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Missing Cases of Herpes Simplex Virus (HSV) Infection of the Central Nervous System When the Reller Criteria Are Applied for HSV PCR Testing: a Multicenter Study

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ABSTRACT Previous studies suggested that herpes simplex virus (HSV) PCR testing can be safely deferred in patients with normal cerebrospinal fluid (CSF) white blood cell (WBC) counts and protein levels as long as they are older than 2 years of age and are not immunocompromised, the so-called Reller criteria. In this multicenter study, we retrospectively assessed the validity of these screening criteria in our setting. A total of 4,404 CSF specimens submitted for HSV PCR testing to the respective microbiology laboratories at the participating hospitals between 2012 and 2018 were included. Six commercially available HSV PCR assays were used across the participating centers. Ninety-one of the 4,404 CSF specimens (2.1%) tested were positive for HSV DNA (75 samples for HSV-1 and 16 for HSV-2). Nine patients failed to meet the Reller criteria, of whom seven were deemed to truly have HSV encephalitis. Overall, no significant correlation between HSV PCR cycle threshold (C_T) values and WBC counts or total protein levels was found. In addition, median HSV PCR C_Ts were comparable between patients who met the Reller criteria and those who did not ($P = 0.531$). In summary, we show that HSV DNA may be detected in CSF specimens with normal WBC and protein levels collected from immunocompetent individuals older than 2 years with HSV encephalitis. Nevertheless, the data also indicate that the number of cases detected could be lowered at least by half if CSF specimens with borderline WBC counts (4 cells/mm^3) as well as children of any age are systematically tested.

KEYWORDS central nervous system infections, cerebrospinal fluid, herpes simplex virus

Herpes simplex virus (HSV) is among the commonest etiological agents of sporadic central nervous system (CNS) infection in immunocompetent patients (1). Timely diagnosis and prompt initiation of acyclovir are crucial for patient survival and recovery (1). Requests for cerebrospinal fluid (CSF) HSV PCR testing, the diagnostic gold standard (1), can be a burden on molecular microbiology laboratory resources; moreover, the results are often noncontributory. Based on previous studies (2, 3), Hanson and

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TABLE 1 PCR assays used at the participating centers and their analytical sensitivity for HSV-1 and HSV-2 detection in cerebrospinal fluid specimens

PCR assay	LOD (copies/ml)[a] for:		Center[b]
	HSV-1	HSV-2	
LightCycler HSV 1/2 ASR real-time PCR (Roche Diagnostics, Indianapolis, IN, USA)	1,000	1,000	HAV, HCUV, HGUA, HGUC
RealCycler Progenie HSVT v3.0 kit (Progenie Molecular, Valencia, Spain)	1,000	1,000	HCGUV, HCUV
RealQuality RS-HSV-1 and HSV-2 (AB Analitica, Padua, Italy)	300	600	HCUV
RealStar HSV PCR kit 1.0 (Altona Diagnostics, San Francisco, CA, USA)	150	600	HGUA, HUPA
Simplexa HSV 1 & 2 Direct kit (Diasorin Molecular Inc., Saluggia, Italy)	250	125	HGUC
FilmArray ME panel (bioMérieux, Marcy-l'Étoile, France)[c]	1,250	1,200	HUPA, HCGUV, HAV

[a]Limits of detection (LODs) were directly provided by the respective manufacturers for the LightCycler HSV 1/2 ASR real-time PCR (TIB Molbiol LightMix kit for the detection of HSV-1/2, catalog no. 40-0378-32, 2015; Roche Diagnostics, Rotkreuz, Switzerland), the Simplexa HSV 1 & 2 Direct kit (REF MOL2150 PI, 2015; Focus Diagnostics, Cypress, CA, USA), and the FilmArray ME panel (FilmArray ME panel instruction booklet, USARFIT-PRT-0276-02, 2016; BioFire Diagnostics, LLC, Salt Lake City, UT, USA). For the remaining HSV PCR assays, LODs were calculated based on the information provided in the respective package insert (RealQuality, RS-HSV 2, RS_HSV-1, code RQ-S07, 2016 [AB Analitica, srl, Padua, Italy]; RealStar HSV PCR kit 1.0, June 2017 [Altona Diagnostics GmbH, Hamburg, Germany]; RealCycler HSVT *in vitro* diagnostic kit, 2012 [Progenie Molecular, Valencia, Spain]) as copies/ml = copies per PCR × (total volume of eluate [100 μl]/total volume for PCR [5 μl]) × volume (200 μl) of extracted CSF.

