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Diagnostic Utility of a Multiplex Herpesvirus PCR Assay Performed with Cerebrospinal Fluid from Human Immunodeficiency Virus-Infected Patients with Neurological Disorders

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We used a multiplex nested-PCR assay for the simultaneous detection in cerebrospinal fluid (CSF) of five human herpesviruses (HVs) (cytomegalovirus [CMV], Epstein-Barr virus [EBV], varicella-zoster virus [VZV], herpes simplex virus [HSV], and human herpesvirus 6 [HHV-6]) in a clinical evaluation of human immunodeficiency virus (HIV)-infected patients with neurological disorders. This method, which has the advantages of being rapid and economical, would be of particular interest for the diagnosis of neurological syndromes caused by more than one HV. We studied 251 CSF samples from 219 patients. HV DNA was demonstrated in 93 (37%) of the CSF samples (34% of the patients). CMV was the HV most frequently detected in our patients (25%), while EBV, VZV, HSV, and HHV-6 DNAs were present in significantly fewer cases (7, 4, 3, and 1%, respectively). When results were compared with the final etiological diagnoses of the patients, the multiplex HV PCR showed high specificity for the diagnosis of CMV and VZV neurological diseases and for cerebral lymphoma (0.95, 0.97, and 0.99, respectively). The sensitivity of the assay was high for CMV disease (0.87), was low for cerebral lymphoma (0.33), and was not evaluable for VZV disease due to the small number of patients with this diagnosis. Nevertheless, detection of VZV DNA had possible diagnostic value in four of the nine cases, and EBV DNA amplification always predicted the diagnosis of cerebral lymphoma in patients with cerebral masses. Detection of HSV DNA was frequently associated with CMV amplification and fatal encephalitis. HHV-6 was not considered to have a pathogenetic role in the three cases in which it was detected. This multiplex HV PCR assay is a specific and clinically useful method for the evaluation of HIV-infected patients with neurological disorders related to HV.

Neurological complications are common in human immunodeficiency virus (HIV)-infected patients. The differential diagnosis includes a wide spectrum of opportunistic infections and tumors, as well as vascular and metabolic complications. In addition, HIV itself can be an important cause of neurological disorders. Unfortunately, clinical, cerebrospinal fluid (CSF), and neuroimaging findings are frequently nonspecific. Furthermore, routine CSF tests such as viral cultures have a very low diagnostic yield. Given these difficulties, it is not surprising that quite frequently a definitive diagnosis has been established only after cerebral biopsy or postmortem examination. In recent years, PCR analysis of CSF has contributed substantially to improving the diagnosis and clinical management of central nervous system (CNS) complications in HIV-infected patients (1, 10).

The human herpesviruses (HVs) are an important cause of CNS disease in HIV-infected patients. Cytomegalovirus (CMV), varicella-zoster virus (VZV), and herpes simplex virus (HSV) types 1 and 2 can cause encephalitis, meningitis, myelitis, or polyradiculitis (13). Epstein-Barr virus (EBV) DNA has been demonstrated by in situ hybridization in virtually all AIDS-related primary CNS lymphomas (PCNSLs). In AIDS patients, CSF PCR has been shown to be a sensitive and specific method for the diagnosis of CNS diseases caused by HVs, particularly HSV and CMV (10, 12, 16). Detection of EBV DNA in CSF has been also useful for the diagnosis of PCNSLs (3, 7, 9, 14).

Infections caused by different HVs may be clinically indistinguishable in AIDS patients, and simultaneous CNS infections caused by different HVs have occasionally been described (12, 18, 19, 23, 24). A multiplex PCR assay with the ability to detect simultaneously different HVs in CSF might therefore be a useful and practical approach to the differential diagnosis of CNS disorders in HIV-infected patients. It offers the advantages of rapidity and economy, particularly in cases in which more than one HV may be etiologically involved. It also offers the possibility of detecting coinfections with several HVs.

We have reviewed retrospectively our experience with the use of a multiplex HV PCR (MHV-PCR) assay for the simultaneous detection and typing of human HVs in the CSF of HIV-infected patients with neurological disorders. The goals

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of our study were (i) to assess the diagnostic utility of an MHV-PCR technique for the diagnosis of HV-related disorders in HIV-infected patients and (ii) to evaluate the relative roles of different HVs as causative agents of neurological complications in HIV-infected patients.

