

West Nile Virus Neuroinvasive Disease

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Abstract

- **Objective:** To summarize the virology, modes of transmission, clinical manifestations, diagnostic studies, and available therapeutic modalities for West Nile virus (WNV) infection.
- **Methods:** Review of the literature.
- **Results:** WNV infection represents one of the most common arboviral diseases worldwide. Although most individuals with WNV infection are asymptomatic, a significant number of patients develop severe neurological disease including meningitis, encephalitis, and acute flaccid paralysis. WNV is primarily transmitted by the bite of infected mosquitoes, but other novel modes of WNV transmission have been reported. Physicians should maintain a high degree of clinical suspicion for WNV infections in patients presenting with an acute febrile illness, especially those with central nervous system manifestations in summer and fall. Treatment remains largely supportive and the emphasis remains on prevention.
- **Conclusion:** WNV infection remains a significant public health challenge.

West Nile virus (WNV) infection now represents one of the most common arboviral diseases worldwide. Although most individuals with WNV infection are asymptomatic, a significant number of patients develop severe neurological disease, including meningitis, encephalitis, and acute flaccid paralysis. Smithburn et al published the first report of neurotropic WNV infection in 1940 and isolated the virus from the blood of a woman with fever residing in the West Nile district of Uganda [1]. Subsequently, the virus became recognized as a cause of meningitis and encephalitis in elderly patients in Israel in the 1950s. Epidemics of WNV infection have been reported in many countries, including South Africa, France, Romania, India and Indonesia. In endemic areas like Egypt, a 40% WNV seroprevalence rate has been described [2].

WNV was not recognized in the Americas until the outbreak of encephalitis in New York City and surrounding areas in 1999 [3]. The Centers for Disease Control and Prevention (CDC) reported 62 human cases that year, with many infected birds and horses reported as well. WNV

quickly spread across the country after its initial introduction with wide geographical distribution. The largest epidemics of neuroinvasive WNV disease ever reported occurred in the United States in 2002 and 2003 [6]. In 2002, the CDC reported a total of 4156 cases in humans, including 284 fatalities. WNV has spread across the Western Hemisphere and is now found throughout the United States, Canada, Mexico, the Caribbean, and parts of Central and South America.

Studies of phylogenetic lineage have suggested that the virus is over 1000 years old, and it has been postulated as one of the possible causes of Alexander the Great's early death based on reports of his exposure to dead ravens prior to the onset of the febrile illness leading to his death [4]. The virus is thought to have developed into 2 lineages [5]. Lineage 1 WN viruses have been associated with clinical human encephalitis (including the U.S. strains). Lineage 2 WN viruses are thought to be maintained in enzootic foci in Africa.

WNV infections remain a significant public health challenge. In this review, we summarize the virology, modes of transmission, clinical manifestations, diagnostic studies, and available therapeutic modalities.

Virology

WNV is an arthropod-borne human pathogen and is classified in the family Flaviviridae and genus Flavivirus. WNV particles are about 50 nm in diameter and contain a single-stranded plus-sense RNA genome that is surrounded by an icosahedral capsid with envelope. The viral particle contains core (C) proteins, and the lipid envelope contains 2 important viral proteins: E (envelope) and M (membrane). The E protein is the major antigenic determinant on virus particles and mediates binding and fusion of the virus during entry into the cell. The M protein is produced during the maturation of the virus within the secretory pathway, and is a small proteolytic fragment of the precursor prM protein [7]. Viral replication starts by the binding of the viral particle through the interaction of E protein with specific receptors that are present on the surfaces of many cells [8]. After

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entry into the cells, the RNA genome serves as mRNA and is translated by ribosomes into 10 mature viral proteins via proteolytic cleavage. These proteins assemble and transcribe a complementary minus-strand RNA from the genomic RNA. The complementary minus-strand RNA in turn serves as a template for the synthesis of the positive-strand genomic RNA. The viral E, preM, and C proteins assemble with the genomic RNA to form progeny virions, which then migrate to the cell surface where they are surrounded by a lipid envelope and are released [9].

