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PCR for Detection of Herpes Simplex Virus in Cerebrospinal Fluid: Alternative Acceptance Criteria for Diagnostic Workup

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The determination of herpes simplex virus (HSV) infection using a PCR assay is one of the most commonly requested tests for analysis of cerebrospinal fluid (CSF), although only a very low proportion of results are positive. A previously reported study showed that selecting only those CSF samples with >5 leukocytes/mm^3 or a protein level of >50 mg/dl was adequate for the diagnostic workup. The aim of the present study was to assess the reliability of alternative acceptance criteria based on elevated CSF white blood cell counts (>10 cells/mm^3). We analyzed all requests for HSV PCR received between January 2008 and December 2011. CSF samples were accepted for analysis if they had >10 cells/mm^3 or if the sample was from an immunocompromised patient or a child aged <2 years. In order to evaluate our selection criteria, we identified those CSF samples with a leukocyte count of 5 to 10 cells/mm^3 or protein levels of >50 mg/dl in order to test them for HSV type 1 and 2 (HSV-1 and HSV-2) DNA. During the study period, 466 CSF samples were submitted to the microbiology laboratory for HSV PCR. Of these, 268 (57.5%) were rejected, and 198 (42.5%) were tested according to our routine criteria. Of the tested samples, 11 (5.5%) were positive for HSV DNA (7 for HSV-1 and 4 for HSV-2). Of the 268 rejected specimens, 74 met the criteria of >5 cells/mm^3 and/or protein levels of >50 mg/dl. Of these, 70 (94.6%) were available for analysis. None of the samples yielded a positive HSV PCR result. Acceptance criteria based on CSF leukocyte counts, host immune status, and age can help to streamline the application of HSV PCR without reducing sensitivity.

Detection of herpes simplex virus (HSV) DNA in cerebrospinal fluid (CSF) using PCR assay has been validated for the diagnosis of central nervous system (CNS) herpes infections. HSV PCR is currently recognized as the reference method (1, 2). This sensitive but expensive test is included in the routine evaluation of many patients with suspected CNS infection, although most of the tests performed yield negative results (3). Therefore, screening for suitable diagnostic samples is necessary.

Acceptance criteria based on elevated CSF leukocyte counts (>5 cells/mm^3) and protein levels (>50 mg/dl) have been proposed as a way to save health care costs without reducing sensitivity (4, 5); however, other acceptance criteria have not been evaluated.

Most patients with viral CNS infection have an abnormal CSF leukocyte count, which usually ranges from 10 cells/mm^3 to 200 cells/mm^3 (6). The CSF leukocyte counts reported in previous studies (1, 2, 5–13) are summarized in Table 1. In our institution, we accepted CSF specimens for HSV PCR testing if they had >10 cells/mm^3. This cutoff was based both on our experience before the study and on data from the studies cited above. We also accepted specimens collected from immunocompromised patients and children younger than 2 years of age.

Clinical laboratories attempt to decrease costs while maintaining the quality of the diagnostic approaches used. As our criteria are stricter than those reported by Hanson et al. (5), we achieved a greater reduction in workload with the consequent laboratory savings. In order to evaluate the quality of our approach, we compared both criteria by performing HSV PCR in rejected specimens with leukocyte counts of 5 to 10 cells/mm^3 or protein levels of >50 mg/dl. We also analyzed the correlation between PCR results and leukocyte counts and protein levels in all CSF specimens submitted to the microbiology laboratory.

MATERIALS AND METHODS

Acceptance criteria. From January 2008 to December 2011, we accepted CSF specimens for HSV PCR analysis if they had an elevated CSF leukocyte count (>10 cells/mm^3) or if the sample was collected from an immunocompromised patient (HIV-positive patient or transplant recipient) or a child younger than 2 years of age. Specimens which did not meet the acceptance criteria were rejected and frozen immediately at −80°C if an adequate volume was available.

In the case of an urgent request by the attending physician for analysis of a specimen, HSV PCR was carried out even if the patient had normal CSF parameters.

