West Nile Virus: Lessons Learned from Outbreaks

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Research interest: optimizing the treatment of RSV infections in transplant recipients, CMV testing, novel pathogens, West Nile virus IFN therapy

The purpose of this talk is to familiarize the reader with the current literature regarding West Nile virus, it's pathogenesis, history of epidemics and spread through the Americas, transmission, clinical manifestations, diagnosis, prognosis, treatment, and prevention strategies.

Introduction

West Nile virus (WNV), an arbovirus in the family *Flaviridae*, was first identified in the blood of a febrile 37 year old Ugandan in 1937 by Smithburn and colleagues while seeking cases of yellow fever in the West Nile district of Uganda.(1) Subsequent investigations revealed that this positive-strand RNA neurotropic virus belonged to the Japanese encephalitis complex of the family Flaviviridae, to which Kunjin virus, Japanese encephalitis virus, St. Louis encephalitis virus, Murray Valley encephalitis virus, and Usutu virus also belong. WNV was first introduced to the US in 1999, and the current 2012 outbreak is the largest since 2003, with over 3,500 cases reported as of September 25,2012, with > 51% of the cases classified as West Nile neuroinvasive disease (WNND). Questions naturally arise, such as:

1) Where did WNV originate and how did it spread to the Americas?
2) What disease manifestations are associated with WNV?
3) Who is most susceptible to severe WNND?
4) How is WNND diagnosed?
5) What therapeutic agents are under investigation for WNND?
6) What are current strategies to prevent WNV infection?

Pathogenesis

WNV virions are spherical with a genome encoding structural proteins (capsid C, membrane prM, and envelope E) as well as 7 nonstructural (NS) proteins involved in genome replication such as the RNA-dependent RNA polymerase NS5. (2) The E glycoprotein is the main target of neutralizing antibodies.

During infection, virions attach to the surface of the host cell, enter via receptor-mediated endocytosis, then fuse with the cell membrane, uncoating the virus and releasing the RNA into the cytoplasm. The RNA is translated into polyproteins and serves as a template for replication. The viruses are assembled on the endoplasmic reticulum, mature during transport and are subsequently released through exocytosis. (2, 3)

Following intradermal injection by a mosquito, the virus replicates in Langerhans cells, which migrate to the lymph nodes, leading to primary viremia. (4) Viral replication is further augmented in the reticuloendothelial system leading to secondary viremia. (5) Viremia usually occurs within a few days of the initial mosquito bite, lasting up to a week, and ending with the appearance of neutralizing anti-WNV IgM. (6) During this time, stimulation of Toll-like receptor (TLR) and tumor necrosis factor (TNF-α) likely mediates changes in the blood brain barrier, allowing for infection of the central nervous system (CNS) where the virus primarily targets neurons. The chemokines CXCL10 and CCL5, and their ligands CXCR3 and CCR5, play an important role in the recruitment of CD8+ and CD4+ T cells and monocytes to the CNS for clearance of the virus, while Interferon α/β help to control CNS infection. (7) Thus through neutralizing antibodies that target the E glycoprotein, the host's humoral immunity limits dissemination of WNV, while cellular immunity via CD8+ and CD4+ T-regulatory cells is required to clear WNV CNS infection. Interferon responses also serve as a key host defense mechanism to control infection, especially early after infection.(8)

Once in the brain, WNV exerts neuropathologic effects with microglial nodules, perivascular cuffing with mononuclear cells, and variable degrees of neuronal loss predominantly in the brainstem and anterior horns of the spinal cord.(9-11) Immunohistochemical staining with anti-WNV antibodies reveal viral antigens focally and sparsely distributed throughout the brainstem and anterior horns, except in severely immunocompromised patients, where extensive antigen staining is visualized throughout the CNS. (12)

History of WNV worldwide

After its discovery, WNV was considered enzoonotic in the Old World, including Africa, the Middle East and Mediterranean, and Asia, primarily associated with asymptomatic or mildly symptomatic disease. (13) From 1937-1950s, WNV outbreaks were episodic, with human, horse and bird infections generally mild. Symptomatic human infection resulted in mild dengue-like illness with fever, malaise, lymphadenopathy, and rash, without neurologic disease manifestations or long-term sequelae. (14)

However, notable outbreaks occurred the 1950-1970s in Israel, the Camargue region of France, and South Africa with fatalities secondary to encephalitis reported. (15-19) Israel's 1952 outbreak affected primarily children < 6 years old, with the highest morbidity in those < 3 years old and no fatalities. Symptoms were mild and recovery was slower in adults than children, but no patients developed long-term sequelae. (20) Severe neurologic manifestations in patients infected with WNV were later reported for the first time in Israel during the 1957 outbreak. (21) The Camargue region of France also experienced notable equine outbreaks, with neurological manifestations predominating such as ataxia and weakness, with 25% mortality. (22) Several human cases of encephalitis were reported (23), with one fatality (18). The 1974 outbreak in South Africa was one of the largest human outbreaks, with > 3000 cases, however patients presented with fever, rash, and polyarthralgia, without neurologic symptoms. (24)