Pathogenesis

The disease is transmitted to humans by the bite of infected mosquitoes. Birds act as amplifying hosts and infect mosquitoes, which then transmit disease to other birds. Humans, horses, and other nonavian vertebrates are incidental hosts. *Culex* mosquitoes are the principal WNV vectors, but other mosquito species have been demonstrated as WNV carriers.

In the southern United States, WNV infection occurs year around, while in the northern United States the infection occurs in summer and early fall. Outbreaks caused by WNV have been difficult to predict but are thought to be linked to hot dry summers [10]. The incubation period following exposure ranges from 3 to 14 days [11] but may be longer (up to 21 days) in organ transplant recipients.

Initial replication of the virus takes place at the site of infection, probably in dendritic cells that subsequently migrate to the lymph nodes where a second round of viral replication occurs [12]. The virus then enters the bloodstream and disseminates and can be isolated from lung, spleen, and lymph nodes [13]. It is unclear if this represents active viral replication or passive uptake in endothelial cells from the periphery. In a small number of patients, the virus crosses the blood-brain barrier and causes inflammation of the brain and spinal cord. Penetration of virus into the central nervous system (CNS) follows stimulation of toll-like receptors and increased tumor necrosis factor- α production, which is thought to increase the permeability of the blood-brain barrier [14].

WNV directly affects neurons, particularly in the nuclei and gray matter of the brain and spinal cord [15,16]. In postmortem immunohistochemical studies of brain specimens from patients with neuroinvasive WNV infection, glial nodules with variable loss of neurons and perivascular cuffing by mononuclear cells have been demonstrated [17]. Examination of spinal cords from patients with flaccid paralysis have demonstrated lymphocytic infiltrates in the nerve roots and within the cord proper along with microglial nodules and neuronophagia [18,19].

Koh et al [20] studied host transcriptional changes and demonstrated that following WNV infection of glial cells, there are changes in the expression of 23 genes, including

an upregulation of genes known to cause apoptosis, resulting in neurologic damage. Greater understanding of these pathways might lead to development of specific therapeutic modalities for this infection.

Recently several reports have indicated that genetic factors may increase susceptibility to WNV infection. Mice models have demonstrated the importance of the chemokine receptor CCR5 as a protective factor against neuroinvasive WNV infection [21]. CCR5- Δ 32 is a deletion mutation variant of the CCR5 gene and is found not infrequently in people of northern European descent. CCR5- Δ 32 homozygosity has been demonstrated in about 1% of healthy U.S. white random blood donors. In an interesting study of 2 independent cohorts of patients with laboratory-confirmed symptomatic WNV infection, Glass et al [22] demonstrated that there is a very significant increase in the prevalence of WNV infections among CCR5- Δ 32 homozygous white persons (4.2%–8.3%). These authors also demonstrated that CCR5- Δ 32 homozygosity was associated with worse outcomes in these patients. CCR5 is a major HIV coreceptor and CCR5 inhibitors have been recently introduced as novel antiretroviral therapeutic agents. The impact of these agents on the acquisition of symptomatic WNV infections remains to be seen.

Modes of Transmission

WNV is primarily transmitted by the bite of infected mosquitoes with humans as the incidental hosts. Studies of WNV epidemics have consistently linked disease to mosquito exposure [23,24]. Other novel modes of WNV transmission have been reported in the last few years and include the following:

Transmission in a Laboratory

In 2002, the CDC reported WNV infections in 2 microbiologists following percutaneous inoculation of WNV in the laboratory [25]. Hence it is recommended that laboratory workers handling WNV-infected materials should use every precaution to minimize contact and aerosol exposure to these materials. The CDC recommends that live WNV should be handled in BSL-3 facilities.