Laboratory record review. We reviewed laboratory records (protein level and leukocyte counts) for all CSF specimens submitted for HSV PCR during the study period. We identified frozen specimens with a leukocyte count of 5 to 10 cells/mm^3 or protein levels of >50 mg/dl in order to test them for HSV types 1 and 2 (HSV-1 and HSV-2) DNA.

HSV PCR analysis. DNA was extracted using a NucliSENS EasyMAG system (bioMérieux, Boxtel, The Netherlands) according to the manufacturer's instructions. HSV-1 and -2 were detected using real-time reverse transcriptase PCR (affigene HSV 1/2 tracer, Cepheid AB, Sweden) in a Stratagene MX3000 thermocycler (Stratagene, La Jolla, CA).

Five positive samples were randomly thawed and tested retrospectively for HSV PCR in order to analyze the viability of HSV DNA after 1 freeze-thaw cycle.

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TABLE 1 Previously reported studies of HSV CNS infection[a]

Reference	Study period	Study design	Study population characteristics	No. of cases/no. of tests performed (%)	No. of HSV cases		Study results	
					Type 1	Type 2	WBCs (count or median [range]) (cells/mm^3)	Patients (n [%] or disease status)
17	1993–1996	Prospective	All age groups	18/49 (36.7)	NA	NA	<5	0 (0)
							5 to 53[b]	4 (25)
							50 to 500	11 (68.7)
							>500	1 (6.3)
14	1995–1997	Retrospective	All age groups	13/716 (1.8)	NA	NA	≤5	2[c]
7	1997	Prospective	All age groups	11/1,339 (0.8)	7	4	26 (23 to 86)	HSVE
							65 (<5[d] to 38)	HSVM
8	1996–2001	Retrospective	All age groups	50/2,759 (1.8)	2	48	202 (2 to 667)	HSVE
							484 (58 to 1,888)	HSVM
9	1999–2000	Retrospective	Patients >10 yr of age	115/249 (46.2)	39	76	41 (0 to 648)	HSV-1
							238 (2 to 1,900)	HSV-2
18	1997–2000	Retrospective case-control study	Adult patients	20/1,174 (1.7)	1	19	475.7 (100 to 1,130)	HSV-2
10	1999–2004	Retrospective	All age groups	7/1,296 (0.5)	7	0	67 (11 to 1,680)	
5	2004–2007	Retrospective	All age groups (immunocompetent patients)	8/109 (7.4)	0	8	240 (180 to 2,200)	
11	5-year period	Retrospective case-control study	Infants (birth to 60 days)	4/88 (4.5)	NA	NA	29 (11 to 91)	
1	1994–2005	Prospective	Children (median age, 4 yr). Immunosuppressed patients excluded	10/322 (3.1)	8	2	34.5 (3[e] to 380)	
6	1992–2006	Retrospective	Adult patients	24/35 (68.6)	NA	NA	22.5 (9.7 to 114.2)	

[a] NA, not available; WBCs, leukocytes; HSVE, herpes simplex virus encephalitis; HSVM, herpesvirus simplex virus meningitis.
[b] The number of patients with a WBC count of 5 to 10 cells/mm^3 and the age and immune status are not available.
[c] The age and immune status of these patients are not available.
[d] A 37-year-old woman had <5 cells/mm^3 in CSF. The clinical presentation and immune status of this patient are not available.
[e] The patient was a 14-year-old girl with generalized tonic-clonic seizures and status epilepticus.

RESULTS

During the study period, 466 CSF specimens were submitted to the microbiology laboratory for HSV PCR testing. According to our screening criteria, 57.5% were rejected.

Accepted specimens. We accepted 198 specimens for HSV PCR testing on arrival at the laboratory. Of these 198 specimens, 156 had >10 leukocytes/mm^3, 32 were accepted based on host characteristics (17 immunocompromised and 15 pediatric patients), and the remaining 10, which did not meet any of our criteria, were considered urgent by the attending physician.

Of the 198 samples tested, 11 samples (5.5%: 7 for HSV-1 and 4 for HSV-2) were found to have HSV DNA. The threshold cycle (C_T) values of the positive samples ranged from 32.6 to 38.7 for HSV-1 and from 30.9 to 35.7 for HSV-2.