MATERIALS AND METHODS

Patients and samples. Two hundred fifty-one CSF samples from 219 HIV-infected patients referred for neurological evaluation were studied. The patients had been monitored in the Infectious Diseases Units of Hospital Ramón y Cajal and Hospital Carlos III in Madrid, Spain, between May 1992 and January 1998. Twenty-seven patients had more than one CSF sample processed (23 had two samples, 3 had three samples, and 1 had four specimens). Six patients had CSF examinations for two different neurological disorders. All patients were monitored until death or are currently attending the outpatient clinics of the above-listed hospitals.

Aliquots of all CSF samples were sent to the Centro Nacional de Microbiología for PCR assay. Additional CSF specimens were processed at each patient's hospital for bacterial, mycobacterial, and fungal cultures. When indicated, tests for syphilis, *Brucella*, or *Cryptococcus neoformans* polysaccharide antigen or cytological examinations were performed on CSF. One hundred eighteen CSF samples were processed for viral culture.

An etiological diagnosis, based on strict preestablished diagnostic criteria that did not include the PCR results, was assigned for each patient. The criteria used for etiological diagnosis included past medical history, clinical presentation, neurological exam, CSF and neuroradiologic findings, and short- and long-term clinical course, including response to specific treatments. HIV-related disorders were included in the diagnosis only if they were the reason for CSF examination, because many patients with other neurological diseases had a previous history of HIV-associated sensory neuropathy or encephalopathy. We will use the term "case" for each CSF sample with its corresponding patient's diagnosis.

Diagnostic criteria. Etiological diagnoses were further classified as probable, definite, or uncertain. In several patients a neurological disease was excluded after diagnostic evaluation.

An etiological diagnosis was classified as definite when (i) a clinically suspected CNS disease was confirmed by CSF culture or cytology, CNS biopsy, or postmortem examination; or (ii) the patient presented with a neurological disorder characteristic of a certain pathogen and had a documented systemic disease caused by the same pathogen, with response to specific treatment and exclusion of other possible etiologies. The only exception to the requirement for documentation of systemic disease was cerebral toxoplasmosis. Only the following disorders were considered characteristic of CMV CNS disease: lumbosacral polyradiculitis, multiplex mononeuritis, and encephalitis with periventriculitis or with symptoms or signs of brain stem involvement. For the diagnosis of VZV CNS disease, only lymphocytic meningitis, acute segmental myelopathy, and acute encephalitis were considered characteristic and only patients with concomitant cutaneous zoster were included.

An etiological diagnosis was classified as probable when it fulfilled the following diagnostic criteria (after exclusion of other possible etiologies): (i) for CMV disease, presentation with a neurological disease characteristic of CMV infection of the CNS by a patient with documented systemic disease caused by CMV; (ii) for VZV disease, presentation with a neurological disease characteristic of VZV infection of the CNS in a patient with concomitant cutaneous zoster; (iii) for HSV disease, presentation with focal encephalitis clinically and radiologically suggestive of HSV encephalitis, with response to acyclovir; (iv) for cerebral lymphoma, presentation with a mass lesion clinically and radiologically suggestive of CNS lymphoma (positive single-photon emission computed tomography with ^{201}Tl when performed), nonresponsive to antitoxoplasma treatment, or meningeal carcinomatosis in a patient with documented systemic lymphoma; (v) for progressive multifocal leukoencephalopathy, presentation with a progressive subacute focal or multifocal neurological disorder with characteristic nonenhancing demyelinating lesions apparent on magnetic resonance imaging; (vi) for HIV-related disorders, presentation with a neurological disease fulfilling established criteria for such disorders (26); and (vii) for other CNS opportunistic infections, presentation with a neurological disease with clinical, radiological, and CSF findings characteristic of a certain pathogen in a patient with documented systemic disease by the same pathogen or responsive to specific treatment.

Patients not fulfilling the criteria for definite or probable diagnosis, or with two or more probable diagnoses, were classified as having uncertain diagnoses, while those without organic neurological disease or with toxic and metabolic disorders were said to have a diagnosis of nonneurological disease.