Thereafter only sporadic cases were reported worldwide in Europe, India, until the mid-1990s, with the first large urban epidemic in Bucharest, Romania, affecting > 800 persons. (25) Further outbreaks were noted in Algeria (26), Morocco(27), Tunisia (28), Italy (29), and Russia (30), with a resurgence in Israel as well as in France during the latter part of the decade. During the 1994 Algeria outbreak, 50 patients presented with high fever and neurological signs, 20 with encephalitis and 8 deaths. Of the 14 clinical cases with WN serologies, 13 were children < 10 years old. (26) On the other hand, in Tunisia in 1997, 173 patients were diagnosed with WN meningitis (WNM) or meningoencephalitis, with 8 deaths, (28) and a median age in one town of 52 with 50% > 60 years old. Most of the patients defervesced by day 4, with resolution of neurologic signs by day 6. However, 3 patients died in one town, with decreased consciousness and rapidly progressive neurologic/respiratory compromise. Two patients had sequelae with tremors, chronic headache. (31)

In Israel from 1998-2000, WNV resurged in horses (1998)(32), geese (1999) (33), and in 2000 more than 400 patients were diagnosed with WNV infection, with over half of the patients diagnosed with encephalitis, followed by WNV fever, then

meningitis. Mortality was higher than usual (14.1%) especially in those > 70 years old. (34, 35) These observations of particularly severe neurologic disease were a marked departure from reports during prior outbreaks. Thus WNV outbreaks were becoming more widespread, and were becoming more of a public health threat with increasing neurologic presentations and death. Only in due time would WNV cross over to the Americas.

WNV in the Americas

In August 1999 patients with encephalitis with profound muscle weakness presented to hospitals in New York (NY), prompting an infectious disease physician to contact the New York City Department of Health. (36) Outbreak surveillance identified 59 patients hospitalized with WNV, with 7 deaths. Interestingly, the median age of patients was older (71, range 5-90), with 63% of the patients with encephalitis, 27% with muscle weakness, and 10% with acute flaccid paralysis (AFP), not seen in prior epidemics. This was the first time the virus was detected in the Americas, and now it appeared to manifest with more severe neurologic symptoms.

Epidemiologic and veterinary studies suggest that infected migratory birds (37), or possibly illicitly imported exotic birds, or less likely infected mosquitoes imported on airplanes and ships, may have introduced WNV to North America. In fact, large numbers of dead and dying birds (wild and captive) at the Bronx Zoo and other parts of NY had been reported immediately prior to initial reports of disease in humans. (37-39) Perhaps the lack of natural immunity to this virus new to North America could explain the deaths in birds and increased virulence in adults. Subsequent analyses revealed it was the same strain as the one implicated in the Israeli outbreaks, with >99.8% homology to a virus isolated from a goose in Israel in 1998 (40).

Since its introduction to the Americas in 1999, WNV has consistently caused outbreaks from 2002-2007, with the current outbreak in 2012 noted as one of the most severe thus far, apart from the 2002 outbreak. Over the years, the WNV NY99 strain present during the NY 1999 outbreak has been replaced by the WN02 strain that appears to be transmitted earlier and is more infectious than its predecessor. (41-43) Its rapid spread through North America can be attributed to 1) increased virulence from a single amino acid substitution in the WNV NS3 helicase, conferring increased viremia and mortality in American crow species (44), as well as a single amino-acid change in the envelope protein conferring greater replication in mosquitoes and increased transmission to birds (45), 2) availability of appropriate mosquito vectors –especially ones that bite both birds and humans (46), 3) a reservoir of WNV-naïve birds, and finally 4) suitable climate conditions with warmer temperatures allowing for increased transmission of the WNO2 genotype (47).

The CDC's ArboNET program tracks reported cases of WNV infection in birds, horses, mosquitoes, and humans, in the United States, with likely underreporting of cases as most patients with asymptomatic or mildly symptomatic WNV infection do not seek medical care. Through 2007, more than 11,000 cases of WNND in 48 states

plus Washington DC and Puerto Rico have been reported to the CDC, with greater than 1000 deaths (15).

Worldwide, since 2000, the reach of WNV has spread, with epidemics in equines and humans reported in countries in Africa (Sudan), the Americas (Argentina (48), Brazil (49), Canada (50), Colombia (51), Cuba (52), Guadeloupe (53), El Salvador (54), Haiti (55), Jamaica (56), Mexico (57)), Asia (India(58)), and Europe (Hungary(59)). WNV isolates in North America belong to the lineage Ia, which originated from Europe and the Mediterranean. It is also the most widespread lineage, while Ib is isolated to Australia, Ic to Southeast Asia, and lineage II to parts of Africa and the Mediterranean. (60) Migrating birds likely propagated the virus from North America throughout the Caribbean into Central America and some parts of South America. The lack of large WNV epidemics in South America may be explained by many factors, including attenuated viral strain and increased avian host availability (61).

