Submitted December 2017; Accepted February 2018

CASE REPORT

Herpes simplex encephalitis: the pitfall of multiple false-negative polymerase chain reactions

J. Heidt[1], M.M. Leembruggen-Vellinga[2], J.W. Dorigo-Zetsma[3], G. Innemee[1]

[1]Department of Intensive Care, [2]Department of Neurology, [3]Department of Medical Microbiology, Tergooi Hospital, Hilversum, the Netherlands

Correspondence

J. Heidt - jheidt@tergooi.nl

Keywords - **encephalitis, herpes simplex virus, polymerase chain reaction**

Abstract

A 78-year-old woman was admitted to our ICU with the clinical picture of herpes simplex encephalitis (HSE). We started treatment with acyclovir but rejected this diagnosis after two negative HSV-1/-2 PCRs on cerebrospinal fluid (CSF), obtained on day 2 and 5. Our patient initially improved, but showed neurological deterioration again after 21 days. To our surprise, a third HSV-1 PCR on CSF obtained on day 23 was positive. Acyclovir was restarted, but she did not recover and died on day 37.

HSE is a severe neurological disease, with high mortality and morbidity. Early recognition, diagnosis and appropriate treatment (acyclovir 10 mg/kg x3/day for 14-21 days) are of upmost importance to provide the patient with the best chance of recovery. It is very important to keep in mind the possibility of multiple negative PCR results.

Introduction

Acute viral encephalitis is a severe neurological disease, with high morbidity and mortality. The definitive diagnosis can be a challenge and can include pitfalls as we present in this case.

Case history

A 78-year-old woman presented to the emergency department with a suspected stroke. She had experienced a 24-hour lasting weakness of her right arm, together with aphasia, dysphagia, a swollen tongue and diaphoresis. She had no relevant medical history, known prescribed medications were omeprazole and losartan/hydrochlorothiazide.

Physical examination showed a Glasgow Coma Scale (GCS) of E4V1M6 (aphasia), a heart rate of 99/minute, a blood pressure of 175/104 mmHg and a tympanic temperature of 37.7oC. There were no signs of meningism or skin abnormalities. Neurological examination showed weakness of the right arm and hyperreflexia of the right side of the body. The swollen tongue, positioned in the left corner of the mouth, and diaphoresis were clearly visible. While being examined, she developed focal rhythmical convulsions of the right side of the face and an altered consciousness.

Table 1. Lumbar puncture results

Lumbar puncture #1 (Day 2 of illness ; day 1 of hospital admission)	Erythrocytes < 5000x10^6/l, leucocytes 57x10^6/l (mononuclear 8%, polymorphonuclear 92%, glucose 5.3 mmol/l (serum 9.0 mmol/l), total protein 0.45 g/l No bacterial growth PCR diagnostics negative: HSV1, HSV2, VZV, enterovirus, parechovirus, *Mycobacterium tuberculosis* complex, *Chlamydophila psittaci, Mycoplasma pneumonia, Legionella pneumophila*
Lumbar puncture #2 (Day 5 of illness ; day 4 of hospital admission)	No erythrocytes, leucocytes 2x10^6/l, glucose 5.7 mmol/l (serum 10.8 mmol/l), total protein 0.36 g/l No bacterial growth. PCR diagnostics negative: HSV1, HSV2, VZV, enterovirus, parechovirus, *Borrelia burgdorferi*, EBV, CMV.
Lumbar puncture #3 (Day 24 of illness; day 23 of hospital admission)	No erythrocytes, leucocytes 6x10^6/l, glucose 4.1 mmol/l (serum 8.3 mmol/l), total protein 0.25 g/l No bacterial growth PCR diagnostics: **HSV-1 POSITIVE**, HSV-2 negative, VZV negative.