The sensitivity, specificity, and predictive values of the PCR assay were calculated according to these diagnoses. For these calculations we excluded cases with an uncertain diagnosis and combined cases with a definite or probable diagnosis. To avoid the possibility of bias, only the PCR result for the first CSF sample was considered when there were two or more CSF examinations in the course of one neurological disorder.

MHV-PCR assay. HV DNA was detected by a multiplex nested-PCR method essentially as previously described (22). For the first amplification, two equimolar mixtures of nondegenerative oligonucleotides were used as primers, whose 3′ ends were aligned with selected consensus regions from the herpesviral DNA polymerase genes. The specific fragments obtained were the substrates for a second, multiplex reaction, for which primers were designed to produce differently sized fragments for each related virus.

To avoid false-positive PCR results due to carryover contamination, clinical-specimen aliquoting, preparation of reagents, DNA extraction, first-round amplification, and the nested PCR were performed in safety cabinets located in separate laboratories, all of them far away from the area where amplified products were analyzed. Each cabinet was equipped with an independent batch of reagents, a set of micropipettes, sterile reagent tubes, and filtered pipette tips. A negative control (H_2O) was included in each PCR run. To prevent false-negative results, a total of 100 molecules of a cloned and purified fragment of the DNA polymerase gene of pseudorabies virus, a nonhuman HV, was added to each extraction tube as an internal control with the aim of both checking the efficiency of DNA extraction and determining the presence of possible inhibitors of amplification in samples. A weak-positive control (sensitivity control), consisting of 100 molecules of cloned and purified genome fragments of the DNA polymerase gene of each human HV, was also included in a single reaction tube. Positive results were confirmed by testing a second fresh aliquot of the same specimen. Only when concordant results were obtained in both aliquots was the specimen considered positive.

Specimen DNA was extracted by proteinase K treatment and heat inactivation as previously described (22). For the first amplification step, 5-μl aliquots of DNA extracts from the clinical specimen were added to a reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 4 mM $MgCl_2$, 200 μM each deoxynucleoside triphosphate, 10 pmol of each (forward and reverse) primer for each HV, and 1.25 U of *Taq* polymerase (total volume, 50 μl). Conditions for this amplification, which was carried out in a PTC-200 thermal cycler (MJ Research, Watertown, Mass.), were as follows: one cycle of 94°C for 2 min; 30 cycles of 94°C for 30 s, 53°C for 1 min, and 72°C for 30 s; and one cycle of 72°C for 5 min. After the first amplification round, 1 μl of the reaction product was added to 49 μl of a second reaction mixture consisting of 60 mM Tris-HCl (pH 8.5), 15 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 200 μM each deoxynucleoside triphosphate, 10 pmol of each (forward and reverse) primer for each HV, and 1.25 U of *Taq* polymerase. Amplifications were performed under the same conditions as were used for the first amplification round, except that the annealing temperature was 47°C. A total of 10 μl of each second-round amplification product was electrophoresed through a 4% high-resolution agarose gel in 0.5× Tris-borate-EDTA buffer and stained with ethidium bromide (0.5 μg/ml).

Statistical analysis of the data was done by means of Fisher's exact test.

RESULTS

Characteristics of patients. The study included 219 patients. Their mean age was 34 years (range, 22 to 59), and 78% were males. The major risk factors for HIV infection were intravenous drug abuse (72%) and unprotected homosexual contact (15%). A CD4 lymphocyte count obtained close to the time of presentation with neurological symptoms was available for 210 patients. The median CD4 count was 25 cells/mm^3 (range, 1 to 783). Only 7% of patients had CD4 lymphocyte counts over 200. Most patients (75%) were at stage C3 of HIV infection according to the Centers for Disease Control and Prevention classification system. Less than 5% of the patients were receiving highly active antiretroviral therapy at the time of presentation with neurological symptoms.

Neurological etiological diagnoses of the patients were classified into three groups (Tables 1 to 3): group A, HV-related diseases (51 cases; 32 with CMV disease, 4 with VZV disease, 14 with CNS lymphoma, and 1 with simultaneous CMV disease and lymphoma); group B, non-HV-related diseases, including cases with a final diagnosis of nonneurological disease (115 cases); and group C, uncertain diagnosis (85 cases). In 25 patients the etiological diagnosis was confirmed pathologically or by CSF culture or cytology. Five additional patients had a definite diagnosis of cerebral toxoplasmosis. Only 2 of the 118 CSF samples cultured for viruses yielded a positive result (CMV in both cases).

