

# Naturally Acquired West Nile Virus Encephalomyelitis in Transplant Recipients

## Clinical, Laboratory, Diagnostic, and Neuropathological Features

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**Background:** In the 2003 West Nile virus (WNV) epidemic, Colorado reported more WNV cases than any other state, including an unprecedented number in organ transplant recipients.

**Methods:** Physicians caring for transplant recipients hospitalized with naturally acquired WNV encephalitis provided data to characterize the clinical symptoms, results of diagnostic studies, and outcomes.

**Results:** Eleven transplant recipients were identified (4 kidney, 2 stem cell, 2 liver, 1 lung, and 2 kidney/pancreas). Seven were directly admitted to 1 of the 2 hospitals in the study, and 4 were referred to 1 of these centers from regional hospitals. All but 1 patient had a prodrome typical of WNV encephalitis in nonimmunosuppressed patients. Ten patients developed meningoencephalitis, which in 3 cases was associated with acute flaccid paralysis. One patient developed acute flaccid paralysis without encephalitis. Six patients had significant movement disorders including tremor, myoclonus, or parkinsonism. All patients had cerebrospinal fluid pleocytosis and WNV-specific IgM in the cerebrospinal fluid and/or serum. Cerebrospinal fluid cytologic studies (n=5) showed atypical lymphocytes, some

resembling plasma cells; however, flow cytometry (n=3) showed that cells were almost exclusively of T-cell (not B-cell or plasma cell) lineage. Magnetic resonance images of the brain were abnormal in 7 of 8 tested patients, and electroencephalograms were abnormal in 7 of 7, with 2 showing periodic lateralized epileptiform discharges. Nine of 11 patients survived infection, but 3 had significant residual deficits. One patient died 17 days after admission, and autopsy findings revealed severe panencephalitic changes with multifocal areas of necrosis in the cerebral deep gray nuclei, brainstem, and spinal cord as well as diffuse macrophage influx in the periventricular white matter. A second patient died of complications of WNV encephalitis 6 months after hospital admission.

**Conclusions:** Naturally acquired WNV encephalitis in transplant recipients shows diagnostic, clinical, and laboratory features similar to those reported in nonimmunocompromised individuals, but neuroimaging, electroencephalography, and autopsy results verify that these patients develop neurological damage at the severe end of the spectrum.

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NATURALLY ACQUIRED West Nile virus (WNV) infection first appeared in North America in 1999.<sup>1,2</sup> Since 1999, the epidemic has spread westward throughout the United States,<sup>3,4</sup> although originally afflicted areas on the Eastern seaboard continue to report new cases.<sup>3</sup> Consequently, WNV has now become stably entrenched in the United States.<sup>5</sup>

Between July 2003 and October 2003, nearly one third of all human WNV cases and 29% of all WNV-associated deaths in the United States occurred in Colorado. There were 2947 confirmed WNV cases and 63 deaths in Colorado; 79% of WNV-infected patients in Colorado had WNV fever, 13% had meningitis, and 8% had encephalitis.<sup>3</sup> Cases were concentrated in 3 north central counties (Boulder, Larimer,

and Weld counties), each of which reported at least 400 cases and accounted for approximately 45% of the total cases in the state. This is a referral area for 2 major medical institutions that provide initial and follow-up care for the overwhelming majority of transplant recipients in Colorado.

Approximately 25 000 individuals receive organ transplants in the United States annually.<sup>6</sup> Solid-organ recipients are treated with long-term immunosuppres-

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sive regimens that put them at increased risk for opportunistic infection. These transplant recipients are among several select patient groups, including elderly individuals or those with malignancies, that

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may be at increased risk for developing neurological or other serious manifestations of WNV infection.

This article details the clinical, laboratory, and neuroimaging features of 11 transplant recipients (4 kidney, 2 stem cell, 2 liver, 1 lung, and 2 kidney/pancreas) hospitalized with WNV encephalitis. This represents the largest experience reported to date with naturally acquired WNV encephalitis in transplant recipients. All patients received their transplant at a time remote (5 months to 15 years) from the onset of WNV infection. None had received blood transfusions or transfusion products in the 3 months prior to infection or were hospitalized at the time of illness onset.

## METHODS

Cases were ascertained through contact with hospital transplant services, medical record review, and the Colorado Department of Public Health and Environment (CDPHE) (Denver). Seven patients were admitted to the University of Colorado Hospital or Presbyterian–St Luke's Hospital (Denver) at the onset of illness, and 4 were transferred to the University of Colorado Hospital from regional hospitals. Diagnosis of WNV encephalitis infection was based on the detection of WNV IgM in the cerebrospinal fluid (CSF) by capture enzyme-linked immunosorbent assay (ELISA) in 6 cases and in the serum alone in 5 cases in accordance with CDPHE criteria.<sup>4</sup> Serologic tests were performed at either the University of Colorado Hospital using Food and Drug Administration–approved capture ELISA (Focus Technology, Cypress, Calif) or by the CDPHE. No patient had a history of encephalitis, Flavivirus vaccination, or travel to endemic areas of Japanese encephalitis virus.

Data were gathered from physician and hospital medical records regarding initial clinical signs and symptoms, neurological illness, and results of studies including WNV serologic tests (11), CSF examination (11), magnetic resonance imaging (MRI) (8), electroencephalography (EEG) (7), CSF WNV polymerase chain reaction (3), and electromyography (3). The CSF cytologic preparations were obtained during the acute phase of illness in 5 patients, and flow cytometry was performed in 3 samples. Two patients died, and 1 underwent a full autopsy. Autopsy tissues were fixed for 2 to 7 days in 10% formalin, routinely processed, and sectioned at 4 to 6  $\mu$ m. Hematoxylin-eosin, Luxol fast blue–periodic acid-Schiff, modified Bielschowsky silver, and immunohistochemical staining for microglia (CD68), macrophages (HAM56), and lymphocytes (CD45, CD3 for T cells, and CD20 for B cells) were conducted on selected sections. Postmortem polymerase chain reaction testing for WNV in the brain, spinal cord, and blood was performed by the Centers for Disease Control (Ft Collins, Colo).

## RESULTS

All cases occurred between July 2003 and October 2003. Patients included 7 women and 4 men aged 32 to 62 years. Two patients had received allogeneic stem cell transplants for non-Hodgkin lymphoma (patient 1) or acute myelogenous leukemia (patient 2). Nine patients had received kidney (4), kidney/pancreas (2), liver (2), or lung (1) transplants for chronic diseases including hypertensive end-stage renal disease (2), diabetes mellitus (2), adult-onset polycystic kidney disease (1), glomerulonephritis (1), alcoholic liver disease (1), hepatitis C (1), or chronic obstructive pulmonary disease (1).