PCR = polymerase chain reaction ; HSV = herpes simplex virus ; VZV = varicella zoster virus ; EBV = Epstein-Barr virus ; CMV = cytomegalovirus

Laboratory results showed a slightly elevated ESR 35 mm/hour, leucocytes 6.5 x 109/l, C-reactive protein 6 mmol/l, haemoglobin 7.0 mmol/l, normal coagulation, sodium 130 mmol/l, potassium 4.0 mmol/l, glucose 9.0 mmol/l, normal kidney function and normal hepatic biochemistry. Brain computed tomography scan (CT scan) was normal. An electroencephalogram (EEG) showed continuous discharges suspicious for epileptic activity, mainly in the left hemisphere. Lumbar puncture was performed, cerebrospinal fluid (CSF) analysis showed an elevated (polymorphonuclear) leukocyte count, while polymerase chain reaction (PCR) for herpes simplex virus (HSV)-1 and -2 was negative *(table 1)*. Acyclovir 550 mg x3/day (10 mg/kg x3/day) was started for suspected viral encephalitis.

Levetiracetam was started for epilepsy. That night, the patient's consciousness deteriorated, with an E1V1 M4 score and progression of the convulsions of her face and right arm. A new brain CT scan showed no abnormalities. Phenytoin was added, and she was admitted to the intensive care unit. At that time the GCS had worsened to EVM-3. She was intubated and mechanically ventilated, and propofol was started to counteract the persistent convulsions.

She was treated with propofol until the convulsions ceased, while the acyclovir, levetiracetam and phenytoin were continued. On admission day 4, no convulsions were observed after discontinuing the propofol and EEG registration showed a diffuse slow background (encephalopathic) pattern without signs of epilepsy. Brain CT scan again showed no abnormalities. A second lumbar puncture was performed, with CSF showing normal biochemistry and a negative PCR for HSV-1 and -2 *(table 1)*. We rejected the diagnosis of herpes simplex encephalitis (HSE), because it seemed highly unlikely with two negative HSV PCRs on CSF. We decided to continue treatment with acyclovir just to be on the safe side, for a total of 10 days. Limbic encephalitis / autoimmune encephalitis (AIE) was considered an alternative explanation. A CT scan of chest and abdomen was performed to screen for teratoma or other neoplasms, as this may cause encephalitis as a paraneoplastic disorder.[1] No such neoplasms were found. Now shifting to the differential diagnosis of AIE, we also decided to start treatment with methylprednisolone 1000 mg a day, for a total of three days. Brain magnetic resonance imaging (MRI) showed bilateral temporal and insular hyperintensity, compatible with infectious or inflammatory changes as seen in limbic encephalitis or HSE *(figure 1, panel A)*.

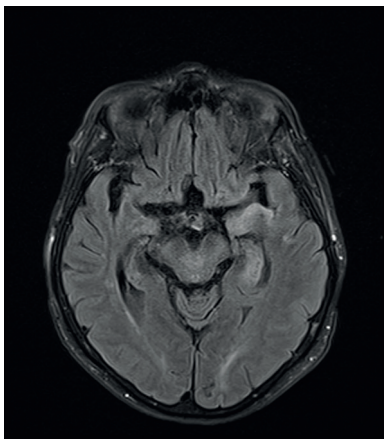

Figure 1 Panel A. Brain MRI
MRI on admission day 7, illness day 8: T2-hyperintensity and mild swelling in the left temporal lobe with abnormal signal on diffusion-weighted images, suggestive for (herpes) encephalitis.

On admission day 8, there seemed to be slight neurological improvement, and on admission day 9, the patient had a GCS of E3Vtube M6. While still in diagnostic uncertainty, but judging HSE to be unlikely after two negative HSV PCRs and clinical improvement with a high dose of steroids, we tended once again towards the differential diagnosis of AIE and started treatment with intravenous immunoglobulin (IVIG) 0.40 mg/kg/day for 5 days. On admission day 10, the patient regained consciousness (E4Vtube M6), after which the acyclovir was discontinued. She was extubated the day after, and re-intubated the same day due to post-extubation stridor, for which she was treated with dexamethasone 10 mg x4/day for 6 days. Because of the urgent need for re-intubation we did not have the chance to inspect the vocal cords with fibre-optic laryngoscopy. On admission day 17, after a week of neurological improvement, we performed a tracheotomy. In the absence of signs of epilepsy for the past 12 days, the phenytoin was discontinued, while levetiracetam treatment was maintained.

