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Persistence of West Nile virus

Melissa N. Garcia [a,*], Rodrigo Hasbun [b], Kristy O. Murray [a]

[a] Section of Pediatric Tropical Medicine, Department of Pediatrics, National School of Tropical Medicine, Baylor College of Medicine and Texas Children's Hospital, Houston, TX 77030, USA
[b] University of Texas Health Science Center at Houston, School of Medicine, Houston, TX 77030, USA

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Abstract

West Nile virus (WNV) is a widespread global pathogen that results in significant morbidity and mortality. Data from animal models provide evidence of persistent renal and neurological infection from WNV; however, the possibility of persistent infection in humans and long-term neurological and renal outcomes related to viral persistence remain largely unknown. In this paper, we provide a review of the literature related to persistent infection in parallel with the findings from cohorts of patients with a history of WNV infection. The next steps for enhancing our understanding of WNV as a persistent pathogen are discussed.

Keywords: West Nile virus; Persistence; Renal outcomes; Neurologic outcomes; Morbidity; Mortality

1. West Nile viral characteristics, acute disease presentation, and epidemiology

West Nile virus (WNV) is a positive-sense single stranded RNA virus from the Japanese encephalitis complex of the Flaviviridae family [34]. Other medically important viruses from the Flaviviridae family include Japanese encephalitis, dengue, yellow fever, and hepatitis C [34]. WNV is a 50 nm spherical enveloped particle containing a 10.8 kb genome. The 3433 amino acid polyprotein codes for viral capsid, envelope, membrane, and nonstructural proteins [10,15]. Virus replication occurs in host cellular cytoplasm [15]. Virions are then assembled in the endoplasmic reticulum and transported in vesicles to the cell surface for exocytosis [10,8,76].

WNV is an avian zoonosis maintained in nature through an enzootic cycle between mosquitoes and avian species. *Culex* species, particularly *Cx. pipiens, Cx. tarsalsis,* and *Cx. quinquefasciatus*, are important mosquito vectors for disease transmission in North America [64]. Avian amplifying hosts vary by geographic location, and over 330 avian species have tested positive for WNV in North America alone [64,17]. However, migratory birds are suspected to be the principal vehicle for the geographic spread of WNV [21,62]. Birds become infected from the bite of an infected mosquito via transmission of virus in the salivary fluid, and immunologically naïve birds can develop viremia for up to 100 days, allowing for a long period of time to infect mosquitoes seeking out a blood meal [17,33]. Horses and humans are considered dead-end hosts, as they are unable to generate a sufficient viral titer for infection of naïve mosquitoes [80]. However, horses and humans are capable of developing an immune response and clinically-apparent disease as a result of infection.

Humans are traditionally infected by the bite of an infected mosquito via transmission of virus in the salivary fluid. Other less common transmission sources include blood transfusion, organ transplantation, congenital, and possibly through ingestion of infectious breast milk [30,52,29,56]. The majority of infected persons do not develop symptoms, while 20% have self-limiting febrile illness, and less than 1% develop acute neuroinvasive disease that can manifest as encephalitis, meningoencephalitis, meningitis, or acute flaccid paralysis

* Corresponding author.
E-mail address: mnolan@bcm.edu (M.N. Garcia).

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[71,70]. There are no specific treatments for symptomatic disease other than supportive care.

The first reported case of WNV infection was described in a febrile woman from the West Nile district of Uganda in 1937 [73]. Sporadic epidemics occurred over the subsequent 60 years, particularly in the Mediterranean basin [41,42]. In 1999, the first outbreak of WNV was described in New York City [48]. Following this initial outbreak, WNV quickly spread across the North American continent resulting in continuous epidemics in 47 contiguous states of the United States, Canada, and Mexico [37,11,65,19,40]. Human cases began to taper off over the subsequent years coinciding with establishment of the disease in an endemic cycle with modest epizootics every 3–4 years [51,37,28,63].

In the summer of 2012, an unprecedented large outbreak occurred in the United States, with Texas being in the center of the majority of virus activity with more than 1800 human cases reported [16]. This outbreak served as a reminder that WNV would continue to cause substantial morbidity and mortality in the United States. To date, more than 41,000 human cases of disease, including 1700 deaths, have been reported to CDC's arbonet surveillance system since WNV was first recognized in New York in 1999 (http://www.cdc.gov/westnile/statsmaps/). Based on the number of neuroinvasive disease cases reported, an estimated 3 million adults have been infected in the United States, with geographic pockets of higher seroprevalence seen in the central plains region [57]. The highest seroprevalence has been reported from North Dakota where 1 per 12 residents is believed to be infected based on blood donor studies [13]. Despite the high disease burden, there are no effective prevention or treatment strategies clinically available. Additionally, the long-term morbidity, including neurological outcomes, is still relatively unknown. Having established a prospective cohort of patients with a history of WNV infection starting in 2002 in Houston, Texas, we have been granted the opportunity to study disease outcomes first hand. Here, we review the evidence of persistence of WNV infection in animal models and published human observational studies, and parallel these studies with our own cohort findings.

