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Review

A Tale of Two Flaviviruses: West Nile Virus and Zika Virus in Solid Organ Transplantation

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Abstract

Flaviviruses can cause epidemics and endemics with substantial public health threat and economic impacts. In the last few decades, many flaviviruses have re-emerged or expanded their territories to new regions or continents, including West Nile virus (WNV) which has become endemic in the US since its arrival in 1999 and Zika virus (ZIKV) which recently spread across the Americas. These events demonstrate the speed with which a vector-borne pathogen can disseminate when introduced into a susceptible population with competent vectors. The threat of flaviviruses has special relevance to the solid organ transplant populations because of the possibility of donor-derived transmission and increased disease severity in immunocompromised patients. This review will focus on the clinical manifestations, diagnosis, treatment and prevention of flavivirus infection, with an emphasis on WNV and ZIKV as they both garnered much attention in the SOT arena when introduced into the US and they epitomize the unpredictability of the epidemiological and clinical features of flaviviruses. Several other flaviviruses will also be discussed when data regarding transplant patients are available.

Keywords
West Nile virus; Zika virus; flavivirus; solid organ transplantation

1. Introduction

The genus Flavivirus, in the family of *Flaviviridae*, has >70 members and includes a number of important human pathogens such as West Nile (WNV), dengue (DENV), yellow fever (YFV), Zika (ZIKV), St. Louis encephalitis (SLEV), Japanese encephalitis virus (JEV) and others. Flaviviruses are small, non-enveloped viruses with a single, positive strand RNA genome of 10-11kb in length. The genome encodes a single polyprotein, which is processed by cellular and viral proteases into 3 structural proteins [a capsid protein (C), an envelope protein (E), and a transmembrane protein (prM)] as well as 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). The non-structural proteins form the viral replication machinery and also modulate the host immune response [1]. Most of the flaviviruses are arboviruses transmitted by mosquitoes or ticks, with birds or non-human mammals as part of the replication cycle. Several flaviviruses (e.g. YFV, DENV and ZIKV), are well-adapted to replication in humans and do not necessarily require other animal hosts for transmission, but for others (including WNV), humans are incidental dead-end hosts, as they do not develop sufficiently prolonged or high-level viremia to play a significant role in viral amplification and transmission [2].

Flaviviruses can cause epidemics and endemics with substantial public health threat and economic impacts [3]. In the last few decades, many flaviviruses have re-emerged or expanded their territories to new regions or continents, likely due to changes of human behaviors and environmental conditions. Dense urbanization, population growth, rapid globalization as well as global climate changes that affect vector prevalence and demography are all likely contributors. After WNV's first arrival to North America in 1999, it subsequently spread to all 48 states of the continental United States (US) of America and has become endemic in the US. The more recent spread of Zika in America further demonstrates the speed with which a vector-borne pathogen can disseminate when introduced into a susceptible population with competent vectors. The threat of flaviviruses has special relevance to the solid organ transplant (SOT) populations because of the possibility of transmission through the transplanted organs and increased disease severity in immunocompromised patients. This review will focus on the clinical manifestations, diagnosis, treatment and prevention of flavivirus infection, with emphasis on WNV and ZIKV as they both garnered much attention in the SOT arena when introduced into the US and they epitomize the unpredictability of the epidemiological and clinical features of flaviviruses. Several other flaviviruses will also be discussed when data regarding transplant patients are available.

2. Epidemiology and Transmission

2.1 West Nile Virus

For WNV, natural transmission is mosquito-borne with the principle vectors being *Culex* spp., while susceptible birds, especially corvids, are the principle vertebrate reservoir. Humans, horses, and many other animal species are incidental hosts only [2]. WNV was first isolated in Uganda in

1937. Several epidemics occurred in the Old World in the subsequent decades, but most cases were asymptomatic or mild. Since the mid-1990s, there were increased frequency and scale of outbreaks in humans and horses, along with increased disease severity in humans, including neurological complications and death [4]. WNV entered the US (and the Western Hemisphere) in August 1999, then subsequently spread throughout the US continent. The majority of WNV infections are asymptomatic and/or unreported, but a previous report estimated that 2-4 million people in the US were infected by this virus [4]. Per data from ArboNET (which is a national arboviral surveillance system managed by the CDC and state health departments), by the end of 2017, more than 48,000 cases have been reported in the US, including approximately 23,000 cases of neuroinvasive disease and 2,164 deaths [5]. For the last 2 decades, WNV activities have fluctuated, likely attributable to a number of factors involving susceptible vectors and vertebrate hosts, as well as environmental factors [6, 7]. In 2018, WNV activities have been detected in 47 states and District of Columbia.

Other than natural transmission through mosquito bites, WNV infections in humans have also been acquired through blood product transfusion [8, 9], breast-feeding [10], transplacental transmission [11], occupational exposure in laboratory workers [12], and SOT, which will be discussed in detail below.

2.2 Zika Virus

ZIKV has 2 distinct transmission cycles and in contrast to WNV, non-human primates and humans appear to be the only vertebrate hosts in nature [13]. The sylvatic cycle involves transmission between non-human primates and forest mosquitoes; the urban cycle involves humans and urban-dwelling mosquitoes with *Aedes aegypti* and *Aedes albopictus* as the principle vectors. Zika was first isolated from a sentinel rhesus macaque in the Zika forest of Uganda in 1947, and the first human case was recognized in Nigeria in 1952. In the next 5-6 decades, only sporadic cases were reported and they were confined to a relatively narrow equatorial belt across Africa and Asia. However, in 2007 explosive human outbreaks were reported on Yap Island, Micronesia, causing ~5,000 infections in a total population of ~6,700 (with an attack rate of ~75%) [14]. In 2013-14, an outbreak in French Polynesia involved 32,000 persons, with an attack rate of 66% [15]. It subsequently spread across the Pacific Islands and entered Latin America in March 2015 in northeastern Brazil. By the end of 2015, there were 440,000 to 1,300,000 suspected cases in Brazil alone, and by March 2016, it affected at least 33 countries and territories in the Americas [16, 17].

ZIKV can be detected in multiple body fluids, including blood [18], semen [19], urine [20], saliva [21], amniotic fluid [22] and breast milk [23]. Other than natural transmission by mosquitoes, other reported modes of transmission include mother-to-fetus transmission during pregnancy, peripartum transmission, sexual transmission, and blood transfusion. Infections acquired through a monkey bite and lab accident have also occurred [24, 25] and breast-feeding is a potential transmission route as well [23].

For the US territories, a total of 37,270 cases of ZIKV were reported by September 2018, with 5,723 cases in the US and District of Columbia [26]. Greater than 99% of those cases in the US territories were acquired through presumed local mosquito-borne transmission. In contrast, those in the US were mostly among travelers returning from affected areas (95%); only 4% acquired infection through presumed local natural transmission and ~1% (52 cases) were thought to be

transmitted by sexual activity. Blood transfusions were thought responsible for a few cases, but no cases of ZIKV transmission by transplanted tissues or organs have been reported to date.

In February 2016, given the alarming rate of its spread through much of Central and South America as well as the Caribbean, the World Health Organization (WHO) declared that ZIKV infection associated with microcephaly and other neurological disorders constitutes a public health emergency of international concern. However, natural transmission of ZIKV in the US seems to have peaked in 2016. There was a marked decrease of cases in 2017, and in 2018 as of September, only 41 ZIKV cases were reported in the US. All 41 cases were in travelers returning from affected areas, with no cases of presumed natural transmission [26].

3. Clinical Syndrome

In general, flavivirus infections are mostly asymptomatic, and symptomatic cases are often self-limiting, with resolution of the illness in about a week. However, severe or life-threatening disease can be seen in a small percentage of patients, depending on the virus type, as well as the age, immune status and co-morbidities of the patients. Previous flavivirus infection might also modulate disease outcome and this phenomenon is best known with DENV. While the first DENV infection is frequently asymptomatic and mild, secondary infection with a heterologous serotype can increase risk for severe disease (e.g. dengue hemorrhagic fever or dengue shock syndrome). Such an increased risk in secondary infection is thought to be due to antibody-dependent enhancement (ADE) of viral infection through IgG engagement of Fc receptors [27, 28]. ZIKV shares a high degree of genetic and structural relatedness with other flaviviruses. In particular, the viral surface glycoprotein envelope (E), which is the major target for neutralizing antibody responses, contains regions that are highly conserved between ZIKV and DENV [29] and high cross-reactivity has been demonstrated in serological assays [30]. DENV or WNV-convalescent plasma has been shown to enhance ZIKV infection *in vitro* and in animal models [31]. It is conceivable that individuals immune to DENV might develop more severe disease when infected with ZIKV through ADE, although a recent study of ZIKV-infected patients in Brazil who were previously exposed to DENV did not demonstrate ADE effects *in vivo* [32].

