Limits of Early Diagnosis of Herpes Simplex Encephalitis in Children: A Retrospective Study of 38 Cases

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The prognosis of herpes simplex encephalitis (HSE) depends on the early and appropriate administration of specific antiviral therapy. We retrospectively reviewed 38 cases of children with proven HSE, to evaluate the reliability of polymerase chain reaction results, according to the time of cerebrospinal fluid (CSF) sampling. Initial negative results were observed in 8 of 33 CSF samples drawn before day 3 of the disease and were significantly associated with a low level of protein and <10 leukocytes/mm3 in the CSF.

Herpes simplex virus (HSV) is the most common cause of acute sporadic focal encephalitis in Western countries. The incidence of HSV encephalitis (HSE) is estimated to be 1–4 cases/106 people/year, and nearly 30% of these occur in patients aged <20 years [1, 2]. The prognosis of the disease has been significantly improved by acyclovir treatment, provided that the drug is administered very early after the onset of symptoms [1, 3]. Early diagnosis is, therefore, crucial for predicting outcome. PCR for HSV DNA performed on CSF is now considered to be the reference standard for establishing the diagnosis of HSE from the first days of the disease and has been widely accepted for routine clinical decision making [4, 5]. Its recent generalized use has widened the previous clinical spectrum of the disease [6] and has greatly facilitated the treatment of patients. Antiviral therapy by acyclovir is recommended immediately after lumbar puncture in any case when a diagnosis of HSE is suspected. However, if the diagnosis strongly relies on biological PCR-based results, the treatment may be interrupted when HSV PCR is negative in children with mild initial symptoms. Several studies have pointed to the existence of negative results of HSV PCR performed on early CSF samples of patients with HSE, in both adults [3, 7] and children [7–10]. In some of these patients, HSV DNA was detected in CSF obtained a few days later [3, 7]. To further evaluate the place of PCR among diagnostic parameters in the context of suspected encephalitis in children, we conducted a multicenter retrospective study to assess the reliability of the initial results of HSV PCR in proven HSE.

Patients and methods. Patients were traced from records kept by the Virology Department of Saint Vincent de Paul Hospital (Paris), where CSF samples from patients with infections of the CNS were prospectively assayed by PCR, according to a procedure published elsewhere [11]. After the exclusion of adult and neonatal cases of HSE, 42 infants and children who presented with clinical and/or radiological signs of meningencephalitis received a diagnosis of HSE from 1990 to 1997. The diagnosis was ascertained in 32 cases by positive HSV PCR in at least 1 CSF sample and in 9 cases by showing the intrathecal synthesis (ITS) of HSV antibodies. In the last case, the virological diagnosis was based on the appearance of HSV IgM and an 8-fold increase in HSV IgG titers on 2 serum samples taken at a 16-day interval.

Information was retrieved from medical records on age, sex, neurological signs and symptoms at admission, electroencephalogram (EEG), cerebral CT scan, and/or MRI results. Biological data were reviewed, including CSF leukocyte count, protein level, IFN-α levels, HSV DNA PCR, and HSV serological test results. The delay between the onset of symptoms and the initiation of treatment and the dose and duration of antiviral treatment were recorded. Day 0 was considered to be the day of the first reported neurological symptoms.

IFN-α was measured in serum and CSF according to a method described elsewhere [12]. PCR for HSV DNA was done as described elsewhere [11], using 100 μL of CSF instead of 200 μL. The PCR assay detected genome equivalents (GEq)/mL for HSV-1 and 2 × 103 GEq/mL for HSV-2. Serological testing was done using ELISA (Behring), and the ITS of HSV-specific IgG was measured by calculating the ratio of serum to CSF IgG [13].

Patients were divided in 2 groups, according to their PCR results. Differences in the values of CSF leukocyte count and protein level between the 2 groups were assessed using the nonparametric Mann-Whitney U test. χ2 and Fisher’s exact tests were used to search for a significant association between PCR.
results and CSF leukocyte count. \(P<.05\) was considered to be significant.