Transmission

WNV depends on the bite of infected mosquitoes for transmission to its reservoir (birds) and occasionally to incidental hosts (humans, equines). The mosquitoes that carry WNV in Africa, the Middle East, and Asia are *Culex univittatus* and *C. pipiens molestus*, however WNV has been detected in up to 43 other mosquito species (62). In the U.S. *C. pipiens, C. quinquefasciatus, C. tarsalis, C. restuans*, and *C. nigripalpus* are the main mosquito vectors (15). *C. pipiens* , the dominant vector in the Northeast and North-central US, *C. tarsalis*, the dominant vector in the Western states, and *C. nigripalpus* the vector in the Southeastern US, shift their feeding behavior from birds in the early summer to mammals and humans as the bird populations decline (63, 64). The virus overwinters in hibernating adult female mosquitoes, waiting until the spring when the mosquitoes emerge and bite birds (65, 66). Female mosquitoes can also transmit WNV to their eggs, which hatch in the spring to mature into WNV infected adult mosquitoes (67). Mosquitoes also serve as effective vectors of spread of WNV, as their saliva enhances transmission and with each feed an infected *Culex pipiens quinquefasciatus* mosquito can inject approx 10^4 plaque-forming units of virus into its host (68, 69).

WNV endemicity is maintained in nature by its cycling between many species of mosquitoes and > 200 species of birds, but it has also been reported in other animals such as alligators, dogs, frogs, alpacas, reindeer, sheep, and wolves. (70) However, bird species such as the corvids (crows, magpies, and jays), house sparrows, house finches, and grackles, serve as the main amplifying hosts, followed by small mammals such as squirrels, chipmunks, and rabbits (15, 71, 72). In particular the American robin (*Turdus migratorius*) has been implicated in promoting the propagation of WNV throughout the United States, as the mosquito preferentially bites this host in the early summer, when WNV amplification is highest. (73) Not only do birds become infected through mosquito bites, but they also can acquire WNV infection by ingesting infected mosquitoes. (70) Once infected, birds may remain viremic for long periods of time (up to 100 days) with the highest levels of viremia (> 10^{10} plaque-forming units per milliliter) reported in passeriforme birds (jays, grackles, finches, crows, sparrows), facilitating

transmission to more than 80% of biting mosquitoes. (74, 75) Note that infected birds may also shed virus from their cloaca and nasopharynx. Once infected, certain bird species develop symptomatic illness, with noticeable bird deaths apparent during the 1998 Israel outbreak, followed by the 1999 NY outbreak. In fact, bird die-offs (crows) can serve as a sentinel event, predicting human outbreaks of WNV (76-78), as the American crow is highly susceptible to WNV infection. (79)

Horses, on the other hand, are not effective vectors of viral transmission, as they cannot sustain the high-level of viremia necessary for continued infection of mosquitoes in order to permit perpetuation of the epidemic. (80) Large outbreaks of WNV infections in horses have been reported (32, 81), with > 15,000 horses in North America with lab-confirmed WNV infection or seroconversion in 2002 alone. With 10% of infected horses developing encephalitis or myelitis and a mortality rate of 28-45%, this is not an insignificant equine disease. (82) Thus WNV vaccines were developed and implemented for the vaccination of horses.

Humans are also incidental hosts, with most cases of WNV infection occurring through infected mosquito bites. Risk factors for transmission due to mosquito bites include factors that serve to increase mosquito populations as well as increase human population density or outdoor human activity, such as seasonality, with most infections occurring in the late summer/early fall when young bird populations decrease so mosquitoes increasingly bite humans (83), outdoor work (84), slow moving water bayous lined with vegetation (85), and forested areas, decreased elevation, rural residence, and wetlands (86).

While mosquito bites remain the mainstay of WNV infection, WNV transmission has also been reported through blood transfusions (87) with 23 patients infected during the 2002 US WNV outbreak, leading to the implementation of nucleic acid testing (NAT) of minipools as part of WNV screening of the blood supply by July 2003. (88) Through universal screening of blood through NAT, from July through October 2003, 877 units of WNV-infected blood were identified among 2.5 million units screened, leading to a rate of 3.5/10,000 with 49% confirmed positive, yielding a sensitivity of 92% and specificity of 99%, with the observation that the prevalence per 10,000 increases in areas and during times of high WNV activity (Colorado 67.7, South Dakota 77.5, Wyoming 74.1, and North Dakota 102). (89) Blood units positive for WNV RNA, and negative for IgM (up to 67% of WNV + NAT donations) are considered most infectious. (90) Also dilution of blood through minipool testing renders detection low levels of viremia difficult, leading to transmission of WNV in one case, later identified through individual unit testing. (91)

If WNV is transmitted through blood donations, then transmission through organ transplants is feasible, with the first cases in the US reported in August 2002 when ¾ recipients of organs from a common donor developed WNV encephalitis. (92, 93) Since then, more reports of WNV transmission through organ transplants (94-96), especially through donors who have received multiple blood products (97), have prompted many organ procurement centers to screen for WNV through NAT in areas and during periods of high WNV activity in the US. (98) In Italy, in areas of high WNV endemicity, WNV IgM capture enzyme-linked immunosorbent assay (99)

as well as NAT has been suggested due to the transmission of WNV despite negative NAT screens. (100)

Transmission of WNV transplacentally from mother to child was reported with the infant presenting with bilateral chorioretinitis and positive CSF for WNV IgM as well as severe cerebral abnormalities noted on MRI of the brain (101). In a review of 71 WNV infected women, approx 6% preterm births and 9% spontaneous abortions were noted, with 11% with major birth defects, including one infant with underlying lissincephaly who developed WNV encephalitis and died. (102) WNV transmission has also been reported through infected breast milk (103), laboratory exposure (104), conjunctival exposure (105), and possibly through a contaminated dialysis machine (106).