Transplacental Transmission

The first reported case of intrauterine WNV infection involved a previously healthy 20-year-old woman in her 27th week of gestation who developed WNV meningoencephalitis [26]. The patient delivered a live infant at 38 weeks of gestation. The infant had bilateral chorioretinitis and severe cerebral abnormalities including bilateral white matter loss and cystic change in the temporal lobe consistent with focal cerebral destruction. The infant was diagnosed with WNV neuroinvasive disease on the basis of blood samples

that were positive for WNV-specific IgM antibodies. The placenta was also found to be positive for WNV by PCR. This case prompted intensified surveillance for WNV infection during pregnancy and screening for WNV infection in infants. This intensified surveillance led to the identification of an additional 77 pregnant women across the United States clinically infected with WNV who delivered 72 live infants [27]. Among these infants, 55 had their cord blood tested for WNV IgM and 54 tested negative. The 1 infant who tested positive for cord blood WNV IgM tested negative for WNV IgM in peripheral serum. One infant who did not have WNV IgM in cord blood subsequently developed WNV meningitis at age 10 days. Two additional cases of WNV were diagnosed among these infants. Paisley et al [28] screened umbilical cord blood from 549 infants for WNV-specific IgM and IgG antibodies and found that 4% tested positive for WNV-specific IgG, and none of the samples tested positive for IgM antibodies. No significant differences were noted in any of these infants with respect to growth parameters or other outcomes measured.

Transmission Through Breast Milk

The CDC reported a case of possible WNV transmission to an infant through breastfeeding [29]. In September 2002, a 40-year-old woman delivered a healthy infant but received blood transfusion for anemia. The blood product was later confirmed to be positive for WNV by polymerase chain reaction. The mother subsequently developed WNV meningitis, and a serum sample from the infant was positive for WNV IgM. The infant was breastfed for 16 days (6 days after symptom onset in the mother) and had minimal outdoor exposure. Although the breast milk was found to be positive for WNV-specific IgM and IgG, viral cultures remained negative and the infant remained healthy. The implications of these findings remain unknown and at this time there is felt to be insufficient data to suggest a change to current breastfeeding recommendations.

Transmission Through Blood Transfusions

Patients with laboratory evidence of WNV infection within 4 weeks after receipt of blood products from viremic donors are considered to have transfusion-related WNV infection. Twenty-three cases of blood product transfusion-related WNV infections were reported in 2002 from 16 WNV viremic donors [30]. Of interest, all of the 16 donors involved were negative for WNV-specific IgM antibodies at the time of donation. Based on these data, U.S. blood collection agencies (BCAs) responded in 2003 by implementing routine donor testing by WNV nucleic acid amplification tests (NATs). Approximately 6 million units of blood were screened with WNV NATs between June and December 2003 and 818 viremic samples were identified [31]. In spite

of these improved measures, 6 cases of transfusion-related WNV infections were reported and were associated with blood products that had viral concentrations too small to be detected by minipool NAT. This led the BCAs to alter their screening policies to test samples by individual NAT in areas with high WNV infection rates. During 2003–2005, these tests allowed detection of more than 1400 WNV viremic donations [32]. Clinicians should remain aware that although these strategies substantially reduce the incidence of transfusion-related WNV infections, they do not eliminate them.

Transmission Through Organ Transplantations

In 2002, 4 cases of WNV infections were demonstrated to have been acquired through organ transplantations [33]. All 4 patients received organ transplants from a single donor who was subsequently demonstrated to have been viremic at the time of organ recovery. All 4 patients became ill, with 3 developing encephalitis, and 1 patient subsequently dying secondary to WNV infection. In 2005 another cluster of transplant-associated WNV infections was reported [34]. WNV infection was confirmed in 3 of 4 recipients of organs from a single donor. Two patients developed neuroinvasive disease and the third had asymptomatic WNV infection. The donor's serum samples tested positive for WNV specific IgM and IgG antibodies. Studies have demonstrated that immunosuppressed organ transplant recipients have a several-fold higher risk of developing WNV neuroinvasive disease as compared to the general population [35]. Hence WNV infections should be considered in all organ transplant patients with febrile illness and neurologic symptoms, especially if clusters of illness are seen.

