Rabies encephalitis presenting with new-onset refractory status epilepticus-Update

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In June 2003, a 15-year-old previously healthy girl with normal developmental history presented to the emergency department with recurrent convulsions. On presentation, she was afebrile, bradycardic, and minimally arousable. She had extensor posturing of the upper extremities and withdrew both lower extremities to pain. Convulsions persisted despite treatment with lorazepam and fosphenytoin. She was intubated for airway protection and was started on a propofol infusion. Urine drug screen was negative and CT head was normal. She was started on empiric intravenous antibacterial and antiviral therapy. Continuous video EEG showed generalized nonconvulsive status epilepticus (SE) that remained refractory to treatment with propofol, phenytoin, and phenobarbital.

PRACTICAL IMPLICATIONS

Rabies encephalitis should be included in the differential diagnosis of new-onset refractory status epilepticus despite a normal MRI and the lack of robust cellular responses in the CSF.

In retrospect, the patient's family reported that she had a single episode of unilateral arm jerking at school followed by transient impairment in awareness 2 weeks prior to presentation and a 1-month history of listlessness and decline in academic performance for which she received no neurodiagnostic workup or treatment. There was no family history of epilepsy, metabolic disorders, or immunodeficiency. The patient resided in rural Northeastern Kentucky. There was no recent travel history, nor was there any known exposure to or bites from potentially rabid animals.

Extensive diagnostic studies performed in peripheral blood and in CSF were all negative (table). CSF obtained 6 hours after presentation showed 5 erythrocytes/ μ L, 8 leukocytes/ μ L (6 lymphocytes, 2 neutrophils), elevated glucose (98 mg/dL), and elevated protein (91 mg/dL). MRI head was obtained 24 hours after presentation. No definite abnormalities were noted on T1 (axial and coronal, both pre- and postgadolinium), T2 and T2 FLAIR (axial and coronal), and diffusion-weighted imaging.

On hospital day 2, she developed rhabdomyolysis, severe lactic acidosis, and hypophosphatemia. These rapidly worsened, and the patient died 3 days after admission. A complete autopsy was performed shortly after. Microscopic examination of formalin-fixed, paraffin-embedded brain tissue demonstrated diffuse intraparenchymal inflammation, focal neurophagia, and rare neurons containing intracytoplasmic inclusions consistent with Negri bodies in the left parahippocampal cortex (figure 1). In situ reverse transcriptase polymerase chain reaction (RT-PCR) of brain tissue, also done in 2003, was positive for rabies virus (RABV) RNA (nested primers GAGTCACTCGAATATGTC, GTATTGCCTCTCTAGCGG, GACATGTCCGGAAGACTG, and CTACAATGGATGCCGAC).¹ Other findings at autopsy included diffuse acute myocarditis and myoglobin cast nephropathy. Of interest is that multiple histoplasma granulomas were observed in lung, liver, and spleen, consistent with concurrent disseminated histoplasmosis.

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Editor's note: Subsequent to the publication of "Rabies encephalitis presenting with new-onset refractory status epilepticus (NORSE)," it was brought to our attention that additional details would make the case report more instructive for our readers. The paper has now been updated to the satisfaction of the editors.

Table List of diagnostic studies performed in peripheral blood or serum, and in CSF

Peripheral blood and serum
Blood cultures
California encephalitis antibody panel
Eastern equine encephalitis antibody panel
Fungal complement fixation
HIV enzyme-linked immunosorbent assay
Mycoplasma pneumoniae antibody panel
Rapid plasma reagin
St. Louis encephalitis antibody panel
Western equine encephalitis antibody panel
CSF
Acid fast stain
Bacterial, viral, mycobacterial, and fungal cultures
Cryptococcal antigen
Epstein-Barr virus PCR
Gram stain
Herpes simplex virus PCR
La Crosse virus PCR
West Nile virus antibody panel
All the studies were negative.

Analyses done by the Centers for Disease Control and Prevention (CDC) in 2003 showed negative serum and CSF RABV antibodies, and negative hemi-nested RT-PCR. Immunohistochemical staining using polyclonal and monoclonal reagents was negative for RABV-specific antigen, though sensitivity of the test can be decreased after formalin fixation.² No *in situ*-based tests were done at the CDC to look for a host response to a virus. No viral genetic studies were performed. The official cause of the patient’s death, as stated in her death certificate from 2003, was “multiorgan failure as a consequence of encephalitis due to rabies infection.”