Clinical presentation

WNV infections are largely asymptomatic, with 80% of patients asymptomatic, 20% with West Nile Fever (WNF), and < 1% with WNND (25, 107-109). According to follow-up interviews of 821 blood donors to the American Red Cross from 2003 to 2008 with confirmed WNV infection, 26% were symptomatic with at least 3/8 indicator symptoms: fever, chills, headache, rash, fatigue, generalized weakness, severe myalgias, arthralgias, painful eyes. (109)

Diagnosis of WNF or WNND as per the CDC Definitions for the diagnosis of arboviral infections is presented in the following table.

	Non-neuroinvasive	**Neuroinvasive**
Clinical criteria	Fever (≥ 100.4°F or 38°C), AND absence of neuroinvasive disease, AND absence of a more likely clinical explanation	Fever (≥ 100.4°F or 38°C), AND meningitis, encephalitis, acute flaccid paralysis, or other acute signs of central or peripheral neurologic dysfunction , AND absence of a more likely clinical explanation
Laboratory criteria	Isolation of virus from, or demonstration of specific viral antigen or nucleic acid in, tissue, blood, CSF, or other body fluid, OR four-fold or greater change in virus-specific quantitative antibody titers in paired sera, OR virus-specific IgM antibodies in serum with confirmatory virus-specific neutralizing antibodies in the same or a later specimen, OR virus-specific IgM antibodies in CSF and a negative result for other IgM antibodies in CSF for arboviruses endemic to the region where exposure occurred, OR virus-specific IgM antibodies in CSF or serum.	

The WNV incubation period ranges 2-14 days, with WNF presenting with acute onset of high fever > 39°C, accompanied by headache, myalgias, muscle weakness, neck pain or stiffness, arthralgias, difficulty concentrating, gastrointestinal symptoms (nausea, anorexia, diarrhea), painful eyes, a nonpruritic generalized maculopapular or roseolar rash, with lymphadenopathy reported more commonly in outbreaks in the Old World. (14, 35, 36, 83, 109-111) Most acute symptoms last for 3-10 days.(109) The maculopapular rash usually presents 5 days after onset of illness, and lasts for approximately a week, occurring more often in

younger patients, with up to 27% reporting dysesthesia and 33% reporting pruritus (112, 113) Interestingly, appearance of rash appears to serve as a potential prognostic factor, with significant decrease in encephalitis, encephalitis plus death, and death, according to a 2002 Illinois Dept of Health study as well as decreased risk of meningitis, encephalitis, and death, according to a 2003 Colorado Dept of Public Health study. (114, 115) Other presentations of WNV infection reported include rhabdomyolysis (116, 117), myocarditis (118), myositis with orchitis (119), pancreatitis (120), fatal hemorrhagic fever (121), and central diabetes insipidus (122).

WNND includes meningitis, encephalitis, and acute flaccid paralysis (AFP) or Guillain-Barré syndrome (GBS). As per published studies, WNND occurs in < 1% of infected persons, with frequencies higher in the elderly or immunocompromised, alcohol abusers, patients with cancer, diabetes, hypertension, and liver disease. (36, 123, 124) WNND in the Americas is rare in children. Transplant recipients in particular appear susceptible to more severe neurologic disease with a calculated rate of WNV meningoencephalitis of 200/100,000 population in transplant vs. 5/100,000 in non-transplant recipients in the Toronto area. (125) Upon sub-classification of WNND, approx 60-70% present with WNE while 30-40% present with WNM, with approx 5-10% in both categories additionally developing AFP (84% poliomyelitis-like and 14% GBS-like, however disease classification and nomenclature is not standardized for WNV- associated AFP). (36, 47, 123, 126, 127)

Patients with WNND can present with clinical signs as seen in patients with WNF, except with the addition of meningeal signs, confusion and altered mental status in the case of encephalitis, or asymmetric weakness in the case of AFP. Cranial nerve palsies (especially cranial nerve 7 leading to facial weakness and cranial nerve 8 contributing to dizziness, vertigo, and nystagmus) are reported in up to 11% of patients with WNND (126-128), while seizures are rare (1-16%). Signs of brainstem involvement such as dysarthria (36, 129) have been reported while cerebellar abnormalities such as gait ataxia are not infrequent, ranging 11-57%. (126, 129, 130) Since the 1999 NYC epidemic, asymmetric upper extremity tremors (present at rest or kinetic), have been reported, with frequencies ranging 12% (36) to up to 80-100% (128). Myoclonus is also frequent (up to 1/3 of patients) and differentiates WNE from the other arboviral encephalitides, while a distinguishing feature of WNV infected patients is the presence of Parkinsonian symptoms (bradykinesia, cogwheel rigidity, postural instability) in up to 2/3 of those with WNND. (128) In addition, unusual presentations attributed to WNV have been reported: WNV-induced stiff-person syndrome possibly due to cross-reactivity between a WNV protein and glutamic acid decarboxylase (131), WNV-associated opsoclonus-myoclonus (129), acute chorea (132), and aseptic meningitis with stuttering (133).