After that, the patient worsened clinically, with an E1Vtrach M1-score on admission day 21 (i.e. three weeks after admission, and almost two weeks after neurological recovery). On day 21 facial twitches re-occurred and treatment with phenytoin and propofol was restarted. EEG registration did not show epileptic activity, only a diffuse and undifferentiated slow background pattern. A second brain MRI was performed, which showed more extensive cortical hyperintensity and partial gadolinium enhancement *(figure 1, panel B)*.

On admission day 23, a third lumbar puncture was performed, and a second course of methylprednisolone treatment was prescribed. This time, the HSV-1 PCR result surprisingly came back positive on admission day 25, with a cycle threshold value of 30 cycles, which was reproduced in a confirmatory test. Steroid treatment was discontinued immediately and acyclovir was restarted. On day 27, after discontinuation of propofol, no

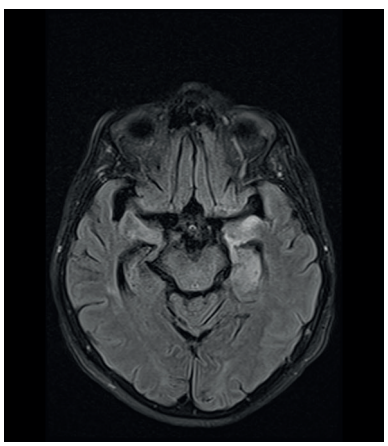

Figure 1 Panel B. Brain MRI
MRI on admission day 21, illness day 22: mild progression of these abnormalities.

more twitches were seen but her consciousness was low, with a GCS of E1Vtrach M4. On admission day 31, an EEG registration showed encephalopathic patterns and no epilepsy. A brain MRI *(figure 1, panel C)* showed a further increase of the cortical and subcortical abnormalities, with mass effect and iron depositions compatible with HSE. Autoantibodies associated with AIE were not found in the CSF samples that were sent to a tertiary academic clinic *(table 2)*, and HSE was assumed to be the final diagnosis. The patient did not regain consciousness. We discussed the troublesome situation and prospects of the patient within our ICU and neurology team, and with the family. In the absence of recovery under antiviral treatment and with no prospect of reasonable and acceptable recovery, we decided to stop treatment on admission day 34. The patient died on day 37. The family did not grant permission for autopsy.

Table 2. Additional diagnostic tests

Laboratory results	Vitamin B1: 97 nmol/l (67-150 nmol/l) Vitamin B6: 64 nmol/l (35-110 nmol/l) Vitamin D: 115 nmol/l (50-250 nmol/l)
Microbiology results	Blood cultures: negative
Serology results	Treponema pallidum IgG + IgM: negative Borrelia burgdorferi IgG + IgM: negative Tick-borne encephalitis virus IgG: equivocal; IgM: negative HIV-1 / HIV-2: negative
Diagnostic tests autoimmune encephalitis (on CFS)	Anti-TPO < 15 kIU/l (< 35 kIU/l) Anti-neural antibodies: negative - VGKC / VGKC-complex (incl. LGII + CASPR2) - NMDA-receptor - Hu - Yo - Ri - Tr - Amphiphysin - CV2 (CRMP5) - Ma1 - Ma2 (TA) - AMPA-receptor - GABAb-receptor - DPPX

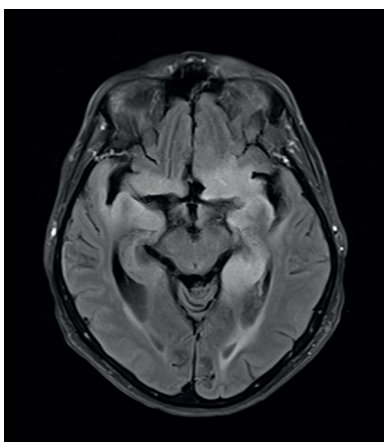

Figure 1 Panel C. Brain MRI
MRI on admission day 31, illness day 32: further progression of bilateral T2-hyperintensity and swelling in the frontotemporal lobes and limbic system.