After original publication of this case report,³ a letter to the editor questioned the diagnosis of rabies encephalitis.⁴ This led, 15 years after the patient’s death, to a review of prior studies including immunohistochemistry done previously to analyze the cellular response to a putative encephalitis.⁵ Additional testing was also conducted by the CDC. These results are described below in chronological order.

Cytokine analyses of the patient’s brain by immunohistochemistry showed marked upregulation of the classic antiviral proteins interleukin 6, interferon-beta, and interferon-gamma in the same exact distribution of rabies viral RNA as

determined by locked-nucleic acid (LNA) *in situ* hybridization. These analyses, done in 2010 for research purposes and in conjunction with documented rabies encephalitis,⁶ were consistent with the results of the *in-situ* RT-PCR done in 2003. Also in 2010, myeloperoxidase expression was markedly elevated in the patient’s brain tissue in the same distribution as the cytokines and viral RNA. Representative images of these data are presented in Figure 2. The protocols for the LNA *in situ* hybridization and immunohistochemistry have been previously published.^{1,7–9} Negative controls included 5 normal brains and brain sections from 5 people with multiple sclerosis, whereas positive controls included a series of viral infections including at least 10 cases of encephalitis due to tick-borne viruses, Coxsackie virus, HIV-1, and rabies virus. The cytokine and myeloperoxidase interpretations were done blinded to the clinical information.

The CDC re-examined remaining tissue samples in 2018, 15 years after the patient’s death. Testing done at this time did not identify Negri bodies but did note structures that could be mistaken for inclusions, such as degenerate cellular material in the cytoplasm. A real-time RT-PCR assay was negative for the presence of RABV nucleic acid. It has been documented that formalin-fixed, paraffin-embedded tissues slide and block storage of >7 years can lead to RNA degradation.¹⁰

Discussion

As many as 40% of cases of SE fail to respond to first- and second-line antiepileptic medications.¹¹ These cases are defined as “refractory status epilepticus” (RSE). When RSE develops in a previously healthy individual and cannot be explained by an obvious underlying cause after initial investigations, the condition is termed “new-onset RSE” (NORSE).¹² In the largest case series published to date, half of all cases of NORSE remained cryptogenic despite extensive investigations. When an underlying etiology was identified, autoimmune and paraneoplastic encephalitides accounted for 70% of cases, while infectious processes were much less frequent.¹²

Rabies encephalitis is an acute CNS infection caused by the RABV, a RNA rhabdovirus. There is involvement of the brainstem and cerebrum, with early predilection for the limbic system that accounts for behavioral changes seen early in the disease. The incubation period, although highly variable, is on average 1–2 months. Once clinical onset of symptoms ensues, rabies encephalitis has a fulminant course and is almost uniformly fatal, with an average survival of less than 6 days.¹³ Although seizures are uncommon,¹⁴ cases of rabies encephalitis presenting with SE have been reported.^{15,16} In the United States, in up to 80% of indigenous cases there is no clear evidence of exposure to a rabid animal.¹⁷ Routine CSF analysis cannot differentiate rabies from other viral encephalitides. Absence of robust cellular responses with either normal or elevated protein in the CSF has been reported in both encephalitic and paralytic rabies.^{14–16,18}

Figure 1 Microscopic findings in left parahippocampal gyrus (hematoxylin and eosin stain)

Although brain MRI in human rabies encephalitis can show nonspecific abnormalities, T2 hyperintensities of the basal ganglia or thalami are often identified.¹⁹ MRI findings may be influenced by the multiple metabolic comorbidities seen in these patients.¹³ Normal MRI, like in our patient, has been reported in other cases of rabies that were diagnosed in the United States.^{15,18,20,21} Similarly, MRI can be unrevealing in many cases of NORSE, where only 62% demonstrate any abnormalities on MRI.¹²

In retrospect, as suggested by the presence of myoglobin cast nephropathy at autopsy, we suspect that the development of rhabdomyolysis in our patient might have been explained at least in part by propofol-related infusion syndrome. Coincidentally, our patient was found to have biopsy-proven disseminated histoplasmosis despite negative complement fixation. Because there was no personal or family history of immunodeficiency, the co-occurrence of both infections and the patient's residence in a geographic area where numerous

caves are found suggested caves as a common source exposure. Although we speculate that bat RABV variant could have been the cause of our patient's infection, no viral genetic studies were originally done for confirmation.