**Results.** The aim of the present study was to evaluate the reliability of early PCR results in HSE. Subsequently, 4 of 42 patients were excluded from analysis because the first PCR was performed after day 15 of the disease or was not done at all. Among the 38 remaining patients, PCR was performed between days 0 and 3 in 33 cases and between days 5 and 11 in 5 other cases. Overall, 32 patients had at least 1 positive PCR result that detected HSV-1 DNA.

Patients included 23 boys and 15 girls (ratio, 1.53) aged 3 months–16 years. Among these, 16 (42%) were aged 3–12 months, 8 (21%) were aged 1–3 years, and 14 (37%) were aged 5–16 years. In 18 cases (47%), nasopharyngeal infections were recorded 1–10 days before the onset of encephalitis. The distribution of clinical symptoms of HSE varied with age. Among the 24 patients aged <3 years, 19 (79%) had partial febrile seizures, which were brachiofacial in 9 patients. One patient had generalized febrile seizures. The seizures were associated with an altered state of consciousness in 11 (58%) patients, with meningeal irritation in 4, altered tonus in 2, and altered behavior in 1. In the other 4 patients, the first symptoms consisted of fever with either meningeal signs in 1 or a focal neurological deficit in 3. These 4 children had seizures the following days. Among the 14 patients aged >5 years, febrile seizures were the first neurological symptoms in only 5 (36%). Seizures were partial in 4 patients, among whom 1 child had previous radiotherapy for a midbrain glioma, and generalized in 1. In 1 child, partial seizures were the single symptom on day 0, and fever was noticed on the following day. Among these 6 patients, 4 (67%) presented an altered state of consciousness, and the others had meningeal irritation, altered behavior, or speech disorders. In the 8 other children aged >5 years, the first symptoms were fever with either meningeal signs (4), headache and emesis (2), or a febrile focal neurological deficit (2). All these children had seizures the following days. Intravenous acyclovir was administered for 10–30 days, at a dosage of 30–70 mg/kg/day. The mean delay between the start of acyclovir and the first reported neurological symptoms was 1.5 days.

Twenty-nine children (76%) had an EEG recording taken during the early phase of the disease, done between days 0 and 1 in 80% of cases. EEG abnormalities were present in all 29 (100%) patients and consisted of nonspecific diffuse slow waves in 9 (31%) patients and focal slow waves in 20 (69%) patients, among whom 11 were strictly focal in the temporal area and 7 displayed typical periodic discharges. Thirty-one (82%) children underwent cerebral imaging during the early phase of the disease, done between days 0 and 1 in 80% of cases. A cerebral CT scan was done for 29 patients, and MRI was done for 2 patients. Brain lesions were observed in 26 (84%) patients and were temporal (4), parietal (6), temporo-parietal (4), frontoparietal (2), parieto-occipital (1), and occipital (1). In 5 patients (4 CT scans and 1 MRI), no necrotic-hemorrhagical lesion was visible on the first neuroimaging, but temporal lesions were obvious the following days.