## PRODROME AND CLINICAL FEATURES

The clinical prodrome, defined as the period between onset of symptoms and neurological disease, ranged from 0.5 to 7 days (mean $\pm$ SD, 3.9 $\pm$ 2.3 days). In 1 patient (patient 10), the symptom onset was indeterminate. This woman had nonspecific colitis with initially negative WNV serologic test results, which lasted several weeks before the onset of encephalitis with subsequent positive findings on WNV serum and CSF serologic tests. One other patient (patient 3) had pancreatitis (lipase maximum, 1127 mIU/mL). In the 10 cases we were able to evaluate, the prodrome duration and symptoms were similar to those reported in immunocompetent patients, including fever (10), nausea or vomiting (7), weakness or lethargy (9), and diarrhea (2). Rash was not noted in any case. Neurological signs and symptoms developed rapidly, reaching a maximum within a few hours to 2 days.

Four patients developed acute flaccid paralysis; all had severe quadriparesis or quadriplegia associated with hypotonia and areflexia with relatively preserved sensation. All required at least brief mechanical ventilation. Three of the 4 patients with severe acute flaccid paralysis also had significant alterations in mental status as well as EEG and neuroimaging findings consistent with associated meningoencephalitis. Two of the 3 patients with acute flaccid paralysis and meningoencephalitis had transient generalized or partial complex seizures. The 7 patients without acute flaccid paralysis all had signs and symptoms of meningoencephalitis. One patient with meningoencephalitis (patient 10), in addition to the 2 patients with acute flaccid paralysis and meningoencephalitis noted previously, also had seizures. Another patient with meningoencephalitis (patient 11) had altered mental status, fever, and headaches but no seizures.

Two patients manifested prominent parkinsonian features including rigidity, cogwheeling, and rest tremor (patients 4 and 5). Both patients also had postural and intention tremors and myoclonus. Two additional patients (patients 8 and 9) had prominent tremors and myoclonus, and 2 had myoclonus alone (patients 7 and 10) without parkinsonian features. Seven patients (patients 1, 3, 4, 6, 7, 8, and 9) required mechanical ventilation (duration, 2 days to >8 weeks) (**Table**). All patients were hospitalized for a minimum of 2 weeks.

## NEUROIMAGING STUDIES

Cranial MRI was performed in 8 patients. Seven of the 8 patients who underwent MRI had abnormalities that were attributed to their WNV infection (exception was patient 2). Two patients had a prominent increased signal in the thalami (**Figure 1A**), basal ganglia, brainstem (**Figure 1C**), or cerebral peduncles (patients 1 and 7) noted between days 3 and 5 of illness, which persisted in patient 7 on MRI studies performed at days 19 and 30. A third patient (patient 9) showed new transient abnormalities in these areas on an MRI study obtained at day 21 that were not present on day 1. Six patients (patients 1, 4, 5, 8, 9, and 10) had areas of increased white matter signal inten-

**Table. West Nile Virus Encephalitis in Transplant Recipients Hospitalized in the 2003 Colorado Outbreak\***

Patient/ Sex/ Age, y	Transplant Type/Symptom Onset Posttransplantation	Treatment	Prodrome Duration, Symptoms, Clinical Course, and Outcome	CSF Profile	WNV Documentation	MRI Findings
1/F/46	Allogeneic stem cell/2 y, 1 mo	CASG 210	6- to 7-d Prodrome: fever, nausea, vomiting, and lethargy at admission (9/18), progressing to flaccid paralysis/poliomyelitis and meningoencephalitis; mechanical ventilation required; coma; death (10/4)	CSF, day 2: WBC=19; RBC=22; PMNs=59%; lymphs=20%; mono/mac=20%; band=1%; protein=81; glucose=59; cytologic tests	CSF, day 2: WNV IgM=4.23; serum, day 1: WNV IgM=2.74	MRI, day 3: bilateral frontal and parietal white matter hyperintensities, (new after reviewing older studies on file) plus T2 hyperintensities in bilateral thalami, cerebral peduncles, and pons (older pons lesion with decreased hyperintensity also noted)
2/F/62	Allogeneic stem cell/8 mo	CASG 210	12-h Prodrome: fever, nausea, vomiting, weakness, cough, and dizziness at admission (9/16), followed by progressive mental status changes and meningoencephalitis; discharged to rehabilitation facility (9/30) with slow improvement	CSF, day 3: WBC=43; RBC=1; PMNs=13%; lymphs=29%; mono/mac=57%; band=1%; protein=75; glucose=97; cytologic tests	CSF, day 3: WNV IgM=3.10; CSF IgG=0.33	MRI, days 4, 8, and 31: devoid of enhancement or new changes (after comparing older studies on file)
3/M/50	Liver/9 y, 3 mo	Omr-IgG-am, interferon alfa-2b, tacrolimus and cyclosporin A held, MMF maintained at low dose	2-d Prodrome: fever, nausea, vomiting, weakness, and abdominal pain at admission (8/30), followed by flaccid paralysis/poliomyelitis with extended intensive care unit stay; mechanical ventilation required; cyclosporin A discontinued, followed by rejection treated with methylprednisolone sodium succinate; patient complained of continued abdominal pain, and lipase level found to be 1127 mIU/mL; mild pancreatitis diagnosed; discharged (9/29) and had slow improvement	CSF, day 5: WBC=64; RBC=7; PMNs=32%; lymphs=53%; mono/mac=13%; band=1%; protein=66; glucose=55; cytologic tests	CSF, day 5: WNV IgM=1.90; PCR negative for WNV; serum, days 5, 8, and 14: WNV IgM=1.13, 5.99, and 4.50; serum, days 9, 10, and 14: WNV IgG=0.69, 1.05, and 1.68	MRI not performed

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sity (Figure 1E). Multiple MRI studies were available in 4 patients (patients 2, 7, 9, and 10). Patient 2 had normal MRI results at days 4, 8, and 31 of illness. Patient 7 showed progressive worsening in studies obtained at days 5, 19, and 30 of illness. Patient 9 developed transient abnormalities in the thalamus, globus pallidus, and brainstem. Patient 10 showed worsening between studies obtained at days 2, 33, and 55 of illness, although diminished leptomeningeal enhancement was noted on day 55.