Possible Dialysis-Related Transmission

A cluster of three patients with confirmed WNV infection were identified who had received hemodialysis on the same day on the same machine at a single center [36]. This cluster was felt to be a possible transmission of WNV associated with the dialysis machine, although this could not be proven conclusively.

Occupational Exposure

In 2002, the Wisconsin Division of Public Health reported 2 cases of febrile WNV infections in workers at a commercial turkey breeder farm [37]. Follow-up serology screening of all employees at this facility revealed a greater incidence of seropositivity as compared with workers from other breeder and nonbreeder farms. The mode of transmission among these workers remains unclear, and novel routes of transmission such as percutaneous, fecal-oral, and respiratory routes have been suggested in addition to mosquito-borne illness. CNS disease in a mouse model following exposure

to WNV aerosols has been demonstrated in the laboratory [38]. Until further information regarding these workers' risk factors for WNV infection is available, clinicians should consider a diagnosis of WNV infection in turkey farm workers with febrile illness, especially if they have CNS manifestations.

Clinical Features

WNV infection has protean manifestations. Most persons with WNV infection (up to 80%) remain asymptomatic. In people with clinical illness, the incubation period ranges from 3 to 14 days [11]. The incubation period may be longer (7–17 days) in organ transplant recipients [33].

The most common clinical manifestation is West Nile Fever (WNF), which is usually a mild illness lasting 3 to 6 days. Patients with this condition present with a febrile illness of sudden onset in conjunction with nonspecific symptoms such as malaise, anorexia, myalgia, nausea, vomiting, and occasionally rash. The rash, if seen, occurs around the 5th day of onset of illness and is usually erythematous and maculopapular (occasionally papular or morbiliform); it is mostly seen on the trunk and is pruritic in about a third of patients [39]. WNF is typically a self-limiting illness.

Neuroinvasive disease is a less frequent manifestation of WNV infection and is seen in 1% to 2% of individuals infected with WNV. The most common neurologic manifestations of WNV are meningitis, encephalitis, and acute flaccid paralysis. Neurologic manifestations of WNV infection are more common in older individuals (> 50 years) and in immunocompromised patients. Uncommon manifestations include ophthalmologic manifestations, Guillain-Barré syndrome, stroke-like presentation, and unilateral brachial plexopathy [40–44].

WNV Meningitis

WNV meningitis (WNM) presents like a classic viral meningitis and is associated with fever, headache, neck stiffness, and photophobia. Cerebrospinal fluid findings in these patients are described below in the diagnostic studies section. WNM is usually self-limited and patients tend to have a favorable outcome [45].

WNV Encephalitis

WNV encephalitis (WNE) is a severe manifestation of WNV infection. Patients present with mental status changes that may range from mild confusion to an unresponsive comatose state. Systemic signs and symptoms including fever, myalgia, and gastrointestinal manifestations such as nausea, vomiting, and diarrhea are commonly seen in these patients. Often these patients demonstrate movement disorders including tremor, myoclonus, and parkinsonism. Tremor is usually postural, and myoclonus involving the

upper extremities and face has been described especially during sleep. Parkinsonian features have been seen in these patients likely secondary to WNV involvement of the substantia nigra [46]. There are several case reports of WNE patients developing cerebellar ataxia with truncal ataxia, intention tremors, and gait instability [47,48]. Seizures have been occasionally described [49]. Cranial nerve palsies are seen in up to 11% of patients [50].

Many patients recover, but adverse events including respiratory failure and even death are seen in patients with WNE. Among survivors, movement disorders, residual focal neurologic deficits, and cognitive deficits have been described. Although many patients have distinct features of WNM or WNE, some patients present with a mixed picture with clinical manifestations of WNM and WNE and are described as patients with WNV meningoencephalitis.

