CONSENSUS

The role of laboratory investigation in the diagnosis and management of patients with suspected herpes simplex encephalitis: a consensus report

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Abstract

As effective therapies for the treatment of herpes simplex encephalitis (HSE) have become available, the virology laboratory has acquired a role of primary importance in the early diagnosis and clinical management of this condition. Several studies have shown that the polymerase chain reaction (PCR) of CSF for the detection of herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2) DNA provides a reliable method for determining an aetiological diagnosis of HSE. The use of PCR in combination with the detection of a specific intrathecal antibody response to HSV currently represents the most reliable strategy for the diagnosis and monitoring of the treatment of adult patients with HSE. The use of these techniques has also led to the identification of atypical presentations of HSV infections of the nervous system and permits the investigation of patients who develop a relapse of encephalitic illness after an initial episode of HSE. A strategy for the optimal use of the investigative laboratory in the diagnosis of HSE and subsequent management decisions is described.

(*J Neurol Neurosurg Psychiatry* 1996;61:339–345)

Keywords: herpes encephalitis; management; herpes simplex; polymerase chain reaction

Herpes simplex encephalitis (HSE) is one of the most common and serious sporadic encephalitides of immunocompetent adults. It may present at any age with an incidence estimated to be between 1 in 250 000 and 1 in 1 000 000 persons a year.[1 2] In adults and in children older than two years, over 90% of cases are caused by herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) is responsible for the remaining cases.[3] Both viruses are widespread in the adult population, with a rate of seropositivity of 60% to 100% for HSV-1 and 10% to 80% for HSV-2. Levels of seropositivity are related to socioeconomic status and geographic area,[1 4–6] Primary HSV infection, reinfection, or virus reactivation may be involved in the pathogenesis of HSE in the immunocompetent adult.[3] In humans the pathogenesis of HSE is only partially understood. However, in experimental animal models entry to the CNS via nerve pathways has been established.[7–10] There is also evidence that HSV can establish a latent infection in the CNS.[11] The host immune response and viral factors such as mutations in viral genes seem to influence transmission, neuroinvasiveness, and neurovirulence.[12 13] The pathological changes associated with severe HSE consist initially of acute inflammation, evolving to haemorrhagic and necrotising lesions. The lesions are characteristically located in the temporal lobes and orbital surface of the frontal lobes, but adjacent frontal, parietal, and occipital lobes and cingulate gyri may also be involved.[14 15] Typically, HSE presents as an acute neurological disease with altered levels of consciousness and non-specific focal neurological signs and symptoms. Generally there is a prodrome of fever, headache, and nausea over a few days. Neurological symptoms such as aphasia, altered olfactory perception, seizures, clouding of consciousness and behavioural changes, usually present on admission, suggest a diagnosis of encephalitis. In untreated patients HSE is rapidly progressive, leading to brain oedema and destruction of vital regulatory centres in the brainstem. In the most severe cases death occurs after seven to 14 days. In a double blind placebo controlled study of treatment of HSE, the mortality in patients treated with placebo was 70% and moderate to severe neurological sequelae affected the great majority of the survivors.[16]

A range of clinical presentations of HSV infection of the nervous system, including mild disease courses, relapsing and remitting encephalitis, or unusual neurological syndromes, sometimes related to specific anatomic locations, have now been described

Unusual presentations of HSV infections of the nervous system

Mild/subacute encephalitis (HSV-1, HSV-2)*[17–22]
Forms resembling psychiatric syndromes (HSV-1, HSV-2)[23–26]
Brain stem encephalitis (HSV-1)[27–32]
Benign recurrent meningitis (HSV-1, HSV-2)[23–40]
Myelitis (HSV-2)[33, 40–45]

*Including patients with AIDS.

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(table). The introduction of nucleic acid based amplification methods, such as the polymerase chain reaction (PCR) has helped, and will continue to help, extend the recognition of such uncommon clinical presentations.

In addition to the variability of presentation, the availability of specific, effective, antiviral therapy (acyclovir), clearly shows the need for a specific and rapid diagnosis. In this context, the diagnostic virology laboratory plays a fundamental part in establishing an aetiological diagnosis, influencing clinical decision making, and aiding in aspects of patient management.