## LABORATORY AND DIAGNOSTIC STUDIES

All patients had at least 1 CSF examination, and 6 patients had multiple ones. Pleocytosis ( $\geq 5$  white blood cells per microliter) was present in all cases, with a range of 5 to 540 cells (mean $\pm$ SD, 89 $\pm$ 152). Four patients (patients 1, 5, 10, and 11) had a polymorphonuclear neutrophil differential of at least 45% on the results of their initial lumbar puncture. One patient (patient 9) had

**Table. West Nile Virus Encephalitis in Transplant Recipients Hospitalized in the 2003 Colorado Outbreak\* (cont)**

Patient/ Sex/ Age, y	Transplant Type/Symptom Onset Posttransplantation	Treatment	Prodrome Duration, Symptoms, Clinical Course, and Outcome	CSF Profile	WNV Documentation	MRI Findings
4/M/56	Liver/15 y	Omr-IgG-am, interferon alfa-2b, tacrolimus held, MMF maintained at lower dose	5-d Prodrome: fever and weakness (no nausea or vomiting) at admission (9/07) to outside hospital; transferred to University of Colorado Hospital (9/09) with hallucinations, neck stiffness, disorientation, meningoencephalitis, parkinsonism, tremors, and myoclonus; mechanical ventilation required; immunosuppression initially held, followed by rejection; treated with methylprednisolone sodium succinate; discharged to long-term care facility with little neurological improvement (10/14); died (3/24).	CSF, day 1: WBC=5; PMNs=3%; lymphs=87%; mono/mac=10%; protein=41; glucose=36 (serum glucose=43); CSF, day 4: WBC=4; RBC=68; protein=48; glucose=76; cytologic tests	Serum, days 4, 9, and 11: WNV IgM=4.18, 4.01, and 3.80; serum, days 9 and 11: WNV IgG=2.48 and 2.70; CSF, day 4: IgM negative and PCR negative for WNV nucleic acid	MRI, day 10: abnormally high signal on FLAIR and T2 studies in left hippocampus without enhancement; mild to moderate central and peripheral white matter lesions also present, but possibly ischemic (no old studies available for comparison)
5/M/61	Lung/2 y	CASG 210, tacrolimus, azathioprine, and dexamethasone were decreased	3-d Prodrome: fever, weakness, disorientation, and spastic movements (no nausea or vomiting) at admission to outside hospital (9/22); transferred to University of Colorado Hospital (9/30) with meningoencephalitis, parkinsonism, tremors, and myoclonus; improved 24-48 h after CASG 210 study drug treatment; discharged (10/08)	CSF, day 1: WBC=45; PMNs=60%; lymphs + mono=40%; protein=75; glucose=82; CSF, day 8: WBC=46; PMNs=5%; lymphs + mono=95%; protein=136; glucose=76	CSF positive for WNV IgM; serum, day 10: WNV IgM=4.01	MRI, day 12: diffuse dural enhancement and thickening, mild brain swelling, and mild patchy areas of predominantly subcortical white matter lesions that were hyperintense on T2-weighted images and FLAIR sequences (no old studies on file for comparison)
6/M/50	Kidney/18 mo	MMF, tacrolimus, held, IVIG, interferon alfa-2b	2-d Prodrome: fever, nausea, vomiting, and myalgia, progressing to headache, blurred vision, altered mental status, seizures, meningoencephalitis, and flaccid paralysis/poliomyelitis; mechanical ventilation required; patient currently paralyzed and ventilator dependent	CSF, day 3: WBC=23; PMNs=29%; lymphs=62%; mono=9%; RBC=0; protein=66; glucose=72; CSF, day 5: WBC=110; PMNs=9%; lymphs=75%; mono=16%; protein=75; glucose=95	CSF, day 3: negative WNV serologic test result; serum, day 1: negative WNV serologic test result; serum, day 8: WNV IgM=6.24; WNV IgG=1.48	CT only; MRI not performed owing to shrapnel in head from old gunshot wound

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persisting pleocytosis (156 cells) at day 17 of illness, and another (patient 10) had persisting pleocytosis at days 40 (38 cells), 52 (39 cells), and 82 (12 cells) of illness. The CSF protein level was elevated in 10 of 11 patients (range,

41-142 mg/dL; mean±SD, 81.8±31.1 mg/dL). The CSF glucose level was normal in every patient except for patient 4, who had simultaneous decrements in the CSF (36 mg/dL) and serum (43 mg/dL) glucose levels.

**Table. West Nile Virus Encephalitis in Transplant Recipients Hospitalized in the 2003 Colorado Outbreak\* (cont)**

Patient/ Sex/ Age, y	Transplant Type/Symptom Onset Posttransplantation	Treatment	Prodrome Duration, Symptoms, Clinical Course, and Outcome	CSF Profile	WNV Documentation	MRI Findings
7/F/59	Kidney/3 y, 4 mo	MMF, cyclosporin A held, IVIG, interferon alfa-2b	3-d Prodrome: fever, nausea, diarrhea, and malaise, progressed to apraxia, bilateral lower extremity weakness, altered mental status, seizures, myoclonus, meningoencephalitis, and flaccid paralysis; mechanical ventilation required; patient currently remains paralyzed but uses tracheostomy collar	CSF, day 1: WBC=16; PMNs=4%; lymphs + mono=96%; protein=60; glucose=67; CSF, day 5: WBC=44; PMNs=5%; lymphs=80%; mono=15%; RBC=5; protein=131; glucose=78	Serum, day 4: WNV IgM=1.44; negative for WNV IgG; serum, day 28: positive for WNV IgM and IgG	MRI, days 5, 19, and 30: day 5 study showed T2 hyperintensities in upper cervical cord, medulla, posterior pons, midbrain, bilateral thalami, basal ganglia, and cerebral white matter, with abnormal meningeal enhancement; day 19 study showed T2 increased signal in bilateral thalami, substantia nigra, striatum, and globus pallidus; day 30 study showed extensive signal abnormalities and enhancement in basal ganglia and thalami
8/F/62	Kidney/7 y	MMF, cyclosporin A held, IVIG, interferon alfa-2b	7-d Prodrome: fever, weakness, myalgias, arthralgias (no gastrointestinal complaints), altered mental status, myoclonus, tremors, and meningoencephalitis; required brief intubation; extubated after 2 d, with dramatic improvement for 2 wk; currently without residual symptoms	CSF, day 1: WBC=540; PMNs=20%; lymphs=60%; mono=20%; RBC=3420; protein=142; glucose=54	Serum, day 2: WNV IgM=3.66; negative for WNV IgG	MRI, day 2: hyperintensities surrounding lateral ventricles
9/F/51	Kidney/pancreas/ 7 y, 10 mo	MMF, cyclosporin A held, IVIG G, interferon alfa-2b, ribavirin	7-d Prodrome: fever (low grade, 100.0°F), nausea, vomiting, fatigue, tremor, diplopia, altered mental status, tremors, and meningoencephalitis; required brief intubation; extubated after 2 d with dramatic improvement but developed tremors and persistent mental status changes that persisted >8 wk after onset; patient eventually returned to baseline mentally, but currently requires bladder self-catheterization	CSF, day 2: WBC=83; PMNs=1%; lymphs=99%; RBC=332; protein=115; glucose=67; CSF, day 2 (repeated): WBC=147; PMNs=3%; lymphs=97%; RBC=12; CSF, day 17: WBC=156; PMNs=0%; lymphs=86%; mono/mac=14%; RBC=70; protein=115; glucose=50	CSF, day 2: WNV IgG not detected; WNV IgM not detected; serum, day 1: WNV IgM=1.80; negative for WNV IgG	MRI, day 1: cerebral white matter hyperintensities without abnormal enhancement; MRI, day 21: subtle abnormal T2 signals in thalami, globus pallidus, and midbrain; MRI, day 45: normal