The main characteristics of the patients with positive HSV PCR results are shown in Table 2. All patients had a clinical syndrome consistent with the PCR result.

Rejected specimens. Of the 268 samples rejected based on our criteria, we identified 74 (27.6%) with leukocyte counts of >5 cells/mm^3 and/or protein levels of >50 mg/dl that would have met the criteria of Hanson et al. (5). Of these 74 samples, 70 (94.6%) remained available for HSV PCR analysis. HSV DNA was not detected in any of these samples.

HSV DNA was detected in the 5 positive samples tested retro-

TABLE 2 Clinical and laboratory characteristics of patients with HSV DNA in CSF

Patient no.	Age/sex	Underlying condition	HSV type	Cell count (cells/mm^3)	Protein count (mg/dl)	Acyclovir therapy (days)	Died
1	47 days/male	Maternal genital herpes	1	2	59	21	No
2	24 yr/female	None	2	68	118	7	No
3	25 yr/female	None	2	120	62	19	No
4	26 yr/female	Asthma	2	210	269	2	No
5	27 yr/female	None	1	17	23	17	No
6	32 yr/male	Charcot-Marie-Tooth II	2	122	160	16	No
7	55 yr/female	HIV/hepatitis C virus	1	5	24	13	No
8	74 yr/male	Type II diabetes mellitus	1	48	199	21	No
9	80 yr/female	None	1	60	54	22	No
10	80 yr/male	Aortic valve disease	1	25	78	9	No
11	15 days/male	None	1	20	221	21	Yes

TABLE 3 Correlation between HSV PCR results and leukocyte counts and protein levels in all CSF specimens (including rejected specimens tested retrospectively)

HSV PCR results	No. of samples	No. of tests performed on reception in the laboratory (no. of positive samples)	No. of specimens rejected on reception in the laboratory	No. of rejected specimens retrospectively tested (no. of positive samples)
Normal protein level	300			
Leukocytes <5 cells/mm^3	218	24[a] (0)	194	0
Leukocytes 5–10 cells/mm^3	23	1 (1)[b]	22	19 (0)
Leukocytes ≥10 cells/mm^3	59	59 (1)	0	0
Protein level of ≥50 mg/dl	166			
Leukocytes <5 cells/mm^3	55	13[c] (1)[d]	42	41 (0)
Leukocytes 5–10 cells/mm^3	14	4[e] (0)	10	10 (0)
Leukocytes ≥10 cells/mm^3	97	97 (8)	0	0
Overall	466	198 (11)	268	70 (0)

[a] Eight pediatric patients, 9 immunosuppressed patients, 7 tested after discussion with the ordering clinician.
[b] HIV-infected patient (patient 7, Table 1).
[c] Five pediatric patients, 7 immunosuppressed patients, 1 tested after discussion with the ordering clinician.
[d] Pediatric patient (patient 1, Table 1).
[e] Two pediatric patients, 2 tested after discussion with the ordering clinician.

spectively for HSV PCR in order to analyze their viability. The C_T values of the retested samples increased slowly compared with those recorded during the initial testing. The initial C_T values of these samples were 30.99, 38.74, 37.78, 35.70, and 32.60. The C_T values of the retested samples were 32.24, 39.92, 41.00, 36.20, and 33.80.

Correlations between PCR results and leukocyte counts and protein levels. The correlations between HSV PCR results and leukocyte counts and protein levels in all the CSF specimens submitted (including rejected specimens tested retrospectively) are presented in Table 3.

All positive samples met our acceptance criteria; 9 had leukocyte counts of >10 cells/mm^3 (irrespective of protein level), 1 was from an immunocompromised patient, and 1 was from a pediatric patient.

DISCUSSION

Our screening criteria for HSV PCR in CSF specimens, which are stricter than those of Hanson et al. (5), are very sensitive and allow a significant further reduction in laboratory workload.

Prompt diagnosis of HSV encephalitis is crucial, because treatment with intravenous acyclovir can decrease morbidity and mortality (14–16). At our institution, as in others (2, 17), the number of HSV PCR assays requested as part of the evaluation of patients with suspected CNS infection has risen. However, most results with CSF are negative.