3.1 West Nile Virus

The incubation period for WNV in general ranges from 2 to 14 days. However, among transplant recipients who acquired WNV through SOT or blood transfusion, incubation time can be prolonged, with a median incubation time of about 13 days [33, 34]. Among those infected, about 80% are asymptomatic [35, 36]; 20-30% develop a mild infection called West Nile fever (WNF), which is mostly self-limiting. Symptoms of WNF may include fever, malaise, lymphadenopathy, periocular pain, gastrointestinal symptoms (such as nausea, vomiting, abdominal pain), myalgia, and headache. Complications such as myocarditis, myositis, orchitis and pancreatitis [37-40] have been reported, but these are rare.

The most severe manifestation of WNV is CNS disease, which develops in only 1 in 150 infected patients in the general population [35], but can be up to 1 in 40 in SOT populations per estimate by one study [41]. Clinical syndromes of CNS involvement include meningitis, encephalitis, and acute flaccid paralysis (AFP). Advanced age is a major risk factor for the development of neuroinvasive disease [42]. Other independent risk factors include male sex, hypertension,

diabetes mellitus, immunosuppressing conditions and cardiovascular disease [43, 44]. Symptoms of WNV encephalitis (WNE) are non-specific, but most patients have fever, headache, and altered mental status, and some also have vomiting, diarrhea and rash [45]. A prominent finding of WNE is muscle weakness with flaccid paralysis and hyporeflexia in up to 30-50% of patients. Cranial neuropathies (e.g. peripheral 7th nerve palsy), optic neuritis, ataxia, movement disorders (e.g. postural or kinetic tremor, myoclonus), and Parkinsonian features (e.g. rigidity or postural instability) are other neurologic findings [45, 46]. Rarely, seizures, increased intracranial pressure, and cerebral edema have also been reported [46].

AFP occurs in 5-15% of patients with neuroinvasive disease and is more common in younger patients, although elderly patients suffer from a higher mortality (up to 22%). AFP can present as a poliomyelitis or more rarely, Guillain-Barré-like syndrome (GBS) [4, 47]. The spectrum of clinical presentations range from single extremity paralysis to flaccid quadriplegia with cranial nerve involvement. Respiratory failure and bladder dysfunction have been observed in 54% and 22% of patients, respectively [48].

Among those with severe illness due to WNV, the overall case-fatality rates range from 3% to 15% and are highest among the elderly [5]. Those with WNV neuroinvasive disease (WNND) can be left with persistent cognitive deficits or neurologic impairment [48, 49]. However, even those with mild WNF may have long-lasting subjective or somatic complaints [50], including tremor, and abnormalities in motor skills and executive functions [51]. After symptomatic WNV infection, more than half of the patients experienced persistent symptoms for more than six months [52, 53].

3.2 Zika Virus

The incubation period for ZIKV infection is ~3-12 days. Similar to other flavivirus infections, most infections result in mild and self-limited illness [13]. For the Yap Island outbreak, only 19% of infected individuals were symptomatic [14]. Symptoms are non-specific and “flu-like”, including transient low-grade fever, maculopapular rash, arthritis or arthralgia. Other reported symptoms include non-purulent conjunctivitis, myalgia, fatigue, headache and retro-orbital pain [14, 54]. A number of neurologic complications have been reported, including GBS [55], acute motor axonal neuropathy, meningoencephalitis, acute myelitis [56, 57] and ophthalmologic disease [58], but compared to WNV, neuroinvasive disease is rare. In the French Polynesian outbreak, incidence of GBS was estimated to be 0.24 per 1000 infections [59]. Non-perinatal ZIKV-associated fatalities have been described, but only rarely [60]. However, while immunocompromised hosts might be at higher risk for severe disease, it is important to note that some of the patients who succumbed to ZIKV infection were previously healthy, or with only mild comorbidities [60-63]. The most devastating clinical syndromes caused by ZIKV are congenital abnormalities, including microcephaly and other CNS malformations, as well as fetal demise [13]. ZIKV has broad tissue tropism, but notably can infect mature neurons as well as neural progenitor cells, which suggest a potential mechanism for causing microcephaly [64, 65].

4. Flavivirus Infections in SOT Recipients

For transplant recipients, mosquito-borne transmission remains the most likely route to acquire flavivirus infection, although transmission through blood transfusion and organ transplantation have been well documented.

Immunocompromised hosts, including transplant recipients, likely experience higher morbidity and mortality from flavivirus infections, as compared to immunocompetent hosts. Among 9 SOT recipients that acquired WNV through blood transfusion [66], all patients developed severe neurological complications, and 2 subsequently died. During the epidemic in Israel in 2000, immunocompromised patients were noted to have a mortality rate of 31%, as compared to 13% among those not immunocompromised [67]. A case series of 11 transplant patients with mosquito-borne WNV reported a mortality rate of 18% [68]. For the 2002 outbreak in Toronto, Canada, the risk of meningoencephalitis in a transplant patient infected with WNV was estimated at 40% as compared to 1% in the general population [69]. These reports studied naturally-transmitted WNV infection in transplant patients, who were mostly months to years post-transplant. SOT patients that acquired WNV through donor-organ transmission might have even higher rates of morbidity and mortality, as the level of immunosuppression is likely enhanced during the immediate post-transplant period.

There are at least 9 clusters of WNV transmission through SOT, involving a total of 9 donors and 26 recipients (Table 1). All cases occurred in the US except 2 in Italy. For the donors, 2 were thought infected through blood transfusion; infection in the other 6 were likely mosquito-borne. Of the 26 recipients, 22 were found infected, and 14 (64%) developed WNV encephalitis resulting in high morbidity and mortality. For hematopoietic stem cell transplant (HSCT) recipients, morbidity and mortality are likely increased as well. Among 7 such patients reported in the literature, 5 died shortly after presentation [70, 71].

For ZIKV, there have not been any cases of transmission through SOT reported to date, but limited data regarding ZIKV infection in SOT recipients suggests that these patients may present with more severe illness. In a recent case series of 4 SOT patients in Brazil (2 liver transplants and 2 kidney transplants) with confirmed ZIKV infection by reverse transcriptase-polymerase chain reaction (RT-PCR), patients presented with abnormal graft function; 75% developed anemia, and 100% developed thrombocytopenia and bacterial infections during the course of illness [72]. A fatal case of a heart transplant patient who developed ZIKV meningoencephalitis presented with severe symptoms of headache, seizures, and hemodynamic instability [73]. After stopping the patient's immunosuppressive regimen, he developed cardiogenic shock and died of a cardiac arrest due to acute cardiac allograft rejection raising further questions about the management strategies of immunosuppressants in this setting.

Experiences with other flavivirus infections in immunocompromised patients are noteworthy. Transmission of DENV through SOT has been reported in DENV endemic areas [74, 75]. While most reported on a single case, Rosso *et al.* [74] described 4 cases of DENV transmission from 2 different donors involving 1 heart recipient and 1 liver recipient in one cluster and 2 kidney recipients in another. All organ recipients presented with fever within the first week after transplant; 3 of the 4 recipients also had thrombocytopenia and lymphopenia. Other signs and symptoms included hepatitis, myalgia, arthralgia and transient encephalopathy. As there are no specific treatments for DENV, these patients received supportive measures and all 4 patients survived.

A number of studies have reviewed the clinical characteristics and outcomes of dengue fever among renal transplant patients [76, 77]. In a cohort of 102 renal transplant recipients in Pakistan with dengue infection, most patients had mild disease with good recovery [76], but in a recent systematic review by Weerakkody *et al.* [77], significantly higher incidence of severe dengue and

higher mortality were noted. In both studies, about 60-70% of patients experienced graft dysfunction during the illness, but most had renal function recovery [76, 77].

Similarly, SLEV may cause more severe illness in immunocompromised patients. Hartmann *et al.* reported 3 cases of SOT recipients with meningoencephalitis caused by SLEV with 1 death [78]. Venkat *et al*. reported a case of possible SLEV transmission via blood transfusion into a kidney transplant recipient [79], but no cases of SLEV transmission through transplanted organs have been reported to date.

In summary, the diagnosis of flavivirus infection in transplant recipients requires a high index of clinical suspicion, when the patient presents with unexplained fever, with or without neurological manifestations, and in particular, when there is known flavivirus activity in the region. Furthermore, the possibility of donor- or transfusion-derived flavivirus infection must be considered during the early post-transplant period.

