**Supplementary material**

Clinical vignettes

Case #1

A 2-month-old previously healthy boy presented with seizures and fever. CSF studies showed 77 white blood cells (WBC)/microliter (87% lymphocytes), elevated protein concentration (78mg/dL) and normal glucose concentration; PCR for herpes simplex virus -1 (HSV-1) was positive. EEG showed periodic lateralized discharges predominant in posterior regions, and the brain MRI showed bilateral T2/FLAIR increased signal in the occipital and temporal lobes (right >left). Intravenous acyclovir (1500mg/m2 daily) was started, and he was admitted to Pediatric Intensive Care Unit for convulsive status epilepticus. Seizures were treated with midazolam, levetiracetam, phenobarbital and phenytoin, achieving good seizure control by day 6 (Video segment 1). Seven days after onset of HSE the patient developed generalized choreoathetosis with prominent orofacial dyskinesias (Video segment 2) without EEG correlate in serial video-EEG recordings, insomnia and periods of irritability alternating with somnolence. The choreoathetosis was severe and persisted for 24 hours, followed by a gradual improvement over the ensuing 60 days. On day 24 after onset of HSE, CSF studies showed 120 WBC/µl (96% lymphocytes), 1568 red blood cells/µl, protein of 249 mg/dL, and normal glucose concentration; HSV PCR was negative, and intravenous acyclovir treatment was stopped. At that point, NMDAR antibodies were detected in CSF (1:40, intrathecal synthesis 75.41) and serum (1:200), but not in CSF and serum obtained at onset of HSE (day 0 and day 5 respectively). Because of continuous neurological improvement, no immunotherapy was initiated. The patient was discharged from hospital on levetiracetam, and oral acyclovir (300 mg/m2 daily). Abnormal movements and insomnia continued to improve (Video segment 3 and 4), and completely resolved two months after HSE onset (Video segment 5). The CSF was normal, HSV-PCR negative, CSF titers decreased (1:20, intrathecal synthesis 2.36), serum NMDAR titers increased (1:3200). At the last follow up (four month after onset of HSE), the patient now 6 month, he is awake, attentive, and has residual deficits in visual tracking that have been attributed to viral-related occipital lesions (PCPC 3).

Case #2

See details on early disease course in Armangue et al. J Pediatr 2013.1 At the last follow-up two years after onset of HSE, the patient is alert and able to interact at age-appropriate level, although she has residual dysphagia and dysphonia due to bilateral opercular necrotic lesions caused by the initial viral infection (PCPC 2). Retrospective study of archived serum obtained at the time of onset of HSE showed no NMDAR antibodies, at day 23 NMDAR antibodies were positive (serum 1/3200, CSF 1/160) demonstrating NMDAR seroconversion after HSE.

Case #3

A previously healthy 6-month-old girl presented with fever, diarrhea and focal seizures. CSF studies showed 10 WBC/µl (76% mononuclear), normal protein and glucose concentration, and positive HSV-1 PCR. Brain MRI showed bilateral increase in T2/FLAIR signal in the temporal lobes (right>left). She was treated with intravenous acyclovir for three weeks (1500mg/m2 daily). Phenobarbital was added because of recurrent seizures 15 days after symptom onset, and she continued to improve. CSF 21 days after symptom onset showed negative PCR for HSV-1 and acyclovir was stopped. One week later (one month after HSE onset), irritability, insomnia, oral dyskinesias and choreoathetosis commenced. Treatment with acyclovir (1500mg/m2 daily) was reinitiated, and the patient was admitted to the Pediatric Intensive Care Unit for a decreased gag reflex, hypoventilation, and episodes of bradycardia. Repeat brain MRI showed encephalomalacia in previously affected areas but no new T2/FLAIR abnormalities. CSF was normal and HSV PCR negative. NMDAR antibodies were found in the CSF but not in the CSF sample obtained at onset of HSE. Treatment with IVIg (0,4g/k/day for 5 days) and methylprednisolone (30mg/k/day for three days) was started. Additional medication included clobazam, valproate and tetrabenazine. No improvement occurred during the next month, she continued with prominent choreoathetosis, unresponsiveness, dysphagia and dysphonia leading to placement of a nasogastric tube and posterior gastrostomy tube. Fifty-two days after HSE, repeat serum/CSF antibody titers showed persistently elevated titers (serum 1/12800, CSF 1/80, intrathecal synthesis 4.71). Screening for an underlying ovarian teratoma was negative. The patient developed a transient syndrome of inappropriate antidiuretic hormone secretion (SIADH). Fifty days after neurological relapse (80 days after onset of HSE), second line immunotherapy was started (rituximab 375 mg/m2 weekly, 4 doses; cyclophosphamide monthly intravenous pulses, first dose: 500 mg/m2, second-sixth dose 750 mg/m2). Two months later (4 months after the onset of neurological relapse), improvement was noted. At the last follow-up 7 months after HSE, the antibody titers have decreased (serum 1/6400, CSF 1/10, intrathecal synthesis 0.59) and all symptoms related to the relapse post-HSE have resolved, but she has residual deficits related to HSE (hemiparesis, difficulty sitting and dysphagia, PCPC 3).