[b]HAV, Hospital Arnau de Vilanova; HCGUV, Hospital Consorcio General Universitario de Valencia; HCUV, Hospital Clínico Universitario de Valencia; HGUA, Hospital General Universitario de Alicante; HGUC, Hospital General Universitario de Castellón; HUPA, Hospital Universitario Peset Aleixandre de Valencia.

[c]The FilmArray meningitis/encephalitis (ME) panel was used for 12 patients.

colleagues (4) suggested that HSV PCR testing can be safely deferred in patients with normal CSF white blood cell (WBC) counts and protein levels ($<$5 cells/mm^3 and $\leq$50 mg/dl, respectively), as long as they are older than 2 years of age and are not immunocompromised, either by HIV infection or transplant—the so-called Reller criteria. This assumption was later validated by another group (5) and was shown to be a cost-effective and safe approach to HSV testing (6). However, the safety of the aforementioned criteria has recently been disputed (7) and counterarguments against it have been put forward (8). The main concern is that a misclassification could delay the administration of acyclovir (7); nevertheless, the application of the Reller criteria was shown not to be harmful (5). In this multicenter study involving eight hospitals from the autonomous community of Valencia, Spain, we retrospectively assessed the validity of the Reller criteria in our setting.

MATERIALS AND METHODS

Cerebrospinal fluid specimens. A total of 4,404 CSF specimens submitted to the respective microbiology laboratories at the participating hospitals between 2012 and 2018 for HSV PCR testing were included in this study. No restrictive acceptance criteria for testing were in place at any of the centers. Specifically, 1,474 specimens were received at the Hospital General Universitario de Castellón (HGUC) from January 2012 to December 2017, 1,058 at the Hospital Clínico Universitario de Valencia (HCUV) from January 2012 to December 2017 (of note, the Hospital Francisco de Borja of Gandía, Valencia, and the Hospital de Sagunto, Valencia, both contribute to the HCUV), 906 at the Hospital Consorcio General Universitario de Valencia (HCGUV) from January 2012 to December 2017, 603 at the Hospital General Universitario de Alicante (HGUA) from January 2012 to December 2017, 204 at the Hospital Universitario Peset Aleixandre de Valencia (HUPA) from January 2014 to December 2017, and 159 at the Hospital Arnau de Vilanova de Valencia (HAV) from January 2013 to March 2018. All the participating centers are tertiary referral hospitals attending both adult and pediatric patients.

Medical and laboratory record review. Demographic information, laboratory data (CSF cellularity and total protein levels), and microbiology laboratory records were retrospectively reviewed for all the patients testing positive for HSV DNA during the study period and were registered in an IBM SPSS Statistics software database (version 20.0; SPSS, Chicago, IL, USA). In addition, the patients' medical charts were reviewed to assess their clinical presentation, comorbid conditions, use of antiviral therapy (if any) and clinical response, CNS neuroimaging by computed tomography (CT) or magnetic resonance imaging (MRI), and electroencephalography (EEG) findings.

Cerebrospinal fluid PCR testing. The following commercially available HSV PCR assays were used across the participating centers: LightCycler HSV 1/2 ASR real-time PCR assays (Roche Diagnostics, Indianapolis, IN, USA), Simplexa HSV 1 & 2 Direct kit (Diasorin Molecular Inc., Saluggia, Italy), RealQuality RS-HSV-1 and HSV-2 (AB Analitica, Padua, Italy), RealCycler Progenie kit (Progenie Molecular, Valencia, Spain), RealStar HSV PCR kit 1.0 (Altona Diagnostics, San Francisco, CA, USA), and the FilmArray meningitis/encephalitis panel (bioMérieux, Marcy-l'Étoile, France). In all cases, the PCRs were performed according to the instructions from the respective manufacturers. Any individual center may have used one, two, or more of these tests either within the same or different time periods. PCR testing was performed within 24 h of receiving the CSF sample; the analytical sensitivity of the PCR assays and their use across institutions are shown in Table 1.