PCR results. HV DNA was demonstrated by PCR in 93 (37%) of the 251 CSF samples and in 74 (34%) of the 219 patients. In 13 cases there was amplification of DNA from two different HVs. PCR was negative for 102 of 115 CSF specimens

TABLE 1. Group A: amplification of DNA from patients with HV-related diseases, according to etiological diagnosis and neurological presentation

Etiological diagnosis[a]	Neurological syndrome(s)[b]	DNA amplified[b]
CMV (12/20)	Encephalitis (16)	CMV (14), CMV + HHV-6 (1)
	Polyradiculitis and encephalitis (3)	CMV (1), CMV + HSV (1), CMV + EBV (1)
	Polyradiculitis (12)	CMV (10)
	Mononeuritis multiplex (1)	—[c]
VZV (1/3)	Meningitis (1)	—
	Encephalitis (2)	VZV (1)
	Myelitis (1)	VZV (1)
Cerebral lymphoma (7/7)	Mass lesion (9)	EBV (4), EBV + CMV (1), CMV (2)
	Meningitis (5)	EBV (1)
Cerebral lymphoma (probable) and cytomegalovirus (confirmed)	Mass lesion and encephalitis (1)	CMV (1)

[a] Values in parentheses are numbers of cases with a definite diagnosis/numbers of cases with a probable diagnosis.
[b] Values in parentheses are numbers of cases.
[c] —, no DNA amplified.

from patients with a diagnosis not related to HV (group B), which gives a global specificity of 0.89 (95% confidence interval [CI], 0.85 to 0.93). This figure was 0.9 (95% CI, 0.86 to 0.94) when only the first CSF sample of each patient was considered. The sensitivity, specificity, predictive values, and likelihood ratios of the test for CMV and VZV neurological disease and for CNS lymphoma, as well as the prevalence of these diseases among the 156 patients with an established etiological diagnosis, are presented in Table 4.

(i) CMV amplification. CMV DNA was amplified from 65 CSF samples obtained from 54 patients (26% of samples and 25% of patients). CMV DNA was amplified from 28 of the 32 CSF samples obtained from patients with neurological CMV disease and from one patient with lymphoma and neurological CMV disease (Table 1). CMV DNA was also amplified from 8 of 135 CSF specimens obtained from the 125 patients whose final diagnoses were not CMV disease (Tables 1 and 2). Of these eight CSF samples, seven came from six patients with documented extraneurological disease (three patients with CMV retinitis) or infection (two patients with positive CMV urine cultures and one whose blood was positive for CMV antigenemia), including two from a patient with cryptococcal meningitis who had polyradicular involvement and who developed encephalitic signs in the late phase of the disease. Thus, it is possible that this patient had concomitant CMV and cryptococcal disease, and consequently the specificity and positive predictive value of the test for CMV disease might be slightly higher than the calculated values.

CMV DNA was detected in 28 of 85 cases of uncertain etiology (group C) (Table 3). Of the 28 cases, 13 had encephalitis (8 of them with signs of brain stem involvement), 5 had polyradiculitis, 2 had both polyradiculitis and encephalitis, and 2 had mononeuritis multiplex. Other clinical presentations of patients with a positive CMV CSF PCR result were subacute dementia (two cases), acute myelopathy (one case), and unspecific encephalopathy (three cases). Thus, in 17 of these cases, the clinical presentation was highly suggestive of CMV neurological disease but the diagnosis was considered uncertain according to our criteria because of the lack of documented systemic CMV disease. Another five cases had systemic CMV disease, but the associated neurological syndrome was considered not characteristic of CMV neurological disease. In nine additional cases there was evidence of systemic CMV infection (CMV was detected by culture or antigenemia in blood in eight cases and in urine in one case). Eleven patients with an uncertain etiological diagnosis and whose

TABLE 2. Group B: amplification of DNA from patients with specific diagnoses not related to HVs, according to etiological diagnosis and neurological presentation