Case #4

A previously healthy 8-month-old boy presented with fever and focal seizures. CSF studies showed 85 WBC/µl (95% lymphocytes), normal protein and glucose concentration, and the PCR for HSV-1 was positive. Brain MRI showed bilateral increase of T2/FLAIR signal in frontal and temporal lobes. He was treated with intravenous acyclovir (1500mg/m2 daily) for three weeks, resulting in substantial recovery, and was discharged on day 21 without neurological symptoms. Twenty-three days after onset of HSE, he developed extreme irritability and insomnia, and treatment with acyclovir (1500mg/m2 daily) was restarted. Two focal seizures were successfully treated with phenytoin, but he developed prominent generalized choreoathetosis. CSF analysis showed 74 WBC (95% lymphocytes), protein concentration of 85 mg/dL, normal glucose concentration and negative HSV-1 PCR. NMDAR antibodies were detected in CSF (1:160, intrathecal synthesis 18.85) and serum (1:3200). Repeat brain MRI showed residual encephalomalacia from the viral infection but no new T2/FLAIR lesions. Screening for an underlying testicular teratoma was negative. Treatment with IVIg (0,4gr/kg/day for 5 days) and methylprednisolone (30mg/kg/day for three days) was started, followed by oral taper of methylprednisolone over 6 weeks. Symptoms did not improve (Video segment 1), and 40 days after onset of HSE, rituximab was started (375 mg/m2 weekly, 4 doses). Repeat NMDAR antibody studies showed mild decrease of CSF titers (1:80, intrathecal synthesis 4.71), and mild increase of serum titers (1:6400), but without significant change of the neurological status. At this time symptoms included, prominent choreoathetosis, unresponsiveness, dysphagia and dysarthria (Video segment 2). On day 70 after HSE, monthly intravenous pulses of cyclophosphamide (1st dose: 500 mg/m2, second dose: 750 mg/m2) were started. He developed pneumonia by *Pneumocystis carinii* requiring one week of mechanical ventilation. Immunosuppression was temporarily discontinued. At the last follow-up 120 days after HSE, the patient has started to improve (more alert, less irritable, PCP4), but still has choreoathetosis and dysphagia.

Case #7

A 41 year-old man presented with confusion and fever. An MRI showed extensive T2/FLAIR abnormalities bilaterally involving temporal, opercular, gyrus rectus and insular regions, with increased signal on diffusion weighted images (DWI), and contrast enhancement. A spinal tap was not performed for concern of increased intracranial pressure. He was treated with intravenous acyclovir for a total of 14 days. Repeat brain MRI nine days after symptom onset showed improvement, he returned to baseline and was discharged without residual symptoms 18 days after onset. Forty-nine days after onset of HSE, he developed confusion, social inappropriate behavior, perseveration, complains of generalized weakness, and double vision. CSF showed 25 WBC/ml (lymphocytic predominance), protein of 65 mg/dL, and normal glucose. Repeat MRI did not show new T2/FLAIR lesions; EEG showed right hemispheric slowing. CSF HSV-PCR performed 61 days after onset of HSE was negative; he had elevated HSV-specific IgG antibody index (4.86) indicating intrathecal antibody synthesis; HSV-1 IgM was negative. NMDAR antibodies were identified in both, CSF (1:640) and serum (1:3200), intrathecal synthesis 40.32.