WNV Flaccid Paralysis

WNV can infect the anterior horn cells of the spinal cord, producing a poliomyelitis-like picture. The incidence of this condition is 3 to 4/100,000 cases [51]. Patients typically present with an asymmetric limb weakness. The first 4 cases were reported in 2002 [52,53]. Limb paralysis occurs within 24 to 48 hours of illness onset and is usually asymmetric. There is usually no associated sensory loss, although paresthesias have been described. Involvement of the respiratory muscles might lead to diaphragmatic paralysis and respiratory failure requiring intubation. Areflexia is not uncommon and occasionally bladder and bowel involvement is seen. Hence, WNV must be strongly considered in the differential diagnosis of patients presenting with asymmetric flaccid paralysis, especially in late summer or fall. Recovery of limb strength in these patients may be slow, and prolonged occupational and physical therapy may be required. A majority of patients do, however, experience substantial improvements in muscle function.

Guillain-Barré Syndrome

Reports of several patients with symmetric ascending weakness with sensory loss have been published [51]. Electromyography studies in these patients reveal presence of a demyelinating sensorimotor neuropathy, which is distinct from the anterior horn cell involvement noted in patients with WNV polio-like syndrome.

WNV Ocular Involvement

Chorioretinal involvement is thought to be a fairly common event during WNV infection. Khairallah et al [43] described the results of ophthalmologic examination in 29 consecutive patients with serologically confirmed WNV infection. The authors demonstrated multifocal chorioretinitis in almost 80% of patients. Most patients were asymptomatic and had

self-limited ocular involvement. Symptomatic patients with chorioretinitis secondary to WNV infection report blurred vision, floaters, and flashes. Most cases occur early on during the course of illness, but delayed disease has been reported. Chorioretinitis is thought to occur due to hematogenous dissemination of virus, and the treatment of this disorder is supportive care; complete resolution is seen in most patients. Case reports of vitritis, uveitis, optic neuritis, and retinal vasculitis in patients with WNV infections have also been published [41,42,54].

Diagnostic Studies

Physicians should maintain a high degree of clinical suspicion for WNV infections in patients presenting with an acute febrile illness, especially those with CNS manifestations in summer and fall. WNV has a wide spectrum of clinical presentations and often presents as a nonspecific illness. Hence laboratory testing is very important in suspected cases to confirm the diagnosis.

Routine Tests

Routine laboratory tests are nondiagnostic in these patients. Patients with WNV infections present with normal or mildly elevated white blood cell counts. Lymphocytopenia, anemia, thrombocytopenia, elevated creatinine kinase, and hyponatremia have been reported [55]. Abnormal liver function tests are not uncommon. No specific constellation of laboratory studies is diagnostic of WNV infection.

Cerebrospinal Fluid

Patients with neuroinvasive WNV disease have nonspecific cerebrospinal fluid (CSF) findings including pleocytosis, mild to moderate elevated protein levels, and normal glucose. Tyler et al [56] reviewed CSF findings in 250 patients hospitalized with WNV-associated CNS disease. Patients with WNV meningitis and encephalitis had a mean of approximately 220 cells/mm³ in CSF. However, 3% of patients with meningitis and 5% of patients with encephalitis had less than 5 cells/mm³ and 8% of both groups had more than 500 cells/mm³. The authors demonstrated that neutrophilic pleocytosis is not a characteristic finding in patients with neuroinvasive WNV disease as had been previously thought, and this finding was demonstrated in less than half of the study population. A greater number of patients in the encephalitis group had elevated CSF protein (47% with protein > 100 mg/dL) in comparison to those with only meningitis (16% with protein > 100 mg/dL). Hypoglycorrhachia was not a common finding and was noted in only 1 patient in the meningitis group.