2. Molecular determinants of acute and persistent infection

Host genetics, host immune responses, and co-morbidities have been implicated in the underlying pathogenesis of acute disease severity. Single-nucleotide polymorphisms in the interferon response pathway, particularly the OAS gene, were found to be associated with symptomatic and neuroinvasive WNV disease [9]. Interleukin-4, a key regulator in adaptive immunity as a B and T cell stimulator, has been demonstrated as being particularly important in distinguishing between asymptomatic and severe human disease [61]. Co-morbidities associated with increased risk for development of neuroinvasive disease include hypertension, diabetes, chronic renal disease, immunosuppressing conditions, cardiovascular disease, history of alcohol abuse, and history of cancer [46,36]. While these factors' influence on acute infection have been well documented, their role in persistent neurologic and renal infection have not been studied.

During acute infection, WNV replicates in dermal cells at the site of inoculation and in draining lymph nodes resulting in a systemic viremia [74]. The exact mechanisms of neuroinvasion are less known but a few hypotheses exist. The blood brain barrier is suspected to be the primary route of invasion due to it being the interface between viremic blood and the brain, and the temporal kinetics of pathogenesis. The blood brain barrier is a highly selective barrier comprised of four main components: endothelial cells, astrocytes, microglial cells and pericytes [1]. Two hypotheses for WNV neuroinvasion mechanisms include transendothelial viral entry of epithelial cells and/or permeability of the blood brain barrier. Transendothelial viral entry has been demonstrated *in vitro* with WNV and Japanese Encephalitis virus, a close relative to WNV, where transcellular vesicle transportation was noted in cerebral endothelial cells [81,38,27]. Unfortunately, the few *in vivo* studies looking at neuroinvasion across these cerebral endothelial cells have not been able to reproduce the same results [12,23].

The second hypothesis of WNV neuroinvasion involves an increased permeability of the blood brain barrier resulting in paracellular migration [66,83]. Tight junctions between cerebral endothelial cells are important in maintaining structural integrity of the blood brain barrier. Two important tight junction proteins responsible for maintaining cellular contact, claudins and occludins, have been seen at reduced levels in WNV post-infection particularly in the presence of pro-inflammatory cytokines [18]. Their reduced levels could be reflective of a disruption of the barrier possibly allowing paracellular migration [74]. With elevated pro-inflammatory cytokines seen years post-infection correlating with neuropsychological symptoms in persons, this neuroinvasive mechanism should be further investigated, as no human studies have been performed [22]. Blood brain barrier leakage post-infection could be measured in neurologically symptomatic WNV patients using contrast magnetic resonance imaging techniques [75].

Immunoglubin M (IgM) antibody is an indicator of active infection that presents within the first 3 days of WNV infection [59]. IgM titers should decline approximately 2–3 months following acute infection, but extended IgM levels could indicate continued activation of the humoral immune response. Multiple studies have shown persistent IgM antibodies at 6 months to 1 year post-infection in both serum and cerebrospinal fluid of WNV infected patients [59,67,53,60,31,14]. With our cohort in Houston, we had the opportunity to longitudinally evaluate serial IgM antibodies over an extended time period (up to 8 years). Unexpectedly, we found a fifth of our patients had detectable IgM antibody levels at both 6 and 8 years post-infection [45]. While IgM levels fluctuated over time, they demonstrated an overall decline. In our cohort, IgM persistence was associated with potentially immunosuppressing social behaviors, such as chronic alcohol abuse and tobacco smoking [45]. Interestingly, we did not find history of

neuroinvasive disease (versus febrile or asymptomatic) associated with persistence of IgM [45]. These findings highlight the need to evaluate IgM persistence in correlation with other chronic conditions, clinical examination findings, and unresolved symptoms associated with original acute WNV disease.

3. Persistent neurological and renal West Nile infection in animal models

Persistent infection with WNV was first documented in rhesus monkeys in 1983 [58]. Investigators found that WNV could be recovered up to 167 days after infection from various tissues, including brain, lymph nodes, spleen, and kidneys. Monkeys were not considered clinically ill at the time of death and many had WNV-neutralizing antibodies detected in serum. Interestingly, the phenotype characteristics of the virus changed over time. In the first 2 months, WNV could be recovered by intracerebral (IC) inoculation of newborn mice or by plaque assay in chick embryo fibroblasts. After that point, the virus recovered from persistently infected monkeys no longer killed inoculated newborn mice, and plaques were not regularly produced in chick fibroblasts. Co-cultivation of trypsinized organ tissue with pig embryo kidney cells allowed for virus detection, although viral cytopathic effect was rarely seen. WNV antigen could only be demonstrated through immunofluorescence of the indicator pig embryo kidney cells. Genetic studies were not done at the time since it was not yet widely available.