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The CSF cytologic examination in 5 patients showed small lymphocytes, varying numbers of neutrophils, monocytes, and reactive and atypical lymphocytes, some

of which resembled plasma cells (**Figure 2A**). Flow cytometry of the CSF was performed in 3 samples using a side light scatter/CD45 gating strategy. The CSF flow cy-



**Table. West Nile Virus Encephalitis in Transplant Recipients Hospitalized in the 2003 Colorado Outbreak\* (cont)**

Patient/ Sex/ Age, y	Transplant Type/Symptom Onset	Treatment	Prodrome Duration, Symptoms, Clinical Course, and Outcome	CSF Profile	WNV Documentation	MRI Findings
10/F/48	Kidney/7 y	MMF, cyclosporin A held after second hospital admission, prednisone decreased when encephalopathy persisted	Indeterminate prodrome†: initial diarrhea for 1 mo, with admission to outside hospital (9/23, day 1); workup included colonoscopy showing nonspecific colitis; confusion and delirium on day 4; workup results negative for infectious etiology; fever resolved and patient discharged although diarrhea persisted; readmitted 1 mo later (10/23) for fever, nausea, vomiting, diarrhea, and weakness; myoclonus, seizures, nuchal rigidity, bilateral lower extremity weakness, and meningoencephalitis on third day of second hospitalization; gradual improvement in mental status but remains encephalopathic	CSF, day 4: WBC=44; PMNs=45%; mono/mac=55%; protein=122; glucose=109; PCR negative for WNV nucleic acid (no CSF WNV IgM testing performed); CSF, day 40: WBC=38; lymphs=92%; protein=79; glucose=116; cytologic tests; CSF, day 53: WBC=39; lymphs=87%; mono/mac=13%; protein=79; glucose=114; CSF, day 81: WBC=12; lymphs=96%; mono/mac=4%; RBC=1; protein=54; glucose=134; cytologic tests	Serum, day 1: negative for WNV IgM and IgG; serum, day 3: negative for WNV IgM and IgG; CSF, day 40: WNV IgM=1:8 (positive >1:1); serum, day 47: WNV IgG=1.99 IgM=3.99; CSF, day 53: WNV IgM=1:4 (positive); CSF, day 81: IgM=1:256	MRI, day 2: mild nonspecific white matter lesions, possible leptomeningeal enhancement in posterior fossa; MRI, day 33: increased central and peripheral frontal and parietal white matter lesions, hyperintense on T2-weighted and FLAIR images; subtle increased signal in right lentiform nucleus; posterior fossa leptomeningeal enhancement; MRI, day 55: resolved leptomeningeal enhancement, mild progression of white matter, and globus pallidus lesions
11/F/32	Kidney/pancreas/5 mo	Sirolimus reduced, tacrolimus and prednisone unchanged	3-d Prodrome: fever, headache, and altered mental status at admission (8/25); meningoencephalitis; rapid improvement with supportive care; discharged (8/31) without residual deficits	CSF, day 1: WBC=93; PMNs=63%; lymphs=13%; mono/mac=24%; RBC=6050; protein=57; glucose=62	CSF, day 1: WNV IgM=3.28; serum, day 2: WNV IgM=2.83	MRI not performed

Abbreviations: CASG 210, placebo-controlled double-blind study sponsored by the Collaborative Antiviral Study Group that compared Omr-IgG-am (an Israeli intravenous immunoglobulin G preparation) (1 dose of 0.5 g/kg) with US intravenous IgG (Polygam; 1 dose of 0.5 g/kg) or saline; CSF, cerebrospinal fluid; CT, computed tomography; FLAIR, fluid-attenuated inversion recovery; IVIG, intravenous immunoglobulin; lymphs, lymphocytes; mac, macrophages; MMF, mycophenolate mofetil; mono, monocytes; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; PMNs, polymorphonuclear leukocytes; RBC, red blood cell count; WBC, white blood cell count; WNV, West Nile virus.

\*All dates refer to 2003. Days for CSF profile and WNV documentation were calculated from day of hospital admission (ie, day 1 = first hospital day). Day 1 represents first day of hospital admission either at outside hospital or at the 2 referral institutions. The CSF glucose and protein concentrations are presented in milligrams per deciliter, and cell counts are presented in cells per microliter. Serum and CSF WNV IgM IgG enzyme-linked immunosorbent assay (ELISA) values less than 0.9 were negative, between 0.9 and 1.1 were equivocal, and greater than 1.1 were positive.

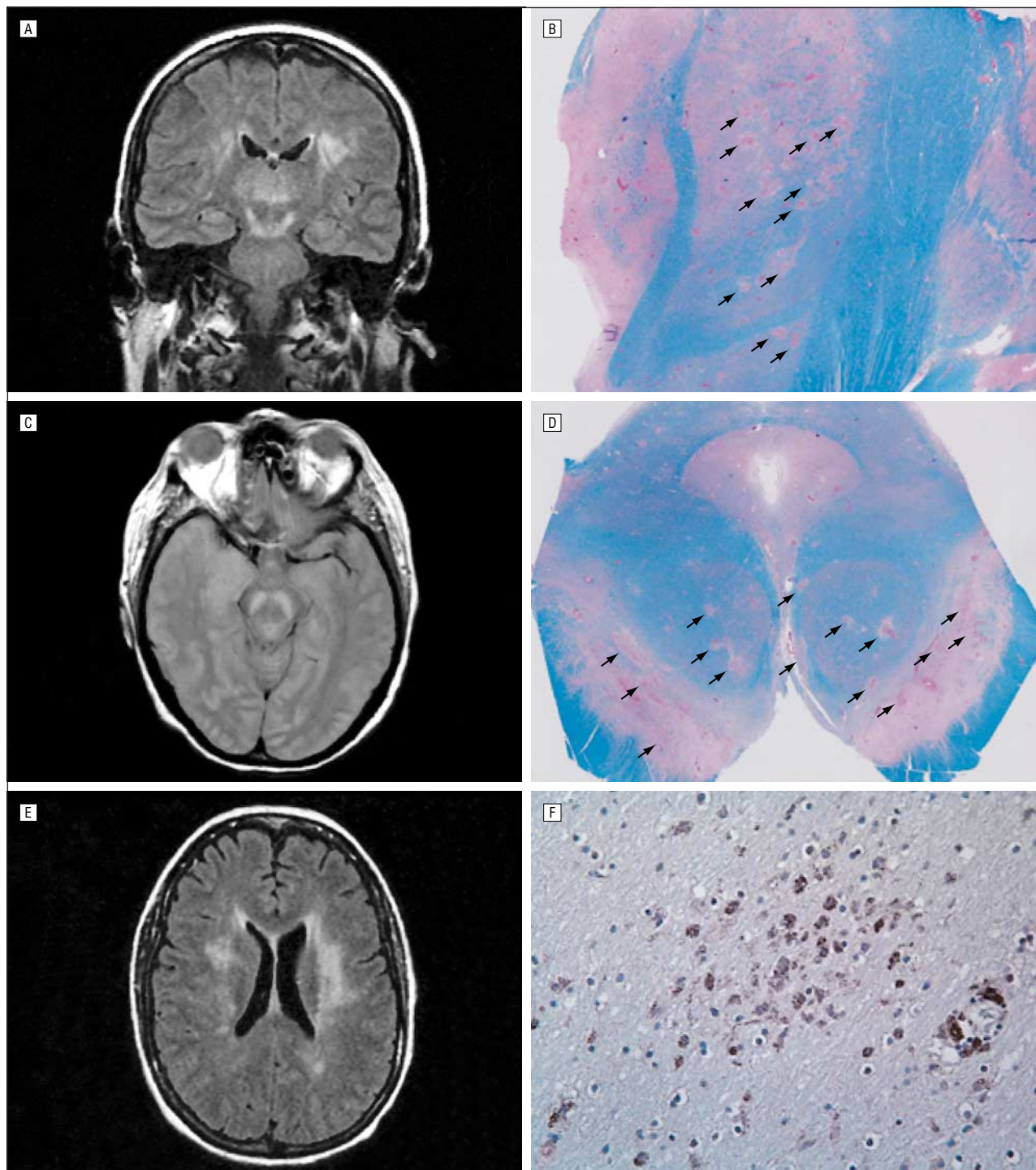
†See text for further details.