He was treated with intravenous acyclovir (600mg/kg iv Q 8 hours) for 21 days. A modest improvement of mental status was noted; however, he had persistent rhythmic movements with the right arm, left face twitching and grimacing, and abnormal tongue movements without EEG correlate. He was treated with phenytoin, levetiracetam, lorazepam, valproate, lacosamide, and clonazepam without clear effect on the abnormal movements. He developed hypoventilation, fever, tachycardia, confusion, agitation, sexual disinhibition, aggressive behavior and sleep dysfunction. Over the next five months, neurological symptoms slowly improved without immunotherapy. He was discharged 9 months after onset of HSE; at the last follow-up three months later (one year after HSE) he was fully recovered (mRS 0).

**Supplementary video legend:**

**Case #1:** Segment 1 (day 5 of HSE)**:**  2-months old boy sleeping on video EEG register without abnormal movements. Segment 2 (day 7 of HSE): Two days later, prominent choreoathetosis with orofacial dyskinesias and tongue movements are noted for first time. Segment 3 (day 12 of HSE): Initial improvement of abnormal movements and level of consciousness is observed. Segment 4, (day 48 of HSE): Mild choreoathetosis is still present but the patient is awake and responsive. Segment 5 (day 88 of HSE): Choreoathetosis has resolved; psychomotor development is appropriate for age except for deficits in visual tracking that have been attributed to viral-related occipital lesions

**Case #2:** Segment 1 (day 38 after onset HSE): 28-months old girl shows prominent generalized choreoathetosis and unresponsiveness, which developed 21 days after onset of HSE. Segment 2 (1 year after HSE): Choreoathetosis has notably improved, residual dysphagia and dysarthria remain but otherwise development is appropriate for age. The patient was treated with steroids, IVIg, rituximab and intravenous cyclophosphamide.

**Case #4:** Segment 1 and 2 (38 and 55 days after HSE): 8-months old boy shows prominent choreoathetosis, orofacial and tongue dyskinesias and unresponsiveness 38 and 55 days after HSE. He is undergoing treatment with immunotherapy and at 90 days after HSE is still severely impaired.

**Supplementary methods**

Identification of patients

All five prospectively studied patients were diagnosed after June 2012 and followed by at least one of the authors. The identification of these patients was due in part to the increased awareness raised by the diagnosis of a patient with relapsing symptoms post-HSE who had NMDAR antibodies,1 and a report suggesting that some patients with HSE develop NMDAR antibodies.2 Since those publications, the number of consultations has steadily increased; we report here the initial 5 patients from whom extensive clinical and immunological information is available, including follow-up with video clips. In addition, we examined available serum and CSF samples of 34 patients with HSE provided by the California Encephalitis Project. Samples were obtained 1-88 days after HSE over a 6-year period as previously described.3

Criteria of definitive or probable HSE

Criteria of definite HSE included development of a typical clinical picture, presence of temporal lobe lesions on MRI, CSF pleocytosis, and positive CSF HSV-PCR. Probable HSE was defined by the same clinical criteria and negative HSV-PCR, but high intrathecal synthesis of HSV antibodies; or typical clinical features and positive HSV-PCR in CSF, but no temporal lobe abnormalities in the MRI.

Clinical scales

Pediatric patients were scored using the Pediatric Cerebral Performance Category scale4 (PCPC, 1=normal, 2=mild disability, 3=moderate disability, 4=severe disability, 5= coma or vegetative state, 6= death). Adult patients were scored using the modified Rankin Scale.5

List of antibodies tested in all patients:

Cell surface/synaptic antigens: NMDA receptor, AMPA receptor, GABA(B)-receptor, mGluR5, dopamine-2 receptor, LGI1, Caspr2, and DPPX proteins.

Intracellular antigens: Hu, CRMP5, Ma1-2, amphiphysin, GAD65.

Tissue based antibody screening

Serum and CSF samples were tested for NMDAR-antibodies following previously reported methods unless indicated otherwise.6 Briefly, non-perfused rat brains were removed, split sagittally, and fixed in 4% paraformaldehyde (PFA) for 1h, cryoprotected with 40% sucrose for 24h, and snap frozen in chilled isopentane. Seven micron-thick sections were then incubated with 0.3% hydrogen peroxide for 20 minutes, with 10% goat serum in PBS for 1h, and then labeled with patient’s or control sample (serial dilutions starting for serum: 1:200; and CSF: 1:2) at 4°C overnight. The next day, sections were incubated with the appropriate secondary antibody for 1h at room temperature and visualized with an avidin-biotin-peroxidase method. To determine IgG subtypes, biotinylated secondary goat anti-human IgG or IgA antibodies at dilution 1:2000 were used (Vector Labs, Burlingame, CA, USA); for IgM antibodies, goat anti-human IgM at dilution 1:1000 was used (Southern Biotechnology, Birmingham, AL, USA).

