The Neuropathology of West Nile Virus Meningoencephalitis

A Report of Two Cases and Review of the Literature

Todd W. Kelley, MD,1 Richard A. Prayson, MD,1 Angela I. Ruiz, MD,1 Carlos M. Isada, MD,2 and Steven M. Gordon, MD2

Key Words: West Nile virus; Meningoencephalitis; Poliomyelitis; Viral encephalitis

DOI: 10.1309/PU4R76JJMG1F81RP

Abstract

West Nile virus (WNV) is an emerging mosquito-transmitted encephalitis virus first recognized in North America in 1999. The pathologic manifestations of WNV infection have not been well defined. This study documents the clinicopathologic features, including autopsy findings, of 2 cases: an 81-year-old man who contracted WNV infection with meningoencephalitis and a polio-like paralysis and a hospitalized 74-year-old woman with meningoencephalitis who acquired WNV through transfusion. The pathologic findings in both cases were marked by perivascular and leptomeningeal chronic inflammation, microglial nodules, and neuronophagia, predominantly involving the temporal lobes and brainstem. These findings also were present in the spinal cord, especially the lumbar region, of the patient with polio-like paralysis. In both cases, most of the inflammatory infiltrate was composed of CD3+ T lymphocytes (a predominance of CD8+ over CD4+ T cells), CD68+ macrophages, and rare CD20+ B lymphocytes. These cases further define the clinical and pathologic spectrum of central nervous system disease in WNV infection.

At the time of submission of this article, the West Nile virus (WNV) outbreak in the United States had resulted in more than 3,000 cases in humans centered throughout the Midwestern states, including Ohio. WNV is a mosquito-borne member of the Flaviviridae family, which also includes the organisms responsible for St Louis encephalitis, yellow fever, and dengue fever. All are arthropod-borne, enveloped, RNA viruses. The natural life cycle of WNV revolves around a reservoir of infected bird populations. Mosquitoes transmit the virus from one bird to another or from birds to mammals, such as humans and horses. This strategy has permitted migrating bird populations to rapidly spread the virus across the continent. Although mosquitoes are the main vector for transmission of the virus to humans, there have been reports of WNV transmission in recipients of WNV-containing blood products and organs and from mother to child through breast milk.1,2

In most healthy adults, WNV infection is asymptomatic or causes a mild flu-like syndrome, characterized by vague constitutional symptoms and fevers that occur after an initial incubation period ranging from 3 days to 2 weeks. In a small minority of infected patients, WNV infection results in meningoencephalitis, which may be accompanied by a neurologic syndrome much like poliomyelitis.3,4 Anecdotally, the major risk factor for this manifestation seems to be advanced age. The WNV poliomyelitis-like syndrome seems to follow a typical course. An early, prodromic phase of fevers and weakness is followed by the acute onset of flaccid paralysis. Typically, extremity reflexes and touch sensation remain intact. Patients often experience mental status changes, signifying involvement of the cerebral cortex. Cerebrospinal fluid (CSF) analysis may show pleocytosis, and
magnetic resonance imaging studies may show increased enhancement of the meninges.\textsuperscript{5} Previous pathologic reports of WNV infection have not included detailed descriptions of the spinal cord.

Large outbreaks of WNV previously have been reported overseas.\textsuperscript{5} The case-fatality rate among patients hospitalized with WNV infection during recent outbreaks in New York, Romania, and Israel ranged from 4\% to 14\%.\textsuperscript{5-7} Since many people infected with the virus have no symptoms, it is difficult to determine the exact percentage of patients in whom the infection progresses to disease and/or death.

We report the autopsy findings in 2 serologically proven cases of WNV infection, including the spinal cord pathology in 1 patient with acute flaccid paralysis syndrome.

Materials and Methods

The gross and histologic findings from 2 serologically proven cases of WNV infection were evaluated. In case 1, a complete autopsy was performed. A brief description of the spinal cord of case 1 has recently been published.\textsuperscript{8} In case 2, the autopsy was limited to the brain only, and the remainder of the organs, including the spinal cord, were unavailable for examination. In both cases, H&E-stained sections were evaluated from the midbrain, pons, and medulla. For the paired central nervous system (CNS) structures, sections were obtained from each side and included the cerebellum, olfactory and optic nerves, hippocampi, mamillary bodies, and the left and right cerebral hemispheres, including each frontal, parietal, temporal, and occipital lobe. For the spinal cord examination in case 1, 4 sections of cervical cord, 4 sections from the thoracic cord, and 5 sections from the lumbar and sacral levels were evaluated. To evaluate potential axonal loss in the spinal cord in case 1, Bodian and Luxol fast blue stains were performed and evaluated on the lumbar spinal cord sections.