Virologic Tests

Nucleic acid amplification tests (NATs) have been imple-

mented to screen all donated blood products since 2003 [57]. However, the utility of these tests in the clinical setting for diagnostic purposes is unclear. Viremia occurs very early in the course of illness and is often absent at the time the patients present with neurologic symptoms. Hence, viral detection by culture-based methods or NATs from serum, CSF, or tissues is often unproductive and not routinely used in establishing the diagnosis. NATs may be of value in testing patients with very recent onset of symptoms as they may still be viremic at the time of presentation [58]. NATs may also be valuable in immunocompromised patients who have prolonged viremia and do not produce an antibody response.

Serologic Tests

Serology is the mainstay for diagnosing WNV disease. IgM antibody capture enzyme immunoassay (MAC-ELISA) is performed to detect WNV-specific IgM antibodies, which appear early in serum and CSF. WNV-specific IgM antibodies can be detected within 8 days of onset of symptoms [59]. Being large molecules, IgM antibodies do not cross the blood-brain barrier, and their detection in CSF indicates local IgM antibody production by lymphocytes as a response to local WNV infection and is diagnostic. On the other hand, WNV-specific IgM antibodies in serum may persist for many months [60], and presence of serum WNV-specific IgM antibodies alone (without IgM antibodies in CSF) does not necessarily indicate recent infection but might serve as a marker for a prior episode of WNV infection. Hence, in order to confirm acute infection based on serology alone, it is necessary to demonstrate a fourfold or higher rise of neutralizing antibody titer between acute and convalescent phase serum samples. It must be borne in mind that cross-reactivity occurs with other JE serogroup viruses, and hence patients recently infected with other flaviviruses (dengue, St. Louis encephalitis) or those who have been recently vaccinated for yellow fever or Japanese encephalitis virus may have a positive serum WNV IgM. In such cases, plaque reduction neutralization tests (PRNTs) may be needed to confirm the diagnosis [59]. PRNT is an assay that detects the reduction of viral plaques by adding serially diluted patient serum and is a sensitive and specific test. Its main limitation is that it is unable to distinguish between IgM and IgG antibodies and hence is unable to distinguish current from past infections if not combined with other ELISA-based antibody testing.

Imaging Studies

CT scans are usually unremarkable in patients with WNV neuroinvasive disease. Magnetic resonance imaging (MRI) scans are nonspecific as well. In a review of MRI findings in 17 patients, Petropoulou et al [61] suggest that WNV commonly affects the basal ganglia, thalami, mesial temporal

structures, brainstem, and cerebellum. Although a diagnosis of neuroinvasive WNV infections cannot be made based on MRI findings alone, the authors suggest that involvement of the mesial temporal lobe on an MRI study in the appropriate clinical setting should lead to the inclusion of WNV infection in the differential diagnosis. In another small study of 17 patients, those with normal MRIs had the best clinical prognosis [62]. There is insufficient evidence at this time to routinely recommend MRIs for diagnostic or prognostic purposes in patients with WNV neuroinvasive disease.

Electroencephalography (EEG)

EEG findings in patients with neuroinvasive WNV infections are generally nonspecific, although up to 86% of these patients demonstrate some EEG abnormalities [63]. An intriguing report of an analysis of EEG studies in 13 patients with serologically confirmed severe WNV meningitis or meningoencephalitis suggested that a pattern of generalized slowing with increased slowing in the anterior regions was specific to this disease, although this has not been conclusively confirmed [64].

Electromyography

EMG studies in patients with asymmetric flaccid paralysis due to WNV infections have demonstrated that the clinical presentation is due to the involvement of anterior horn cells of the spinal cord and are comparable with EMG findings in patients with acute paralytic poliomyelitis [53,65].

Treatment

Although various agents are being studied for the treatment of WNV infection, none have proven efficacious to date. Current therapy essentially comprises supportive measures such as close clinical monitoring, intravenous hydration, symptomatic treatment for headache and myalgias, and respiratory support, including mechanical ventilation when needed. Once the acute illness has resolved, physical therapy plays an integral role in restoring muscle strength and function.