Available leftover CSF specimens from patients not meeting Reller criteria, which had been cryopreserved and not previously thawed, were retrieved for further testing with the FilmArray meningitis/encephalitis panel. This is an automated system that allows 14 targets to be simultaneously detected in a single CSF sample. The commercial source of the PCR assays used to detect other microbial agents in CSF specimens is indicated where appropriate.

Definitions. Abnormal CSF WBC and protein levels were defined as ≥5 cells/mm³ and >50 mg/dl, respectively (1, 9, 10). HSV meningitis was defined by the detection of HSV DNA in CSF and the presence of a compatible clinical picture (acute onset of fever, headache, photophobia, and neck stiffness, either accompanied or not by nausea and vomiting) (9, 10). Moreover, a clinical response to targeted antiviral therapy was considered to support the diagnosis. A diagnosis of HSV encephalitis was based on the detection of HSV DNA in CSF and the presence of compatible clinical signs and symptoms and neuroimaging (T2 hyperintense lesions, with or without enhancement) when available (10–14); a characteristic CSF cytobiochemical profile, EEG findings, and a response to antiviral agents with intrinsic activity against HSV were deemed to support the diagnosis of HSV encephalitis (10–14). HSV meningoencephalitis diagnosis was based upon the detection of HSV DNA in CSF in the presence of clinical and neuroimaging features of both meningeal and parenchymal disease (1).

Two infectious diseases specialists from the infectious diseases unit of the HCUV independently reviewed and collected relevant information from patients with HSV DNA detected in CSF either meeting the Reller criteria or not to ascertain the etiological involvement of HSV. Any discrepancies were discussed until consensus was achieved.

Statistical analysis. The differences between medians were compared using the Mann-Whitney U test, and correlations between variables were assessed using the Spearman's rank test. Two-sided exact *P* values are reported, and *P* values of ≤0.05 were considered statistically significant. The statistical analyses were performed using IBM SPSS Statistics, version 20.0 (SPSS, Chicago, IL, USA).

RESULTS

Herpes simplex virus PCR results. Ninety-one (2.1%) of the 4,404 CSF specimens tested were positive for HSV DNA. HSV-1 was detected in 75 samples (1.70%) and HSV-2 in 16 (0.36%). The median age of the patients who tested positive (49 female and 42 male) was 52 years (range, 0 to 91 years). The rate of HSV DNA detection ranged from 1.3% to 3.9% of the submitted CSF specimens across all the centers. The LightCycler HSV 1/2 ASR real-time PCR assay ($n = 36$) and the RealCycler Progenie kit ($n = 34$) were the most frequently used assays for HSV detection.

Eighty-two of the ninety-one patients (90%) met the Reller criteria, and the diagnosis of CNS HSV infection was deemed to be unarguable in 77 of the 82 patients, who presented with encephalitis ($n = 47$), meningitis ($n = 19$), or meningoencephalitis ($n = 11$). HSV-1 or HSV-2 DNA was detected in the CSF samples from 68 or 14 patients, respectively (Table 2). As for the remaining 5 patients meeting the Reller criteria, there were 3 cases of Guillain-Barre syndrome which might not have been causally related to HSV (HSV-1 was detected in CSF specimens by using the LightCycler HSV 1/2 ASR real-time PCR assay yielding PCR cycle threshold [C_T] values of 32.6, 37.0, and 35.6), one case of *Neisseria meningitidis* meningitis (HSV-1 was detected by means of the RealStar HSV PCR kit 1.0 yielding a C_T value of 38.2), and one case of CMV encephalitis in an HIV-1 infected patient (HSV-1 was detected by using the RealStar HSV PCR kit 1.0, with a C_T value of 38.2).

Of those meeting Reller criteria, elevated CSF WBC counts (≥5 cells/mm³) were seen in 68 patients and high protein levels (>50 mg/dl) were observed in 65 patients; both abnormalities were documented in 53 patients. There were two patients with normal protein levels and WBC counts: one was aged less than 1 year and the other was an HIV-1-infected adult.