Cell-based assays

The CBA for NMDAR was performed as described previously.6 In brief, HEK293 cells were transfected with the NR1 and NR2B subunits of the NMDAR. Twenty-four hours after transfection, cells were fixed in 4% PFA for 10 minutes, permeabilized with 0.3% Triton X-100 (Sigma-Aldrich, St Louis, MO, USA) and incubated with 1% bovine-serum-albumin (BSA) for 1.5 hours. HEK cells were then incubated with patient’s or control samples (serum: 1:40; CSF: 1:2) at 4°C overnight. The next day, cells were labeled with a mouse monoclonal NR1 antibody (1:20 000; Millipore, Billerica, MA, USA) for 1h at room temperature, followed by the corresponding Alexa Fluor 488 and 594 secondary antibodies against human IgG (γ), IgM (µ) and mouse IgGs (1:1000; Molecular Probes, Invitrogen, Eugene, OR, USA) or human IgA (α) (1:1000; Jackson ImmunoResearch, Pennsylvania, USA). CBAs for AMPA receptor,7 GABA(B) receptor,8 mGluR5,9 dopamine-2 receptor,10 LGI1,11 Caspr212 and DPPX13 were performed as previously described.

Epitope analysis was done with NR1 mutants as previously described.14 In brief, G369I and G369S are single point mutations where glycine 369 was replaced by an isoleucine or serine, respectively. The “top lobe construct” carries a deletion of residues 26-140 and 275-349 in the top lobe of the amino-terminal domain (ATD). The ATD-TM4 construct keeps the ATD region and the transmembrane domain 4 (TM4), missing the in-between regions (S1, S2, and TM1-3) corresponding to residues 401-792. HEK293 cells were transfected with either wild-type NR1/N2B, the indicated NR1 mutants or NR2A, NR2C subunits and treated as described above. For transfection with ATD-TM4 construct, the commercial antibody used was a rabbit polyclonal antibody against amino acid 918-938 (dilution 1:2000; G8913, Sigma).

Embryonic rat hippocampal neurons were cultured and the reactivity of patients’ antibodies with live neurons assessed as previously described.6 In brief, 21 days in vitro rat hippocampal neurons were incubated with patients’ CSF (1:5) or serum (1:200) at 37**°**C for one hour, washed in cold PBS, fixed and subsequently incubated with Alexa Fluor 488 secondary antibody against human IgG (1:1000; Molecular Probes, Invitrogen, Eugene, OR, USA). Microscopy was done as previously described.6

**Supplementary results**

Epitope analysis of NMDAR antibodies in post-HSE

All samples from patients with post-HSE and NMDAR antibodies showed reactivity with NR1 subunit of the NMDAR, but not with NR2A, NR2B or NR2C. The reactivity with NR1 was abolished in all cases by the G369I mutation. Deletion of amino acids 26-140 and 275-349 of the NR1 subunit corresponding to the “top-lobe” of the ATD abolished reactivity in 6/8 and reduced reactivity in 2/8 post-HSE patients. These findings, indicating the presence of a major epitope in G369, and the variable effect of the top-lobe deletion are similar to those reported in classical anti-NMDAR encephalitis.13 In summary, the epitope repertoire in patients with NMDAR antibodies post-HSE is similar to that found in anti-NMDAR encephalitis.

**Supplementary Tables**

Supplementary Table 1: Characteristics of a retrospective cohort of 34 patients with HSE