Etiological diagnosis[a]	Neurological syndrome(s)[b]	DNA amplified
HIV associated (0/40)	Dementia (25)	EBV (1)
	Chronic myelopathy (8)	CMV + HSV (1)
	Neuropathy (7)	HHV-6 (1)
PML[c] (1/22)	Focal leukoencephalopathy (23)	CMV (1)
Tuberculosis (7/4)	Meningitis (11)	EBV (2), VZV + EBV (1)
Toxoplasmosis (5/0)	Brain abscess (5)	CMV (1)
Cryptococcosis (8/0)	Meningitis (6)	VZV (1)
	Meningitis and polyradiculitis (2)	CMV + HSV (1), CMV (1)
Candida albicans (1/0)	Meningitis	EBV + HHV-6 (1)
Listeria monocytogenes (1/0)	Meningitis	HSV (1)
Spinal astrocytoma (2/0)	Chronic myelopathy (2)	—[d]
Nonneurological disease (24/0)	Seizures (13)	—
	Sepsis (9)	—
	Tension headache (1)	—
	Myalgia (1)	—

[a] Values in parentheses are numbers of cases with a definite diagnosis/numbers of cases with a probable diagnosis.
[b] Values in parentheses are numbers of cases.
[c] PML, progressive multifocal leukoencephalopathy.
[d] —, no DNA amplified.

TABLE 3. Group C: amplification of DNA from patients with uncertain diagnoses, according to neurological presentation

Neurological syndrome(s)[a]	DNA amplified[a]
Encephalitis (18)	CMV (9), CMV + HSV (3)
Encephalitis and polyradiculitis (2)	CMV (2)
Polyradiculitis (11)	CMV (5)
Leukoencephalopathy (7)	EBV (1)
Subarachnoid hemorrhage (3)	—[b]
Dementia (3)	CMV (1), CMV + VZV (1), EBV (1)
Nonspecific encephalopathy (17)	CMV (3), VZV (3), EBV (2)
Neuropathy (8)	CMV (2), VZV (1)
Mass lesion and encephalitis (1)	CMV + EBV (1)
Mass lesion (2)	—
Cerebral infarction (2)	VZV (1)
Meningitis (2)	—
Trigeminal neuralgia (1)	—
Chronic myelopathy (4)	VZV (4)
Acute myelopathy (2)	CMV (1)
Optic neuropathy (2)	—

[a] Values in parentheses are numbers of cases.
[b] —, no DNA amplified.

CSFs exhibited CMV amplification received anti-CMV treatment, and four of them improved clinically.

CMV DNA amplification from CSF was highly significantly associated with a previous history of systemic CMV disease or infection ($P < 0.001$). CMV DNA was amplified in 47 (59%) of the 80 samples from patients with documented extraneurological CMV disease or infection. However, of 17 cases with diagnoses different from neurological CMV disease but with a previously diagnosed systemic CMV disease (9 cases) or infection (8 cases), PCR was positive for CMV in only four. This proportion of amplification did not differ significantly from that of patients without systemic CMV disease or infection (17 of 154). In patients with known CMV disease, the probability of CMV amplification in CSF was not influenced by previous antiviral maintenance therapy.

(ii) EBV amplification. EBV DNA was amplified in 17 CSF samples (7%) from 15 patients (7%). It was detected in 6 of 15 CSF samples from 12 patients with a diagnosis of definite or probable cerebral lymphoma or lymphomatous meningitis (Table 1). One patient with a pathologically proven PCNSL had three consecutive CSF samples from which EBV DNA was amplified. EBV DNA was also amplified from CSF specimens obtained from 6 of 151 cases with other diagnoses (Tables 1 and 2) and of 5 cases with uncertain diagnoses (Table 3). In only one of these cases was the neurological disorder a cerebral mass.

When we considered exclusively patients with contrast-enhancing intracranial mass lesions detected on neuroimaging, EBV DNA was amplified in 5 of 10 CSF samples (three of eight patients) with probable or confirmed PCNSL and in none of the five cases of cerebral toxoplasmosis (positive predictive value, 1).