As noted above, some discrepancies related to the patient's diagnosis of rabies led to re-evaluation of the case 15 years after her death. Even though the official cause of death listed in her death certificate is rabies encephalitis, the CDC and the Kentucky Department of Health (DOH) did not classify this as a case of human rabies given that (1) there was no documented history of animal exposure, (2) the clinical presentation was considered atypical, (3) testing for rabies showed negative serum and CSF RABV antibodies, hemi-nested RT-PCR, and immunohistochemistry. On the CDC's evaluation of remaining tissue samples in 2018, the presence of Negri bodies vs degenerate cellular material in the cytoplasm was questioned. For these reasons, the CDC did not include this patient in their national rabies database.

Figure 2 Molecular correlates of rabies encephalitis. Findings in left parahippocampal gyrus (hematoxylin and eosin stain)

(A) Rabies RNA was detected in cells with the cytologic features of neurons with both RT in situ PCR (not shown) and with locked-nucleic acid probes (A). Note lack of signal in serial section with scrambled probe (B). High expression of interferon γ (C), interleukin 6 (E), and myeloperoxidase (F) was evident in the same distribution of the rabies virus. Negative controls included no signal for interferon γ in the normal brain (D), and myeloperoxidase signal only in scattered peripheral blood mononuclear cells in the case brain where rabies virus was not detected (G), or in a case of multiple sclerosis (H).

It is important to point out that evidence consistent with rabies encephalitis in this patient included a positive in situ RT-PCR of brain tissue, elevated myeloperoxidase expression, and cytokine analyses showing marked upregulation of the classic antiviral proteins interleukin 6, interferon β , and interferon γ in the same exact distribution of rabies viral RNA as determined by 2 different tests: LNA in situ hybridization and in situ RT-PCR.^{1,22,23} In situ RT-PCR for RABV is more sensitive than RT-PCR because of the dilution effect of the viral-negative cells which outnumber the viral-infected cells many fold.⁷ Moreover, studies have underscored the point that the classic antiviral cytokine response that shows the same geographic distribution of the causative virus is the definitive proof of the viral infection¹ and, as a corollary, would rule out diseases that do not induce the antiviral response. The CDC and the DOH offered 3 possible alternative diagnoses (“immune disorder, mitochondrial disorder, and Lyme carditis”). None of these would be associated with the molecular signature of acute viral encephalitis, as demonstrated in this case. Furthermore, elevated myeloperoxidase expression, as seen in our patient, is present in viral encephalitis but not in autoimmune or noninfectious encephalitis.²³ Taken together, our patient’s laboratory findings are consistent with a diagnosis of viral encephalitis with rabies as the infectious agent.

In conclusion, a diagnosis of fatal viral infection, when autopsy brain tissue is available, should be corroborated by a robust host response and not rely just on viral detection after RNA/DNA/protein extraction. Indeed, the host response is as responsible for the pathophysiology as the viral load in any case of fatal viral encephalitis.^{1,7,22,23} Corroborative tests, especially in situ-based tests, are an important way to ensure specificity and should be incorporated into the standard diagnostic protocols for rabies. Their use could potentially broaden the definition of viral encephalitis in general and rabies encephalitis in particular.

Author contributions

M.F. Villamar: case concept and design, acquisition of data, interpretation of data, manuscript writing. V.D. Smith: case concept and design, acquisition of data, interpretation of data, critical revision of manuscript for intellectual content. J.H. Smith: case concept and design, interpretation of data, critical revision of manuscript for intellectual content. D. Wilson: case concept and design, interpretation of data, critical revision of manuscript for intellectual content. G.J. Nuovo: acquisition of data, interpretation of data, critical revision of manuscript for intellectual content.

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