Diagnosis of HSE

HSE should be suspected in any patient in whom the clinical presentation suggests acute encephalitis.

Low density lesions are demonstrable by CT of the brain in about 70% of cases a few days after onset of symptoms.[46] Fronto-basal and temporal lesions can be shown by MRI as hypointense lesions on T1 weighted images and hyperintense lesions on proton density and T2 weighted images at an earlier stage than by CT.[47 48] This suggests that MRI is the imaging method of choice in HSE. The lesions found by both these methods are, however, only suggestive of, but not specific for, HSE.

Similarly, EEG provides only limited diagnostic data; typically presenting with non-specific slow wave activity during the first five to seven days of illness.[49] Later, more characteristic paroxysmal sharp waves or triphasic complexes with a temporal predominance can be found.[49–51]

Analysis of CSF during the acute phase of HSE generally shows a mild to moderate pleocytosis, ranging from 5 to 500 cells/mm^3, consisting mainly of mononuclear cells. Protein content is either normal (< 0·5 g/l) or may be increased up to 2 g/l.[2 52]

Data derived from the currently available imaging methods, together with EEG recordings and the information obtained from standard CSF analysis, may support a diagnosis of HSE. However, none of these procedures can provide data sufficient to establish an aetiological diagnosis of HSE. A specific diagnosis of HSE can only be achieved by virological studies of the CSF or by examination of brain tissue obtained by biopsy or at postmortem.

Detection of HSV DNA in CSF

HSV DNA can be detected in the CSF of patients with HSE by PCR. This technique is based on the use of oligonucleotides (primers), which recognise and anneal specifically to a target DNA. Subsequently, a DNA polymerase synthesises copies of DNA molecules complementary to the DNA fragment delimited by a primer pair. In a PCR reaction, through repeated cycles of DNA denaturation and synthesis, 10^6 or more copies of the target DNA are produced.[53] The amplified DNA can be visualised using either ethidium bromide stained agarose gels or by hybridisation with a specific internal probe.

After transfer to a nitrocellulose or nylon membrane or detection using an enzyme linked immunosorbent assay (ELISA) based system, the amount of DNA can also be estimated. Because of the exquisite sensitivity of PCR for detecting low copy numbers of nucleic acid molecules, the implementation of measures to ensure that clinical specimens are not contaminated (for example, by microbial contamination from the local environment, from attending medical staff, and during the PCR test procedure) are mandatory.[54 55]

Many different PCR protocols have been developed for the detection of HSV DNA. Although most cases of HSE are caused by HSV-1, HSV-2 is responsible for 5% to 10% of cases. Procedures for the identification of both viruses are therefore required. HSV-1 or HSV-2 DNA can be directly identified and differentiated by the use of type-specific primers (type-specific PCR).[22] Primers specific for HSV-1 or HSV-2 can be used together in the same PCR assay, allowing the simultaneous detection of either virus (multiplex PCR).[56] Alternatively, DNA sequences in genes common to different herpesviruses, including HSV-1 and HSV-2, can be amplified and the PCR product identified using specific probes or endonuclease cleavage (group-specific PCR).[57–59] Primers for the specific amplification of either HSV-1 or HSV-2 have been chosen in the glycoprotein D or in the UL42 region for HSV-1,[60 61] and in the glycoprotein G gene for HSV-2.[22] Primers for the identification of DNA regions common to both HSV-1 and HSV-2 have been chosen in the glycoprotein D and the polymerase genes[57–59]. The sensitivity of a particular PCR assay varies according to the amount of sample processed, the DNA preparation techniques, primers, buffers, and PCR cycling conditions, and whether a single or a "nested" PCR technique is used. In a nested PCR, the products of the first amplification are reamplified with a second set of primers, nested between the first two. This procedure increases the sensitivity and the specificity of detection. Standardisation of sensitivity and specificity between different laboratories is essential to provide reliable and reproducible data. Establishing such standardisation is an objective of the present EU Concerted Action on Virus Meningitis and Encephalitis.