(iii) VZV amplification. VZV DNA was detected in 14 (6%) samples from nine (4%) patients. VZV DNA was amplified in 2 of 4 cases of VZV disease (Table 1), in 2 of 164 cases with non-VZV-related diagnoses (Tables 1 and 2), and in 10 cases (five patients) with uncertain diagnoses (Table 3). Two of these patients had neurological syndromes that may have been caused by VZV: one patient had multiple cerebral infarctions, and another (four CSF samples) had a chronic nonsegmentary myelopathy temporarily associated with recurrent thoracic zoster. The other three patients had nonspecific syndromes: peripheral neuropathy, nonspecific encephalopathy, and dementia. Overall, VZV amplification had a possible diagnostic value for four of the nine patients in whom it was detected.

There was a correlation between a positive PCR for VZV in CSF and a history of zoster ($P = 0.0024$). This association was stronger if the zoster had occurred concurrently with or in the 3 months previous to the neurological evaluation ($P < 0.001$). Nevertheless, in patients with previous or concurrent zoster but with non-VZV-related neurological diagnoses, VZV DNA was not detected more frequently than in cases without previous herpetic lesions, even if only patients with recent or concurrent cutaneous zoster were considered.

(iv) HSV amplification. No patient had a clinical diagnosis of HSV-related neurological disease. HSV was detected in seven cases (3%). In three patients there was another clinical etiological diagnosis. HSV was amplified together with CMV in four additional patients with encephalitis. These four patients died from the encephalitic process soon after the CSF examination, and three of them had had a previous CSF sample from which CMV was amplified.

(v) HHV-6 amplification. The human herpesvirus 6 (HHV-6) genome was detected in three patients (1%), always without apparent clinical significance.

(vi) Double amplification. In 13 cases (5.2%), DNAs from two different HVs were simultaneously amplified. The cases of CMV and HSV coamplification have already been described. CMV and EBV were detected in a patient with cerebral lymphoma, in a patient with CMV polyradiculitis and encephalitis, and in a patient with encephalitis with brain stem involvement and a concomitant cerebral mass of uncertain etiology. CMV and HHV-6 were amplified in a case of probable CMV encephalitis. CMV and VZV were detected in a patient with a nonspecific encephalopathy of uncertain etiology who had simultaneous CMV retinitis. EBV–HHV-6 and EBV-VZV were amplified in two patients with candidal and tuberculous meningitis, respectively.

(vii) Reproducibility. Of 27 patients who had more than one CSF examination, 25 had concordant PCR results in DNA amplifications in sequential samples. Several patients with con-

TABLE 4. Prevalence of CMV and VZV neurological diseases and cerebral lymphoma among 156 patients with established etiological diagnosis: sensitivity, specificity, predictive values, and likelihood ratios of CMV, VZV, and EBV DNA amplification

Diagnosis	Prevalence (%)	Sensitivity[a]	Specificity[a]	Value for[b]:			
				PPV	NPV	LRPT	LRNT
CMV disease	19.8	0.87 (0.75–0.99)	0.95 (0.91–0.99)	0.82	0.97	14.4	0.03
Cerebral lymphoma	7.7	0.33 (0.05–0.60)	0.97 (0.94–1)	0.5	0.95	11	0.68
VZV disease	2.6	0.5 (0.01–0.99)	0.99 (0.98–1)	0.5	0.99	50	0.51

[a] Values in parentheses are 95% CIs.
[b] Abbreviations: PPV, positive predictive value; NPV, negative predictive value; LRPT, likelihood ratio of the positive test; LRNT, likelihood ratio of the negative test.

cordant positive amplifications in sequential CSF samples had a second coamplification in one of the samples. As mentioned above, in four patients with CMV detection, HSV DNA was additionally detected in a subsequent CSF sample (three patients) or in a previous one (one patient). One patient with cerebral lymphoma had three consecutive CSF samples that were positive for EBV DNA amplification, and in the third one, coincident with the development of CMV retinitis, CMV was also amplified. The two cases with discordant amplifications had a negative result followed by a positive one (CMV and EBV).