The antiviral agent ribavirin and interferon alpha, which are both effective against hepatitis C virus, also a member of the family Flaviviridae, have shown *in vitro* activity against WNV in cell culture and animal models [66,67]. Their efficacy however, has not been proven in human studies. Kalil et al [68] reported outcomes in 2 patients with WNV encephalitis who were treated with interferon alpha 2-b therapy for 2 weeks. Both patients in this report demonstrated substantial improvement in mentation on the second day of therapy with near complete recovery at their 9-month follow-up. Although the results in this small study were optimistic, we must remain cautious as there have already been reports of failure of interferon therapy in this setting as well [69].

An observational study of a WNV outbreak in Israel in 2000 suggested that therapy with ribavirin was associated with an increased mortality [70]. However, patients in this cohort had more severe disease (with a 14.1% death rate) and ribavirin was administered to very ill patients, which may have resulted in skewed results. Randomized placebo-controlled studies are needed to determine the efficacy of these agents in the treatment of neuroinvasive WNV infection.

Passive therapy with immunoglobulins has been shown to abort or mitigate Flavivirus infections in animal models [71]. Case reports of successful treatment of WNV neuroinvasive disease in Israel with intravenous immunoglobulin (IVIG) have been published [72]. It should be noted that due to endemicity of WNV in Israel, Israeli IVIG has been shown to have high titers of anti-WNV antibodies, whereas North American IVIG preparations have variable titers of anti-WNV antibody [72,73].

An elegant study of the utility of IVIG in the treatment of WNV infection in mouse models by Ben-Nathan and colleagues [74] suggests that IVIG preparations containing high titers of anti-WNV antibody may neutralize virus and may be an effective therapeutic option. A randomized placebo-controlled clinical trial to determine the efficacy of Israeli IVIG is currently ongoing and may shed more light on this subject [45]. Humanized monoclonal anti-WNV antibodies are being studied in the treatment of neuroinvasive WNV infection in animal models [75].

The applicability of this research to clinical practice remains limited at this time. In the mouse model, the IVIG was administered very early in the course of infection, which may not be feasible in clinical practice. Also, at this time, exact titers of anti-WNV antibodies in IVIG cannot be easily determined by treating clinicians.

Other investigational therapies for neuroinvasive WNV that are currently being considered include minocycline [76], WNV antisense oligomer [77], inhibitors of cellular enzymes like 2-thio-6 azauridine, mycophenolic acid, 6-azaridine triacetate, cyclopentycytosine, pyrazofurin, 6 azauridine (Gea-Banacloche 34), and other novel pharmacological agents targeting macrophage migration inhibitory factor [78].

Prognosis

WNV infection has varying degrees of clinical outcomes ranging from rapid complete recovery to long-term morbidity and occasionally mortality. Mortality is seen mostly in the elderly population and in those who are immunocompromised. Overall mortality in WNV neuroinvasive disease is in the range of 8% to 18% and is mostly seen in patients with WNE and usually not seen in patients with WNM [79,80].

Bode et al [80] published a descriptive study reviewing the risk factors, course, and prognosis of WNV infection in

221 patients in Colorado. Among these 221 patients, 103 had WNM, 65 had WNE, and 53 had WNF alone. In this series, mortality was exclusively seen in patients with WNE (18%). Among patients with WNE, 40% developed limb weakness and more than 30% required mechanical intubation. Risk factors associated with mortality included age over 50 years, immunosuppression, endotracheal intubation, hypertension, and prior stroke. In this study, 76% of patients with WNM or WNF were discharged home without need for additional care as compared to 25% of patients with WNE.