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Case # | AGE | GEN-DER | DEFINITE/PROBABLE HSE\* | HSV TYPE | NEUROLOGICAL SYMPTOMS | MRI FLAIR/T2 CHANGES OF TEMPORAL LOBE | ONSET OF HSE TO CSF (days) | ANTIBODIES DETECTED¶ IN CSF | ONSET OF HSE TO SERUM (days) | ANTIBODIES DETECTED¶ IN SERUM |
| 6 | 45 | F | DEF | 1 | H,S,W | + | 74 | NMDAR (IgG, IgM) | n/a | n/a |
| 7 | 41 | M | PROB | 1 | L | + | 61 | NMDAR (IgG, IgM) | 61 | NMDAR (IgG, IgM) |
| 8 | 82 | M | DEF | 1 | H,L,Fo,S,W,LOC | + | 4 | Neuronal surface | 42 | NMDAR and Additional neuronal surface |
| 9 | 74 | M | DEF | 1 | L,I,S,W,MoD | + | 1 | - | 1 | - |
| 10 | 85 | F | DEF | 1 | n/a | + | 1 | Neuronal surface (IgG, IgM) | 1 | - |
| 11 | 13 | M | DEF | 1 | L,A | + | 1 | - | 2 | - |
| 12 | 60 | F | DEF | 1 | n/a | + | 1 | - | 1 | - |
| 13 | 63 | F | DEF | 1 | Fo,S,C | + | 2 | - | 2 | - |
| 14 | 85 | M | DEF | 1 | L,S | + | 2 | - | 14 | - |
| 15 | 86 | F | DEF | 1 | L,I,LOC | n/a, CT normal | 2 | - | 2 | - |
| 16 | 53 | F | DEF | 1 | H,S | + | 3 | - | n/a | n/a |
| 17 | 58 | F | PROB | 1 | H,S | + | 3 | - | 3 | - |
| 18 | 0 | M | DEF | 1 | Fo,S | +/multi-focal | 3 | - | 3 | - |
| 19 | 66 | M | PROB | 1 | H,L,A,Fo,C,W | + | 4 | Neuronal surface | 13 | - |
| 20 | 3 | M | DEF | 1 | L,S | n/a | 4 | - | 4 | - |
| 21 | 25 | M | DEF | 1 | H,S | +  + | 4 | - | 4 | - |
| 22 | 0 | F | DEF | 1 | L,Fo,S | + | 4 | - | 4 | - |
| 23 | 46 | F | PROB | 1 | Fo | + | 4 | - | 5 | - |
| 24 | 17 | M | DEF | 1 | H,L | + | 5 | - | 5 | - |
| 25 | 14 | F | DEF | 1 | H,L,I,A | + | 5 | - | n/a | n/a |
| 26 | 87 | M | DEF | 1 | L,I,W,Aph | + | 5 | Neuronal surface | 6 | Neuronal surface |
| 27 | 1 | F | DEF | 1 | L,Fo,S,LOC | +/multi-focal | 5 | - | 18 | - |
| 28 | 55 | M | PROB | 1 | H,L,I,A,S | + | 6 | Neuronal surface | 24 | - |
| 29 | 56 | F | PROB | 1 | L,W,My | n/a, CT normal | 7 | - | 7 | - |
| 30 | 1 | F | DEF | 1 | L,I | + | 10 | Neuronal surface (gG, IgM) | 26 | Neuronal surface |
| 31 | 0 | M | DEF | 1 | L,I,S,W | + | 12 | Neuronal surface (IgG, IgM) | n/a | n/a |
| 32 | 32 | M | PROB | 1 | A,Fo | + | 13 | - | 3 | - |
| 33 | 54 | M | PROB | 1 | L,I,Fo,S,C,W | + | 17 | - | 31 | - |
| 34 | 85 | F | PROB | 1 | L,I,Fo,S | + | 18 | Neuronal surface | 20 | Neuronal surface |
| 35 | 7 | M | DEF | 1 | L,Fo,S,W | + | 20 | Neuronal surface | 16 | - |
| 36 | 11 | F | DEF | 1 | L,Fo,W,LOC | + | 20 | - | 19 | - |
| 37 | 1 | M | DEF | 1 | L,I,A,S,LOC | - | 22 | - | 3 | - |
| 38 | 66 | M | DEF | 1 | L | + | 22 | Neuronal surface | 23 | Neuronal surface |
| 39 | 20 | F | PROB | 1 | L,Fo,S,W | + | 88 | - | 88 | - |

**\*** DEF: definite HSE, PROB: probable HSE; ¶only IgG unless indicated otherwise; A: ataxia, F: female, Fo: focal neurologic signs, H: headache, I: irritability/confusion, L: lethargy, LOC: reduced level of consciousness, M: male, Men: meningitis, MoD: movement disorder, My: myoclonus, n/a: not available, Phot: photophobia, S: seizures, W: weakness.

