Journal of Clinical Microbiology, Feb. 1995, p. 484–486 Vol. 33, No. 2
0095-1137/95/$04.00+0
Copyright © 1995, American Society for Microbiology

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

I. W. FONG,[1]* C. B. BRITTON,[2] K. E. LUINSTRA,[3] E. TOMA,[4] and J. B. MAHONY[3]

Department of Medicine, St. Michael's Hospital, University of Toronto, Toronto, Ontario,[1] Department of Pathology and Regional Virology and Chlamydiology Laboratory, St. Joseph's Hospital, McMaster University, Hamilton, Ontario,[3] and Department of Microbiology & Infectious Diseases, Hotel Dieu de Montreal, Montreal, Quebec,[4] Canada, and Neurological Institute, Columbia University, New York, New York[2]

Received 28 July 1994/Returned for modification 3 October 1994/Accepted 22 November 1994

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 neuropathological 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 amplimer of JCV and a 176-bp amplimer 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 JCV-cloned DNA (pMad-1-TC), and detected by enhanced chemiluminescence. JCV DNA was distinguished from BK virus DNA by *Bam*HI 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 sarcoidosis 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

* Corresponding author. Mailing address: St. Michael's Hospital, 30 Bond St., Toronto, Ontario M5B 1W8, Canada. Phone: (416) 864-5746. Fax: (416) 864-5870.

TABLE 1. CNS diseases of control patients without PML

Condition (*n*)	No. of patients
HIV or AIDS related (21)	
Cryptococcal meningitis	2
AIDS encephalopathy	3
Disseminated tuberculosis or meningitis	3
HIV psychosis	1
CNS toxoplasmosis	2
Cerebrovascular accident	1
Cytomegalovirus encephalitis	2
HIV aseptic meningitis	1
Lymphoma	4
Neurosyphilis	1
Transverse myelitis	1
Non-HIV related (27)	
Head injury	4
Dementia	3
Spinal cord compression	2
Seizures	1
Aseptic meningitis	2
Multiple sclerosis	1
Idiopathic gliosis of brain	1
Guillain-Barré syndrome	3
Hydrocephalus	3
Cerebrovascular accident	2
Severe migraine or headache	2
Lymphoma	1
Epidural tumor	1
Spinal stenosis	1

had AIDS with cytomegalovirus encephalitis on brain biopsy, but no autopsy was obtained. The second patient had gliosis of the brain on biopsy, but the HIV serology was negative, although his $CD4^+$ lymphocyte count was 260 cells per mm^3. The sensitivity of PCR for detecting JCV DNA in CSF from patients with confirmed cases of PML was 74% (95% CI, 56 to 92%). The specificity was 95.8% (95% CI, 90 to 100%). The positive predictive value was 89.5% (95% CI, 77 to 100%), and the negative predictive value was 88.5% (95% CI, 80 to 100%).

In this study, we evaluated PCR testing of CSF for JCV DNA by using confirmed PML cases and showed that this approach is useful for confirming this diagnosis; nevertheless, it missed one-fourth of the cases. PCR is a very sensitive method for the detection of JCV DNA. Previous studies have reported the detection of JCV DNA in peripheral blood lymphocytes (14) and brain tissue (11, 17) of HIV-positive and -negative patients with and without PML, but they lacked specificity for the diagnosis of PML.

The results of our study indicate that PCR detection of JCV DNA in CSF is moderately sensitive but highly specific. We used PEP1 and PEP2 primers and PCR targeted to the early region large T antigen, with a reported sensitivity of 100 genome copies after 40 cycles of amplification (1). Our results are similar to those of Gibson et al. (5), who found that CSF from 10 of 13 (76.9%) patients with PML was positive for JCV DNA by PCR, while CSF from 0 of 42 controls was positive. Moret et al. (10) also detected JCV in CSF from 9 of 12 (75%) patients with clinical PML (using the same primers we used), but the diagnosis was confirmed histopathologically for only 4 patients. Similarly, Weber et al. (16) recently reported that PCR was 82% (23 of 28) sensitive and 100% specific by using the primer pair JC36 and JC39 and targeting the large T region with the addition of carrier DNA before extraction. In the study by Weber et al., only 13 of 28 PML cases were confirmed by histopathology, whereas all 23 of our cases were confirmed cases of PML. In contrast to our results, a previous report by Tornatore et al. (14) found that only 3 of 10 patients with PML had JCV in their CSF and Henson et al. (6) detected JCV in only one of six CSF samples from PML patients. The variability in these results may be due to methodological differences or alternatively to differences in the patients studied. To ensure that no true positives were missed, we retested all specimens by PCR by using a different method of specimen preparation (10), and the results were the same.

In our study, 2 of 48 CSF samples from controls were positive for JCV; one of these patients had AIDS and 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%) 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.

