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CASE REPORT

Antibody and Viral Nucleic Acid Testing of Serum and Cerebrospinal Fluid for Diagnosis of Eastern Equine Encephalitis

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Eastern equine encephalitis diagnostic serum antibody can appear 6 days after the onset of symptoms, and its numbers can increase 4-fold in 4 days, arguing for early and frequent serum testing. In populations where cerebrospinal fluid viral nucleic acid testing sensitivity and specificity remain undetermined, cerebrospinal antibody testing should also be performed.

CASE REPORT

Eight patients with eastern equine encephalitis were identified. These patients were among the approximately 440 patients with encephalitis reported to the Department of Health of the State of New York per year from 1966 to 2014. One patient became ill in 1971 (1), one in 1983 (2), one in 2009 (3–5), two in 2010 (3–5), one in 2011 (5), and two in 2014 (6). All patients had the onset of symptoms in late summer. Patients 1, 2, and 6 were in a pediatric-age range of 1 month to 7 years. Patient 7 and patient 8 were in an adult-age range of 40 years to 64 years. Patients 3, 4, and 5 were in a geriatric-age range of 65 years to 75 years. All patients lived in or visited two counties known to have mosquitoes or horses that harbor eastern equine encephalitis virus periodically (7). Hospital charts, death certificates, autopsy reports, county health department records, and state health department regional office records, all obtained under public health laws and in accordance with health codes, were reviewed. Physical examination signs seen in these patients are listed in Table 1. Cerebrospinal fluid had cell counts and glucose and protein levels consistent with meningoencephalitis (Table 2). Various serum and cerebrospinal fluid antibody and nucleic acid tests had been performed between 1971 and 2014 (Table 3). Details of the commercially available nucleic acid testing method, used with these patients, have been published previously (8–10).

Patient 1 met the Centers for Disease Control and Prevention case definition (11) for a confirmed case by having, along with symptoms or signs of encephalitis, a 4-fold rise in a hemagglutination inhibition titer in serum (1, 3, 4).

Patient 2 met the Centers for Disease Control and Prevention case definition (11) for a presumptive case by having hemagglutination inhibition antibody and neutralizing antibody in serum without a 4-fold rise in titer in serum (2–4).

Patient 3 met the Centers for Disease Control and Prevention case definition (11) for a confirmed case by detection of viral genomic material by PCR in cerebrospinal fluid (3–5).

Patient 4 met the Centers for Disease Control and Prevention case definition (11) for a presumptive case by having antiviral antibody shown by immunofluorescence in serum and for a confirmed case by having antiviral immunoglobulin M antibody in cerebrospinal fluid.

Patient 5 met the Centers for Disease Control and Prevention case definition (11) for a confirmed case by detection of viral genomic material by PCR in cerebrospinal fluid (3–5).

Patient 6 met the Centers for Disease Control and Prevention case definition (11) for a confirmed case by detection of viral genomic material by PCR in cerebrospinal fluid (5).

Patient 7 met the Centers for Disease Control and Prevention case definition (11) for a confirmed case by results showing detection of viral genomic material by PCR in cerebrospinal fluid (6) and for a confirmed case by having antiviral immunoglobulin M antibody in cerebrospinal fluid and in serum.

Patient 8 met the Centers for Disease Control and Prevention case definition (11) for a confirmed case by results showing detection of viral genomic material by PCR in cerebrospinal fluid (6).

Antibody testing. Diagnostically useful serum antibody was present as early as 6 days after the onset of symptoms in patient 1. Antibody was present on hospital day 1 or 2 in patients 2, 4, and 6. This appearance of diagnostically useful serum antibody occurred approximately twice as fast as the 2 to 3 weeks after the onset of symptoms previously seen with Venezuelan equine encephalitis (12). With herpes simplex virus encephalitis, virus-specific immunoglobulin G has been detected in cerebrospinal fluid as early as day 6 after the onset of symptoms or signs of encephalitis (13). The serum antibody titer can increase 4-fold over 4 days, as observed in our first patient (patient 1). Cerebrospinal fluid immunoglobulin M may increase relatively rapidly, considering the titer of $<$1:1 on hospital day 1 in patient 7 versus the titer of 1:32 on hospital day 2 in patient 4. Diagnostic cerebrospinal fluid immunoglobulin M antibody levels were present as early as 9 days after the onset of symptoms and as early as hospital day 2, as observed in our patient 4. In one published case (14) of eastern equine encephalitis, in which the serum antibody level rose only 2-fold, from 1:10 to 1:20 over an unspecified time period, that patient had three opportunistic infections, indicating an underlying deficiency of the immune system, possibly AIDS, an emerging disease in 1984, which would account for a diminished antibody response.