Discussion

Acute viral encephalitis is a severe neurological disease, with a high morbidity and mortality. HSV-1 is a common cause, present in up to 12% of all cases of encephalitis.[2-4]

Differentiating between encephalopathy and encephalitis on clinical presentation is difficult. Encephalopathy can be caused by infectious agents, severe illness (such as sepsis) and several metabolic disturbances, and can present as delirium. Criteria for encephalitis are altered mental state for more than 24 hours in the absence of evidence for other causes, and at least two of these following criteria: fever, (new) convulsions, new focal neurological impairment, pleocytosis in the CSF, radiographic abnormalities and/or typical changes of the EEG.[5]

Neurological symptoms of HSE are typical for the location of preference of the virus in the frontotemporal lobes of the brain: disorientation, dysphagia, altered mental state, behavioural changes, amnesia, headache and convulsions.[6,7]

Early diagnostic procedures are of upmost importance, but should never delay the start of treatment. Laboratory results can show leucocytosis and elevated C-reactive protein, but are aspecific. Early CSF analysis is essential. Nowadays PCR on CSF is used to diagnose HSE. It can be negative in the acute phase and does not exclude HSE. PCR on illness day 2-10 has a sensitivity of 96-98% and a specificity of 95-99%.[8] In case of a traumatic lumbar puncture, the PCR can be less reliable, because the porphyrin of haemolysed erythrocytes can inhibit the PCR.[4]

Radiography is another important diagnostic tool. MRI is superior to CT imaging for diagnosing HSE. On MRI, abnormalities can be seen in the frontal and temporal lobes, with a hypo-intense signal on the T1-weighted and a hyper-intense signal on the T2-weighted and FLAIR sequences. In the early phase, cytotoxic oedema causes hyperintensity on the diffusion weighted imaging.[9] EEG registration can show nonspecific encephalopathic changes, periodical discharges, focal slow background pattern or epileptic activity.[10]

Treatment of HSE consists of acyclovir 10 mg/kg x3/day and, if needed, intensive monitoring and supportive measures on a high care or brain care unit. Because of reports of relapses after 10 days of treatment with acyclovir, it is recommended to continue treatment for 14-21 days.[11-13] Untreated HSE has a high mortality rate, approximating 70%. Treatment with acyclovir dramatically decreases the mortality rate to 11-15%.[6,7]

Throughout the greater part of the ICU stay and treatment of this patient, we faced a diagnostic dilemma. Although we had a high clinical suspicion and radiographic images showed abnormalities suggestive of HSE, no HSV-DNA was detected in the CSF obtained on days 2 and 5. Because a PCR taken between days 2-10 has the highest sensitivity and specificity to detect HSV, we decided to pursue other differential diagnoses as well (autoimmune encephalitis),[14,15] and treated the unproven HSE with acyclovir for 10 days. In hindsight it would probably

have been wise to treat for a total of 14-21 days, although the outcome of this severe HSE would not necessarily have been different. After the HSV-PCR of the third CSF sample was found positive, all HSV-PCRs on the three original CSF samples were repeated in our microbiology laboratory, as well as in a reference laboratory. The results were the same, with a reproducible HSV-DNA load found only in the third sample. Cases with the first HSV PCR negative have been described before; a similar case with a positive second HSV-1 PCR result on day 30 was described in 2015.[16] To our knowledge, no cases have been described with two negative HSV PCR results on CSF.

It is debatable whether the treatment with methylprednisolone and its immunosuppressive effects affected the outcome of this case in a negative way. Reactivation of HSV resulting in HSE has mainly been described in immunocompromised patients treated with chemotherapy and corticosteroids.[17-19] Where adjunctive treatment with dexamethasone in bacterial meningitis is standard of care,[20] beneficial effects in HSE remain unclear. Several case reports describe a beneficial effect of dexamethasone in HSE.[21-23] As high loads of pro-inflammatory cytokines such as interleukin (IL)-6 in CSF are associated with poor outcome in patients with HSE, corticosteroids could be beneficial by inhibiting IL-6.[24] At the same time, we know little about the possible negative immunosuppressive effects of adjunctive therapy with corticosteroids in HSE. Future studies should provide us with definitive clinical proof.[25] A multicentre randomised placebo-controlled trial (GACHE trial) started a couple of years ago will probably address the value of corticosteroids in HSE.[26] Because of low recruitment, the GACHE trial was stopped prematurely and publication is on the way. Also, currently a new randomised controlled trial (DexEnceph) is being performed,[27] which will probably give more guidance on this topic in the future. Until that moment, adjunctive dexamethasone treatment can be considered when there is evidence of progression despite acyclovir treatment.[22,23]