5. Diagnosis

5.1 West Nile Virus

5.1.1 Laboratory and Imaging Findings

In patients with WNV infection laboratory findings include relatively normal to elevated total leukocyte count with associated lymphocytopenia and anemia [80, 81]. Hyponatremia can also be seen, especially in patients with encephalitis. Initial studies of cerebrospinal fluid (CSF) in general show a lymphocytic predominant pleocytosis (generally <500 cells/mL), but elevated neutrophils has also been reported [82]. Protein levels are usually elevated (generally <150 mg/dL), while glucose levels remain normal. These findings follow similar trends in the immunocompromised populations [41, 80], although the CSF WBC counts in transplant patients with WNE are minimally elevated as compared to immunocompetent patients (mean CSF WBC 86 cells/mm^3 versus 227 cells/mm^3) [34]. In the case series of naturally acquired WNV encephalitis in transplant patients reported by Kleinschmidt-DeMasters *et al.* [68], pleocytosis was present in all cases (range 5-540 cell/μl; mean±SD, 89±152). In contrast, among the 14 patients with organ-transmitted WNE (Table 1), CSF data were available for 9 patients and 3 did not have any pleocytosis.

Imaging findings in WNV encephalitis tend to be non-specific. Magnetic Resonance Imaging (MRI) findings share similarities with many other inflammatory and infectious processes, including those of SLEV and JEV. Patients with WNND can have normal neuroimaging studies, but abnormalities, when present, are primarily seen in areas of the basal ganglia, thalamus, cerebellum, and brainstem [83]. Some patients may also have involvement of the mesial temporal structures. For patients with extremity weakness, abnormalities can be seen in the grey matter of the spinal cord, more pronounced in the ventral horns, as well as the conus medullaris and the cauda equina. Abnormalities seen by MRI may be progressive, transient and/or migratory, and the transient nature of the imaging abnormalities might explain the negative studies in some patients [84]. Overall, while the MRI findings in WNV tend to be ambiguous, certain MRI findings, such as deep grey matter or mesial temporal lobe involvement, should prompt inclusion of WNV on the differential diagnosis, especially if the clinical picture, epidemiologic factors and mosquito exposures are taken into account. Additionally, the use of electromyography and nerve

conduction studies have been used to distinguish between WNND and GBS where there is a predominance of demyelinating lesions with axonal changes seen in WNV infection [85].

5.1.2 Laboratory Diagnosis

The laboratory diagnosis of WNV remains challenging and often requires second level testing. The currently recognized diagnostic criteria from the European Union include patients with clinical presentation of fever or encephalitis or meningitis in an endemic area with at least one laboratory criterion: (i) isolation of WNV from blood or CSF, (ii) detection of WNV RNA in blood or CSF, (iii) identification of WNV specific antibody response (IgM) in CSF, or (iv) high titer WNV IgM and detection of IgG, and confirmation by neutralization [86]. Similarly, the CDC guidelines recommend studies on blood or CSF for detection of IgM antibodies, plaque reduction neutralization tests (PRNT) using acute and convalescent phase serum samples, viral culture, or RT-PCR for the diagnosis of WNV [5].

Nucleic Acid Testing (NAT). Detection of WNV RNA can be performed through NAT using RT-PCR. The viremic stage of the disease however tends to occur when the patient is asymptomatic making this detection method less sensitive. About 70-80% of WNV patients have undetectable viral RNA in blood at the time of symptom onset [87]. The window for detection in both blood and CSF is approximately 2-18 days after infection, but extended duration of viremia has been shown to occur in SOT patients [88]. Despite its limited sensitivity, positive RT-PCR allows for rapid diagnosis and can be performed on multiple different sample types including blood, CSF, urine, plasma and tissues. Urine samples tend to have higher viral loads than plasma and are more frequently positive in symptomatic patients [89]. WNV shedding in the urine may also persists for months after the initial infection and can be a useful tool in confirming the diagnosis [90]. NAT should be performed routinely in the immunocompromised host population (especially those taking rituximab) given their limited ability to mount an antibody response [91] and possible prolonged viremic period [88].

Serology. Detection of IgM and IgG is one of the most widely used methods for the diagnosis of WNV given the timing and brief duration of the viremic phase of infection. Serological testing can be performed by enzyme-linked immunosorbent assay (ELISA), immunofluorescence assay (IFA), neutralization test and by hemagglutination-inhibition assay. IgM antibodies become detectable in serum on average 4 days after the initial infection but can persist for months or even > 1 year [92]. In one study, up to 17% of patients had detectable IgM levels 1 year after the acute infection [93]. IgG levels usually follow 3-4 days after IgM and are often detectable life-long. In transplant recipients, the development of antibodies might be delayed [68]. For instance, the liver recipient reported by Morelli *et al.* [94] developed a WNV IgM antibody response by day 26 post-transplant, but did not develop IgG seroconversion to WNV for more than 4 months after transplantation. Given the prolonged timelines for serology detection, IgM, IgG, and RT-PCR can be tested concurrently [95]. Since IgM does not typically cross the blood-brain barrier (BBB) but is produced locally within the CSF by infiltrating lymphocytes, a positive IgM in the CSF is usually indicative of CNS infection. However, it is conceivable that serum IgM may enter the CSF in the setting of infection or inflammation that disrupts the BBB in general; thus, finding of IgM in the CSF needs to be interpreted with caution in the correct clinical setting. The sensitivity of this test in CSF is also high given that a large proportion of patients with CNS disease will have detectable IgM within 8

days of the onset of symptoms [96]. A limitation of serology testing is cross-reactivity with other flavivirus species which can co-circulate with WNV as natural transmission and among populations which have been vaccinated against JEV, YFV and now possibly DENV [97]. A previous external quality assurance evaluation of WNV serology showed that IgM testing for WNV had a sensitivity of 50% and a specificity of 95%, and IgG had a sensitivity of 86% and a specificity of 69% [98] demonstrating the limitations of the current diagnostics available. Thus, for diagnosis of flavivirus infections, including WNV, a careful assessment of the patient's travel, exposure and vaccination history as well as the local epidemiology of flaviviruses is crucial.

Positive serological results for WNV by EIA or IFA can be confirmed by PRNT, which is considered the gold standard for distinguishing the serodiagnosis in flaviviruses [98]. This assay improves the specificity of the serology testing by measuring the binding of the antibodies in patient's sera with a laboratory WNV strain [99]. If neutralizing antibodies are present, they will bind to the virus thus inhibiting its entry into cells and preventing plaque formation. This test is also useful in detecting acute infection, by measuring a four-fold increase in titer between an acute and convalescent sample taken at least 2 weeks apart. However, the sensitivity of this test suffers especially early on during the infection when neutralizing antibody titers may be low or in patients with blunted immune responses. Additionally, this assay requires a biosafety level-3 (BSL-3) facility and is available only in reference laboratories [5].

Viral Culture. Culturing WNV requires a class II biological safety cabinet located within a BSL-3 facility [5]. The virus itself can be propagated in tissue culture of mammalian or mosquito cells. Time to cytopathic effect is 2-7 days depending on the initial viral load of the patient [100]. While isolation of WNV can be performed on multiple different sample types including serum, CSF, urine, and tissues, this diagnostic method is less sensitive than NAT. It is also not routinely available, and is not practical nor recommended for making a clinical diagnosis.

NS1. NS1, a nonstructural protein of flaviviruses that functions in the immune evasion of the virus [101], is secreted from infected cells during acute infection and can be detected in acute phase serum. While this is done regularly for other flaviviruses including DENV [102], there are currently no WNV-specific commercial NS1 Ag tests available. However, ELISA tests for NS1 have been shown to detect early stage WNV infections and may be a useful adjunct to current diagnostics for acute infection [103, 104].

5.2 Zika Virus

5.2.1 Laboratory and Imaging Findings

ZIKV infection has been implicated in a broad range of neurologic complications in adult patients including GBS, meningoencephalitis, transverse myelitis, and acute disseminated encephalomyelitis [105]. CSF findings in these patients appear to be similar to other viral encephalitides. In a case report of fatal ZIKV-associated encephalitis the initial CSF studies demonstrated a pleocytosis with a cell count of 10 cells/mm^3 (80% lymphocytes), glucose of 48 mg/dL, and elevated protein of 110 mg/dL [61]. In a case of ZIKV-associated meningoencephalitis in a heart transplant patient, the initial CSF showed a cell count of 2 cells/mm^3 (100% lymphocytes), normal glucose, and elevated protein, but repeat CSF analysis one week later showed 58 cells/ mm^3 (100% lymphocytes), elevated protein level to 105.37 mg/dL and a CSF to blood glucose ratio of 0.41 [73].