The PCR technique has been used for the detection of HSV DNA in CSF from patients with suspected HSE for at least six years. Several retrospective and prospective studies have clearly established PCR as the method of choice for obtaining an early aetiological diagnosis.[22 57–59 61–73] As HSV DNA is usually detectable in the CSF at the onset of neurological symptoms, it enables an early, non-invasive diagnosis to be achieved. In the largest series studied to date, HSV-1 or HSV-2 DNA was detected by a nested PCR assay in 82 and six patients respectively out of 93, giving a sensitivity of 95%.[22] Diagnosis was confirmed by HSV isolation or antigen detection from brain tissue, or by demonstration of a specific HSV intrathecal antibody production.

The specificity in this study was assessed on CSF samples from 60 patients with non-HSE encephalitis. None of these samples were found to be HSV PCR positive.[22] The use of PCR in this context represents one of the most successful diagnostic test procedures ever described in clinical neurology. To date the limited data available suggest that the HSV PCR remains positive for at least five days after the initiation of acyclovir therapy. Data from PCR obtained from patients who are receiving acyclovir must therefore be interpreted with caution.

It is still a matter of debate whether the HSV DNA detected in CSF is derived from intact virions or from viral DNA not associated with mature virions.[57] Detection of HSV DNA is, however considered to be associated with viral replication and HSV infection of the CNS.[60]

Demonstration of a humoral immune response within the CNS

The intrathecal synthesis of antibody against HSV during the course of HSE can be demonstrated by testing CSF and serum samples collected at the same time. An increased CSF:serum quotient for the HSV antibody titre, adjusted for CSF-blood barrier integrity, indicates a specific intrathecal antibody response.[74] Since the first studies in the early 1970s, different methodological approaches have been used for the identification and titration of HSV specific immunoglobulins IgG, IgA, and IgM. The use of methods such as ELISA and isoelectrofocusing and immunoaffinity mediated capillary blotting are associated with high sensitivity.[66 74-80] By the simultaneous determination of albumin, total IgG, IgA, and IgM in CSF and blood, the relative integrity of the CSF-blood-barrier can be assessed using one of the several established formulae.[74 81] The calculation of the antibody specificity index (ASI) permits an accurate assessment of intrathecal antibody synthesis. In this way, the demonstration of a specific intrathecal antibody production is possible even when the CSF-blood barrier is severely disrupted.[74 81] In HSE an ASI $\geqslant 1{\cdot}5$ is usually reached seven to 10 days after the onset of symptoms. Procedures based on the use of capture assays, of reference antibodies (that is, directed against other microbial agents), and antibody production by CSF lymphocytes have also been successfully used to show an HSV specific intrathecal immune response.[82-84]

The evaluation of an intrathecal antibody response against HSV should always be interpreted with care because of the possible cross-reaction with varicella zoster virus (VZV) antibodies during VZV infection of the CNS, and of a polyspecific antibody response as found in multiple sclerosis and certain other autoimmune diseases of the CNS.[74 77 83 85]

Determination of intrathecal antibody synthesis does not serve as a primary diagnostic tool, but can provide data to support PCR results obtained in the acute encephalitic phase. However, in prolonged and chronic cases—that is, when more than 10–14 days have elapsed or when antiviral therapy has been initiated several days earlier—measurement of intrathecal antibody synthesis is the test of choice.

Other methods of HSE diagnosis by CSF analysis

Because positive results are obtained in less than 5% of cases of adult HSE, virus isolation from CSF is not considered a useful diagnostic procedure.[3] To date, laboratory tests for the detection and measurement of viral antigens in CSF[86] are not sufficiently sensitive to be of value for the routine laboratory investigation of CSF in HSE.[73]

The concentrations of cytokines and other markers of immune activation in CSF have also been evaluated in patients with HSE. These include interleukin-6, interferon-γ, tumour necrosis factor-α, neopterin, β-2-microglobulin, and several other molecules.[22 87-89] Although useful in the study of pathogenesis the relevance of altered concentrations of these markers in HSE remains to be fully evaluated. However, α-interferon is a fully evaluated marker of virus infection and is present in the CSF of up to 95% of cases of HSE.