Immunohistochemical studies were performed on selected tissue blocks from the more severely involved areas using a modified avidin-biotin complex technique with a Ventana Benchmark automated system (Ventana Medical Systems, Tucson, AZ). The following antibodies were evaluated: glial fibrillary acidic protein (GFAP; polyclonal, dilution 1:600, DAKO, Carpinteria, CA), CD68 (clone PG-M1, dilution 1:10, DAKO), CD20 (clone L26, dilution 1:50, DAKO), CD8 (clone 144B, dilution 1:20, DAKO), CD4 (clone 1FC, dilution 1:10, Novoceastra, Newcastle upon Tyne, England), and CD3 (polyclonal, undiluted, Zymed, South San Francisco, CA). Appropriate positive and negative controls were evaluated for each immunostain. The medical records were reviewed for relevant clinical information.

Results

Clinical Features

Case 1

An active 81-year-old man with a medical history of abdominal aortic aneurysm (repaired) and hypertension sought care because of a 3-day history of fevers and the acute onset of nausea, vomiting, and ataxia. He stated that he began to feel unwell after a day of golfing.

CSF analysis at an outside institution showed a WBC count of 1,444/µL (reference range, 0-3/µL) with 85\% neutrophils (reference range, 0\%-3\%) and a glucose level of 54 mg/dL (3.0 mmol/L; reference range, 50-75 mg/dL [2.8-4.2 mmol/L]). Other details regarding the analysis were not available.

The patient was treated empirically with broad-spectrum antibiotics for presumed bacterial meningoencephalitis. While awaiting the results of serologic testing for WNV, his neurologic symptoms worsened with the development of flaccid paralysis. He also experienced mental status changes and became minimally responsive to commands. A magnetic resonance imaging study of his brain and spinal cord showed abnormal enhancement in the meninges of the brain and cauda equina, but findings otherwise were unremarkable.

During the hospital course, his peripheral WBC count rose from 8,900 to 16,900/µL (8.9-16.9 × 10\(^9\)/L; reference range, 4,000-11,000/µL [4.0-11.0 × 10\(^9\)/L]). A second lumbar puncture was performed 4 days after the first. This showed a WBC count of 47/µL with 3\% neutrophils and 76\% lymphocytes, a protein level of 206 mg/dL (reference range, 15-45 mg/dL), and a glucose level of 71 mg/dL (3.9 mmol/L). Respiratory distress developed, and, in accordance with the patient’s advanced directive, family members did not want him placed on a mechanical ventilator; he subsequently died. WNV-specific IgM antibodies were detected in the CSF by enzyme-immunoassay (EIA).

Case 2

A 74-year-old woman with a history of coronary artery disease and a 3-vessel coronary artery bypass grafting procedure 6 years ago sought care because of chest pain. She underwent a coronary artery bypass grafting reoperation. Postoperatively, she developed multiple bacterial infections and deep venous thromboses, and heparin therapy was started. Subsequently, she received multiple blood products. On postoperative day 5, an encephalitic clinical picture developed with confusion, increasing lethargy, and somnolence, and she was unresponsive to most commands. Generalized rigidity of the limbs and neck with a resting tremor of the upper extremities were noted. CSF analysis 5 days before death showed a WBC count of 2/µL with 87\% lymphocytes, a protein level of 76 mg/dL, and a glucose level of 151 mg/dL (8.4 mmol/L). Viral serologic EIA results were positive for WNV.
Pathologic Features

Macroscopic Features

Gross examination revealed that the brain, spinal cord, and dura were essentially unremarkable in both cases, except for evidence of mild cerebral edema in case 1 (brain weight, 1,390 g). Otherwise, there was no evidence of herniation, and the brain surfaces did not appear clouded or purulent. The autopsy of case 2 was limited to the brain only. In case 1, examination of the other organ systems showed the presence of a large, organizing, pulmonary thromboembolus, likely the cause of the patient’s respiratory distress. Other gross findings included moderate centriacinar emphysema, severe left ventricular hypertrophy, and mild aortic atherosclerosis. There was no hepatomegaly or splenomegaly.

Microscopic Features: Case 1

Histologic examination of the CNS showed the presence of a chronic inflammatory infiltrate of variable density. The infiltrate was focally most severe in the temporal lobes, brainstem, and basal ganglia. Focal, chronic leptomeningitis also was observed. The CNS infiltrates were characterized predominantly by lymphocytes. Immunohistochemical analysis of the temporal lobe and lumbar spinal cord specimens showed that most of the lymphoid cells in both areas had a T-cell immunophenotype (CD3+); only rare (<5% of the lymphoid cell population) CD20+ B lymphocytes were observed.

There was a slight predominance of CD8+ T cells over CD4+ T cells in parenchymal locations. Leptomeningeal and perivascular infiltrates were composed almost exclusively of CD4+ (>80% of the lymphoid cell population) T cells. Most of the involved parenchymal vessels were situated within the gray matter.