Patients may also report visual problems ranging from eye pain (109) to blurry vision or photophobia. Patients have been described with optic neuritis, vitritis, multifocal chorioretinitis, retinal hemorrhage, hemorrhagic vasculitis, iritis, and uveitis, with one study reporting ocular abnormalities (such as retinal hemorrhage, focal retinal vascular sheathing, retinal vascular leaking, and optic disc

swelling in 69% of 29 consecutive patients undergoing ophthalmologic examination. (134-136)

Perhaps one of the most marked features of WNND in the Americas is the presentation of AFP, also described as "poliomyelitis". Noted first in the 1999 NYC outbreak, AFP manifests as acute onset asymmetric limb weakness that may be the only presentation of WNND or may accompany or follow WNE or WNM, affecting younger patients in general with an incidence of 4 cases/100,000 during WNV epidemics. (36, 128, 137, 138) Patients are areflexic or hyporeflexic, some with bowel/bladder dysfunction, without sensory loss, and may develop fasciculations in the involved limbs later in the course, as this condition is usually irreversible. A reversible presentation of severe muscle weakness without accompanying meningoencephalitis has been reported, probably secondary to spinal cord edema and transient anterior horn cell dysfunction. (138) In addition, cases of myasthenia gravis have been reported in patients with WNV AFP but a definite association remains to be confirmed. (139)

Finally, WNND cases involving the peripheral nerves, as in GBS (140), brachial plexopathy (141), as well a diaphragmatic paralysis due to phrenic nerve involvement (142) have been reported. WNV GBS is distinguished from AFP as it presents later in the course of illness, is symmetric, involves sensory loss, is not accompanied by encephalopathy or CSF pleocytosis, and is characterized by slowed nerve conduction velocity on nerve conduction studies. (143)

Laboratory

Patients with WNV infections usually do not have markedly elevated white blood counts, with counts approx 10,600/mm^3(36, 126), and lymphocytopenia reported in 4 patients at a single institution during the 1999 NYC outbreak (144). Lab abnormalities such as mild anemia, thrombocytopenia, elevated liver function tests, hyponatremia, were reported during an Israeli outbreak in 2000 (35), with 24% of patients with elevated AST documented in the 2000 NY and NJ outbreak (126) and in up to 40% of patients with WNE in Houston (145). Elevated lipase was noted in 65% of patients without clinical signs of pancreatitis in one series (146). Elevated CK was not only reported in patients with rhabdomyolysis (116, 117), but in > 50% of patients hospitalized with WN infection in Houston in one case series (145). However, further studies are needed to determine if the elevated CK is a result of the tremors or myoclonus in patients with WNND, or due to myositis.

Analysis of cerebrospinal fluid (CSF) of 250 patients with serologically-confirmed WNND, revealed that 95% of patients with WNE and 97% of patients with WNM have CSF pleocytosis (> 5 cell/ mm^3), with neutrophil predominance in 41% of the encephalitis and 48% of the meningitis cases. (147) Low glucose is rarely seen, and 99% of encephalitis and 90% of the meningitis patients had elevated CSF protein (> 40mg/dl). Elevated CSF pleocytosis (with large percentage of neutrophils), and elevated total protein were also noted in multiple other studies. (36, 126-128) In addition, reactive lymphocytes appearing as plasma cells (148)and Mollaret cells (149) have been identified in the CSF of patients with WNND.

Diagnosis of WNV infection per CDC guidelines requires isolation of virus, rise in serological titers, or demonstration of antigen or nucleic acid in tissues. Viral

isolation is not routinely performed, as most cases of WNND are based on detection of WNV-specific antibodies in serum, CSF, or both. WNV ELISAs are commercially available and are also performed at major reference labs. Of note, WNV IgM cross-reactivity may occur in the case of recent Yellow Fever or Japanese Encephalitis vaccination, or exposure to Japanese encephalitis, St. Louis encephalitis, Dengue virus, or Tick-Borne encephalitis virus. (150-152) Therefore confirmation of WNV IgM positive tests may be necessary via plaque reduction neutralization tests (PRNTs) as they are more specific for WNV but require more time to perform. (153) PRNTs are performed at the state public health laboratories or the Centers for Disease Control (CDC). Demonstration of WNV-IgM in the CSF is diagnostic of WNND as it does not readily cross the blood brain barrier and may present in the CSF 1-2 days earlier than in serum but usually clears within a week (154-156), although reports of positivity up to 7 months exist (155). WNV IgM develops within 4-7 days of symptom onset, and may persist up to 2 months in up to 50% of patients (156), with most clearing by 5 months (157), although there are reports of positivity up to 500 days after infection (158). Once patients develop WNV IgG antibodies, immunity is not guaranteed as one study evaluating blood from viremic donors demonstrated that despite the formation of WNV-specific IgM or IgG, their blood was able to transmit infectious WNV RNA in vitro.(159) Further studies are needed to determine the efficacy of WNV IgG in protection against re-infection.