Supplementary Table 2: IgG NMDAR antibody titers, intrathecal synthesis of IgG NMDAR antibodies, and presence or absence of IgM and IgA antibodies

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Case # | Onset of HSE to sample (Days) | Sample type | IgG\* NMDAR antibody titers | Intrathecal IgG NMDAR antibody synthesis§ | IgM\*\* NMDAR antibodies | IgA\*\* NMDAR antibodies |
| 1 | 24 | Serum | 1:200 | 75.41 | negative | negative |
| 24 | CSF | 1:40 | positive | negative |
| 2 | 23 | Serum | 1:3200 | 18.85 | positive | negative |
| 23 | CSF | 1:160 | positive | negative |
| 3 | 52 | Serum | 1:12800 | 4.71 | positive | negative |
| 52 | CSF | 1:80 | positive | negative |
| 4 | 23 | Serum | 1:3200 | 18.85 | positive | negative |
| 23 | CSF | 1:160 | positive | positive |
| 5 | 45 | Serum | 1:800 | 34.46 | positive | negative |
| 45 | CSF | 1:160 | positive | positive |
| 6 | n/a | Serum | n/a | n/a | n/a | n/a |
| 74 | CSF | 1:40 | positive | negative |
| 7 | 61 | Serum | 1:3200 | 40.32 | positive | negative |
| 61 | CSF | 1:640 | positive | negative |
| 8 | 42 | Serum | 1:320 | n/a | negative | negative |
| 4 | CSF | negative | negative | negative |

CSF: cerebrospinal fluid, NMDAR: N-methyl-D-aspartate receptor, n/a: not available. §calculated according to15 normal value ≤2.4. \*IgG NMDAR antibody titers were calculated using serial dilutions of CSF or serum on immunohistochemistry with rat brain sections as reported,6 except in case #8 that due to concomitant reactivity against other unknown neuronal surface antigens NMDAR antibody titers were calculated with cell based-assays. \*\*IgM and IgA NMDAR antibodies were determined using cell-based assay (serum 1:40, CSF 1:2) and with immunohistochemistry on rat brain sections (serum 1:200, CSF 1:2).

Supplementary Table 3: Clinical characteristics of retrospectively identified HSE cases with NMDAR antibodies

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| # | AGE SEX | HSV-1 ENCEPHALITIS | | | | | | TIME TO RELAPSE (DAYS) | RELAPSE | | | | | | OUTCOME (Time after HSE onset) |
| SYMPTOMS | CSF | MRI LESIONS | HSV-PCR | TREAT-MENT | NMDAR ANTI-BODIES | SYMPTOMS | CSF | MRI: NEW T2 LESIONS | HSV-PCR | NMDAR ANTI-BODIES | TREAT-MENT |
| 6 | 45 y, f | Fever, headache, confusion, psychosis, seizures. | WBC 327 prot. 91 | bilateral temporal | + | Acyc | n/a | 38 | Memory dysfunction, personality changes | n/a | n/a | n/a | + | n/a | residual complex partial seizures |
| 7 | 41 y, m | Mental status change, lethargy, confusion | WBC 25 prot. 65 | bilateral temporal | -§ | Acyc | n/a | 49 | Confusion, choreic-like movements/seizures, autonomic and respiratory dysfunction | WBC 25 prot. 65 | - | - | + | Acyc | 9 months hospitalization12 months: complete recovery |
| 8 | 82 y, m | Fever, headache, seizures, aphasia, confusion, coma, Bell’s palsy, hemiparesis | WBC 98 prot. 137 | bilateral temporal | + | Acyc | - | 120 | Cognitive decline | missing | missing | n/a | + | n/a | n/a |

§ high intrathecal HSV IgG. Acyc: acyclovir, CSF: cerebrospinal fluid, HSV: Herpes simplex virus, HSE: Herpes virus simplex encephalitis, f: female, m: male, NMDAR: N-methyl-d-aspartate receptor, n/a: not available/not done, PCR: polymerase chain reaction, prot.: CSF total protein in mg/dL, WBC: white blood cells/µl, y: years

**Supplementary Figure 1: Antibody positivity related to the time of onset of HSE**

Time from onset of HSE (in days) comparing patients with and without neuronal surface antibodies in serum (A) and CSF (B). The vertical lines depict the median. Note that antibodies are mainly detected in samples obtained after 1 week from onset of HSE (black symbols). Grey symbols represent samples obtained during the first week of disease.

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