MRI imaging in the setting of ZIKV associated neurologic diseases tend to be non-specific and similar to those associated with other flaviviruses such as WNV and JEV including increased intensity in the spinal cord, middle peduncle, and anterior horns [105]. Other findings of ZIKV encephalitis include diffuse and confluent lesions in the basal ganglia, thalamus, and white matter [61]. When associated with GBS, the most common findings include post-contrast enhancement of the conus medullaris and cauda equina nerve roots. In addition, T2-hyperintensity and post-contrast enhancement of the lumbar spinal ganglia may be seen bilaterally [105, 106]. However, given the lack of specificity of these findings the diagnosis of ZIKV should be made with additional clinical and laboratory testing.

5.2.2 Laboratory Diagnosis

Methodologies for diagnosing ZIKV are similar to those for WNV. Given the overlap in symptoms and geographic locations of ZIKV and other arboviruses such as DENV and chikungunya (CHIKV), early suspicion and testing is important for appropriate diagnosis. Due to issues of cross-reacting antibodies among flaviviruses, it is likely that the seroprevalence of ZIKV cases has been underestimated.

Serology. ZIKV can be detected by NAT in the sera within 5-7 days after infection and may be detected in other body fluids such as urine and semen for a longer period [107]. Given this narrow window, while a positive test is sufficient for the diagnosis of ZIKV, a negative test in the appropriate clinical setting requires further work up [108]. For patients presenting later on in the course of illness measurement of ZIKV IgM is recommended. This test has several limitations given the known cross-reactivity of ZIKV with other circulating viruses in endemic areas and PRNT can also be employed for confirmation. For instance, a positive ZIKV diagnosis would require a positive ZIKV PRNT titer and a negative DENV PRNT titer [109].

NAT. In the SOT population there are unique considerations given concerns for reduced antibody production and possible increase in the duration of viremia [110]. Therefore, testing with both serology and NAT on suspected cases may be useful. Additionally newer technologies such as multiplexed assays for the detection of ZIKV, CHIKV, and DENV are being developed to enhance diagnostic accuracy [111]. There is some evidence that ZIKV likely adheres to red blood cells making detection in whole blood preferable to plasma [112]. Detection of virus in urine and semen is also prolonged as compared to blood samples. It has be shown that ZIKV can persist in the urine up to 20 days after loss of detection in blood, and in semen for several weeks to months [113, 114]. The WHO currently recommends obtaining whole blood/serum and/or urine for NAT testing in the acute phase of illness [115].

NS1. ZIKV NS1 ELISA assays are another modality for acute phase diagnosis. NS1 is secreted by infected cells into the blood stream leading to elevated NS1 specific antibody titers. These antibodies often have a high specificity and limited cross-reactivity with other similar viruses [116, 117]. Further broad-based assays are being developed to help differentiate between these viral febrile illnesses given their similar presentations. Metagenomic next generation sequencing (mNGS) has more recently been used for diagnosis and for identification of co-infections in this setting. This technology can be used on the same patient samples by extracting nucleic acids and constructing NGS libraries for sequencing [118, 119].

New technologies are also being developed for point-of-care diagnostics including a paper microfluidic chip platform [120] and a FDA approved ZIKV point-of-care serology test [121]. These technologies have the potential to improve diagnosis of ZIKV in resource limited settings.

6. Treatment

Treatment of flaviviruses is mostly supportive and there is no specific antiviral therapy available. In patients on immunosuppression there may be some benefits to lowering or removing the immunosuppressive regimen to improve both the humoral and cellular immune responses to the virus [91]. Since WNV can cause debilitating or life-threatening infection, several modalities of treatment have been tested or employed in animal studies and human cases [122]. In contrast, non-perinatal ZIKV infection is relatively mild and treatment remains mostly supportive.

6.1 Intravenous Immunoglobulin (IVIG) and Monoclonal Antibodies

IVIG is a therapeutic preparation of normal human IgG obtained from pooled plasma from thousands of healthy blood donors. Besides the direct neutralizing activities against specific pathogens, IVIG may also confer protective effects through its anti-inflammatory and immunomodulating properties [123]. The activity of IVIG against any specific pathogens depends on the prior exposure of the donors to the microbes as well as immunity obtained through vaccination. Thus, IVIG preparation from different areas or countries can have substantial variability in their capacity in neutralizing different flaviviruses [124]. Successful use of IVIG to treat WNV infection has been reported in several cases, including SOT recipients [125-127]. In all these cases, the patients had severe neuro-invasive disease from WNV, but recovered after prompt administration of IVIG. However, there might be a reporting bias and cases of failure have been noted [128]. A phase I/II randomized, placebo-controlled trial that evaluated the safety and efficacy of hyperimmune IVIG (Omr-IgG-am) in the treatment of patients with or at high risk for progression to WNV encephalitis was completed in 2007 (ClinicalTrials.gov: NCT00069316), but no results have been reported to date.

After infection by flaviviruses, invasion of the CNS may occur in a few days, and the timing of IVIG administration might be crucial. A number of animal models demonstrated clear-cut protection by IVIG when the animals are treated during the viremic phase, before or shortly after inoculation with WNV [129, 130]. In clinical studies, most patients that responded to IVIG treatment were treated early in the course, or before the development of neurological symptoms [131, 132]. Thus, the timing of IVIG administration may be critical to its efficacy [127]. However, most patients are no longer viremic when they present [133, 134] and therefore, might not respond to IVIG treatment. Other than the timing of starting IVIG, the route of administration may be important as well [128]. IgG enters the blood-brain barrier at only low level, and that might explain the relative ineffectiveness of IVIG treatment when CNS involvement is already evident. In cases of GBS caused by ZIKV, IVIG is primarily used as a non-specific modality for GBS, but not as a specific treatment of ZIKV.

Antiserum or monoclonal antibodies against flaviviruses are known to have neutralizing properties and can protect against flavivirus infection *in vivo*. Humanized monoclonal antibodies against WNV have been developed [122]. A humanized monoclonal antibody against WNV E protein (MGAWN1) was found safe and generally well-tolerated in healthy human subjects in a

phase I clinical trial [135], but a phase II study to evaluate its safety and efficacy in patients with WNV infection was terminated due to inability to enroll (ClinicalTrials.gov: NCT000927953). A number of monoclonal antibodies with neutralizing properties against ZIKV have also been developed and they can protect against lethal ZIKV challenge or maternal-fetal transmission, infection and disease in animal models [136, 137]. A first-in-class monoclonal therapeutic against ZIKV, tyzivumab, is currently in a phase I clinical trial (NCT03443830). ZIKV immune globulin (ZIKV-IG) is also being developed as a therapeutic intervention against ZIKV infection. A phase I clinical trial is being conducted to evaluate the safety and pharmacokinetics of ZIKV-IG in healthy adult volunteers (NCT03624946).

There is a theoretical concern for ADE of infection, during which subneutralizing concentrations of antibodies bound to a flavivirus can enhance infection by facilitating uptake into Fc-receptor-bearing cells [138]. However, as discussed previously, the significance of ADE among non-DENV flavivirus infections is unclear.

6.2 Antiviral Compounds

There have been substantial efforts in the development of broad-spectrum antiviral agents for flavivirus infections. Since all flaviviruses share similar genomic organization and replication mechanisms, targeting certain conserved viral proteins or enzymes might confer antiviral-activity across a number of flaviviruses. The most promising potential viral targets include the NS3 protease and NS5 polymerase. Other potential viral targets may include the capsid protein, NS4B, the E glycoprotein and so on. Host molecules exploited by the flaviviruses as part of their replication cycle can also be targeted. This subject has been thoroughly reviewed by Boldescu *et al.* [139]. However, these molecules are in the preclinical stage and none have been tested in human clinical trials to date.

For WNV, the only antiviral compound tested in human cases is ribavirin [140]. Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboximide) is a synthetic nucleoside analog with *in vitro* and *in vivo* activities against a number of DNA and RNA viruses. Its mechanism of action may include competitive inhibition of inosine monophosphate dehydrogenase, lethal mutagenesis after incorporation into the viral genome as well as its non-specific immunomodulatory properties [141-143]. Ribavirin has some activities again flaviviruses *in vitro*, but only with very high doses [122, 144]. As such, clinical use would be limited by its side effects and toxicities [145]. Its low lipid solubility, which leads to ineffective passage through lipoid membranes would argue against its efficacy in treating CNS infections [144]. A hamster WNV model and human experiences suggest that ribavirin treatment may actually increase mortality [140, 146], although arguably, ribavirin was offered to patients with advanced disease and later in the course of illness [140].