We are grateful to the following physicians for sending us CSF samples for analysis: P. Mueller, St. Michael's Hospital, Toronto, Canada; S. Walmsley, The Toronto Hospital, Toronto, Canada; and J. Gill, Foothills Hospital, Calgary, Canada. We are also grateful to D. Bajhan for her assistance in preparation of the manuscript.

REFERENCES

1. **Arthur, R. R., S. Dagostin, and K. V. Shah.** 1989. Detection of BK virus and JC virus in urine and brain tissue by the polymerase chain reaction. J. Clin. Microbiol. **27:**1174–1179.
2. **Berger, J. R., B. Kaszovitz, J. D. Post, and G. Dickinson.** 1987. Progressive multifocal leukoencephalopathy associated with human immunodeficiency virus infection. Ann. Intern. Med. **107:**78–87.
3. **Brouqui, P., C. Bollet, J. Delmont, and A. Bourgeade.** 1992. Diagnosis of progressive multifocal leucoencephalopathy by PCR detection of JC virus from CSF. Lancet **339:**1182.
4. **Fleiss, J. L.** 1981. Statistical methods for rates and proportions, 2nd ed., p. 218. John Wiley, New York.
5. **Gibson, P. E., W. A. Knowles, J. F. Hand, and D. W. G. Brown.** 1993. Detection of JC virus DNA in the cerebrospinal fluid of patients with progressive multifocal leukoencephalopathy. J. Med. Virol. **39:**278–281.
6. **Henson, J., M. Rosenblum, D. Armstrong, and H. Furneaux.** 1991. Amplification of JC virus DNA from brain and cerebrospinal fluid of patients with progressive multifocal leukoencephalopathy. Neurology **41:**1967–1971.
7. **Holman, R. C., R. S. Janssen, J. W. Buehler, M. T. Zelasky, and W. C. Hooper.** 1991. Epidemiology of progressive multifocal leukoencephalopathy in the United States: analysis of national mortality and AIDS surveillance data. Neurology **41:**1733–1736.
8. **Kwok, S., and R. Higuchi.** 1989. Avoiding false positives with PCR. Nature (London) **339:**237–238.
9. **Mahony, J. B., K. E. Luinstra, J. W. Sellors, D. Jang, and M. A. Chernesky.** 1992. Confirmatory polymerase chain reaction testing for *Chlamydia trachomatis* in first-void urine from asymptomatic and symptomatic men. J. Clin. Microbiol. **30:**2241–2245.
10. **Moret, H., M. Guichard, S. Matheron, C. Katlama, V. Sazdovitch, J.-M. Huraux, and D. Ingrand.** 1993. Virological diagnosis of progressive multifocal leukoencephalopathy: detection of JC virus DNA in cerebrospinal fluid and brain tissue of AIDS patients. J. Clin. Microbiol. **31:**3310–3313.
11. **Quinlivan, E. B., M. Norris, T. W. Bouldin, K. Suzuki, R. Meeker, M. S. Smith, C. Hall, and S. Kennedy.** 1992. Subclinical central nervous system infection with JC virus in patients with AIDS. J. Infect. Dis. **166:**80–85.
12. **Redfearn, A., R. A. Pennie, J. B. Mahony, and P. B. Dent.** 1993. Progressive multifocal leukoencephalopathy in a child with immunodeficiency and hyperimmunoglobulinemia M. Pediatr. Infect. Dis. J. **12:**399–401.
13. **Telenti, A., W. F. Marshall, A. J. Aksamit, J. D. Smilack, and T. F. Smith.** 1992. Detection of JC virus by polymerase chain reaction in cerebrospinal fluid from two patients with progressive multifocal leukoencephalopathy. Eur. J. Clin. Microbiol. Infect. Dis. **11:**253–254.
14. **Tornatore, C., J. R. Berger, S. A. Houff, B. Curfman, K. Meyers, D. Winfield,**

and E. O. Major. 1992. Detection of JC virus DNA in peripheral lymphocytes from patients with and without progressive multifocal leukoencephalopathy. Ann. Neurol. **31:**454–462.

15. **Trotot, P. M., R. Vazeux, H. K. Yamashita, C. Sandoz-Tronca, J. Mikol, C. Vedrenne, J. B. Thiébaut, F. Gray, M. Cikurel, G. Pialoux, and R. Levillain.** 1990. MRI pattern of progressive multifocal leukoencephalopathy (PML) in AIDS. J. Neuroradiol. **17:**233–254.
16. **Weber, T., R. W. Turner, S. Frye, B. Ruf, J. Haas, E. Schielke, H.-D. Pohle, W. Lüke, W. Lüer, K. Felgenhauer, and G. Hunsmann.** 1994. Specific diagnosis of progressive multifocal leukoencephalopathy by polymerase chain reaction. J. Infect. Dis. **169:**1138–1141.
17. **White, F. A., III, M. Ishaq, G. L. Stoner, and R. J. Frisque.** 1992. JC virus DNA is present in many human brain samples from patients without progressive multifocal leukoencephalopathy. J. Virol. **66:**5726–5734.