tometry (patient 10; day 40) showed a population of mature T cells expressing the immunophenotypic markers CD3 (93%), CD4 (66%; helper T cells), CD5 (92%; normal T cell marker), and CD8 (25%; suppressor T cells), consistent with a reactive picture. The B cell population was negligible. A second analysis (day 82) was similar. In patient 1, the vast majority of cells (day 2) were T cells positive for CD5 (53%) and CD4 (39%) or CD8 (22%). A negligible number of CD19-positive B cells were present.

The CSF WNV IgM antibody was detectable in 6 of 9 tested patients. The 3 patients with a nondetectable antibody had CSF serologic tests performed at days 2 to 4 of illness (patients 4, 6, and 9). Patients 4 and 9 had nega-

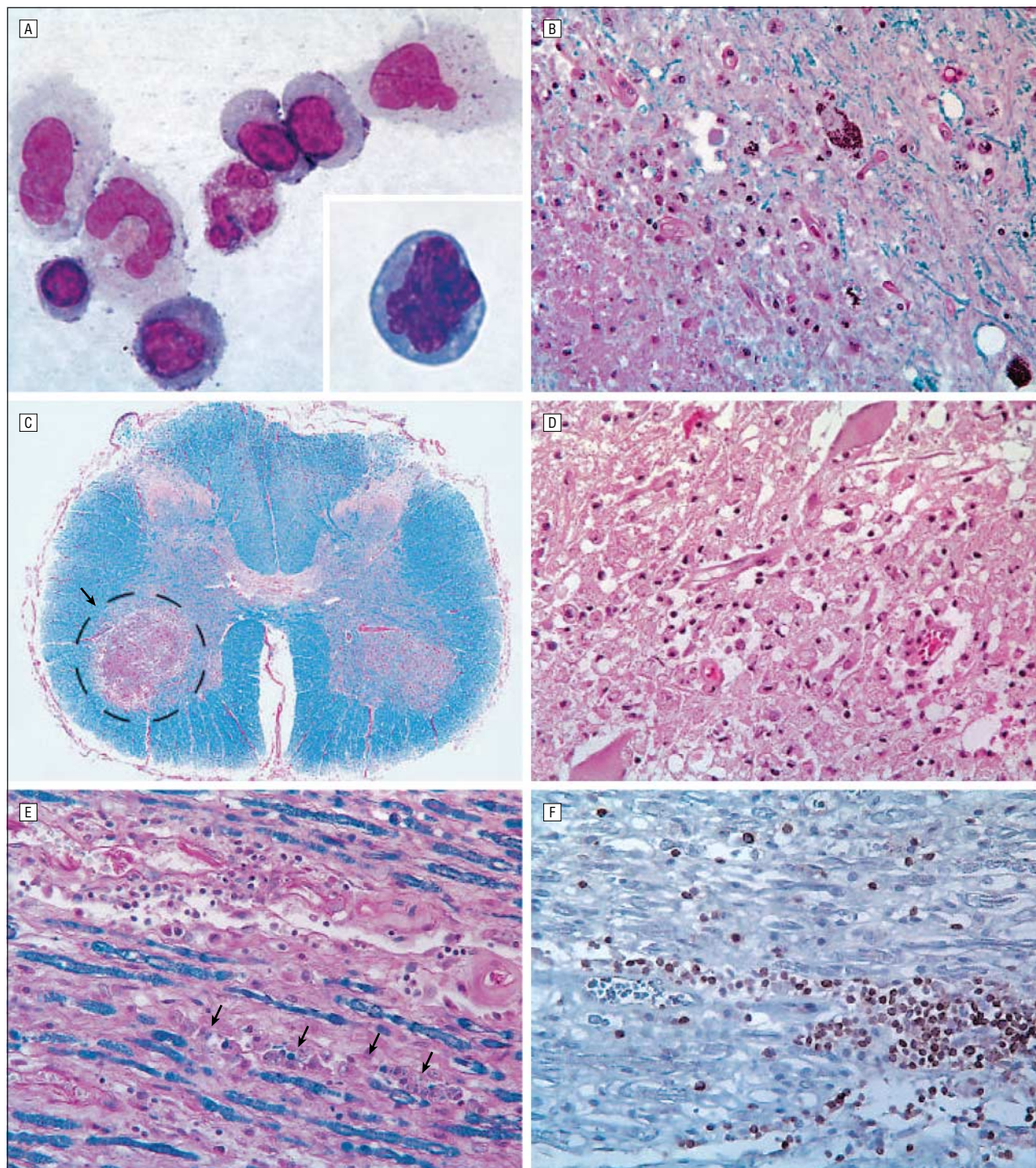
tive CSF WNV IgM results at a time when their serum WNV IgM results were positive. Patient 10 had no detectable serum WNV IgG or IgM at days 1 and 3, with a detectable antibody at days 40, 47, and 53. Five of the 11 patients had multiple serum serologic tests, and all showed seroconversion and/or increases in IgG titers (Patients 3, 4, 6, 7, and 10). The results of CSF polymerase chain reaction were negative in 3 of 3 patients on whom it was performed.