During the course of the disease, 86 CSF samples were taken from the 38 patients included in the study (mean, 2.2 samples/patient). For 33 patients, HSV PCR was performed on CSF obtained between days 0 and 3. In 28 (85%) of 33 cases, PCR was positive in at least 1 CSF. However, HSV was detected in the first CSF obtained from only 25 (76%) of 33 patients. In 9 of 33 patients, PCR was also performed after day 3. PCR was positive in 5 CSF samples obtained on days 4, 5, 9, 11, and 21, respectively, and was negative in 5 CSF samples drawn on days 8, 9, 14, and 25, respectively. From days 0 to 3, IFN-\(\alpha\) levels were elevated (\(>2\) IU/mL) in at least 1 CSF sample from 26 of 31 patients. In 31 of 33 cases, IFN-\(\alpha\) dosage and HSV PCR were assayed comparatively. HSV PCR was positive in 22 (85%) of 26 CSF samples with elevated IFN-\(\alpha\) levels and in 2 (40%) of 5 CSF samples with normal IFN-\(\alpha\) levels. The protein level in the CSF was elevated (\(>0.50\) g/L) in at least 1 CSF sample from 13 (40%) of 33 patients. In 30 of 33 cases, the protein level and HSV PCR were analyzed comparatively. The mean protein level in 8 PCR-negative CSF samples was 0.215 (\(\pm 0.097\)) g/L, whereas it reached 0.71 (\(\pm 0.43\)) g/L in 20 PCR-positive samples. Therefore, the protein level was significantly lower (\(P = .0007\)) when PCR was negative. The WBC count in the CSF was elevated (>5 cells/mm\(^3\)) in at least 1 CSF sample from 28 (88%) of 33 patients. In 32 of 33 cases, the WBC count in the CSF and HSV PCR were analyzed comparatively. The mean value was 29 ± 52/mm\(^3\) in 8 PCR-negative CSFs, whereas it was 229 ± 304/mm\(^3\) in 24 PCR-positive samples. Therefore, the WBC count was significantly lower (\(P = .0011\)) when PCR was negative. Of interest, among 19 CSF samples obtained on day 0, HSV-1 DNA was retrieved from all CSF samples that contained >15 WBC/mm\(^3\), except from 1 CSF sample that contained predominantly polymorphonuclear cells (table 1; patient 1). In contrast, HSV DNA was not detected in 5 CSF samples that contained <10 WBC/mm\(^3\) (patients 2–5 and 7). In patients 2 and 7, however, HSV-1 DNA was amplified from a second CSF drawn on day 3. HSV-1 was detected in all 5 CSF samples on day 1 and in 5 of 6 CSF samples on day 2. Finally, PCR was positive in 2 of 3 CSF samples tested on day 3. In the third patient (patient 8), PCR was positive on a second sample obtained on day 9. A statistical analysis revealed a significant association between the negative results of PCR and <10 WBC/mm\(^3\) in the CSF, either when considering PCR tests done on day 0 (\(\chi^2 = 14.702, P = .0001\); corrected \(P = .001\); Fisher’s exact test, \(P = .0005\)) or PCR done between days 0 and 3 (\(\chi^2 = 17.615, P < .0001\); corrected \(P = .0002\); Fisher’s exact test, \(P = .0002\)). For the last 5 patients in the study, the first HSV
Table 1. Data for patients with herpes simplex virus (HSV)–negative PCR from days 0–3.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Clinical data</th>
<th>First cerebral imaging findings</th>
<th>EEG findings</th>
<th>CSF findings</th>
<th>WBCs/μL</th>
<th>RBCs/μL</th>
<th>Protein, g/L</th>
<th>IFN, IU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7 months</td>
<td>F, L brachiofacial seizures</td>
<td>Normal</td>
<td>R, rhythmic activity</td>
<td>51^a</td>
<td>1500</td>
<td>0.32</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>16 months</td>
<td>F, L brachiofacial seizures, altered state of consciousness</td>
<td>R, temporal lesion</td>
<td>R, slow waves</td>
<td>4</td>
<td>520</td>
<td>0.26</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>22 months</td>
<td>L, hemicorporeal seizures</td>
<td>R, parieto-occipital lesion</td>
<td>R, rhythmic activity</td>
<td>8</td>
<td>16</td>
<td>0.17</td>
<td>&lt;2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>8 months</td>
<td>F, R brachiofacial seizures</td>
<td>Normal</td>
<td>Bilateral, temporal slow waves</td>
<td>4</td>
<td>0</td>
<td>0.40</td>
<td>&lt;2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>9 months</td>
<td>F, R brachiofacial seizures, oral herpes</td>
<td>Bilateral temporal lesion</td>
<td>Diffuse, slow waves</td>
<td>9</td>
<td>0</td>
<td>0.20</td>
<td>&lt;2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3 years</td>
<td>L, clonic seizures, altered state of consciousness</td>
<td>R, parietal lesion</td>
<td>Diffuse, slow waves</td>
<td>151^b</td>
<td>2100</td>
<td>0.17</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>9 months</td>
<td>L, hemicorporeal seizures</td>
<td>R, parieto-occipital lesion</td>
<td>R, parietal, slow waves</td>
<td>1</td>
<td>2</td>
<td>0.10</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>7 years</td>
<td>L, hemicorporeal seizures, altered state of consciousness</td>
<td>Midbrain glioma, no new lesion</td>
<td>R, slow waves</td>
<td>1</td>
<td>0</td>
<td>0.23</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