Nine patients (representing 10% of the HSV-positive cases and 0.2% of all the submitted CSF specimens), namely, three children and six adults, failed to meet the Reller criteria. HSV-1 DNA was detected in 7 of these patients and HSV-2 DNA in the remaining 2 (Table 2). Six of these nine CSF samples had been screened with the RealCycler Progenie kit, two with the LightCycler HSV 1/2 ASR real-time PCR assay, and one with the RealQuality RS-HSV-1 and HSV-2 kit.

Features of patients who tested positive for HSV in CSF but did not meet Reller criteria. As shown in Table 3, all 9 patients who failed to meet the Reller criteria had encephalitis (one of them also presented with signs and symptoms of meningitis). None of these patients had received acyclovir therapy prior to CSF HSV PCR testing. Conventional bacterial cultures, as well as additional PCR testing for a variety of microbial

TABLE 2 Cerebrospinal fluid HSV PCR positive results across participating centers from patients who met or did not meet the Reller criteria

HSV PCR assay	No. of DNA-positive CSF specimens			
	Patients meeting the Reller criteria[a]		Patients not meeting the Reller criteria	
	HSV-1	HSV-2	HSV-1	HSV-2
HGUC[b]				
LightCycler HSV 1/2	14	2	1	1
Simplexa HSV 1 & 2 Direct kit	2	0	0	0
HCUV[c]				
RealCycler HSVT v3.0	13	1	1	1
LightCycler HSV 1/2 ASR	6	0	0	0
RealQuality RS-HSV-1 and HSV-2	6	3	1	0
HCGUV[d]				
RealCycler HSVT v3.0	12	1	4	0
HGUA/ LightCycler HSV 1/2	5	1	0	0
RealStar HSV PCR kit 1.0	2	0	0	0
HUPA[e]				
RealStar HSV PCR kit 1.0	2	5	0	0
FilmArray Panel	1	0	0	0
HAV[f]				
LightCycler HSV 1/2	5	1	0	0

[a]Cerebrospinal fluid white blood cell counts ≥5 cells/mm^3 or protein levels >50 mg/dl in patients older than 2 years of age and not immunocompromised, either by HIV infection or transplant.
[b]HGUC, Hospital General Universitario de Castellón.
[c]HCUV, Hospital Clínico Universitario de Valencia.
[d]HCGUV, Hospital Consorcio General Universitario de Valencia.
[e]HUPA, Hospital Universitario Peset Aleixandre de Valencia.
[f]HAV, Hospital Arnau de Vilanova.

agents other than HSV, yielded negative results. Two infectious disease specialists thoroughly reviewed the clinical charts of these patients and concluded that 7 of these 9 cases were attributable to HSV (Table 3, patients 2, 3, 5, 6, 7, 8, and 9). This conclusion was based on these patients' clinical presentations, EEG, and neuroimaging findings, and in 5 cases, their response to acyclovir. Nevertheless, doubts were raised about the etiological implication of HSV in cases 1 and 4. In patient 1, the signs of encephalopathy were eventually attributed to a CNS vasculopathy, but a definitive etiological diagnosis could not be made for patient 4.

Leftover CSF specimens which had been cryopreserved and not previously thawed were available for 5 of the 9 patients (Table 3, cases 1, 2, 4, 7, and 9) and were retrieved for further testing with the FilmArray meningitis/encephalitis panel. These analyses confirmed the presence of HSV-1 DNA in three specimens (patients 2, 7, and 9) but failed to do so in the remaining two samples (patients 1 and 4). Interestingly, these 2 cases had originally yielded high C_T values (41.1 and 41.0, respectively) via the RealCycler Progenie kit. All 5 CSF specimens tested negative for all the other targets included in the panel, and of note, CSF samples from 4 of the 7 patients with confirmed CNS HSV infection contained 4 cells/mm^3 and normal protein levels, and another (case 5) was from a 13-year-old child.