DISCUSSION

The human HVs should always be included in the differential diagnosis in HIV-infected patients of certain neurological syndromes: aseptic meningitis, polyradiculitis, acute myelitis, and acute encephalitis. Rapid diagnosis of these conditions is critical since HV infections are potentially treatable. Detection of DNA in CSF by PCR has been shown to be a sensitive and specific method for the rapid diagnosis of CNS HV infections (10). A multiplex PCR with the capacity to detect simultaneously, in a single run, all of the HVs would simplify diagnostic procedures and decrease their costs. It would also offer the theoretical advantage of detecting coinfections. However, few studies have explored the use of multiplex PCR techniques for HIV-infected patients. Roberts and Storch have recently reported good results for a multiplex PCR assay used for the differential diagnosis of toxoplasmosis and PCNSL (21).

Our study shows that an MHV-PCR performed on CSF is a useful diagnostic technique for the evaluation of HIV-infected patients with suspected HV-related neurological diseases. The fact that our PCR can amplify multiple target sequences did not seem to decrease its specificity significantly, since we found a global specificity of 0.89. The high rate of reproducibility of the results shown for patients with serial CSF examinations further supports the clinical validity of the results obtained.

In our study, HV DNA was detected in the CSF specimens of more than one-third of HIV-infected patients with neurological disorders. Although severely immunodepressed patients and patients presenting with clinical findings compatible with CMV neurological disease are probably overrepresented in our population, this reflects appropriately, in our opinion, the actual clinical setting in which diagnostic studies for HV are most frequently needed. CMV was the HV most frequently detected in our patients (25%), while EBV, VZV, HSV, and HHV-6 DNA were present in significantly smaller proportions of cases (7, 4, 3, and 1%, respectively). Globally, our results are in agreement with other studies that have searched for HV DNA in CSF from HIV-infected patients with neurological disorders (11, 15, 20).

An important limitation of our study was the relatively small number of patients (25 patients) with a definite diagnosis established by CSF culture or pathological examination. To overcome this limitation, we used strict criteria for the etiological diagnosis of the neurological disorders studied. Consequently, a large number of cases were excluded from the evaluation of the PCR results because they were considered of uncertain etiology, but the clinically established etiological diagnoses were believed to be accurate.

The MHV PCR had a high sensitivity (0.87), a high specificity (0.95), and a high negative predictive value (0.97) for CMV neurological disease. These results are in accordance with those of recent studies (2, 11, 16, 25). The relatively low positive predictive value in our study (0.82) was due to the amplification of CMV DNA in CSF samples from six patients with documented extraneurological CMV infection or disease who exhibited neurological disorders not related to CMV. In one of these patients, a concomitant CMV infection could not be ruled out. Other studies did not detect CMV DNA in CSF of patients with only extraneural CMV disease (2). We cannot exclude the possibility that peripheral-blood contamination of CSF samples could explain some of our false-positive results for CMV DNA amplification. CMV DNA was also amplified in 22 cases with encephalitis, polyradiculitis, or mononeuritis multiplex in which clinical diagnoses of CMV neurological disease were not established, mainly due to the absence of previous CMV disease. We believe that many of these cases were probably caused by CMV, since 17 of these patients presented with a clinical picture highly suggestive of CMV disease. It has been reported that PCR of DNA in CSF is more reliable than clinical features in the diagnosis of CMV neurological disease in patients with AIDS (16).

We found a highly significant association between a previous history of systemic CMV disease and the amplification of CMV DNA in CSF. Because our diagnostic criteria for the diagnosis of CMV neurological disease included a previous history of systemic CMV disease, we evaluated the possibility that a positive CSF CMV PCR might be simply a consequence of systemic CMV disease. However, CMV amplification was no more frequent for patients with a previous history of systemic CMV disease who presented with a non-CMV neurological disease than for patients without systemic CMV disease. Therefore, the association of systemic CMV disease and CMV amplification in this population with neurological disorders probably reflects the prevalence of CNS CMV disease in this group of patients (4).