The relative homogeneity of ZIKV strains may improve the rapidity of identifying drug targets. There are ongoing efforts towards development of anti-ZIKV therapeutics including repurposing approaches which have identified potentially useful compounds (e.g. emricasan that inhibits caspase function to reduce apoptosis and bromocriptine that inhibits the ZIKV NS2B-NS3 protease *in vitro*) [147, 148], but larger scale *in vivo* studies are still needed to evaluate their efficacy [149]. Nucleoside/nucleotide analogs have also been explored as ZIKV therapeutics. These drugs tend to have better safety profiles by targeting viral proteins. Several drugs in this family, including sofosbuvir, ribavirin, favipiravir, and 7-deaza-2’-C-methyladenosine (7DMA), have been evaluated

for their anti-ZIKV effects [150]. Sofosbuvir was shown to reduce ZIKV replication and infection in cell culture and demonstrated a mortality benefit in mice [151]. 7DMA has been shown to reduce cytopathic effects and overall cell death *in vitro* as well as delayed disease progression in a murine model [152]. However, other than antiviral properties, these drugs may also need to cross the BBB and/or the placental barrier to be clinically useful.

6.3 Other Strategies

Interferons (IFNs) play important roles in defense against viral infections. Interestingly, all of the flaviviruses examined thus far (including WNV, ZIKV, DENV, YFV and JEV) can antagonize IFN-I responses by preventing JAK-STAT signaling, suggesting an important evolutionary advantage for these viruses to interfere with the host's interferon response [153].

IFN-α has been studied in several clinical trials against flavivirus diseases. A small pilot study showed efficacy of IFN-α-2b against meningoencephalitis due to SLEV [154], but a larger randomized double-blind placebo-controlled trial of IFN-α-2a for JEV did not show any improved outcome [155]. For WNV, only case reports exist, and experiences of both success [156, 157] and failure [158] have been reported. However, similar to the use of IVIG, failure of IFN in treating WNV might be under-reported due to publication bias. The IFN pathway has also been studied in the setting of ZIKV with the aim of developing new therapeutics. Administration of IFN leads to upregulation of interferon stimulatory genes (ISGs) which have an immune modulatory effect. A subset of these ISGs termed interferon inducible transmembrane proteins (IFITMs) have been shown to play a role as restriction factors to prevent ZIKV infection [159]. For example, IFITM3 showed a reduction in ZIKV replication by inhibiting the formation of the fusion pore by altering aspects of the plasma membrane [160, 161]. Another ISG with potential antiviral effects is cholesterol-25-hydroxylase (CH25H) enzyme [162]. IFN activation leads to increased expression of CH25H which converts cholesterol into 25-hydroxycholesterol (25HC). 25HC regulates the induction of retinoic acid-inducible gene-I in macrophages which can inhibit viral particle entry [163]. However, while there are several promising targets in this pathway, the side effects of IFN therapy make treatment in the setting of pregnancy challenging.

For SOT patients, IFN therapy may potentially induce an immunoreactive state and increase the risk of graft rejection [164]. For instance, the long-term use of IFN-α-2b has been associated with an increased risk of acute rejection in renal transplant recipients [165]. In contrast, 4 renal transplant recipients with WNE in a single cohort received short courses of IFN (4-17 days) and did not have any significant deterioration of their renal graft function [68].

Other anti-flavivirus treatment strategies also include RNA interference-based intervention and antisense technology. These approaches have been shown to protect mice against flaviviruses, including WNV and JEV [166, 167]. A phase I/II randomized-blinded study in humans was initiated in 2004 to evaluate the safety and efficacy of an antisense compound AVI-4020 targeting WNV (ClinicalTrials.gov: NCT00091845). However, the study was terminated due to a limited pool of eligible WNV patients.

Among all the flaviviruses, DENV is currently most wide-spread throughout the world, infecting ~50-100 million people annually, with an estimate of about half a million deaths from fatal sequelae of DENV infection, such as dengue hemorrhagic fever and dengue shock syndrome. As such, much effort has been devoted to developing effective treatment for DENV infection and

many drugs or molecules have been tested in the clinical setting. A detailed description of these therapeutics is beyond the scope of this article [168], but treatment modalities efficacious for DENV would likely benefit drug development for other flaviviruses as well.

7. Prevention

7.1 General Approach

For natural WNV or ZIKV infection through mosquitoes, preventive strategies that can reduce this route of transmission include avoiding outside activities when it is prime mosquito feeding time (e.g. night time and dawn for *Culex* species, but day time for *Aedes* species), application of insect-repellents (e.g. N,N-diethyl-m-toluamide, DEET), wearing permethrin treated clothing/gear or long-sleeved shirts and long pants, elimination of stagnant waters as breeding sites and avoiding travel to endemic areas if possible. Integrated mosquito management by local governments is also a crucial component to control mosquitoes. Methods employed include mosquito surveillance, larval control by source reduction and larvicide application, adult mosquito control by targeted or space spraying and so on [169].

7.2 Screening Blood Products

Screening of blood-donors can also help reduce acquisition of flaviviruses through transfusion of blood products. The FDA has issued guidance to test the blood supply for WNV and ZIKV, but not for other arboviruses such as DENV or CHIKV [170]. For WNV [171], the FDA recommends testing donor samples by NAT in "minipools" (MP-NAT), and if reactive, followed by testing of the individual donations (ID-NAT). Year-round screening should be performed on donor samples with either MP-NAT or ID-NAT; however, ID-NAT screening is recommended during periods of high WNV activity.

For ZIKV [172], the FDA had initially issued guidance in February 2016 recommending deferral of donors who reported a diagnosis of ZIKV infection, or might be at risk for ZIKV through travel or sexual contact. For areas with active transmission, the FDA had recommended discontinuing local blood collections and obtaining blood components from unaffected areas of the US. However, with the first local mosquito-borne cases of ZIKV reported in Florida in July 2016 and the concern for potential rapid spread of ZIKV in the US, the FDA revised its recommendation in August 2016 that ID-NAT should be performed on all blood donations to minimize the risk of ZIKV transmission through blood transfusion. Saá *et al.* have recently analyzed this approach and found it costly with a low yield [173]. With ~4 million donations screened for ZIKV infection by ID-NAT, 160 were initially reactive and 9 were confirmed positive. In total, the cost of identifying these "true-positives" through ID-NAT was 5.3 million USD each. The Blood Products Advisory Committee subsequently reviewed the available data on ZIKV blood donor screening and the evolving ZIKV epidemiology in the US, and determined that the current incidence and prevalence of ZIKV did not support continued universal ID-NAT. Thus, in July 2018, the FDA issued revised guidance to recommend the use of MP-NAT year-round in all US states and territories, but with defined criteria to switch to ID-NAT when local mosquito-borne ZIKV transmission is suspected or documented [172].

7.3 Screening Organ Donors

The Organ Procurement and Transplant Network (OPTN) in the US currently does not require WNV laboratory testing of all organ donors. Instead, organ procurement organizations are advised to defer deceased donors with encephalitis, meningitis, or acute flaccid paralysis of undetermined etiology from regions with reported WNV activity [174, 175]. Current OPTN policy requires that living donors be evaluated for risk of transmitting WNV to recipients. As most WNV infections are asymptomatic, laboratory screening is recommended during periods of human WNV activity where the donor lives, works or travels. Screening of living donors with WNV-NAT should be performed as close to the time of donation as possible [176]. Recommendations for identification of potentially WNV-infected SOT donors has been outlined by Singh and Levi [177].

As most people infected with WNV remain asymptomatic, prevention of WNV transmission through organ transplantation relies on the exclusion of donors with viremia. However, among the 9 clusters of organ-transmitted WNV, screening by NAT alone would have only identified 5 donors as acutely infected with WNV (Table 1). This certainly underscores the fact that current methodologies for donor screening for WNV are imperfect. Clinicians evaluating donors for organ transplantation or taking care of transplant recipients need to carry a high index of suspicion for WNV infection when the patient presents with a febrile illness and neurological symptoms, especially during the peak season of transmission in endemic areas.

For ZIKV, OPTN recommends that either deceased or living donors with exposure to ZIKV and compatible symptoms should be used with caution. Living donors with symptoms could be tested for ZIKV with the assistance of state health departments. Testing for DENV and CHIKV would be appropriate as well. No testing is currently available in a time frame likely to assist in the decision of whether or not to procure organs from a potential deceased donor, but an active viral infection will most likely rule out a donor as a viable option [178].