Seven of the 11 patients had at least 1 EEG performed during their hospitalization (patients 1, 4, 6, 7, 8, 9, and 10), all of which showed abnormalities. Diffuse slowing in the  $\theta$  and/or  $\delta$  range was seen in all patients but was more prominent frontally in 2 patients



**Figure 1.** A, Coronal fluid-attenuated inversion recovery (FLAIR) image (time to repeat [TR], 8802 milliseconds; echo time [TE], 115.5 milliseconds; inversion time [TI], 2200 milliseconds; number of excitations [NEX], 0.5) shows an area of abnormally increased signal in the thalami, substantia nigra (extending superiorly toward the subthalamic nuclei), and white matter. B, Corresponding tissue section from the same patient at autopsy 15 days later shows innumerable ovoid foci of necrosis and pallor throughout the thalamus and subthalamic nucleus (arrows). Whole-mount section; Luxol fast blue–periodic acid-Schiff for myelin; original magnification  $\times 3$ . C, Axial proton density image (TR, 2666.6 milliseconds; TE, 13.8 milliseconds; NEX, 2.0) at the level of the midbrain shows the increased signal in the substantia nigra bilaterally. D, Corresponding tissue section at autopsy illustrates multifocal involvement of the substantia nigra with nearly 50% of the area destroyed; red nuclei are clearly affected on the pathological specimen. Whole-mount section; Luxol fast blue–periodic acid-Schiff for myelin; original magnification  $\times 3$ . E, Axial FLAIR image (TR, 8802 milliseconds; TE, 125 milliseconds; TI, 2200 milliseconds; NEX, 0.5) at the level of the lateral ventricle bodies shows an increased signal within the white matter bilaterally. A scan performed approximately 5 months earlier demonstrated an abnormal signal in the left periventricular white matter. This increased once West Nile virus encephalitis developed, and the lesions in the right cerebral white matter (left side of photograph) were new. F, Photomicrograph taken from the right periventricular white matter shows numerous macrophages, both in perivascular areas (lower right) and diffusely throughout the white matter (center) (HAM 56 immunostaining for macrophages, with hematoxylin-eosin counterstain, original magnification  $\times 200$ ).





**Figure 2.** A, Patient 2. Cerebrospinal fluid cytologic preparation demonstrates large monocytes (2 at left and 1 at upper right), a multilobed neutrophil (center), large reactive lymphocytes (2 cells to the immediate right of the neutrophil), and a plasmacytoid lymphocyte (lower center), which are seen in varying numbers in the cerebrospinal fluid in most cases of West Nile virus encephalitis. Cytospin preparation; Wright-Giemsa, original magnification  $\times 1000$ . Inset: Patient 10. Atypical lymphocytes with hyperchromatic convoluted nuclei. Filter preparation; Wright-Giemsa, original magnification  $\times 1500$ . B, Patient 1. Higher-power photomicrograph of substantia nigra damage (see Figure 1C and D) illustrates zonal necrosis (lower left), macrophage influx, and only a few remaining pigmented neurons. Luxol fast blue–periodic acid-Schiff for myelin; original magnification  $\times 200$ . C, Patient 1. Whole-mount section of the spinal cord illustrates bilateral poliomyelitis found at autopsy, with discrete ovoid areas of particularly severe anterior horn cell damage, example at left (encircled). Luxol fast blue–periodic acid-Schiff for myelin; original magnification  $\times 7.5$ . D, Patient 1. Higher-power photomicrograph of the anterior horn cell area shows a few surviving neurons in a sea of macrophages. Hematoxylin-eosin; original magnification  $\times 200$ . E, Patient 1. Dorsal nerve roots focally demonstrate ongoing myelin breakdown (arrows) indicative of nerve root damage in addition to the poliomyelitis illustrated in Figure 2C and D. Luxol fast blue–periodic acid-Schiff for myelin; original magnification  $\times 100$ . F, Patient 1. Dorsal nerve root from the same area illustrated in Figure 2E contains numerous CD3-positive (brown chromogen) T cells and virtually no CD20-positive B cells (red chromogen); this was the most inflammatory area identified in the neuropathological examination. Dual-label immunohistochemistry with hematoxylin-eosin counterstain.



(patients 6 and 9). In 2 patients (patients 6 and 7), we noted triphasic slow waves typical of those seen in metabolic encephalopathies. Seizures occurred in 3 patients but were transient and easily controlled. Two of these patients (patients 6 and 7) had periodic lateralized epileptiform discharges (PLEDs) noted on at least 1 EEG. In the 3 patients with seizures, sequential EEGs obtained at different points during their illness showed an evolution from diffuse slowing to PLEDs, followed by gradual resolution of EEG abnormalities.

Four of 11 patients (patients 1, 3, 6, and 7) rapidly progressed to acute flaccid paralysis. In 2 of these patients (patients 1 and 3), nerve conduction and electromyographic studies were performed. Patient 1 showed absent or severely reduced amplitude of the compound muscle action potentials (CMAPs) with relatively preserved amplitude of the sensory nerve action potentials in the involved limbs. Conduction velocities were either normal or mildly slowed in nerves with reduced CMAPs. There was no evidence of denervation in a study conducted at day 10 of illness. Electromyographic and nerve conduction studies performed in patient 3 one month after the onset of illness showed severely decreased amplitude of the CMAPs with moderate decrements in conduction velocity in the involved nerves. Sensory nerve action potential amplitudes were also reduced but to a lesser degree than the CMAPs. Occasional spontaneous activity (fibrillations and positive sharp waves) was seen in all involved muscles. These findings were felt to be consistent with a predominantly motor axonopathy or anterior horn cell disorder with associated denervation. Test results provided evidence of anterior horn cell damage rather than Guillain-Barré syndrome. No evidence of denervation was seen on electromyographic studies in patient 10, who had meningoencephalitis but no acute flaccid paralysis.

### TREATMENT

Treatment varied greatly among the cohort, precluding any analysis of efficacy. Patients 3 and 4 received Omr-IgG-am, a trademarked Israeli intravenous immunoglobulin (IVIg) preparation with a high-titer antibody against WNV, with Food and Drug Administration and institutional review board approval as part of an Investigational New Drug application. These patients were also treated with interferon alfa-2b (3 million U/d subcutaneously for 7 to 17 days). Patients 1, 2, and 5 were participants in Collaborative Antiviral Study Group trial 210, a placebo-controlled double-blind study currently in progress that is comparing Omr-IgG-am (1 dose of 0.5 g/kg) with US IVIg (Polygam) (1 dose of 0.5 g/kg) or saline. Four additional patients (patients 6, 7, 8, and 9) were treated with US IVIg (1 dose of 1 g/kg) and interferon alfa-2b (3 million U/d<sup>2</sup> for 3 to 6 days). One patient also received ribavirin (patient 9) (400 mg twice a day for 8 days), and 2 patients (patients 10 and 11) received no specific antiviral therapy.

Immunosuppressive therapy was either reduced or held in all solid-organ recipients. Maintenance therapy for the 2 stem-cell recipients was not altered. The 2 patients who had undergone liver transplants (patients 3 and 4) had

their cyclosporin A or tacrolimus stopped and mycophenolate mofetil reduced. All patients with kidney transplants had the doses of their immunosuppressive medications reduced or held. One patient with a kidney transplant (patient 10) had mycophenolate mofetil and cyclosporin A discontinued when she first developed seizures and encephalopathy, but her symptoms failed to improve until her steroid dosage was decreased as well.