*NOTE.* EEG, electroencephalogram; F, fever; L, left; ND, not done; R, right.

^a Of these, 55% were polymorphonuclear cells.

^b Of these, 74% were leukocytes.

PCR was performed between days 5 and 11. HSV was detected in 4 CSF samples obtained on days 5, 7, 9, and 11, respectively, that had a high leukocyte count (45–130 cells/mm³), whereas PCR was negative in 1 CSF sample obtained on day 9, containing 7 leukocytes/mm³.

HSV serological testing was done for 32 patients. A diagnosis of primary HSV infection based on seroconversion was established in 6 cases. In 16 cases, HSV-specific IgG was present at onset of symptoms, and HSE was interpreted as secondary to either reactivation or reinfection. In 10 cases, serum samples were drawn too late during the course of the disease to accurately date the start of infection. HSV-specific ITS was seen in all 18 investigated cases.

**Discussion.** The present retrospective study describes 38 children with proven HSE treated with acyclovir during a 7-year period marked by the novel use of HSV PCR as a reference standard for diagnosis. By reviewing their clinical and biological features, we underline the difficulties encountered in assessing PCR-based HSE diagnosis at the very onset of the disease in the pediatric population.

In our study population, HSE was predominantly observed in infants aged 3 months–1 year. A high proportion of severe HSV infections during this period of life could be related to the disappearance of passive immunity from maternally acquired HSV-specific antibodies. Of interest, the male-to-female sex ratio was 1.53. Epidemiological studies of HSE in adults showed an equal distribution among male and female patients [3, 14]. However, a recent report of HSE in children also described more cases in boys than girls [2]. Although the retrospective design of our study hampers interpretation, a higher susceptibility of male infants and children to HSE, compared with other infectious diseases, can be hypothesized. Larger series are required to confirm this result.

The clinical spectrum of HSE has been redefined since the beginning of the use of HSV PCR for diagnosis [15]. Our results confirm that, in children beyond the neonatal period, the range of clinical symptoms is wider than has been previously evaluated. Indeed, febrile seizures were observed as first neurological symptoms in 79% of children aged 3 months–3 years, compared with 38% of children aged >4 years. In these latter patients, other diagnoses were primarily considered on the first days of the disease, which could have led to adverse consequences on therapeutic management. Moreover, neuroimaging showed characteristic temporal lesions in only 60% of cases, whereas involvement of other lobes was observed in 40% of cases. This proportion of frontal, parietal, or occipital lesions is higher than has been observed in adults [3] and could suggest a more varied route of access of the virus to the CNS in young patients [16].