PCR cycle threshold values, white blood cell counts, and protein content in cerebrospinal fluid specimens. HSV PCR C_T values were retrieved from 77 CSF specimens (68 obtained from patients meeting Reller criteria); overall, we found no significant correlation between C_T values and WBC counts (Fig. 1A) or total protein levels (Fig. 1B). A lack of correlation was also observed when C_T values provided by the RealCycler Progenie kit ($n = 31$) and by the LightCycler HSV 1/2 ASR real-time PCR assay ($n = 28$) were analyzed separately ($P = 0.092$ and $P = 0.470$ for leukocytes, respectively; $P = 0.573$ and $P = 0.784$ for protein levels, respectively). Because of the limited sample

TABLE 3 Demographic, laboratory, and clinical findings in patients who tested positive for HSV DNA in CSF and did not meet the Reller criteria

Patient no./age (yr)/sex[a]	Center[b]/HSV PCR assay	HSV type (C_T)	Total protein content (mg/dl)	WBC[c] (cells/mm^3)	Clinical syndrome/outcome	Suggestive signs of HSV encephalitis in EEG or neuroimaging[d]	Response to acyclovir therapy	Additional PCR tests that yielded negative results[e]
1/66/F	HCGUV/RealCycler Progenie kit	1 (41.1)	41.3	2	Encephalitis/death	No	No	VZV, CMV, EBV, HHV-6
2/23/F	HCGUV/RealCycler Progenie kit	1 (24.7)	23.0	4	Meningoencephalitis/survival	No	Yes	CMV, EBV, MTB
3/70/M	HCGUV/RealCycler Progenie kit	1 (36.2)	27.0	1	Encephalitis/death	No	No (one dose administered)	MTB
4/3/M	HCGUV/RealCycler Progenie kit	1 (41.0)	25.2	1	Encephalitis/survival	No	No	None
5/13/F	HGUC/LightCycler HSV 1/2 ASR	1 (36.1)	24.0	1	Encephalitis	Yes (EEG and MRI)	Yes	EV, VZV, EBV
6/65/F	HGUC/LightCycler HSV 1/2 ASR	2 (36.4)	31.0	4	Encephalitis/death	Yes (MRI)	No (one dose administered)	None
7/9/M	HCUV/RealQuality RS-HSV-1 and HSV-2	1 (37.0)	14.0	4	Encephalitis/survival	Yes (EEG and MRI)	Yes	VZV, HHV-6
8/67/F	HCUV/RealCycler Progenie kit	2 (21.1)	29.0	4	Encephalitis/survival	Yes (EEG)	Yes	HHV-6, CMV, MTB, TG
9/80/F	HCUV/RealCycler Progenie kit	1 (25.2)	17.0	2	Encephalitis/survival	Yes (MRI)	Yes	EV

[a]M, male; F, female.

[b]HCUV, Hospital Clínico Universitario de Valencia; HCGUV, Hospital Consorcio General Universitario de Valencia; HGUC, Hospital General Universitario de Castellón.

[c]WBC, white blood cell count.

[d]EEG, electroencephalography; MRI, magnetic resonance imaging.

[e]CMV, cytomegalovirus; EBV, Epstein-Barr virus; EV, enterovirus; HHV-6, human herpesvirus-6; MTB, *Mycobacterium tuberculosis* complex; TG, *Toxoplasma gondii*; VZV, varicella-zoster virus. Leftover cryopreserved CSF specimens from patients 1, 2, 3, 4, 7, and 9 were tested by the FilmArray meningitis/encephalitis panel (bioMérieux, Marcy-l'Étoile, France), which targets the following microbial agents: *Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitides*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, CMV, EV, HSV-1, HSV-2, HHV-6, human parechovirus, VZV, and *Cryptococcus neoformans/gattii*. Real-time PCR assays used at HCGUV to screen VZV, CMV, EBV, HHV-6, and MTB were purchased from Progenie Molecular (Valencia, Spain). At HCUV, screening for EV, VZV, HHV-6, and TG was conducted using real-time PCR assays from Progenie Molecular, whereas the Artus *M. tuberculosis* PCR kit (Qiagen GmBH, Hilden, Germany) and the CMV RealTime CMV PCR (Abbott Molecular, Des Plaines, IL, USA) were used for screening of MTB and CMV, respectively. PCR assays purchased from Progenie Molecular were used at HGUC for EV, EBV, and VZV screening.