Conclusion

HSE is a severe neurological disease with a high mortality and morbidity. Early recognition, diagnosis and appropriate treatment are of upmost importance to provide the patient with the best chance of recovery. It is very important to keep in mind the possibility of one or, as in our case, even two negative results of HSV PCR in the CSF. In case of proven HSE, or in case of high clinical suspicion and/or radiographic abnormalities pathognomonic for HSE, the duration of acyclovir treatment should be at least 14 days. In case of severe neurological illness, antiviral treatment should be continued for 21 days. Repeating lumbar puncture HSV PCR when in diagnostic doubt is recommended, even after >10 days in the disease process. Adjunctive dexamethasone treatment can be considered when there is evidence of progression despite adequate acyclovir treatment.

Acknowledgments

The authors would like to thank X.Y.D. Beele (Department of Radiology, Tergooi Hospital, Hilversum, the Netherlands) for providing the MRI images and S. Heidt (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, the Netherlands) for creating and providing *figure 2*.

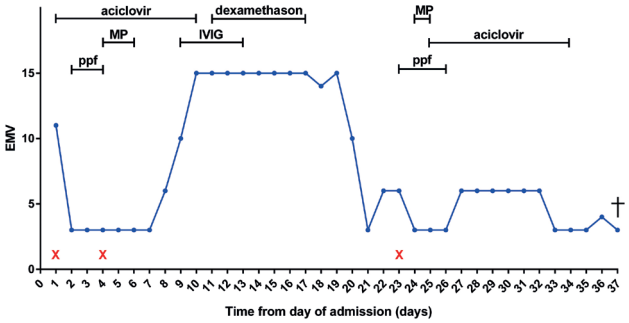

Figure 2. Timeline showing Glasgow Coma Score and treatment interventions throughout ICU admission
MP = methylprednisolone; IVIG = intravenous immunoglobulin, ppf = propofol. The red X's represent the lumbar punctures. In hindsight and with knowledge of the definitive diagnosis, the slow deterioration after the first treatment with acyclovir was stopped becomes quite obvious

Disclosures

All authors declare no conflict of interest. No funding or financial support was received.

References

1. Sillevis Smitt PA, Polman SK, de Beukelaar JW, et al. Three patients with a paraneoplastic neurological syndrome: the significance of paraneoplastic antibodies. Ned Tijdschr Geneeskd. 2007;151:874-80.
2. Huppatz C, Durrheim DN, Levi C, et al. Etiology of encephalitis in Australia, 1990-2007. Emerg Infect Dis. 2009;15:1359-65.
3. Khetsuriani N, Holman RC, Anderson LJ. Burden of encephalitis-associated hospitalizations in the United States, 1988-1997. Clin Infect Dis. 2002;35:175-82.
4. Nijsse B, Jacobs BC, Murk JL. Richtlijn acute virale (meningo-)encephalitis (2012, revisie 2017). Erasmus MC, afdeling neurologie.
5. Venkatesan A, Tunkel AR, Bloch KC, et al. Case definitions, diagnostic algorithms, and priorities in encephalitis. Consensus statement of the international encephalitis consortium. Clin Infect Dis. 2013;57:1114-28.
6. Raschilas F, Wolff M, Delatour F, et al. Outcome of and prognostic factors for Herpes simplex encephalitis in adult patients. Results of a multicenter study. Clin Infect Dis. 2002;35:254-60.
7. Riancho J, Delgado-Alvarado M, Sedano MJ, et al. Herpes simplex encephalitis: clinical presentation, neurological sequelae and new prognostic factors. Ten years of experience. Neurol Sci Off Ital Soc Clin Neuro-physiol. 2013;34:1879-81.
8. Kennedy PGE. Viral encephalitis. J Neurol. 2005;252:268-72.
9. Kiroglu Y, Calli C, Yunten N, et al. Diffusion-weighted MR imaging of viral encephalitis. Neuroradiology. 2006;48:875-80.
10. Bradshaw MJ, Venkatesan A. Herpes simplex virus-1 encephalitis in adults: pathophysiology, diagnosis, and management. Neurotherapeutics. 2016;1-16.
11. Skelly MJ, Burger AA, Adekola O. Herpes simplex virus-1 encephalitis: a review of current disease management with three case reports. Antivir Chem Chemother. 2012;23:13-8.
12. Tunkel AR, Glaser CA, Bloch KC, et al. The management of encephalitis: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis. 2008;47:303-27.
13. Solomon T, Michael BD, Smith PE, et al. Management of suspected viral encephalitis in adults – Association of British Neurologists and British Infection Association National Guidelines. J Infect. 2012;64:347-73.

