significance of JC virus DNA levels in cerebrospinal fluid of patients with HIV-associated progressive multifocal leukoencephalopathy. Clin Infect Dis 40: 738-744.