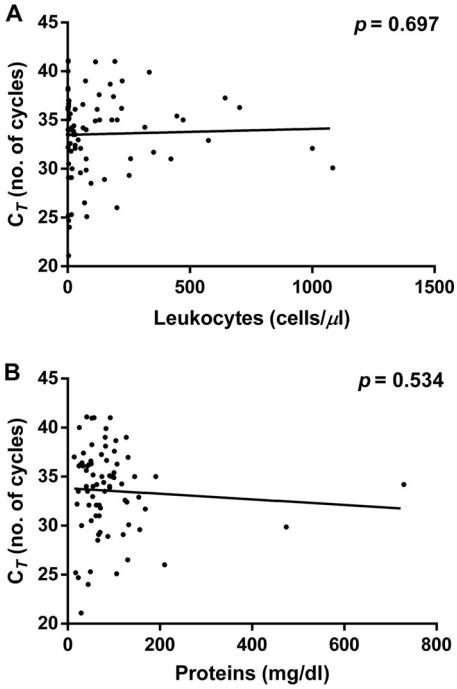

FIG 1 Overall correlation between herpes simplex virus (HSV) PCR cycle threshold (C_T) values and white blood cell (WBC) counts (A) and protein levels (B) in cerebrospinal fluid (CSF) samples from patients who tested positive for HSV-1 or HSV-2 DNA.

sizes, no meaningful statistical analysis could be performed using C_Ts obtained from the remaining PCR assays used in this study (data not shown). In addition, the median HSV PCR C_Ts were comparable between CSF samples from patients who met the Reller criteria and samples from those who did not ($P = 0.531$). A subanalysis of C_Ts specifically derived from the RealCycler Progenie kit also failed to show a statistically significant difference ($P = 0.841$).

DISCUSSION

Applying laboratory and host immune-status criteria for HSV PCR testing of CSF fluids may help to rationalize the use of this technique in clinical practice. The reliability of elevated WBC count (>10 cells/mm^3) acceptance criteria in patients with no documented immunosuppressive condition was first suggested in a single-center study (14) but has not since been confirmed in other studies. However, screening criteria incorporating CSF cytobiochemical parameters (WBC counts and total protein levels) and host factors have been successfully applied for guiding HSV PCR testing. In this context, the Reller criteria (4), which are based on previously reported data (2, 3), have been validated by another group (5). The finding of normocellular CSF fluids in patients with confirmed HSV encephalitis in some series is not extraordinary (15–17); nevertheless, very few cases of patients with CNS HSV infection who do not meet the Reller criteria have been documented (15). In our view, previous studies assessing the validity of the Reller criteria were hindered by their single-center design (2–4); in this sense, the strength of this current study stems from its multicenter design, including a large number of patients.

Overall, the rate of detection of HSV DNA in our cohort (2.1%) was comparable to that in other recent series (10) and varied slightly across the participating centers. A total of 9 patients who tested positive for HSV in CSF failed to meet the Reller criteria. Nevertheless, this fact must be qualified, because 2 cases (from the HCGUV) were resolved as not being causally linked to HSV; the HSV PCRs from these 2 patients yielded high C_T values (41.1 and 41.0) but the routine PCR assay used at that center (the RealCycler Progenie kit) called the specimens positive nonetheless. Furthermore, these

specimens tested negative when they were reassayed using the FilmArray meningitis/encephalitis (ME) panel using unmanipulated cryopreserved CSF fluids in a nonclinical microbiology laboratory (to avoid potential contamination with environmental HSV DNA amplicons); thus, it is likely that these were originally false-positive results. It is of note that HSV PCR testing was performed within 24 h of receiving the CSF sample; in this context, it has been estimated that in approximately 5% to 10% of adults with proven HSV encephalitis initial CSF findings may be normal. This figure may even be higher in immunosuppressed patients and children (18). The occurrence of clinical (and perhaps analytical) false-positive CSF HSV PCR results was previously reported for 7 patients who did not meet the Reller criteria (5). Of note, in our series, this may have also occurred in a few patients ($n = 5$) who did meet the Reller criteria.