Brain biopsy

Until the introduction of PCR, the identification of HSV infection by analysis of brain tissue obtained by biopsy represented the only conclusive method for the in vivo diagnosis of HSE. Despite the possibility of sampling error, the sensitivity and specificity of HSV isolation from brain tissue for the diagnosis of HSE have been estimated as being 96% and 99% respectively.[90] The risk of complication associated with brain biopsy has been calculated as being of the order of 3%, and a proportion of these complications may prove to be fatal.[90-92] However, these optimal data for sensitivity and specificity depend to a large extent on neurosurgical expertise and the use of relevant imaging facilities to ensure that the biopsy site is correctly located. Given the much less invasive nature of a lumbar puncture and its repeatability, together with the sensitivity and specificity of HSV-PCR, it is clear that PCR analysis of CSF is currently the method of choice for the diagnosis of HSE. In cases with an unresolved diagnosis or when there is no clinical improvement, brain biopsy remains an option that requires serious consideration.[92]

Treatment

The recommended antiviral treatment for HSE is a 10 day course of acyclovir given intravenously at a dosage of 10 mg/kg every eight hours.[2 93] To be effective, treatment must be initiated as early as possible during the disease. Two large studies in the mid 1980s compared the efficacy of acyclovir versus vidarabine.[2 93] The mortality was significantly lower among patients receiving acyclovir than

in those treated with vidarabine (19% *v* 50%–55%). Patients left with minor or no sequelae were 38%–56% of patients treated with acyclovir and 13% of those receiving vidarabine. The efficacy of acyclovir seemed to be higher in those patients treated early after onset of disease. Acyclovir is well tolerated either alone or in combination with other treatment regimens, but should be used with care in patients with renal impairment.

The currently accepted treatment protocol has been established from controlled clinical trials. However, because recurrences of encephalitis have been reported after the standard 10 day acyclovir regimen,[65 94–96] alternate treatment regimes are currently being considered—for example, 15 mg/kg every eight hours, and/or 14 to 21 days of treatment. Long term oral acyclovir treatment after the acute episode of HSE, and treatment with new antiviral drugs giving better drug bioavailability, are also under consideration (for example, famcyclovir and valacyclovir).

To reduce cerebral oedema corticosteroids are sometimes given to patients with HSE. Their use remains controversial. In cases with a massive brain oedema, hyperventilation, osmotherapeutics, thiopentone, or 4-γ-hydroxybutyric acid loading are also used. Supportive therapy, including respiratory assistance, maintenance of salt and water balance, and control of seizures, is required in the acute phase of the disease.

The role of the virus laboratory in the diagnosis and clinical management of HSE

HSE should be suspected in all patients presenting with an acute encephalitis. An HSV infection should also be considered in other disorders of the nervous system, such as brainstem encephalitis, ascending myelitis, and benign recurrent meningitis (table).

After clinical examination, neuroimaging and EEG can be helpful in the differential diagnosis. Blood and CSF samples must be collected on admission. The CSF should be aliquoted at the bedside, and an aliquot sent immediately to the diagnostic virology laboratory for the detection of HSV DNA by PCR. Other aliquots are used for differential cell count, determination of glucose and protein content, and for specific HSV intrathecal antibody production. Any additional laboratory investigations which may help to resolve the differential diagnosis must be considered at this time. It is therefore essential that direct communication between the laboratory and attending clinician is established as early as possible.

Although 50 μl–200 μl CSF would suffice for PCR analysis in most laboratories, it must be kept in mind that other investigations (for example, serology, virus culture, PCR for detection of other micro-organisms) may also be required, particularly when HSV-PCR is negative. Therefore at least 1 ml of CSF should be collected for the virus laboratory. Strict procedures to maintain the sterility of CSF during lumbar puncture and the subsequent handling of samples are crucial to avoid contamination, which may cause false PCR positive results. Owing to the relative stability of DNA in CSF, samples for PCR analysis may be sent to the laboratory at room temperature. If the samples cannot be delivered within one day, it is preferable to store them at −20°C. (Note: viral DNA has been demonstrated in CSF samples stored at −20°C or +4°C for several years.) Currently available PCR tests can provide a result within less than 24 hours after receipt of the CSF sample. Because an early aetiological diagnosis is essential to ensure appropriate patient treatment and management, a PCR test result must always be reported to the clinicians as rapidly as possible.