### AUTOPSY RESULTS

Patient 1 died of acute pneumonia on hospital day 17. The brain was grossly normal but microscopically contained small multifocal areas of necrosis and macrophage influx in nearly every sampled section of the subcortical gray matter, brainstem, cerebellum, and spinal cord. Microglial clusters and neuronophagia were found in the anterior horn, cerebral neocortex, hypothalamus, and hippocampus. Numerous ovoid foci of damage pockmarked the thalami and subthalamic nuclei (Figure 1B) as well as the substantia nigra and red nuclei (Figure 1D and Figure 2B), directly corresponding to the abnormalities seen on MRI studies (Figures 1A and C). The periventricular and deep right cerebral white matter (Figure 1E) showed diffuse macrophage infiltration but minimal necrosis and only mild myelin pallor (Figure 1F). Other affected sites included the tegmentum of pons and basis pontis, medulla, cerebellar molecular layer, and spinal cord anterior horns (Figure 2C). Silver stains identified few axonal swellings except in the basis pontis, which demonstrated pontine leukodystrophy. Focal ongoing myelin breakdown in the dorsal nerve roots attested to nerve root damage in addition to the gray matter damage in the spinal cord (Figure 2E). Although only scant perivascular lymphocytic collections, almost all of which were T-cell lymphocytes according to dual CD3/CD20 immunostaining, were present in the central nervous system sections, T-cell inflammation (Figure 2F) in the nerve roots was more conspicuous (Figure 2F). The WNV genome was detected using postmortem polymerase chain reaction with a viral load equivalent to approximately 2000 plaque-forming units per milliliter in the spinal cord and brain and at a lower level in the blood. Patient 4 died 6 months after hospital admission, but no autopsy was performed.

### COMMENT

The 11 patients reported in this article represent the largest series to date of WNV encephalitis in transplant recipients.<sup>7-15</sup> Our findings are likely applicable to other immunocompromised patients.<sup>16-18</sup> The initial clinical symptoms in transplant recipients included fever, weakness, myalgia, gastrointestinal complaints, and altered mental status. Both the duration and nature of the prodromal symptoms were similar to those reported in immunocompetent patients with the possible exception of rash, which was not noted in our cases, and the 1 patient with a potential prolonged gastrointestinal illness.<sup>1</sup>

Studies of the CSF were performed in all cases. The CSF protein ranged from 41 to 142 mg/dL (mean±SD,

81.8 ± 31.1 mg/dL; normal, 15-45 mg/dL), comparable with values reported in the study by Nash et al.<sup>1</sup> All 11 patients manifested an initial pleocytosis, with 4 patients having a polymorphonuclear neutrophil count of at least 45%, a feature described previously.<sup>1,11</sup> The initial CSF or serum samples were positive for WNV IgM in 6 of our 9 tested patients. Notably, patient 10 had negative CSF polymerase chain reaction, serum WNV IgM, and IgG serologic test results during the acute onset of her altered mental status. The CSF WNV IgM level was not obtained at that time. Although occasionally transplant recipients described in the literature have been noted to have borderline<sup>10</sup> or initially negative<sup>9,10</sup> serum or CSF<sup>9,17</sup> WNV IgM test results, most transplant recipients show the CSF profile expected for immunocompetent individuals, implying that they are able to mount appreciable humoral immune responses to WNV infection. Atypical reactive lymphocytes, some of which resembled plasma cells, were seen in the CSF of 5 patients with WNV encephalitis, as previously noted.<sup>12</sup> However, flow cytometric analyses of 3 CSF samples verified that the cells were predominantly of T-cell lineage (CD4 > CD8), with negligible numbers of B cells and plasma cells.

The MRI results were abnormal in 7 (87%) of 8 patients in whom studies were performed, a higher incidence than previously reported in immunocompetent patients.<sup>1,18</sup> The most common MRI finding in our series was patchy areas of increased signal intensity in the deep or periventricular white matter, which correlated with regions of ill-defined myelin pallor and diffuse macrophage influx in patients who underwent autopsy. However, white matter lesions of this type might also have been the result of preexisting ischemic injury or immunosuppressive therapy. Three patients had typical MRI features of WNV encephalitis, including abnormalities in the thalamus, basal ganglia, or upper brainstem. The prominence of MRI abnormalities in our cohort and other immunocompromised patients<sup>8,10,11,17</sup> suggests that more severe injury may occur in immunocompromised compared with immunocompetent WNV-infected individuals.

Evidence of severe WNV infection was found at autopsy in patient 1, who had multifocal areas of necrosis throughout the thalami, subthalamic nuclei, brainstem (especially the substantia nigra, red nuclei, and pons), and spinal cord anterior horn. Previous case reports of WNV encephalitis in transplant recipients have also suggested an increased clinical severity of disease.<sup>9-11,17</sup> Most autopsy reports in immunocompetent patients have shown considerably less damage,<sup>19-23</sup> although severe damage can occur.<sup>24</sup> The mortality rate in our series (18%) was higher than that reported in both hospitalized immunocompetent patients (12%)<sup>1</sup> and unselected Colorado patients with meningitis or encephalitis (10%).

All patients who had EEGs manifested a diffuse slowing of variable severity consistent with generalized encephalopathy. Two patients showed PLEDs, and 2 patients had triphasic slow waves, patterns not previously reported in WNV infection.<sup>18,25</sup> Transient seizures occurred in 30% of our cases. The MRI and EEG findings coupled with the 1 autopsy report suggest that WNV encephalitis in transplant recipients is more severe than would be expected in immunocompetent individuals.

Once disease developed in these hospitalized patients, the symptoms, duration of hospitalization, requirements for mechanical ventilation, and long-term outcome all fell into the severe end of the spectrum for what has previously been reported for immunocompetent patients with WNV encephalitis.<sup>18</sup> Four of our 11 patients made excellent recoveries, returning to baseline functional status at evaluation 3 months postinfection (patients 2, 5, 8, and 11). One patient (patient 9) made a slow recovery and still has persistent tremors and mental status changes. Four patients have not improved and are in long-term care facilities (patients 3, 6, 7, and 10), 2 of whom remain ventilator dependent (patients 6 and 7). Patients with severe acute flaccid paralysis have shown only a minimal improvement in weakness. Two patients died, one (patient 1) on day 17 and the other (patient 4) 6 months after hospital admission.