As ZIKV can persist in tissues, it is uncertain the amount of time that a donor with a positive NAT for ZIKV should be deferred. The FDA, however, has defined a 4-week deferral period for blood donation after travel to a ZIKV endemic area. For donors of human cells, tissues, and cellular and tissue-based products (HCT/P), the FDA requires a 6-month deferral for both living and cadaveric donors [179]. For deceased organ donors, no automatic deferral is recommended at this time [178].

For regions endemic for other flaviviruses, such as DENV, the incidence of transmission through transplanted organs is difficult to estimate, as screening of donors for these infections is not routinely performed. A few cases of DENV infections were reported in the immediate post-transplant periods [75, 180, 181], but in some cases, whether the recipients were infected by natural transmission or donor organs could not be easily determined. In particular, several of these cases involved live kidney or liver donors, who tend to be relatives of the recipients. It is thus conceivable that both the donors and their recipients might have shared similar vector exposure prior to the elective organ transplant. As described above, 2 clusters of DENV transmission through SOT were reported by Rosso *et al.* [74]. As a consequence, their hospital in Cali, Colombia implemented screening with NS1 antigen detection in donors during DENV outbreaks. To date, no additional cases have been detected since then (Rosso, personal communication).

Table 1 West Nile Virus (WNV) transmission through solid organ transplantation (SOT).

Year Location [Reference]	Donor or Recipient Age (year)/Sex	Trans-planted Organ	Mode of donor WNV acquisition	Clinical diagnosis of recipients	WNV testing					Treatment of recipients	Outcome of recipients
					NAT/PCR		Serology		Others		
					CSF	Serum	CSF	Serum			
2002 Georgia, USA [182]	Donor 20 F		Blood transfusion			(+)		IgM(-)	Serum (+) viral culture		
	Recipient 1 31 F	Kidney		WNV encephalitis			IgM(+)	IgM(+/-)		Supportive	Alive, with neurologic deficit
	Recipient 2 8 M	Kidney		WNV encephalitis			IgM(-)	IgM(-)	Brain at autopsy PCR(+)/ IHC(+)/ viral culture(+)	Supportive	Died
	Recipient 3 63 M	Heart		WNV encephalitis		(+)	IgM(+)	IgM(+)		Supportive	Alive
	Recipient 4 71 F	Liver		WNV fever				IgM(+)		Supportive	Alive
2005 New York, USA [131]	Donor NA		Mosquito-borne			(-)		IgM(+) IgG(+)			

	Recipient 1 NA	Liver		WNV encephalitis	(+)		IgM(+)	IgM(+)		Omr-IgG-am	Comatose
	Recipient 2 NA	Lung		WNV encephalitis	(-)		IgM(+) IgG(+)	IgM(+) IgG(+)		Omr-IgG-am	Comatose
	Recipient 3 NA	Kidney		Asymptomatic		(+)		IgM(-) IgG(+)		Omr-IgG-am	Alive
	Recipient 4 NA	Kidney		Not infected		(-)		IgM(-) IgG(-)		Omr-IgG-am	Alive
2008 Louisiana, USA [183]	Donor 18 M		Blood transfusion			(-)		IgM(-) IgG(-)	Blood donor to organ donor (+)IgM		
	Recipient 62 M	Heart		WNV encephalitis			IgM(+)	IgM(+)		Supportive	Alive, with neurologic deficit
2009 Italy [94]	Donor 78 F		Mosquito-borne			(+)					
	Recipient 25 F	Liver		Asymptomatic		(+)		IgM(+)		FFP, Omr-IgG-am	Alive
2009 California, USA [127]	Donor 53 M		Possibly mosquito-borne			(+)		IgM(-)			

	Recipient 55 M	Liver		WNV encephalitis		(-)	IgM(+)	IgM(+)		IVIG	Alive
2009 USA [175]	Donor 50 M		Mosquito-borne			(+)		IgM(+) IgG(+/-)			
	Recipient 1 55 M	Kidney		WNV encephalitis						NA	Alive
	Recipient 2 54 F	Kidney		Not infected						NA	Alive
	Recipient 3 49M	Liver		Not infected						NA	Alive
2010 USA [175]	Donor 55 M		Mosquito-borne			(+)		IgM(-) IgG(+)			
	Recipient 1 NA	Kidney		WNV encephalitis						NA	Died
	Recipient 2 NA	Kidney		Asymptomatic						NA	Alive
	Recipient 3 NA	Liver		Not infected						NA	Alive
2011 Italy [184]	Donor NA		NA			(-)		IgM(+) IgG(+)			
	Recipient 1 NA	Kidney		WNV encephalitis	(+)	(+)	IgM(+) IgG(+)	IgM(+) IgG(+)		IVIG with high WN titer	Critically ill
	Recipient 2 NA	Kidney		WNV encephalitis	(+)	(+)	IgM(+) IgG(+)	IgM(+) IgG(+)		NA	Critically ill
	Recipient 3	Heart		Asymptomatic		(-)		IgM(-)		NA	Alive and

	NA							IgG(-)			well
	Recipient 4 NA	Liver		Asymptomatic		(-)		IgM(+) IgG(+)		NA	Alive and well
	Recipient 5 NA	Lung		Neurological symptoms due to drug toxicity		(+)		IgM(+) IgG(+)			Neurological symptoms due to drug toxicity
2011 California, USA [34]	Donor 56 M		Mosquito-borne			(-)		IgM(+) IgG(+)	Tissues (+)PCR		
	Recipient 1 59 M	Kidney		WNV encephalitis	(+)	(+)	(-)	(-)		IVIG, IFN,	Died
	Recipient 2 51 M	Kidney		WNV encephalitis	(+)	(+)	(-)	IgM(+)		IVIG, IFN, FFP	Alive, no residual neurologic deficits
	Recipient 3 59 M	Lung		WNV encephalitis	(+)	(+)	IgM(+) IgG(+)	(-)		IVIG, IFN	Died
	Recipient 4 63 M	Liver		Asymptomatic	(+)	(-)	(-)	IgM(-) IgG(+)		IVIG, ribavirin	Alive and well

M, male; F, female; NA, not available; NAT, nucleic acid testing; PCR, polymerase chain reaction; CSF, cerebral spinal fluid; IHC, WNV specific immunohistochemical staining; FFP, fresh frozen plasma; IVIG, intravenous immunoglobulin; IFN, interferon alpha-2b.

In general, it is recommended that for donors with a cause of death clinically compatible with an arborvirus infection, donations should be used with great caution.

7.4 Vaccines

A detailed discussion of vaccines against flaviviruses is beyond the scope of this review. Currently, several WNV vaccines are licensed for use in horses, but no WNV or ZIKV vaccines are available for human use. A number of WNV and ZIKV vaccines are currently in development using various strategies, including mRNA- or DNA-based, inactivated, live-attenuated, viral vector-based vaccines and so on [185-187]. A number of these vaccines are in phase I or phase II clinical trials. Universal WNV or ZIKV vaccination in the US is not likely to be cost-effective [188], given the sporadic nature of WNV and limited spread of ZIKV at this time. However, certain high-risk populations, such as those with immunodeficiency and/or residence in endemic areas, may benefit from a safe and effective vaccine.

7.5 Travel Recommendations for SOT Recipients

Travel advice for SOT recipients has been recently reviewed [189, 190]. Among the flaviviruses, vaccines approved for human use in the US are available for YFV and JEV, but they are either contraindicated or with uncertain efficacies for immunocompromised patients. The only available vaccine for YFV is a live-attenuated vaccine (YF-Vax) and is contraindicated post SOT due to the risks of prolonged viremia and encephalitis [189, 190]. Immunocompromised persons should avoid travelling to areas where yellow fever is endemic [191]. For JEV vaccines, both live-attenuated and inactivated versions have been marketed, but only an inactivated Vero-cell culture-derived vaccine (JE-VC; manufactured as Ixiaro®) is currently available in the US. While this vaccine is safe for SOT recipients, data on its immunogenicity and efficacy in SOT or other immunocompromised populations are lacking.

In general, the epidemiology of flaviviruses is a constantly changing landscape. SOT recipients and their physicians are encouraged to consult the CDC's travel webpage [192] for updated information on areas at risk for ZKV, YFV, JEV, WNV and other arboviruses. Taking steps to prevent mosquito bites remains the most important measure to protect against flavivirus infection when traveling to endemic areas, as discussed above.