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TABLE 1 Day of onset of symptoms and physical examination signs in eastern equine encephalitis

Symptom, observation, or outcome	Hospital day of occurrence for patient:							
	1	2	3	4	5	6	7	8
Symptoms prior to hospitalization[a]								
Fever or chills	−1	−6	−1	−7	−1	−6	−1	−1
Body ache	None	−6	−4	−7	None	None	−1	None
Vomiting	None	−6	−3	−1	−4	−3	−1	None
Diarrhea	None	None	−1	None	−4	None	None	−2
Headache	None	−1	−1	−1	None	−5	−1	−2
Speech difficulty	None	None	−1	−1	−1	None	None	−1
Lassitude	−1	−1	None	−1	−1	−3	−7	None
Seizure, localized or generalized	−1	−2	None	None	−1	−4	None	None
Face weakness	None	None	None	None	−1	None	None	None
Arm weakness	None	None	None	−7	−1	None	None	None
Leg weakness	None	None	None	−7	−1	None	None	None
Physical examination observations in hospital[b]								
Ophthalmoplegia or paresis	2	2	1	1	1	1	1	3
Meningismal signs	None	1	1	1	None	1	1	2
Focal or general motor seizure	1	1	5	None	None	1	1	None
Focal motor paresis or plegia	3	None	1	1	1	None	4	8
Decorticate flexion signs	13	2	None	None	2	1	None	3
Decerebrate extension signs	None	2	None	None	2	1	None	None
Parkinsonian tremor	17	None	None	None	None	None	None	None
Parkinsonian rigidity	9	None	1	None	4	1	None	6
Stupor, arousable	1	1	1	1	1	1	None	None
Coma, unarousable	4	2	7	3	4	3	None	None
Edema, pitting	3	2	3	4	4	None	None	None
Edema, nonpitting	4	None	3	6	4	3	None	None
Cyanosis	2	None	None	None	None	6	None	None
Staccato hiccups	None	None	5	5	7	None	None	None
Apneic episode	3	2	7	None	12	4	None	None
Clinical outcomes								
Discharge from hospital	22	2	12	7	12	6	9	16
Death	641[c]	2	12	7	12	6	No	No

[a] Each negative number designates the number of days prior to admission to the hospital that a symptom was first noted.
[b] Each number designates the day in the hospital when a physical examination sign was first noted; the day of admission to the hospital was considered to be day 1 in the hospital.
[c] Death occurred 641 days after admission to the hospital (619 days after discharge from the hospital) in a chronic nursing facility.

Nucleic acid testing. The published literature contains no evaluation of the sensitivity and specificity of testing cerebrospinal fluid for viral nucleic acid by PCR for diagnosing eastern equine encephalitis in humans. A negative cerebrospinal fluid viral nucleic acid test result was not unexpected, given the inability to culture eastern equine encephalitis virus from cerebrospinal fluid or from blood (15). In a study of 2,963 patients with encephalitis from 1966 to 1977, in New York state, one patient with eastern equine encephalitis virus was detected, using serum antibody testing (16). In subsequent studies of 3,485 patients with encephalitis from 1997 to 2003 (9) and 2,357 patients with encephalitis from 2004 to 2007 (8), eastern equine encephalitis virus was not detected by viral nucleic acid testing of cerebrospinal fluid. The sensitivity and specificity of viral nucleic acid testing compared to those of antibody testing for eastern equine encephalitis were not determined in these studies. Cerebrospinal fluid viral nucleic acid testing for eastern equine encephalitis warrants comparison with the traditional standard of antibody testing. Although our sample size was small, it included every known patient who had eastern equine encephalitis in New York state during that time period, 1966 to 2014.

Clinical utility. Among our patients who had both an antibody test and a nucleic acid test, namely, patients 3, 4, 6, 7, and 8 (Table 3), patient 3 had what might have appeared to be a false-negative serum antibody test result. The reason for this may be that the specimen was obtained relatively early in the course of disease, on hospital day 1 (Table 3, patient 3), 4 days after the onset of symptoms (Table 1, patient 3), before antibody was able to be produced. Patient 4 had what might have appeared to be a false-negative cerebrospinal nucleic acid test result. The reason for this may be that the specimen was obtained later in the course of disease, on hospital day 2 (Table 3, patient 4), 9 days after the onset of symptoms (Table 1, patient 4), after a population of host cells was exhausted as part of the natural history of the disease. The sensitivity and specificity of these tests would be able to be calculated (17) if further research were to be performed, using higher numbers from stored or new specimens.