Loeb et al [81] reported on the physical and mental function outcome of 156 patients with WNV infection. In this study, it is interesting to note that with respect to a favorable mental outcome, no difference was seen between patients with and without neuroinvasive disease. In terms of physical recovery, however, patients with WNE required a longer time to functional physical recovery as compared with those without neuroinvasive disease, although all patients eventually recovered to the same extent. Overall, physical and mental functioning returned to normal levels in these patients approximately 1 year after the initial illness. Recovery was impeded in patients with preexisting comorbid conditions. A major limitation of this study is that patients did not undergo any neuropsychological testing. Other researchers have suggested that cognitive and functional recovery in patients with WNE might not be complete [82]. Klee et al [83] reported that only 37% of 42 patients in their study considered themselves to have recovered fully. Fatigue, memory, and concentration difficulties are frequently seen in these patients, and neuropsychological testing will be a very important tool in future studies.

Some studies have suggested that patients with WNE have considerable short- and long-term morbidity and mortality [82]. Movement disorders such as tremor, myoclonus, and parkinsonism persisting for many years have been reported following acute infection [82].

Although many patients with WNV-associated flaccid paralysis continue to improve within the first year, some may have residual weakness. Some authors have suggested using motor unit number estimation (MUNE) as a tool to help in prognostication of muscle function recovery [84]. These studies, however, included very small numbers of patients and larger studies will be needed to validate these findings before they can be used in general practice.

Prevention

WNV is a major public health burden and there is a lot of ongoing research looking at methods of preventing WNV infections. WNV vaccine research is in the forefront. The protective role of vaccine in the prevention of WNV infections has been established in animals, with various types of vaccines currently marketed for veterinary use (inactivated

vaccine, modified live vaccine, and live-chimera vaccine).

There are several ongoing clinical studies to test the efficacy of different types of WNV vaccines in humans. However, the sporadic nature of WNV epidemics and the asymptomatic nature of illness in the majority of patients raises questions regarding vaccine cost-effectiveness in mass immunization programs. That being said, WNV vaccine may be a valuable tool for use in special populations such as in the elderly and in immunocompromised hosts in whom WNV is known to cause severe illnesses with high morbidity and mortality.

ChimeriVax-WN02 is a promising agent currently undergoing phase II clinical trials scheduled for completion in 2009. ChimeriVax-WN02 is a chimeric live attenuated recombinant vaccine that is produced using 17D yellow fever vaccine strain with genes coding for immunogenic WNV structural proteins (pre-membrane [prM] and envelope [E] protein) [85]. Chimeric West Nile/dengue virus vaccine is another molecularly engineered live attenuated vaccine developed from dengue virus type 4 and includes prM and E protein genes of WNV. Its protective role has been established in animal models and it is undergoing phase I clinical trial in humans [86].

A WNV DNA vaccine encoding prM and E protein genes is under development, with encouraging results from phase I studies [87]. Other novel vaccines are also under development.

Until WNV vaccines become available for commercial use, control of mosquito vectors and prevention of mosquito bites remain the mainstay of prevention. The CDC recommends using mosquito repellents (DEET, Picaridin, oil of lemon eucalyptus, IR3535) when outdoors, wearing clothes with long sleeves and long pants and instituting measures to eliminate mosquito breeding grounds by eliminating sources of stagnant water. Most states also have public health programs for mosquito elimination and control.

Screening of all donated blood products by using nucleic acid amplification tests is another public health measure instituted to prevent transfusion-related WNV infections.

Conclusion

Following the New York epidemic in 1999, WNV infection has spread across the entire continental United States. Through case series and case reports, we have become aware of new modes of transmission of this virus as well as previously unknown clinical manifestations. Clinicians must have a high index of suspicion for the presence of this infection in the appropriate epidemiologic setting. Although the majority of patients with WNV infection are asymptomatic, there is substantial morbidity and mortality associated with this illness. At this time, treatment remains largely supportive and the emphasis remains on prevention. More research

is needed to help us further understand the pathophysiology of these infections, which will hopefully lead to the development of safe and effective agents for treatment. Anti-WNV vaccine development remains the hope of the future.

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