8. Summary

Despite both being mosquito-borne, WNV and ZIKV have apparently taken different courses in the US; WNV has become endemic since its introduction in 1999, but ZIKV activity has apparently peaked in 2016 after its arrival, with no local mosquito-borne transmission in 2018 to date. Regardless, both viruses have emerged among susceptible hosts with unexpected virulence and new clinical syndromes, while limited preventive and therapeutic options are available. Immunocompromised populations are particularly vulnerable and may experience increased morbidity and mortality to flavivirus infections. The potential of donor-derived transmission in SOT remains a concern. To respond to the threat of these viruses, a multi-pronged approach that includes research on viral, host, ecologic and entomologic factors, development of improved diagnostics and therapeutics, as well as effective public health strategies, is urgently needed.

As pointed out by Fauci and Morens [193], pandemic expansion of arboviruses previously restricted to remote ecologic niches is a potential new disease-emergence phenomenon. Physicians caring for SOT populations must be vigilant against such threats and be prepared to manage transplant recipients amidst such endemics or epidemics.

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Competing Interests

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References

1. Lindenbach BD, Murray CL, Thiel H, Rice CM. Flaviviridae. In: Knipe DM, Howley PM, editors. Fields virology. 6th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2013.
2. Komar N. West Nile virus: epidemiology and ecology in North America. Adv Virus Res. 2003; 61: 185-234.
3. Thomas SJ, Endy TP, Rothman AL, Barrett AD. Flaviviruses. In: Bennett JE, Dolin R, Blaser MJ, editors. Principles and Pracice of Infectious Diseases. 2. 8th ed. Philadelphia, PA: Elsevier Saunders; 2015.
4. Murray KO, Walker C, Gould E. The virology, epidemiology, and clinical impact of West Nile virus: a decade of advancements in research since its introduction into the Western Hemisphere. Epidemiol Infect. 2011; 139: 807-817.
5. CDC. https://www.cdc.gov/westnile/index.html. 2018.
6. Petersen LR, Fischer M. Unpredictable and difficult to control--the adolescence of West Nile virus. N Engl J Med. 2012; 367: 1281-1284.
7. Lindsey NP, Staples JE, Lehman JA, Fischer M. Surveillance for human West Nile virus disease - United States, 1999-2008. MMWR Surveill Summ. 2010; 59: 1-17.
8. CDC. Investigation of West Nile viiurs infection in recipients of blood transfusion. MMWR Morb Mortal Wkly Rep. 2002; 51: 973-974.
9. Meny GM, Santos-Zabala L, Szallasi A, Stramer SL. West Nile virus infection transmitted by granulocyte transfusion. Blood. 2011; 117: 5778-5779.

10. CDC. Possible West Nile virus transmission to an infant through breast-feeding: Michigan, 2002. MMWR Morb Mortal Wkly Rep. 2002; 51: 877-878.
11. CDC. Intrauterine West Nile virus infection: New York, 2002. MMWR Morb Mortal Wkly Rep. 2002; 51: 1135-1136.
12. CDC. Laboratory-acquired West Nile virus infections--United States, 2002. MMWR Morb Mortal Wkly Rep. 2002; 51: 1133-1135.
13. Petersen LR, Jamieson DJ, Powers AM, Honein MA. Zika Virus. N Engl J Med. 2016; 374: 1552-1563.
14. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med. 2009; 360: 2536-2543.
15. Cao-Lormeau VM, Roche C, Teissier A, Robin E, Berry AL, Mallet HP, et al. Zika virus, French polynesia, South pacific, 2013. Emerg Infect Dis. 2014; 20: 1085-1086.
16. Baud D, Gubler DJ, Schaub B, Lanteri MC, Musso D. An update on Zika virus infection. Lancet. 2017; 390: 2099-2109.
17. Metsky HC, Matranga CB, Wohl S, Schaffner SF, Freije CA, Winnicki SM, et al. Zika virus evolution and spread in the Americas. Nature. 2017; 546: 411-415.
18. Musso D, Nhan T, Robin E, Roche C, Bierlaire D, Zisou K, et al. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. Euro surveillance: bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2014; 19.
19. Atkinson B, Hearn P, Afrough B, Lumley S, Carter D, Aarons EJ, et al. Detection of Zika virus in Semen. Emerg Infect Dis. 2016; 22: 940.
20. Gourinat AC, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of Zika virus in urine. Emerg Infect Dis. 2015; 21: 84-86.
21. Musso D, Roche C, Nhan TX, Robin E, Teissier A, Cao-Lormeau VM. Detection of Zika virus in saliva. J Clin Virol. 2015; 68: 53-55.
22. Calvet G, Aguiar RS, Melo ASO, Sampaio SA, de Filippis I, Fabri A, et al. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. Lancet Infect Dis. 2016; 16: 653-660.
23. Colt S, Garcia-Casal MN, Pena-Rosas JP, Finkelstein JL, Rayco-Solon P, Weise Prinzo ZC, et al. Transmission of Zika virus through breast milk and other breastfeeding-related bodily-fluids: A systematic review. PLoS Negl Trop Dis. 2017; 11: e0005528.
24. Grischott F, Puhan M, Hatz C, Schlagenhauf P. Non-vector-borne transmission of Zika virus: A systematic review. Travel Med Infect Dis. 2016; 14: 313-330.
25. Lazear HM, Diamond MS. Zika virus: New clinical syndromes and its emergence in the western hemisphere. J Virol. 2016; 90: 4864-4875.
26. CDC. https://www.cdc.gov/zika/index.html. 2018.
27. Halstead SB. Pathogenesis of dengue: challenges to molecular biology. Science. 1988; 239: 476-481.
28. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, et al. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. J Infect Dis. 2000; 181: 2-9.

29. Priyamvada L, Hudson W, Ahmed R, Wrammert J. Humoral cross-reactivity between Zika and dengue viruses: implications for protection and pathology. Emerg Microbes Infect. 2017; 6: e33.
30. Dejnirattisai W, Supasa P, Wongwiwat W, Rouvinski A, Barba-Spaeth G, Duangchinda T, et al. Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with zika virus. Nat Immunol. 2016; 17: 1102-1108.
31. Bardina SV, Bunduc P, Tripathi S, Duehr J, Frere JJ, Brown JA, et al. Enhancement of Zika virus pathogenesis by preexisting antiflavivirus immunity. Science. 2017; 356: 175-180.
32. Terzian ACB, Schanoski AS, Mota MTO, da Silva RA, Estofolete CF, Colombo TE, et al. Viral load and cytokine response profile does not support antibody-dependent enhancement in Dengue-Primed Zika virus-infected patients. Clin Infect Dis. 2017; 65: 1260-1265.
33. Pealer LN, Marfin AA, Petersen LR, Lanciotti RS, Page PL, Stramer SL, et al. Transmission of West Nile virus through blood transfusion in the United States in 2002. N Engl J Med. 2003; 349: 1236-1245.
34. Winston DJ, Vikram HR, Rabe IB, Dhillon G, Mulligan D, Hong JC, et al. Donor-derived West Nile virus infection in solid organ transplant recipients: report of four additional cases and review of clinical, diagnostic, and therapeutic features. Transplantation. 2014; 97: 881-889.
35. Mostashari F, Bunning ML, Kitsutani PT, Singer DA, Nash D, Cooper MJ, et al. Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey. Lancet. 2001; 358: 261-264.
36. Zou S, Foster GA, Dodd RY, Petersen LR, Stramer SL. West Nile fever characteristics among viremic persons identified through blood donor screening. J Infect Dis. 2010; 202: 1354-1361.
37. Buber J, Fink N, Bin H, Mouallem M. West Nile virus-induced pancreatitis. Travel Med Infect Dis. 2008; 6: 373-375.
38. Khouzam RN. Significant cardiomyopathy secondary to West Nile virus infection. South Med J. 2009; 102: 527-528.
39. Pergam SA, DeLong CE, Echevarria L, Scully G, Goade DE. Myocarditis in West Nile virus infection. Am J Trop Med Hyg. 2006; 75: 1232-1233.
40. Smith RD, Konoplev S, DeCourten-Myers G, Brown T. West Nile virus encephalitis with myositis and orchitis. Human Pathol. 2004; 35: 254-258.
41. Kumar D, Prasad GV, Zaltzman J, Levy GA, Humar A. Community-acquired West Nile virus infection in solid-organ transplant recipients. Transplantation. 2004; 77: 399-402.
42. Huhn GD, Austin C, Langkop C, Kelly K, Lucht R, Lampman R, et al. The emergence of west nile virus during a large outbreak in Illinois in 2002. Am J Trop Med Hyg. 2005; 72: 768-776.
43. Jean CM, Honarmand S, Louie JK, Glaser CA. Risk factors for West Nile virus neuroinvasive disease, California, 2005. Emerg Infect Dis. 2007; 13: 1918-1920.
44. Murray KO, Koers E, Baraniuk S, Herrington E, Carter H, Sierra M, et al. Risk factors for encephalitis from West Nile Virus: a matched case-control study using hospitalized controls. Zoonoses Public Health. 2009; 56: 370-375.
45. Debiasi RL. West nile virus neuroinvasive disease. Curr Infect Dis Rep. 2011; 13: 350-359.
46. Sejvar JJ, Marfin AA. Manifestations of West Nile neuroinvasive disease. Rev Med Virol. 2006; 16: 209-224.
47. Jeha LE, Sila CA, Lederman RJ, Prayson RA, Isada CM, Gordon SM. West Nile virus infection: a new acute paralytic illness. Neurology. 2003; 61: 55-59.

