Diagnostic Value of Detecting JC Virus DNA in Cerebrospinal Fluid of Patients with Progressive Multifocal Leukoencephalopathy

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JC virus DNA was detected by PCR in the cerebrospinal fluid of 17 of 23 (73.9%) patients with confirmed cases of progressive multifocal leukoencephalopathy and 2 of 48 (4.2%) controls without progressive multifocal leukoencephalopathy. The sensitivity and specificity of this PCR were 74 and 95.8%, respectively, while the positive and negative predictive values were 89.5 and 88.5%, respectively.

JC papovavirus (JCV) is a human polyomavirus that causes a severe demyelinating disease of the central nervous system (CNS), progressive multifocal leukoencephalopathy (PML). This disease is seen primarily in immunosuppressed patients and has been increasingly recognized since the AIDS epidemic began (2, 7). A diagnosis of PML can be suspected from the presence of primarily white matter lesions by computerized tomography or magnetic resonance imaging of the brain, but a brain biopsy is required for confirmation of the diagnosis (15).

PCR has been used to detect JCV DNA in brain tissue, peripheral blood lymphocytes, and cerebrospinal fluid (CSF) (1, 3, 5, 10, 13, 14) of immunocompromised and immunocompetent patients with and without PML. This study was implemented to determine the value of PCR for detecting JCV DNA in the CSF of patients with PML, most of whom had acquired AIDS also.

CSF specimens were obtained from the following three groups of patients: (i) AIDS patients and two other patients without human immunodeficiency virus (HIV) with PML confirmed by histopathology of brain tissue obtained by biopsy or autopsy; (ii) AIDS patients with no pathological or magnetic resonance imaging evidence of PML but with other neuro-pathological diseases, e.g., cryptococcal meningitis, toxoplasmosis, etc.; (iii) HIV-negative patients without evidence of PML but with other neurological diseases, e.g., brain tumor, meningitis, etc. For the purpose of analysis, the results of groups 2 and 3 were combined to represent the results of the control group.

CSF specimens were tested for the presence of the human polyomaviruses JC virus and BK virus by PCR with PEP1 and PEP2 primers, which produce a 173-bp ampler of JCV and a 176-bp ampler of BKV, as described previously (1). Briefly, unseparated CSF (cells and fluid) specimens (50 μl) were boiled for 10 min, and aliquots (10 μl each) were tested directly by PCR for 40 cycles of amplification which consisted of 1.5 min of denaturation at 94°C, 1.5 min of annealing at 55°C, and 2 min of extension at 72°C. The amplified product was analyzed by gel electrophoresis on 2% agarose with ethidium bromide staining, probed with fluorescein-11-dUTP-labelled JC virus cloned DNA (pMad-1-TC), and detected by enhanced chemiluminescence. JCV DNA was distinguished from BK virus DNA by BamHI restriction digestion of the amplified product, yielding fragments of 120 and 53 bp (1). To ensure that all positive specimens were detected, all CSF specimens were tested a second time by the same PCR protocol but with a slightly different specimen preparation procedure. For the second PCR, 0.3 ml of CSF was treated by the method of Moret et al. (10) to selectively precipitate DNA from the specimen without chloroform-phenol extraction. This method involved boiling a 0.3-ml aliquot of CSF for 5 min with 1 μg of carrier salmon sperm DNA and precipitation with sodium acetate-ethanol. Pellets were resuspended in 30 μl of water, and 10 μl was used for PCR.

Three positive controls (1, 10, and 100 fg of DNA) and two negative controls were run with each PCR. Positives were retested blindly. The sensitivity of PEP1 and PEP2 in our hands is 10 fg of JCV DNA (10). The specificity of the PEP1 and PEP2 primers has been confirmed by Arthur et al. (1). Special precautions were taken to minimize contamination of specimens by amplified product (8, 9).

95% confidence intervals (CI) were derived for all results expressed as rates or proportions. The sensitivity, specificity, and positive and negative predictive values of this test were calculated according to the method of Fleiss (4).

CSF samples from 23 patients with confirmed cases of PML and from 48 controls were examined. Twenty-one control patients were HIV infected and had various neurological diseases (Table 1). Twenty-seven controls were not HIV infected and had the neurological conditions listed in Table 1. Two of the patients with histologically confirmed cases of PML were not HIV infected. One had sarcomiosis and was treated with steroids, and the second had congenital immunodeficiency syndrome (previously reported [12]). The remaining 21 PML patients had AIDS. Seventeen (73.9%) of 23 CSF specimens from patients with PML were positive for JCV DNA (95% CI, 56 to 92%). However, three (17.6%) of these samples were weakly positive and were detected only after DNA hybridization of PCR products. In the control group, 2 (4.2%) of 48 samples were positive for JCV DNA (95% CI, 0 to 10%). Further testing confirmed that these were true positives and were not due to contamination. Both of these were detected only by DNA probing of PCR products. One of these patients...
had AIDS with cytomegalovirus encephalitis on brain biopsy, but no autopsy was obtained. The second patient had glialosis of the brain on biopsy, but the HIV serology was negative, although he had AIDS with cytomegalovirus encephalitis on brain biopsy. Cross-contamination was excluded because repeat testing of the original specimens was also positive, and strict containment procedures were used to avoid contamination. It is conceivable that these two patients had subclinical JCV infection of the CNS or had a few focal lesions of PML which were missed on biopsy. Even with these two false-positive cases, the specificity of the test (96.9%) was excellent.

In summary, CSF examination for JCV DNA by PCR is a useful test for confirming the diagnosis of PML. A negative test, however, does not exclude this diagnosis, and brain biopsy should be reserved for cases with suspicious white matter lesions on computerized tomography scan or magnetic resonance imaging in which JCV is not detected by PCR.

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