Of note, antibody development in transplant recipients may be delayed, therefore PCR testing may used for the diagnosis of WNND as transplant recipients exhibit delayed viral clearance from the CSF and serum (160). WNV PCR testing is not recommended for the diagnosis of WNND in immunocompetent patients, as peak viremia usually occurs 3-4 days prior to the development of symptoms and usually resolves prior to the onset of symptoms, resulting in low sensitivity in the CSF (57%) and serum (14%). (161)

Radiology/Other studies

Neuroimaging studies are generally not required for the diagnosis of WNND, as the clinical symptoms plus the CSF results and WNV serologic studies confirm the diagnosis. CT scans of the head are usually normal, while MRI findings range from unremarkable MRI readings, to abnormal findings (in up to 20-70% of patients with acute WNND) on diffusion-weighted (DW) images in the corona radiata or internal capsule, or fluid attenuated inversion recovery (FLAIR) or T2-weighted sequences, mainly in the cortical gray and white matter, cerebellum, basal ganglia, thalamus, mesial temporal lobes, substantia nigra, leptomeninges, internal capsule, pons, and midbrain).(47, 162, 163) Patients with WNV AFP may have MRI abnormalities involving the anterior horns and anterior roots. (163, 164) A West Nile Virus MRI Registry was established by the Centers for Disease Control and the Louisiana State University Health Sciences Center Orleans for the further elucidation and characterization of MRI abnormalities in patients with WNND. (165)

Electroencephalographic abnormalities are reported in 57-86% of cases, with generalized slowing (most prominent in the anterior or temporal regions) and intermittent temporal slowing (166, 167) as the most common findings,

occasionally coupled with triphasic waves (168). Seizures and signs of status epilepticus are rare. (128)

Electromyography/nerve conduction studies in patients with AFP demonstrate signs consistent with acute paralytic poliomyelitis and anterior horn damage: 1) normal sensory action potentials, 2) normal/reduced amplitude compound motor action potentials, 3) widespread fibrillation potentials, and 4) normal nerve conduction velocities. (169, 170)

Prognosis

Mortality, primarily in patients with WNND, appears to be higher in the elderly, and patients with underlying diseases such as cancer and immunosuppression, hypertension, diabetes, chronic kidney disease, and history of alcohol abuse. (36, 110, 124, 171, 172) Risk factors for progression from encephalitis to death are absence of CSF pleocytosis, renal insufficiency, need for intubation and mechanical ventilation, presence of myoclonus or tremors, and loss of conciousness. (145) Mortality is generally higher in patients with WNE (20-35% in elderly) and WN-AFP (10-50%) compared to <1% in patients with WNM. (173) Risk factors for delayed mortality (months or longer after acute illness) are autoimmune disease, use of tobacco, encephalitis during acute WNV illness, and endotracheal intubation during acute illness. (174)

In surviving patients, many follow-up studies reveal persistent cognitive, physical, or psychological deficits, especially in patients with WNE or WN-AFP. In fact, up to one year after initial WNV infection, over half of subjects may have objectively measurable neuropsychological impairment in at least 2 cognitive domains (psychomotor speed, memory, attention, executive, and visuospatial abilities). (175) The following table summarizes the most common reported symptoms with their durations.

	Watson (111) (n=98) > 30 days	**Klee (176) (n=36) 18 months**	**Haaland (n = 116) 9 months**	**Patnaik (n=656) 5-7 months**	**Carson (n=15) 11-16 months**
Fatigue	50%	64%	NA	NA	60%
Headache	11%	36%	NA	13%	33%
Myalgias	21%	40%	NA	40%	53%
Muscle weakness	40%	56%	NA	46%	47%
Neck pain/stiffness	20%	NA	NA	13%	NA
Difficulty concentrating	25%	33% @ 1 yr	42%	NA	13% word-finding
Memory problems	NA	NA	42%	NA	47%
Joint pains	25%	31%	NA	NA	33%
Photophobia	4%	NA	NA	12%	NA
Difficulty walking	NA	42%	NA	NA	20%
Insomnia	NA	36%	NA	NA	13%
Depression	NA	44% @ 1 yr	NA	NA	24% mod-severe

Functional recovery may be slow in patients with WN infection, especially WNE, while it occurs variably patients with AFP. Of 98 patients with WNF, 63% reported that it took > 30 days to "get back to normal", and up to 25% had difficulty concentrating, while 50% reported fatigue. (111) 30-56% of patients with WNV infection during the 1999 NYC outbreak, on follow-up 18 months later, reported difficulty with normal daily activities such as shopping, preparing meals, doing laundry, light housekeeping or heavy chores, or using transportation. (176) Of 221 patients hospitalized with WN disease in Colorado in 2003, 29% of those with WNE, 18% died, 29% were discharged to a rehabilitation facility, 17% to a long-term care (LTAC) facility, 15% to home with assistance, and 20% to home without assistance, while no patients with WNM died, 6% were discharged to rehab, 3% to LTAC, 11% to home with assistance, and 80% to home without assistance. (123) A survey of 15 patients with WNND 8 months later revealed that 73% were home and functioning independently, 20% were home but dependent on assistance, and 7% remained in rehabilitation. (128) In this study, severity of illness did not predict poor outcomes, as 5/7 patients with Glasgow Coma Scale score of ≤ 12 returned to baseline functional levels within 4 months. 100% of the patients with WNM recovered fully, while patients with WNE and AFP had residual tremors or parkinsonism. The patients with AFP demonstrated no recovery of limb function, with the lowest overall functional scores. In fact, patients with WNV-AFP demonstrate variable recovery, with patients with more than 50% normal motor unit number estimation (MUNE) on initial EMG more likely to recover, even if the initial MUNE did not seem to correlate with presenting disease severity. (177) Another interesting observation is that those patients with normal MR images or abnormalities only on DW images may have a better prognosis than those with abnormalities on FLAIR or T2WI, and those with meningeal involvement may have severe residual neurologic deficits, while those with spinal cord and nerve-root abnormalities may have moderate-to-severe residual neurologic deficits. (162) Thus individualized patients rehabilitation plans are required to enable WNND patients with residual deficits to achieve the highest functional outcomes. (178)