All patients ($n = 7$) with a final diagnosis of HSV CNS infection who failed to meet the Reller criteria presented with encephalitis ($n = 6$) or meningoencephalitis ($n = 1$), whereas meningitis or meningoencephalitis clinical syndromes were much more common among patients who did meet these criteria. In this respect, CSF abnormalities have long been reported to be of greater magnitude during the course of HSV meningitis than in that of HSV encephalitis (1, 11).

It is conceivable that using HSV PCR assays with increased analytical sensitivity may make the identification of patients who do not meet the cytobiochemical Reller criteria more likely. In our series, however, HSV DNA detection in all but one of the CSF samples with normal WBC counts and protein levels was achieved by using two PCR assays (RealCycler Progenie kit and the LightCycler HSV 1/2 ASR real-time PCR assay) displaying the highest limits of detection (LODs; >1,000 copies/ml for both HSV-1 and HSV-2) among those used in the study. In this context, data from a recently published study (19) indicate that the CSF HSV DNA load in proven CNS HSV infections is usually well above 1,000 copies/ml, particularly in adult patients (median, 5.4 log10 copies/ml); yet, 6 of 25 CSF samples tested in another study had <200 copies/ml (19). In addition, CSF HSV DNA load may not correlate with the magnitude of CSF cytobiochemical abnormalities. In support of this assumption, (i) we found no correlation between HSV PCR C_T values, WBC counts, and protein levels, regardless of whether pooled or PCR assay-specific C_T values were considered in the analysis. In line with this, the ranges of CSF WBC counts and protein levels were comparable between patients with confirmed CNS HSV infections displaying high (>10,000 copies/ml) HSV DNA content in CSF and those with low content (<10,000 copies/ml), as determined by a quantitative real-time PCR assay (20). (ii) The overall median HSV PCR C_T values were not significantly different between CSF specimens from patients that met the Reller criteria and those from patients that did not; nevertheless, the scant number of cases in the latter group limits the conclusions that can be made. Collectively, these data suggest that failure to detect HSV DNA in CSF specimens from patients that do not meet the Reller criteria are unlikely, but possible, when using commercially available HSV PCR assays whose LODs are within the range of those used in the current study.

Cost-effectiveness is a factor which determines whether given laboratory screening criteria should be routinely implemented or not. Hauser and colleagues (6) showed that application of the Reller criteria for CSF HSV PCR testing is cost-effective if fewer than 1 in 200 patients deferred from PCR in fact test positive for HSV CNS infection. Hence, in our setting, we would have met this stipulation because only 1 in 629 patients would have been otherwise misclassified.

Cost-effectiveness aside, the key question is whether the lack of early recognition of HSV CNS infection cases by applying Reller criteria for HSV PCR testing is medically acceptable. Several facts should be gauged to address this issue. First, treatment with acyclovir is usually started once clinical presentation and imaging findings suggest viral encephalitis, even in the absence of CSF cytobiochemical abnormalities and without waiting for confirmation of HSV by PCR (21, 22). Second, CSF HSV PCR may be negative early on in cases of HSV encephalitis (1). Third, in our series, the positive predictive value of HSV PCR for the diagnosis of CNS infection was 93% in patients meeting Reller criteria, whereas it was substantially lower (77%) in patients not meeting them; thus,

CSF HSV PCR testing outside Reller criteria may favor the inappropriate use of acyclovir in some patients. Fourth, our data indicated that the number of missing cases of encephalitis would be substantially reduced (to only 2 cases) if CSF specimens with 4 WBCs/mm^3 as well as children of any age (<18 years old) were systematically tested. Moreover, acyclovir would likely have been started in one of these two patients (Table 3, case 9) on clinical and MRI findings grounds, regardless of whether HSV PCR testing had been performed. Fifth, the implementation of this screening policy would allow clinicians the option of bypassing the rule if considered appropriate. Taking into consideration all the above, we advocate the use of Reller criteria as long as adult patients with CSF displaying borderline WBC counts and children are not deferred from HSV PCR testing.

In our view, the current study has two main limitations: first, its retrospective design; and second, the use of multiple PCR assays (with variable LODs) across the participating centers rather than a single PCR with an optimal (low) LOD. Further studies are warranted to validate the screening strategy ("modified Reller criteria") proposed herein.

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