The volume of CSF required for the demonstration of intrathecally produced HSV-specific antibodies varies according to the technique used. At least 1 ml CSF should be sent and stored as for the PCR sample; 5 ml–10 ml blood must always be collected at the same time as the CSF and both sent to the laboratory.

In clinical practice, the initiation of acyclovir therapy is mandatory as soon as a diagnosis of encephalitis, suggestive of HSE, is reached. Treatment must also be initiated in all atypical cases in which a positive PCR test result for HSV DNA in the CSF is obtained. This is an important consideration, as the possibility of unusual presentations of HSE must always be taken into account. Concomitant antibiotic therapy should also be given until a diagnosis of bacterial meningitis has been ruled out.

If a first CSF is PCR negative but the clinical suspicion of HSE remains high, a further CSF sample should be re-examined by PCR to exclude an initial false negative result. It should always be remembered that a PCR negative result can be obtained from patients with HSE who are already receiving acyclovir.[60] Conversely, a negative HSV CSF result on PCR suggests an alternative aetiological cause of the encephalitis, and demands further laboratory tests. In such cases, the withdrawal of acyclovir therapy should be considered. However, such action should only be taken after a critical evaluation of all the available diagnostic and clinical data.

At the end of treatment or as indicated by the individual clinical course, further paired CSF and serum samples for detection of HSV DNA and HSV specific antibodies must be obtained. The post-treatment CSF sample is generally negative for HSV DNA,[58 60] whereas analysis of the CSF/serum pair at this time generally shows significant HSV specific antibody production (for example, an HSV-ASI > 1·5, usually > 10).[74] In patients with whom intrathecal production of HSV specific antibodies cannot be demonstrated after two or three weeks, the diagnosis should be reconsidered.

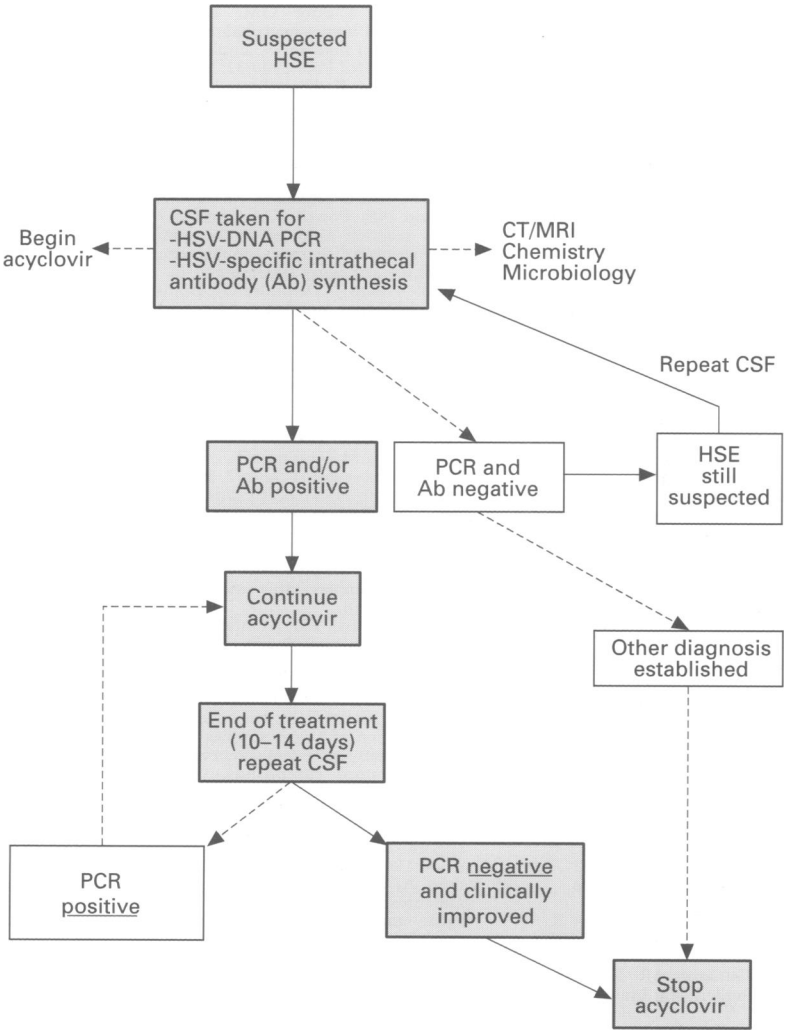
If CSF remains PCR positive after a first course of treatment, acyclovir should be continued until there is virological or clinical