The patients in this series received a variety of treatments for WNV encephalitis, and the uncontrolled and nonblinded nature of the series precludes analysis of the efficacy of specific therapies. There is currently no known effective treatment for WNV infection. We generally treated patients by (1) reducing immunosuppression specifically involving tacrolimus,<sup>1</sup> cyclosporin A, and steroids; (2) nonspecific antiviral therapy (interferon alfa-2b)<sup>2</sup>; and (3) passive immunization with anti-WNV antibody-containing IVIG when available.<sup>3</sup> Long-term use of interferon alfa-2b has been associated with an increased risk of acute humoral renal transplant rejection,<sup>26</sup> although no significant deterioration in renal graft function was noted in the patients in this study during short (4-17 day) courses of treatment. Patients treated with Omr-IgG-am received it late in the course of infection, precluding analysis of its potential efficacy in short-term treatment. The effect of our treatment on the natural history of the disease is unclear. The results of the ongoing randomized placebo-controlled Collaborative Antiviral Study Group 210 trial comparing Omr-IgG-am, an Israeli IVIG preparation with high-titer anti-WNV antibody, with US IVIG G (Polygam) or saline should provide more definitive information regarding the utility of passive immunization.

The major limitation of our study is that no data currently exist in our state to document the number of transplant recipients in Colorado who may have contracted the virus but only developed subclinical or mild clinical infections. Seroprevalence studies are now in progress to answer this question. The major strength of our study is the number of cases we report, which provides perspective to single case reports in the literature that may be biased toward fatal examples. We have shown that when neurological disease develops in transplant recipients owing to WNV, it manifests on the severe end of the spectrum.

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## REFERENCES

- Nash D, Mostashari F, Fine A, et al, and the 1999 West Nile Outbreak Response Working Group. The outbreak of West Nile virus infection in the New York City area in 1999. *N Engl J Med*. 2001;344:1807-1814.
- Asnis DS, Conetta R, Teixeira AA, Waldman G, Sampson BA. The West Nile virus outbreak of 1999 in New York: the Flushing Hospital experience. *Clin Infect Dis*. 2000;30:413-418.
- Centers for Disease Control and Prevention Web site. Available at: [www.cdc.gov/ncidod/dvbid/westnile/](http://www.cdc.gov/ncidod/dvbid/westnile/). Accessed June 12, 2004.
- Colorado Public Department of Health and Environment Web site. Available at: [www.cdphe.state.co.us/dc/zoosis/wnv/vnvhom.html](http://www.cdphe.state.co.us/dc/zoosis/wnv/vnvhom.html). Accessed June 12, 2004.
- Morse DL. West Nile virus—not a passing phenomenon. *N Engl J Med*. 2003;348:2173-2175.
- Scientific Registry of Transplant Recipients. Fast facts about transplants. Available at: [www.ustransplant.org/facts.html](http://www.ustransplant.org/facts.html). Accessed June 12, 2004.
- Hardinger KL, Miller B, Storch GA, Desai NM, Brennan DC. West Nile virus-associated meningoencephalitis in two chronically immunosuppressed renal transplant recipients. *Am J Transplant*. 2003;3:1312-1315.
- Bosanko CM, Gilroy J, Wang AM, et al. West Nile virus encephalitis involving the substantia nigra. *Arch Neurol*. 2003;60:1448-1452.
- Hiatt B, DesJardin L, Carter T, Gingrich R, Thompson C, de Magalhaes-Silverman M. A fatal case of West Nile virus infection in a bone marrow transplant recipient. *Clin Infect Dis*. 2003;37:e129-e131.
- Hong DS, Jacobson KL, Raad II, et al. West Nile encephalitis in 2 hematopoietic stem cell transplant recipients: case series and literature review. *Clin Infect Dis*. 2003;37:1044-1049.
- Solomon T, Fisher AF, Beasley DW, et al. Natural and nosocomial infection in a patient with West Nile encephalitis and extrapyramidal movement disorders. *Clin Infect Dis*. 2003;36:e140-e145.
- Carson PJ, Steidler T, Patron R, Tate JM, Tight R, Smego RA Jr. Plasma cell pleocytosis in cerebrospinal fluid in patients with West Nile virus encephalitis. *Clin Infect Dis*. 2003;37:e12-e15.
- DeSalvo D, Roy-Chaudhury P, Merchen T, et al. West Nile virus encephalitis in organ transplant recipients. Abstract presented at: International Symposium of Neurology; September 5, 2003; Baltimore, Md.
- Hamdan A, Green P, Mendelson E, Kramer MR, Pitlik S, Weinberger M. Possible benefit of intravenous immunoglobulin therapy in a lung transplant recipient with West Nile virus encephalitis. *Transpl Infect Dis*. 2002;4:160-162.
- Huang C, Slater B, Rudd R, et al. First isolation of West Nile virus from a patient with encephalitis in the United States. *Emerg Infect Dis*. 2002;8:1367-1371.
- Spiegel R, Miron D, Gavriel H, Horovitz Y. West Nile virus meningoencephalitis complicated by motor aphasia in Hodgkin's lymphoma. *Arch Dis Child*. 2002;86:441-442.
- Kumar D, Ramesh Prasad GV, Zaltzman J, Levy GA, Humar A. Community-acquired West Nile virus infection in solid-organ transplant recipients. *Transplantation*. 2003;76:1-4.
- Sejvar JJ, Haddad MB, Tierney BC, et al. Neurologic manifestations and outcome of West Nile virus infection. *JAMA*. 2003;290:511-515.
- Armstrong WS, Bashour CA, Smedira NG, et al. A case of fatal West Nile virus meningoencephalitis associated with receipt of blood transfusions after open heart surgery. *Ann Thorac Surg*. 2003;76:605-607.
- Sampson BA, Armbrustmacher V. West Nile encephalitis: the neuropathology of four fatalities. *Ann N Y Acad Sci*. 2001;951:172-178.
- Agamanolis DP, Leslie MJ, Caveny EA, Guarner J, Shieh WJ, Zaki SR. Neuropathological findings in West Nile virus encephalitis: a case report. *Ann Neurol*. 2003;54:547-551.
- Doron SI, Dashe JF, Adelman LS, Brown WF, Werner BG, Hadley S. Histopathologically proven poliomyelitis with quadriplegia and loss of brainstem function due to West Nile virus infection. *Clin Infect Dis*. 2003;37:e74-e77.
- Kelley TW, Prayson RA, Ruiz AI, Isada CM, Gordon SM. The neuropathology of West Nile virus meningoencephalitis: a report of two cases and review of the literature. *Am J Clin Pathol*. 2003;119:749-753.
- Omalu BI, Shakir AA, Wang G, Lipkin WI, Wiley CA. Fatal fulminant pan-meningoencephalitis due to West Nile virus. *Brain Pathol*. 2003;13:465-472.
- Gandelman-Martón R, Kimiagar I, Itzhaki A, Klein C, Theitler J, Rabey JM. Electroencephalography findings in adult patients with West Nile virus-associated meningitis and meningoencephalitis. *Clin Infect Dis*. 2003;37:1573-1578.
- Baid S, Toloff-Rubin N, Saidman S, et al. Acute humoral rejection in hepatitis C-infected renal transplant recipients receiving antiviral therapy. *Am J Transplant*. 2003;3:74-78.