Not only are patients with WNND affected physically, but also psychologically. Depression plays a prominent role after infection with WNV, with studies reporting from 23-44% of patients admitting to depression for up to many months after their initial infection. (148, 176, 179, 180) In one study, up to 24 percent of patients with WNND developed moderate-to-severe depression up to approx 11-16 months after infection (181), while another reported up to 75% of patients with mild-to-severe depression, with up to 35% requiring antidepressants and 10% counseling (179).

In addition to neuropsychological symptom persistence in patients with WNV infection, there is evidence that WNV may persist in tissues long after infection. First, in 1983 WNV was detected in various tissues of monkeys 5-6 months after encephalitis had resolved despite the presence of neutralizing antibodies. (182) The viral phenotype had evolved, with less apparent virulence. WNV was also detected in the brain and kidneys of infected hamsters (183-185) with persistent asymptomatic shedding of 10^2-10^4 plaque-forming units of

infectious virus/ml in the urine for up to 8 months after infection (184, 185), also with phenotype attenuation (186). WNV persisted in multiple tissues (skin, spinal cord, brain, lymphoid tissues, kidney, and heart) of asymptomatic mice up to 6 months after infection. (187) In humans, WNV was detected in the brain tissue and CSF of patients up to 3 weeks after acute infection (12), and more recently WNV RNA and antigens were detected in postmortem samples up to 99 days after onset of illness in an immunocompromised patient (188). Interestingly, WNV RNA has been reported in the urine of 20% (5/25) of patients with WNV infection for up to 7 years after initial illness, with 2/5 patients with renal failure, although infectious virus could not be cultivated from the urine. (189) Most recently, further investigation of the same cohort determined that 40% had chronic kidney disease (CKD), with 40% in Stage I-II disease, with a notable lack of traditional risk factors for CKD and history of WNND independently associated with CKD. (190) Further studies are needed to determine if there is truly any association between WNV infection and CK

Treatment

There are currently no FDA-approved therapies available for the treatment of WNND in humans. Treatment is largely supportive, with a recommendation of hospitalization for those with WNND as compared to those who are asymptomatic or with mild symptoms consistent with WNF. Multiple investigational agents have been or are currently being evaluated.

Ribavirin is a guanosine analogue that competitively inhibits inosine monophosphate dehydrogenase (191), and inhibits WNV replication and cytopathic effect in cell culture at high doses (192, 193), yet treatment of WNV-infected hamsters with ribavirin increased mortality (194). In addition, during an outbreak in Israel in 2000, 41% mortality was noted in those receiving ribavirin compared to 10% in those did no (OR of 6.7 for death; $p = < 0.0001$). (109) In addition, two patients receiving ribavirin and pegylated interferon for Hepatitis C still developed WNV infection (195).

Interferon (IFN) inhibits growth of WNV in vitro and administration of IFN-α2b appears to protect mice and hamsters from WNV infection when given within 4-6 hours prior to viral challenge (196). Additionally, reduction of mortality from WNV infection is demonstrated when IFN is administered before viral challenge, but not when given 2 days after infection(194). This poses a challenge as most patients seek treatment after infection and the development of symptoms. Anecdotal reports of treatment of WNV infections with IFN-α are currently available with variable results ranging from improvement in symptoms to no change.(129, 197) Non-randomized, non-blinded treatment of patients with St. Louis encephalitis during an outbreak situation appeared to reduce complications. (198) Unfortunately the only randomized placebo-controlled trial evaluating IFN-α2b spearheaded by Dr. Rahal in New York closed due to low patient accrual (verbal communication with Dr. Wehbeh). A randomized, double-blind, placebo-controlled trial evaluating IFN-αn3 was initiated also by Dr. Rahal's group, but has been on hold also due to low patient accrual (verbal communication with Dr. Wehbeh). As noted previously, two hepatitis C patients who received pegylated interferon plus ribavirin developed WNV infection. (195) Finally, a randomized double-blind placebo controlled trial

comparing IFN-α2a to placebo was performed on 1112 children with suspected or documented Japanese encephalitis virus infection without improvement in outcome. (199) Randomized double-blind placebo-controlled multicenter studies enrolling patients with WNND are required to demonstrate efficacy and safety in a rigorous manner.