improvement. An inadequate response to acyclovir may be associated with an HSE relapse. It remains to be determined whether this may be the consequence of a high viral load or of an incomplete or abnormal immunological response or the development of resistance to acyclovir. Resistance of HSV to acyclovir as a cause of treatment failure in immunocompetent patients with HSE has not yet been reported.

Summary

At the present time, PCR analysis of the CSF for the detection of HSV DNA, together with the detection of intrathecally produced HSV-specific antibodies, play a fundamental part in the diagnosis of HSE and for considering treatment strategies. Detection of HSV specific DNA by PCR is useful for the diagnosis of HSE early in the course of encephalitis (usually up to 10 days after onset of symptoms). Later, the demonstration of an intrathecal antibody production provides further evidence supporting a diagnosis of HSE. The diagnostic algorithm proposed (figure) indicates how to make optimal use of these techniques in the clinical management of HSE.

Some aspects regarding the efficacy of acyclovir remain problematic and require further clarification. After a standard 10 day course of treatment, clinical and virological relapses of HSE have been described. Furthermore, a number of patients do not recover completely after therapy and are left with severe sequelae such as aphasia, behavioural changes, and epilepsy. To prevent relapse and other such sequelae higher dosage and longer duration of acyclovir treatment—namely, 14 or 21 days—may prove to be appropriate. No final decision has yet been made on the most appropriate treatment strategy for HSE. What is firmly established at the present time is that obtaining an aetiological diagnosis as early as possible, together with immediate initiation of acyclovir therapy, will lead to the best possible clinical outcome for patients with HSE.



Diagnostic algorithm for management of patients with suspected HSE. As patients may present with raised intracranial pressure immediate lumbar puncture may not be possible. In these circumstances start of acyclovir treatment before lumbar puncture is warranted.

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NEUROLOGICAL STAMP

Claude Bernard (1813–78)

The great French physiologist Claude Bernard was born in the same year as Livingstone. Bernard was the son of a poor wine grower who began writing plays to earn money but turned to medicine on the advice of a literary critic. He began his brilliant career by assisting Magendie and becoming involved in his research on spinal nerves. His works in neurophysiology included the description, origin, and function of the chorda tympani and studies of the function of the cervical sympathetic system. While investigating the function of the submandibular gland, he showed that the sympathetic nerve was the constrictor of the blood vessels and the chorda tympani the dilator. So the fundamental facts of vasomotor physiology became known. He demonstrated that simple reflex movements were due to the influence sensory roots exerted on the motor roots and that a puncture of the floor of the fourth ventricle caused diabetes. The piqûre diabétique has been interpreted by many as being the result of excessive stimulation of secretory nerves to the liver.

Claude Bernard was the first to demonstrate that curare blocked the nerve stimulation of muscle while the muscle itself remained directly excitable and he carried out important work on the action of other drugs, including the opium alkaloids.

Bernard's wife and daughters deserted him when he refused to give up experimental medicine for a more lucrative practice of his profession. Towards the end of his life he published his famous *Introduction à la médecine expérimentale (1927)*. The book discusses the importance of the constancy of the internal environment, refutes the notion of the "vital force" to explain life, and emphasises the need in planning experiments for a clear initial hypothesis which has then to be proved. Because of this work he was elected to the French Academy in 1869. In 1878 Charles Edouard Sequard was appointed to the Chair of Medicine at the College de France, in succession to Claude Bernard.

He has been philatelically honoured as a great physician and founder of modern physiology. His was a national funeral, the first ever granted to a scientist in France. France issued this stamp (Stanley Gibbons 648, Scott B89) in 1939. The surtax was used for supporting unemployed intellectuals.

L F HAAS