14. de Bruijn MA, van Sonderen A, Sillevis Smitt PA, et al. Richtlijn diagnostiek en behandeling van auto-immuun encephalitis. Erasmus MC, afdeling neurologie; 2016.
15. Wingfield T, McHugh C, Vas A, et al. Autoimmune encephalitis: a case series and comprehensive review of the literature. Q J Med. 2011;104:921-31.
16. Buerger KJ, Zerr K, Salazar R. An unusual presentation of herpes simplex encephalitis with negative PCR. BMJ Case Rep. 2015;2015.pii:bcr2015210522.
17. Jacobs DH. Herpes simplex virus encephalitis following corticosteroids and cranial irradiation. Neurology. 1999;52:1108-9.
18. Saito M, Kiyozaki H, Obitsu T, et al. Herpes simplex virus-1 encephalitis induced by chemotherapy and steroids in an esophageal cancer patient: a case report. BMC Cancer. 2016;16:233.
19. Silvano G, Lazzari G, Resta F, et al. A herpes simplex virus-1 fatal encephalitis following chemo-radiotherapy, steroids and prophylactic cranial irradiation in a small cell lung cancer patient. Lung Cancer. 2007;57:243-6.
20. de Gans J, van de Beek D. European dexamethasone in adulthood bacterial meningitis study investigators: dexamethasone in adults with bacterial meningitis. N Engl J Med. 2002;347:1549-56.
21. Mesker AJ, Bon GG, de Gans J, et al. Case report: a pregnant woman with herpes simplex encephalitis successfully treated with dexamethasone. Eur J Obstet Gynecol Reprod Biol. 2011;154:231-2.
22. Lizarraga KJ, Alexandre LC, Ramos-Estebanez C, et al. Are steroids a beneficial adjunctive therapy in the immunosuppressed patient with herpes simplex virus encephalitis? Case Rep Neurol. 2013;5:52-5.
23. Musallam B, Matoth I, Wolf DG, et al. Steroids for deteriorating herpes simplex virus encephalitis. Pediatr Neurol. 2007;37:229-32.
24. Kamei S, Taira N, Ishihara M, et al. Prognostic value of cerebrospinal fluid cytokine changes in herpes simplex virus encephalitis. Cytokine. 2009;46:187-93.
25. Openshaw H, Cantin EM. Corticosteroids in herpes simpex virus encephalitis. J Neurol Neurosurg Psychiat. 2005;76:1469.
26. Martinez-Torres F, Menon S, Pritsch M, et al. Protocol for German trial of acyclovir and corticosteroids in herpes-simplex-virus-encephalitis (GACHE): a multicenter, multinational, randomized, double-blind, placebo-controlled German, Austrian and Dutch trial [ISRCTN45122933]. BMC Neurol. 2008;8:40.
27. DexEnceph: A study of dexamethasone in adults with Herpes Simplex Virus (HSV) encephalitis. (Accessed 5 February 2018, at http://www.dexenceph.org.uk and http://www.isrctn.com/ISRCTN11774734)