Although the CSF of patients with HSE usually exhibits a pleocytosis containing predominantly lymphocytes and moderately elevated protein levels, samples drawn within the first hours of the disease may be normal or reveal a predominance of polymorphonuclear cells [3, 17]. In the present study, 4 (21%) of 19 CSF samples drawn on day 0 contained <10 cells/mm³ [3]. During the past 10 years, techniques based on the amplification of viral genomes by PCR have provided the most sensitive tools for the diagnosis of viral encephalitis. In our
study, 8 HSV PCR–negative results were observed out of 33 patients: 6 on day 0, 1 on day 2, and 1 on day 3. Therefore, our results show that early PCR detected HSV-1 DNA in 79% of children with HSE. Moreover, HSV-1 was successfully amplified in only 74% of cases on day 0 of disease. In addition, 4 of 5 CSF samples tested positive between days 5 and 11. Early negative HSV PCR results during HSE have been recorded repeatedly [3, 7]. However, the proportion of negative PCR results obtained in children is surprisingly higher than that observed in an adult population tested during the same period of time [3]. Indeed, only 3 of 93 adult patients had negative HSV PCR results on an initial normal CSF sample drawn at onset of HSE. In those 3 cases, a second sample drawn a few days later tested positive for HSV-1 PCR. Negative results of HSV PCR could theoretically be due to a lack of sensitivity of the method. The level of detection of HSV-1 DNA by our in-house PCR was ~3 pfu [11], or 200 Geq/mL. A similar level of detection coincided with a sensitivity of 98% when PCR was assayed on CSF samples from biopsy-proven cases of HSE [18]. Another hypothesis is that HSV probably was in the brain and caused symptoms of encephalitis, although it was not in the CSF at the time of the first lumbar puncture. Experimental data have suggested that HSV may be absent from the CSF during the earliest phase of the disease [19]. The negative result from one CSF sample that contained mostly polymorphonuclear cells, which might indicate an early stage of the disease [17, 19], supports this hypothesis. Moreover, although these values do not reach statistical significance, we observed that false-negative PCR results were more frequent at day 0 (26% of assays) than after this date (14% of assays). In addition, in 3 children, HSV-1 DNA was found in a second CSF sample after a negative result was obtained in a first CSF sample that contained <10 leukocytes/mm³. Finally, a statistically significant correlation was found between the presence of <10 cells/mm³ in the CSF and HSV PCR–negative results, whether analyzed on day 0 or among all samples. A normal cell count in the CSF was indeed associated with negative HSV PCR results in pediatric [20, 21] as well as in adult patients [15, 22]. These observations are in agreement with comparative analyses of the efficacy of HSV PCR in CSF pellets and supernatants [23] and with in situ hybridization studies [24], which led to the conclusion that HSV is mainly detected in CSF cells. However, in one patient in our study, HSV DNA was not retrieved from an early CSF sample that contained a pleocytosis made of lymphocytes. A recent report has also underlined negative PCR results on 3 similar CSF samples [7]. In an animal model of HSE, the presence of inflammatory cells in the CSF was shown to precede the presence of detectable HSV DNA in the CSF [19].

Overall, for 3 patients in the present series, neither initial biological nor radiological testing was suggestive of HSE. The CSF leukocyte count was normal in 2 of 3 patients and contained predominantly polymorphonuclear cells in the last 1. Whatever the mechanism that explains the absence of cells in the CSF and the negativity of PCR at onset of HSE in children, we want to draw attention to the fact that a careful interpretation of initial negative HSV PCR results is warranted in the context of clinical, EEG, and/or radiological features suggestive of encephalitis. In the pediatric setting, acyclovir is most often administered as soon as HSE is suspected. Antiviral therapy should not be interrupted solely on the basis of the results of PCR done on the CSF. The biological diagnosis of HSE is unambiguously confirmed by a delayed ITS of specific antibodies, because this synthesis is constant and represents the hallmark of a previous infection of the CNS by HSV [4]. This marker should be sought in all undiagnosed encephalitis cases. Several studies have unambiguously shown that the prognosis of HSE is significantly improved by acyclovir treatment, provided that the delay between the onset of symptoms and the administration of the drug is brief. This is of particular importance in infants and children, in whom lesions of the developing brain due to HSV may have devastating consequences. HSV PCR is presently considered to be the reference standard for an early diagnosis of HSE. We want to insist on the possibility of negative biological data that may lead to the interruption of appropriate therapy. In these cases, a second CSF sample should be drawn within the first days of the disease, and antiviral therapy should be administered as long as the diagnosis has not been excluded.

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