The argument can be made that antibody determinations, as well as nucleic acid determinations, should be performed for the

TABLE 2 Cerebrospinal fluid cell count and glucose and protein levels in specimens from a patient harboring eastern equine encephalitis[a]

Cerebrospinal fluid parameter	Value or result for patient:							
	1	2	3	4	5	6	7	8
Total no. of leukocytes (per mm^3)	615[b]	53	130	94	21	130	1734	193
No. of polymorphonuclear leukocytes (per mm^3)	605	44[b]	10[b]	60[b]	1[b]	35[b]	1317[b]	29[b]
No. of mononucleated leukocytes[c] (per mm^3)	10	6[b]	120[b]	25[b]	20[b]	95[b]	416[b]	164[b]
% polymorphonuclear leukocytes	98[b]	88	8	64	4	27	76	15
% mononucleated leukocytes[c]	2[b]	12	92[b]	27	96	73	24	85
No. of erythrocytes (per mm^3)	16	43	30	0	680	15	ND, 2[d]	175
Glucose level (mg per 100 ml)	73	84	69	123	103	83	70	68
Protein level (mg per 100 ml)	187	77	83	144	106	25	117	56
Gram stain	NOS	NOS	NOS	NOS	NOS	NOS	NOS	NOS
Lactate dehydrogenase level (U per liter)	ND	ND	ND	ND	ND	ND	ND	ND
Bacterial culture	NG	NG	NG	NG	NG	NG	NG	NG
Specimen obtained on hospital day(s):	1	1	1	2	2	1	1, 6[d]	1

[a] CSF, cerebrospinal fluid; NOS, no organisms seen; NG, no growth; ND, not done.
[b] Result derived by calculation from laboratory reports of cell percentages or cell counts.
[c] Mononucleated leukocytes consist of the combination of lymphocytic leukocytes plus monocytes plus eosinophils.
[d] Result from a specimen obtained on hospital day 6.

diagnosis of eastern equine encephalitis. In cerebrospinal fluid, the argument can be made that antibody testing for eastern equine encephalitis should not be abandoned, because viral nucleic acid testing sensitivity and specificity have not been accurately and precisely determined and published, and that nucleic acid testing should not replace antibody testing for the diagnosis of eastern equine encephalitis. Performing both antibody and nucleic acid tests can be recommended. Such testing should eventually accu-

TABLE 3 Serum and cerebrospinal fluid antibody and nucleic acid testing in eastern equine encephalitis[a]

Parameter[b]	Value(s) or result for patient:							
	1	2	3	4	5	6	7	8
Serum								
EEEV antibody hemagglutination inhibition titer(s)	1:320, 1:1,280, and 1:5,270[c,d]	1:40[e]	ND	ND	ND	ND	ND	ND
EEEV antibody neutralization titer	ND	1:80[e]	ND	ND	ND	ND	ND	ND
EEEV immunoglobulin M antibody immunofluorescence titer	ND	ND	ND	ND	ND	ND	≥1:256[f]	ND
EEEV immunoglobulin G antibody immunofluorescence titer	ND	ND	<1:16[f]	1:16[f]	ND	1:32[f]	1:512[f]	≥1:256[g]
WNV immunoglobulin M antibody enzyme-linked immunoassay titer	ND	ND	ND	ND	−[f]	ND	−[f]	ND
WNV immunoglobulin G antibody enzyme-linked immunoassay titer	ND	ND	ND	ND	ND	−[f]	−[f]	ND
Specimen obtained on hospital day	5, 8, 12[c]	2	1	2	2	1	6	61[g]
Cerebrospinal fluid								
EEEV immunoglobulin M antibody	ND	ND	ND	1:32 [f]	ND	ND	<1:1[h,i], 1:1[j,f]	ND
EEEV immunoglobulin G antibody	ND	ND	ND	ND	ND	ND	1:1[j,f]	ND
EEEV PCR	ND	ND	+[k]	−[k]	+[k]	+[k]	+[j,k]	+[k]
WNV immunoglobulin M antibody	ND	ND	−[k]	ND	ND	ND	−[j,k]	ND
WNV immunoglobulin G antibody	ND	ND	+[k]	ND	ND	ND	ND	ND
WNV PCR	ND	ND	−[k]	−[k]	−[k]	−[k]	−[j,k]	−[k]
HSV PCR	ND	ND	−[k]	−[k]	−[k]	−[k]	−[j,k]	−[k]
Specimen obtained on hospital day(s)	1	1	1	2	2	1	1[h], 6[j]	1

[a] +, positive; −, negative; ND, not done.
[b] EEEV, eastern equine encephalitis virus; WNV, West Nile virus; HSV, herpes simplex virus.
[c] Specimens were obtained on hospital days 5, 8, and 12, respectively.
[d] Testing for antibody was performed at the Virus Laboratory, Department of Health of the State of New York, Albany, NY.
[e] Testing for antibody was performed at the Centers for Disease Control, Atlanta, GA.
[f] Testing for antibody was performed at the Diagnostic Immunology Laboratory, Department of Health of the State of New York, Albany, NY.
[g] Specimen was obtained on day 61 after admission to hospital, when the patient was at home after discharge from the hospital.
[h] Specimen was obtained on hospital day 1.
[i] Testing for antibody was performed by Laboratory Corporation of America, Raritan, NJ.
[j] Specimen was obtained on hospital day 6.
[k] Testing for nucleic acid was performed at the Viral Encephalitis Testing Laboratory, Department of Health of the State of New York, Albany, NY, using a commercially available nucleic acid method, as previously published (Dupuis et al. [8]; Huang et al. [9]; Hull et al. [10]).