Focally, perivascular and infiltrating macrophages (CD68+) were observed and were most notable in the aforementioned most severely affected sites. Scattered gray matter microglial nodules were present throughout the CNS and were most numerous in the lumbar spinal cord. GFAP immunostaining highlighted the presence of gliosis in the more affected areas. There was a mild, focal CD8+ infiltrate involving anterior nerve roots, particularly in the lumbar cord region. Bodian and Luxol fast blue stains highlighted a mild loss of axons in the anterior nerve roots of the lumbar cord. Rare Marinesco bodies were identified in the substantia nigra. No viral inclusions were identified. There was no evidence of cranial nerve involvement.

Microscopic Features: Case 2

The histopathologic findings in the brain of case 2 were similar to those of case 1. As in case 1, there was a mononuclear inflammatory infiltrate of variable intensity, composed predominantly of CD3+ lymphocytes, which was most severe in the brainstem, temporal lobes, and basal ganglia. A milder leptomeningitis, compared with that in case 1, also was present. The inflammatory infiltrate was composed predominantly of CD3+ T lymphocytes, and, similar to case 1, CD20+ B lymphocytes were rare (<5% of the lymphoid cell population).
Population). Parenchymal and perivascular inflammatory infiltrates were a prominent feature in case 2, and, in contrast with case 1, there was a slight predominance of CD8+ T cells in both areas. Similar to case 1, most of the involved parenchymal vessels were located in the gray matter. There also were focal, parenchymal, and perivascular collections of CD68+ cells in case 2, but they were less numerous than in case 1. The presence of microglial nodules was noted throughout the brain, but they were most numerous in the brainstem. GFAP staining showed the presence of gliosis in the most affected areas. Marinesco bodies and viral inclusions were not identified. There was no evidence of cranial nerve involvement.

Discussion

In a report of 4 WNV infection autopsy cases, in which all of the patients exhibited prominent symptoms of fatigue or weakness, the distribution of parenchymal and perivascular inflammation was more severe in the brainstem, with variable involvement of the cerebellum and cortex. The presence of microglial nodules was reported in all cases, leptomeningitis in 2 of 4 cases, and cranial nerve root involvement in 2 of 4 cases. Immunohistochemical analysis of viral antigens showed prominent staining in neurons of the brainstem and spinal cord and an absence of staining in other organ systems. Although histologic descriptions of the spinal cord were not given, previous descriptions of the pathology of the brainstem and cerebrum in WNV infection were similar to those that we report herein. A notable difference was the relative paucity of B lymphocytes (CD20+) in the brain and spinal cord of the cases reported herein compared with the description by Sampson et al. Overall, the pathologic description of WNV infection is nonspecific, and serologic testing is likely to remain the standard for diagnosis.

The spectrum of clinical illness due to WNV infection continues to be defined but should be in the differential diagnosis of summer meningoencephalitis syndromes. The flaccid paralysis of WNV poliomyelitis-like syndrome also may manifest in a manner similar to the ascending paralysis of Guillain-Barré syndrome. CSF studies showing high protein levels in the absence of pleocytosis should raise the clinical suspicion of both entities, although the frequency of this pattern of pathology in WNV infection is unclear.

Currently, the diagnosis of WNV in humans is confirmed through an EIA test for the presence of IgM to WNV in a serum or CSF sample. However, techniques for detecting the virus in tissues via reverse transcriptase–nested polymerase chain reaction assays exist. Although WNV–specific antibodies are not yet commercially available, there is sufficient cross-reactivity with antibodies to other flaviviruses, such as the St Louis encephalitis virus, to permit immunohistochemical analysis for the presence of WNV with this surrogate marker (unpublished observation).

The Centers for Disease Control and Prevention has determined that 1 of our patients (case 2) acquired WNV infection through blood transfusion during hospitalization for coronary artery disease. Encephalitis developed after she received multiple blood and platelet transfusions after cardiac surgery. One unit of packed RBCs from a donor who...
subsequently was determined to have acute WNV infection had been transfused during the perioperative period.

The pathologic findings of WNV infection reported herein and in previous reports illustrate a predilection for involvement of the brainstem and the anterior horn of the spinal cord in patients with cord-related symptomatology. Recent clinical reports have detailed a syndrome much like poliomyelitis arising from WNV infection. This is the first histopathologic description of the spinal cord in the setting of this recently described phenomenon. The presence of neuronophagia, inflammation, and microglial nodules in the spinal cord corroborate the clinical observation of a progressive, flaccid, extremity paralysis as a manifestation of WNV infection mimicking poliomyelitis. Similarly, patients with WNV infection and encephalitic or rhomboencephalitic clinical manifestations exhibit a comparable pattern of pathology that seems to preferentially involve the brainstem and cerebrum, especially the temporal lobe.

From the Departments of 1 Anatomic Pathology and 2 Infectious Diseases, Cleveland Clinic Foundation, Cleveland, OH.

Address reprint requests to Dr Prayson: Dept of Pathology, L25, Cleveland Clinic Foundation, 9500 Euclid Ave, Cleveland, OH 44195.

References