The frequency of VZV DNA detection (4%) among our patients is within the range reported in the literature (2.5 to 7%) (5, 8). Only two of four cases with a diagnosis of neurological VZV disease had a positive PCR for VZV. The small number of patients with a diagnosis of VZV disease precludes any conclusion about the sensitivity of the test for this diagnosis, as shown by the wide 95% confidence interval, but its specificity is high. In our series, VZV DNA amplification was possibly of diagnostic value for four of the nine patients in whom it was detected. Previous studies evaluating VZV CSF PCR have reported conflicting results (5, 8). We found that a previous history of zoster was significantly associated with VZV DNA amplification in CSF, even in cases in which the previous history was remote. However, the proportion of VZV CSF DNA amplification in patients with neurological diseases not related to VZV who had a previous history of cutaneous zoster was similar to that in patients without a previous history of zoster. Therefore, it seems that in a significant number of cases the amplification of VZV in CSF from patients with neurological disorders probably indicates a pathogenetic role of VZV in the patient's neurological disease.

Our results for EBV DNA amplification with regard to the diagnosis of CNS lymphoma (PCNSL and lymphomatous meningitis) were disappointing due to a low sensitivity and a low positive predictive value. This is in contrast with the results of other series, which show a sensitivity ranging from 80 to 100% (3, 7, 9, 11, 14). When we evaluated only patients with cerebral masses or abscesses, the detection of EBV DNA had a high positive predictive value for the diagnosis of probable or definite PCNSL. The inclusion in our study of four patients with meningeal dissemination of systemic lymphoma, which is associated with EBV less frequently than is PCNSL (17), did not influence the diagnostic value of the MHV-PCR. The low sensitivity obtained compared to those of other series could have been due to an inaccurate diagnosis of probable PCNSL

in four patients with negative PCR results or, alternatively, to the different primers used. The estimated detection level of our technique is less than 10 genomes of EBV DNA (6). Cinque et al. (9) and De Luca et al. (14) used primers for the EBNA1 gene, with a detection level of four genomes, while Arribas et al. (3) used primers that amplified a segment of the *Bam*HI-W region with a reported level of sensitivity of one EBV genome. On the other hand, the specificity in our series was comparable to that in previous reports. In 10 patients, EBV amplification seemed not to have any relationship to the CNS disease. In several studies (3, 11, 20), EBV DNA was also detected in CSF from patients with other diseases. The clinical significance of these amplifications is unknown. In one study (11), EBV DNA was detected in CSF in some patients for whom lymphoma lesions were not seen in neuroimaging studies, suggesting that the detection of EBV DNA in the CSF might be a preclinical marker of disease. We did not find similar cases, although this may have been due to a shorter follow-up period or to the lack of autopsy studies.

CNS infections caused by HSVs are uncommon in AIDS patients and are often clinically and pathologically atypical (12). None of our patients had a clinical diagnosis of herpetic encephalitis, and HSV was detected in only 3% of our cases. Interestingly, in all but one case, HSV DNA was amplified simultaneously with that of CMV, a finding described by other authors (11, 12). CMV and HSV coinfections of the CNS have been reported in AIDS patients (18, 19, 23, 24). HSV type 1 or 2 infection has been demonstrated in 5 to 16% of AIDS cases with CMV encephalitis (4, 24). Five of our patients with concomitant HSV and CMV DNA amplification had encephalitis.

The significance of HHV-6 DNA detection in CSF remains unclear. In our series it was detected on only three occasions, and in none of these cases did it appear that HHV-6 had a pathogenic role in the patient's neurological disease. Other studies have also found that HHV-6 is rarely detected in CSF of HIV-infected patients (20). Currently there is no evidence indicating that HHV-6 can cause neurological disease in these groups of patients.

In conclusion, our MHV-PCR for CSF is a specific technique for the evaluation of HIV-infected patients with suspected neurological diseases related to HV, comparable to conventional PCRs aimed at detecting single HV sequences. In spite of some limitations of the MHV-PCR, the advantages of rapidity, economy, and ability to detect coinfections, together with its possible utility also in the differential diagnosis of viral CNS diseases in patients without HIV infection (6), could make the use of this technique preferable to conventional PCR. It is highly sensitive and has a high negative predictive value for CMV neurological disease. Although the diagnostic value of VZV, HSV, and HHV-6 DNA amplification in HIV-infected patients is still unclear, it may be an important diagnostic aid for selected patients. The sensitivity of amplification of EBV DNA for the diagnosis of CNS lymphoma is low but may support this diagnosis in patients with cerebral masses. Further refinement of the technique for detection of EBV DNA might improve its diagnostic utility.

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