mulate enough data for determinations of sensitivity and specificity in actual clinical practice, in different populations of patients with various pretest probabilities of having this disease. One reason for the paucity of data for determining sensitivity and specificity was that some tests were cancelled by a laboratory. The issue of cancelling by a laboratory of tests ordered by a clinician as a policy needs discussion.

This is analogous to the diagnosis of herpes simplex virus encephalitis; false-negative PCR test results of cerebrospinal fluid can occur early in the course of the disease (18). With herpes simplex encephalitis, testing of cerebrospinal fluid for antiviral immunoglobulin M antibodies contributed to making a diagnosis in two-thirds of patients (19), with the caveat that immunoglobulin M and immunoglobulin G antibodies in cerebrospinal fluid were not found in the first week of disease (13). Commercial herpes simplex virus enzyme-linked immunosorbent assays for immunoglobulins G and M have shown sensitivities of between 89% and 98% and specificities of between 82% and 100% (20), so nucleic acid testing has a role in improving the diagnosis of disease. Nucleic acid testing for herpes simplex virus in cerebrospinal fluid has not had sensitivity and specificity determined in relation to antibody testing (21). It has been stated previously that herpes simplex virus nucleic acid and antibody tests of serum and cerebrospinal fluid are complementary and that both should be used (21). It can be argued that using both would increase the probability of making a correct diagnosis of eastern equine encephalitis.

Regarding the utility of performing both antibody and nucleic acid testing for diagnosis, among 191 patients with symptomatic West Nile virus who had both serum-specific antibody testing and plasma-specific nucleic acid testing, 36% were diagnosed by nucleic acid testing alone, 49% by antibody testing alone, 9% by both, and 8% by neither (22). With West Nile virus, cases may go unrecognized if only nucleic acid testing or only immunoglobulin M testing is requested. With blood donors, among serum specimens tested for West Nile virus, 5% of nucleic acid-negative specimens were immunoglobulin M positive and 1% of immunoglobulin M-negative specimens were nucleic acid positive ($P < 0.05$). And among cerebrospinal fluid specimens, 8% of nucleic acid-negative specimens were immunoglobulin M positive and 0% of IgM-negative specimens were nucleic acid positive ($P < 0.05$) (23).

Regarding the timing of testing, in patient 4 with eastern equine encephalitis, in cerebrospinal fluid analyses, the nucleic acid test for eastern equine encephalitis virus was negative on a specimen that was obtained 9 days after the onset of symptoms also. This finding may be analogous to results of a study of 284 patients with symptomatic West Nile virus, for whom all nucleic acid tests of plasma for West Nile virus were negative by day 9 after the onset of symptoms (22).

In cases of patients with encephalitis and suspicion of a viral etiology, serum antibody should be tested repeatedly and frequently, because antibody can appear as early as the first hospital day and the titer can increase a diagnostically significant 4-fold within 4 days. The importance of repeated testing of serum needs to be emphasized, because in this series of patients, only 1 of 8 had serum antibody tested more than once, to determine if there was a 4-fold increase of titer, making a definitive diagnosis. A positive serum titer should not be dismissed when a cerebrospinal fluid nucleic acid test result is negative, keeping in mind that a cerebrospinal test for nucleic acid is expected to be negative early in the course of a case of viral encephalitis (18). When a cerebrospinal fluid nucleic acid test result is negative, serial testing of serum is a way to make a definitive diagnosis when a repeated lumbar puncture for a test of cerebrospinal fluid would not be considered. More frequent, possibly daily, testing in patients could lead to knowledge of when such tests would be expected to become positive and negative during the course of the disease. Daily testing for antibody cannot be considered excessive in comparison with the all-too-common iatrogenic loss of blood. For example, complete blood counts were performed one to three times per day, to the extent that the hemoglobin level decreased from 11 to 7 g per deciliter during 12 days in hospital, with patient 5. The early appearing and rapidly increasing antibodies in serum and cerebrospinal fluid allow diagnosis within days rather than weeks. Timely sequential diagnostic testing for both nucleic acid and antibody is particularly important in eastern equine encephalitis, which can have a fatal outcome within days, thereby precluding convalescent testing for antibody.

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We confirm that we all have participated in the concept and design of the study, analysis and interpretation of data, and drafting or revising of the manuscript, have approved the manuscript as submitted, and are responsible for the reported research.

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