Previous studies (3, 4) suggest screening CSF cell count and protein values before performing HSV PCR in order to rationalize the use of this test. To date, the only criteria proposed have been elevated CSF leukocyte counts (>5 cells/mm^3) and/or protein levels (>50 mg/dl) (3). In addition, Hanson et al. (5) included host immune status and patient age as a part of the criteria. Other acceptance criteria have not been evaluated. In the present study, we compared the reliability of acceptance criteria based on a leukocyte cutoff of >10 cells/mm^3 and host characteristics (age and immune status) that met criteria reported elsewhere (9).

While a cutoff of >10 cells/mm^3 is debatable, it was based on our experience before the study and on the observation that in previously reported studies of HSV CNS infection the median CSF leukocyte count ranged from 22 to 484 cells/mm^3 (Table 1). In these studies (3–5, 8, 9, 11–13, 17–19), cases of HSV CNS infection with <10 cells/mm^3 in CSF were anecdotal. In addition, in these few cases, the age and immunological status of the patients were not always available (4, 12). It is noteworthy that none of the HSV PCR-positive specimens had <10 cells/mm^3 in the study by Hanson et al. (5). Normal CSF findings in early states of HSV encephalitis have rarely been reported in the literature (20). In addition, false-negative PCR results can occur when the test is performed too early (7, 21). In this situation, classic clinical features are the most important criteria for identifying HSV encephalitis.

In previously reported studies, HSV CNS infections were found in 2 to 4% of patients (5, 10, 11). Using our criteria, we detected a positivity rate of 5.5%. All CSF specimens that yielded positive results met our acceptance criteria. It is noteworthy that 10 samples that did not meet our acceptance criteria on arrival at the laboratory were accepted because the attending physician considered them urgent, although none of the 10 patients finally presented a positive HSV PCR result.

By applying our criteria, we tested 10% fewer specimens than if we had used the criteria of Hanson et al. (9). We rejected almost 60% of the specimens submitted, with consequent savings in laboratory costs (€52/test, €14,000). In the present study, the analysis of rejected specimens did not reveal any missed cases of HSV CNS infection. These findings suggest that the application of more stringent acceptance criteria enabled us to reduce the number of tests performed while increasing the percentage of positivity.

We agree with Hanson et al. (5) that the inclusion of host immune status is particularly important, because HSV encephalitis has been reported in immunocompromised patients with normal CSF parameters (10). In the present study, 1 of the patients with positive HSV PCR results was immunosuppressed.

The revised criteria could be limited by the need to have the patient's clinical history (immunocompetence and age); however, the time taken to review laboratory and medical records corresponding to the PCR requested and to freeze the remaining CSF samples was shorter than the time required to process and test the

samples (data not shown). Despite the possibility of reviewing the electronic medical history, communication between the laboratory and the physician remains essential. In the study by Hanson et al. (5), a CSF specimen with normal parameters belonging to a patient with advanced HIV disease that was erroneously excluded because the laboratory lacked accurate information proved to be positive in the retrospective analysis. In our laboratory, we use the electronic service to report rejection of a sample to the attending physician; if the physician has a high clinical suspicion or information about the immune status that the laboratory lacks, he/she can contact us to request the HSV PCR.

Our study was subject to a series of limitations. First, not all of the rejected specimens were available for retrospective testing. Second, the revised criteria are based on a limited number of positive samples. Nevertheless, despite these limitations, our criteria could reasonably be applied to select suitable specimens for HSV PCR, which would reduce laboratory costs. We are performing an ongoing study of screening CSF before HSV PCR to evaluate the impact on the cost savings resulting from decreased use of antivirals and reduced hospital stay.

In conclusion, the application of acceptance criteria based on leukocyte counts (>10 cells/mm^3), host immune status, and patient age would enable us to identify suitable specimens for HSV PCR testing and, in turn, reduce the number of PCR tests performed without missing cases of HSV infection.

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We declare that we have no conflicts of interest.

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