More recently, persistent WNV infection of both the brain and the kidneys was demonstrated in experimentally infected hamsters [77,79,72]. Hamsters developed chronic infection of epithelium of the distal renal tubules and shed 10^2 to 10^4 plaque-forming units of infectious virus/ml in the urine for up to 8 months [77,79]. Infectious virus could be recovered using a co-cultivation technique on fresh kidney tissue [79]. Interestingly, attenuated virus cultured from hamster urine was found to preferentially establish a persistent renal infection when inoculated into mice [68]. The findings provide evidence for a quasispecies concept for viral persistence based on viral mutations selective for specific tissue tropism. In addition to renal tropism of WNV, persistent infection of the central nervous system (CNS) in hamsters has also been documented [79].

In immune-competent mice, infectious WNV was shown to persist for a month in all mice, and WNV RNA could still be detected in 12% of mice up to 6 months post-infection [4]. Persistence of infection was found to be tissue dependent, with skin, spinal cord, brain, lymphoid tissue, kidney, and heart being affected. Infectious virus could not be recovered after 4 months. Interestingly, persistent infection was found to be subclinical. As a means of understanding how WNV can persist in the central nervous system, Diniz et al. infected primary murine neuronal and neuroglial cells with WNV and found rapid cytopathic effect among neuronal cells [20]. Interestingly, cell death was not observed in astrocytes. Instead, infectious virus continued to be produced by the infected astrocytes for 114 days, leading to the theory that astrocytes could play a critical role in the development of persistent CNS infection in the host.

4. Neurological consequences from West Nile infection in humans

Similar to other neuroinvasive viral infections, WNV patients continue to report symptoms and display measurable abnormalities on neurological examinations post-infection [14,24,55]. Somatic complaints have been consistently noted among WNV-positive uncomplicated fever and neuroinvasive disease cases several years post-infection [25,14,24,3,69,82]. Survival analysis of recovery rates suggests that recovery plateaus around two years post-infection, with 40% of study participants continuing to report symptoms related to their infection 8 years later [32,82,44]. While somatic symptoms are anticipated in neurologically impaired WNV patients, recent evidence has suggested that patients who presented with non-neuroinvasive febrile illness also suffer a high prevalence of specific somatic complaints post-infection.

Interestingly, fatigue, depression, excessive sleep, and word-finding difficulty have been reported at a higher frequency among non-hospitalized febrile cases than hospitalized neuroinvasive cases at post-infection evaluations [14,49,22,32]. Depression and fatigue objectively measured on validated questionnaires have differed in prevalence by disease severity. Beck Depression Inventory II and Center for Epidemiologic Studies Depression scales have exhibited depression among febrile patients at higher or equivocal levels compared to neuroinvasive patients [49,14,47,7]. Conversely, one study found higher levels of depression among neuroinvasive patients at one year post-infection using an alternate depression scale (Depression Anxiety Stress Scale) [39]. Inherent differences between populations and depression scales used could influence the opposing outcomes between this one study and other published literature. Modified Fatigue Impact Scale and Fatigue Severity Scale have consistently shown higher or similar rates of fatigue that impairs daily activities among febrile and neuroinvasive WNV patients [39,14,22]. In our cohort, prolonged fatigue was found to be statistically correlated to anti-viral and pro-inflammatory cytokines post-infection [22].

A few probable explanations exist for the high prevalence of fatigue and depression among febrile WNV patients. First, febrile patients could be misdiagnosed cases with actual neurologic involvement. Second, febrile patients might be less likely to return for follow-up care resulting in mismanagement of neurologic sequelae. Third, febrile patients are more likely to be healthier and younger prior to infection than neuroinvasive cases, and new onset disabilities from WNV infection could be more notable in their daily activities. Regardless of the causality, all symptomatic WNV patients appear to be at risk for long-term somatic sequelae.

Neurologic impairment measured by neurologic examinations, electromyograms, nerve conduction studies and magnetic resonance imaging has been noted post-infection. A cohort of 40 patients in Colorado, USA found 52% of cohort

patients had abnormal neurologic exams 6.5 years post-infection with decreased or absent limb reflexes, tremor in upper limb, muscular atrophy, flaccid paralysis, and gait abnormality the most prevalent findings [24]. We found similar abnormal neurologic findings when assessing 118 patients in our cohort (manuscript under review).

Physical impairment resulting from cerebral injury is to be expected following WNV neuroinvasive disease; however, continual impairment could be a result of persistent central nervous system infection, and serial cerebrospinal fluid analysis and neurologic exams could help differentiate neurologic sequelae outcomes. Additionally, to our knowledge, age-matched controls have not been evaluated to determine influence of advanced age on neurologic outcomes.