Passive transfer of high-titer WNV-specific immunoglobulins in mice and hamsters confer protection (200, 201), with maximal protection when the antibody is administered prior to CNS entry of the virus, although some protection is offered (202). Pooled high-titer Israeli immunoglobulins, marketed under Omr-IgG-am^{Tm}, administered to mice even up to 4 days after infection with WNV, offers a significant protective effect, especially in immunosuppressed mice. (203) Omr-IgG-am^{Tm} has been used anecdotally in small numbers of patients (especially transplant recipients) with either some (202, 204-206) or no benefit (207). A phase 2 NIH/Collaborative Antiviral Study Group multicenter, randomized, double-blind, placebo-controlled trial evaluating the safety and efficacy of Omr-IgG-am^{Tm} in the treatment of WNV disease was initiated 2003 and closed 2011, with results pending. Of note, Omr-IgG-am^{Tm} was voluntarily recalled 11/7/2011 due to possibility of increased thrombotic events likely due to a manufacturing process, and was cleared by the Israeli Ministry of Health on 7/26/12 for use after the company improved its manufacturing process to remove thrombosis-generating agents, however FDA clearance is currently pending. In addition, immunoglobulin therapy has certain disadvantages due to the fact that it is a pooled blood product with risk of other transmitted infections, titers of WNV-specific antibodies may vary, and its volume may be a deterrent for patients with cardiac or pulmonary compromise.(208)

Monoclonal antibodies are currently being studied as treatment options, with the humanized monoclonal antibody E16 (targeting the envelope protein) showing efficacy in reducing paralysis and mortality in mice, even with a single dose administered 5 days after WNV infection. (209, 210) Administration of E16 monoclonal antibody prevented the development of acute flaccid paralysis in hamsters, even when administered many days after infection (211). MGAWN-1 humanized monoclonal antibody prevents mortality in hamsters (212).

Multiple other therapeutic agents are currently being investigated. RNA interference involves the cellular degradation of RNA, and two studies have demonstrated that siRNA administered to mice reduces WNV viremia, leading to partial protection against lethal challenge. (213, 214) Antisense oligomers are being used to modulate viral gene expression, and several demonstrate inhibitory activity in cell culture (215, 216). A phase I/II clinical trial evaluating one such agent, AVI-4020, was terminated 2009 due to low numbers of eligible WNV patients. Antiviral peptides targeting the E protein, when administered to mice reduce WNV viremia and lethality (217). Additionally, might there be a role for protease inhibitors (218)? Finally, high-throughput screening of compounds to identify potential WNV inhibitors is underway, with small molecule anti-WNV probes in development (208, 219, 220). In effect, multiple different therapeutic agents are under Investigation, but proving efficacy and safety in vivo will require large-scale, multi-center randomized double-blind placebo-controlled trials that are open long-term,

in the event that significant WNV human epidemics will occur in the study center areas.

Prevention

As there are no approved treatment options for WNND, preventing exposure to potential WNV-infected mosquitoes is the mainstay of avoidance of infection. This can be achieved through personal measures such as wearing appropriate insect spray and avoiding outdoor activities when mosquitoes are most active, as well as community-wide measure such as ground and aerial spraying.

The Centers for Disease Control educates the public on the 5 "Ds": staying indoors between Dusk and Dawn, Draining standing water, Dress to protect with long-sleeved shirts and trousers when outdoors, and using mosquito repellants like DEET.(47) Decreased time spent outdoors during WNV season, using appropriate insect repellant, and draining flooded basements appears to reduce the incidence of WNV infection in certain locales. (107, 221) Personal protection can reduce the risk of WNV infection during an epidemic by up to 50% (222). Studies have also demonstrated that the risk of WNV exceeds the risk of exposure to acute or sub chronic amounts of insecticides (223). Aerial spraying has been shown result in significant decrease in mosquito populations (224) and to be cost-effective in an area previously burdened with a WNV epidemic with a cost-benefit analysis demonstrating that only 15 cases of WNND prevented render the spraying efforts worthwhile (225).

Screening the blood supply using NAT and screening potential organ donors via NAT and clinical assessment, during times of heightened WNV activity also help prevent further transmission, as discussed earlier.(88, 98)

Finally, as WNV establishes endemicity in the Americas, vaccines are being developed as a way to prevent severe WNND in those infected. As WNV infections pose a significant equine burden, 3 vaccines have been approved for horses. There are no human vaccines approved to date, however phase I and II trials are underway evaluating different types of human vaccines, (DNA vaccines, live-attenuated chimeric vaccines, recombinant viral vector vaccines, purified protein vaccines) but these are not ready for widespread human use yet. (2, 47)

Conclusion

WNV, an Old World virus arriving in New York in 1999 and now establishing endemicity in the Americas, causes significant morbidity and mortality in the elderly and the immunocompromised. Transmission occurs primarily through the bite of infected mosquitoes, leading to viremia the development of neurologic symptoms (meningitis, encephalitis, AFP) in < 1% of persons infected. However, patients who are older, with certain chronic medical conditions, or immunocompromised, are at risk of more severe disease or death. Survivors report multiple neurologic and psychiatric problems up to years after infection, and there is intriguing evidence of possible persistence of WNV infection. No FDA-approved treatments currently exist, and the mainstays of prevention are avoiding the bites of infected mosquitoes, however, vaccines are currently under development. With each epidemic we are learning more about the evolving pathogenesis of WNV, the ecological factors that

serve to perpetuate it's spread, and notably the host risk factors for increased morbidity and mortality, but further studies are needed regarding therapeutic options for WNND, how to address the long-term neuropsychiatric effects of WNV infection, and to elucidate whether or not WNV establishes persistence and contributes to chronic end-organ disease.

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