5. Renal consequences of West Nile in people

In conjunction with the publications supporting evidence of virus persistence in the kidneys in animal models, the first report of human viruria was described [78]. The culmination of these reports led us to investigate the possibility of viruria and renal pathology in our Houston cohort. From preliminary evaluations, we found 20% of cohort patients positive for WNV RNA in urine by PCR [38], and 40% of patients met clinical guidelines for chronic kidney disease several years post-infection [43,50]. Interestingly, 83% percent of those with advanced stages of chronic kidney disease were found positive for viral RNA in urine [50].

Of the original cohort evaluated for renal morbidity, 73% (102/139) were available for repeat testing over the subsequent three years. Of the 13 patients with a glomerular filtration rate (GFR) less than 60 mL/min/1.73 m^2 at original evaluation, 8 were still available for repeat testing (3 had died and 2 were lost-to-follow-up). Of these eight patients, 88% (7/8) still had a GFR less than 60 mL/min/1.73 m^2 at their follow-up evaluation with a median change of −2 mL/min/1.73 m^2 (data not published). One patient originally had a GFR of 52 mL/min/1.73 m^2 and increased to a GFR of 62 mL/min/1.73 m^2 over a two year time point. Finally, of those who met the clinical guidelines for chronic kidney disease 75% (41/55) were available for repeat testing. They had a median GFR change of −2 mL/min/1.73 m^2 (range −23 to +33 mL/min/1.73 m^2) over the subsequent three years. Our cohort demonstrates consistent impaired renal functioning over time.

Multiple recent studies have investigated presence of viral RNA and renal pathology with mixed results [5,24,6,43,50,54]. Urine is a known complex media, containing multiple PCR inhibitors and external factors affecting sample preservations that create multiple challenges for diagnostic capacities. Serial testing of urine in our cohort exemplifies the complexity of this approach, with seemingly random fluctuations between being positive and negative test results for viral RNA by PCR. Current efforts in our lab are targeted at understanding whether WNV infection can result in persistent renal infection in a subset of patients similar to what has been reported among multiple animal models and determine the clinical impact of WNV on kidney health.

Surveillance during an outbreak in Italy found that symptomatic (neuroinvasive and uncomplicated febrile illness) acutely infected patients had detectable WNV RNA in their urine for up to 31 days post-onset, longer than viremia (20 days post-onset) [5]. Viral load in urine was higher in neuroinvasive patients than in febrile patients; however, non-neuroinvasive febrile patients excreted RNA in urine longer than their counterparts [5]. Interestingly, paired samples of plasma and urine from this patient population found 83% of febrile patients had detectable RNA in urine but no evidence of viremia [5]. Unfortunately, kidney evaluations were not reported.

This paper has implications that patients presenting with uncomplicated febrile illness can also have evidence of viruria, which has differed somewhat from our cohort. We found renal morbidity significantly more likely to be associated with a history of neuroinvasive disease [50]. It is probable that both symptomatic WNV groups exhibit viruria in acute infection; however, neuroinvasive patients progress onto renal morbidity, possibly due to the higher likelihood of comorbidities like hypertension and diabetes and possibly persistent renal infection as seen in animal models of infection. Improved methods for detection of virus in renal tissue and urine and continued evaluation of GFR and renal injury biomarkers as compared to age-, race/ethnicity-, and gender-matched controls would greatly add to the understanding of WNV-related kidney pathology. Renal biopsies evaluated by PCR and electron microscopy would also bring added value, although these samples are understandably harder to obtain for research purposes on human subjects.

6. Mortality

More than 1700 fatal acute cases of WNV infection have been reported [2]; however, the impact of clinical disease on life expectancy still needs to be defined. Recent evidence has implicated that WNV infection can result in excessive mortality among infected populations. Higher than expected standardardized mortality rates have been noted up to four years post-acute infection in two cohorts [35,26]. Interestingly, 20% of deaths in a Colorado cohort listed renal disease as a contributing cause of death [35]. The long-term consequences of infection should continue to be monitored to examine increased risk of premature mortality in patients.

7. Concluding remarks

WNV is an arboviral disease that has historically caused significant morbidity and mortality worldwide. Following the 2012 outbreak in the United States, it is fair to state that WNV is an established disease of public health importance. With recent advancements on understanding the long-term neurologic and renal outcomes of patients with a history of WNV infection, it is imperative that clinicians begin to monitor for the development of long-term sequelae. We propose in this review that WNV infection has the potential to establish itself as a chronic disease, with continued sequelae that manifests with neurologic and renal impairment.

Conflict of interest

The authors have no conflicts of interest to declare.

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