48. Saad M, Youssef S, Kirschke D, Shubair M, Haddadin D, Myers J, et al. Acute flaccid paralysis: the spectrum of a newly recognized complication of West Nile virus infection. J Infect. 2005; 51: 120-127.
49. CDC. Acute flaccid paralysis syndrome associated with West Nile virus infection - Mississippi and Louisiana, July-August 2002. MMWR Morb Mortal Wkly Rep. 2002; 51: 862-828.
50. Sejvar JJ. The long-term outcomes of human West Nile virus infection. Clin Infect Dis. 2007; 44: 1617-1624.
51. Carson PJ, Konewko P, Wold KS, Mariani P, Goli S, Bergloff P, et al. Long-term clinical and neuropsychological outcomes of West Nile virus infection. Clin Infect Dis. 2006; 43: 723-730.
52. Cook RL, Xu X, Yablonsky EJ, Sakata N, Tripp JH, Hess R, et al. Demographic and clinical factors associated with persistent symptoms after West Nile virus infection. Am J Trop Med Hyg. 2010; 83: 1133-1136.
53. Weatherhead JE, Miller VE, Garcia MN, Hasbun R, Salazar L, Dimachkie MM, et al. Long-term neurological outcomes in West Nile virus-infected patients: an observational study. Am J Trop Med Hyg. 2015; 92: 1006-1012.
54. Heang V, Yasuda CY, Sovann L, Haddow AD, Travassos da Rosa AP, Tesh RB, et al. Zika virus infection, Cambodia, 2010. Emerg Infect Dis. 2012; 18: 349-351.
55. Dos Santos T, Rodriguez A, Almiron M, Sanhueza A, Ramon P, de Oliveira WK, et al. Zika virus and the guillain-barre syndrome - Case series from seven countries. N Engl J Med. 2016; 375: 1598-1601.
56. Carteaux G, Maquart M, Bedet A, Contou D, Brugieres P, Fourati S, et al. Zika virus associated with meningoencephalitis. N Engl J Med. 2016; 374: 1595-1596.
57. da Silva IRF, Frontera JA, Bispo de Filippis AM, Nascimento O. Neurologic complications associated with the Zika virus in Brazilian adults. JAMA Neurol. 2017; 74: 1190-1198.
58. Kodati S, Palmore TN, Spellman FA, Cunningham D, Weistrop B, Sen HN. Bilateral posterior uveitis associated with Zika virus infection. Lancet. 2017; 389: 125-126.
59. Cao-Lormeau VM, Blake A, Mons S, Lastere S, Roche C, Vanhomwegen J, et al. Guillain-barre syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. Lancet. 2016; 387: 1531-1539.
60. Azevedo RS, Araujo MT, Martins Filho AJ, Oliveira CS, Nunes BT, Cruz AC, et al. Zika virus epidemic in Brazil. I. Fatal disease in adults: Clinical and laboratorial aspects. J Clin Virol. 2016; 85: 56-64.
61. Soares CN, Brasil P, Carrera RM, Sequeira P, de Filippis AB, Borges VA, et al. Fatal encephalitis associated with Zika virus infection in an adult. J Clin Virol. 2016; 83: 63-65.
62. Swaminathan S, Schlaberg R, Lewis J, Hanson KE, Couturier MR. Fatal Zika Virus Infection with Secondary Nonsexual Transmission. N Engl J Med. 2016; 375: 1907-1909.
63. Zonneveld R, Roosblad J, Staveren JW, Wilschut JC, Vreden SG, Codrington J. Three atypical lethal cases associated with acute Zika virus infection in Suriname. IDCases. 2016; 5: 49-53.
64. Tang H, Hammack C, Ogden SC, Wen Z, Qian X, Li Y, et al. Zika virus infects human cortical neural progenitors and attenuates their growth. Cell Stem Cell. 2016; 18: 587-590.
65. Song BH, Yun SI, Woolley M, Lee YM. Zika virus: History, epidemiology, transmission, and clinical presentation. J Neuroimmunol. 2017; 308: 50-64.
66. Mezochow AK, Henry R, Blumberg EA, Kotton CN. Transfusion transmitted infections in solid organ transplantation. Am J Transplant. 2015; 15: 547-554.

67. Weinberger M, Pitlik SD, Gandacu D, Lang R, Nassar F, Ben David D, et al. West Nile fever outbreak, Israel, 2000: epidemiologic aspects. Emerg Infect Dis. 2001; 7: 686-691.
68. Kleinschmidt-DeMasters BK, Marder BA, Levi ME, Laird SP, McNutt JT, Escott EJ, et al. Naturally acquired West Nile virus encephalomyelitis in transplant recipients: clinical, laboratory, diagnostic, and neuropathological features. Arch Neurol. 2004; 61: 1210-1220.
69. Kumar D, Drebot MA, Wong SJ, Lim G, Artsob H, Buck P, et al. A seroprevalence study of west nile virus infection in solid organ transplant recipients. Am J Transplant. 2004; 4: 1883-1888.
70. Brenner W, Storch G, Buller R, Vij R, Devine S, DiPersio J. West Nile virus encephalopathy in an allogeneic stem cell transplant recipient: use of quantitative PCR for diagnosis and assessment of viral clearance. Bone Marrow Transplant. 2005; 36: 369-370.
71. Kitagawa MG, Ettinger N, Breen D, Erklauer J, Chang E, Herce H, et al. Transmission of West Nile virus through a hematopoietic stem cell transplant. J Pediat Infect Dis Soc. 2018; 7: e52-e54.
72. Nogueira ML, Estofolete CF, Terzian AC, Mascarin do Vale EP, da Silva RC, da Silva RF, et al. Zika virus infection and solid organ transplantation: A new challenge. Am J Transplant. 2017; 17: 791-795.
73. Schwartzmann PV, Ramalho LN, Neder L, Vilar FC, Ayub-Ferreira SM, Romeiro MF, et al. Zika virus meningoencephalitis in an immunocompromised patient. Mayo Clin Proc. 2017; 92: 460-466.
74. Rosso F, Pineda JC, Sanz AM, Cedano JA, Caicedo LA. Transmission of dengue virus from deceased donors to solid organ transplant recipients: case report and literature review. Brazil J Infect Dis. 2018; 22: 63-69.
75. Gupta RK, Gupta G, Chorasiya VK, Bag P, Shandil R, Bhatia V, et al. Dengue virus transmission from living donor to recipient in liver transplantation: A case report. J Clin Exp Hepatol. 2016; 6: 59-61.
76. Nasim A, Anis S, Baqi S, Akhtar SF, Baig-Ansari N. Clinical presentation and outcome of dengue viral infection in live-related renal transplant recipients in Karachi, Pakistan. Transpl Infect Dis. 2013; 15: 516-525.
77. Weerakkody RM, Patrick JA, Sheriff MH. Dengue fever in renal transplant patients: a systematic review of literature. BMC Nephrol. 2017; 18: 15.
78. Hartmann CA, Vikram HR, Seville MT, Orenstein R, Kusne S, Blair JE, et al. Neuroinvasive St. Louis encephalitis virus infection in solid organ transplant recipients. Am J Transplant. 2017; 17: 2200-2206.
79. Venkat H, Adams L, Sunenshine R, Krow-Lucal E, Levy C, Kafenbaum T, et al. St. Louis encephalitis virus possibly transmitted through blood transfusion-Arizona, 2015. Transfusion. 2017; 57: 2987-2994.
80. Nash D, Mostashari F, Fine A, Miller J, O'Leary D, Murray K, et al. The outbreak of West Nile virus infection in the New York City area in 1999. N Engl J Med. 2001; 344: 1807-1814.
81. Weiss D, Carr D, Kellachan J, Tan C, Phillips M, Bresnitz E, et al. Clinical findings of West Nile virus infection in hospitalized patients, New York and New Jersey, 2000. Emerg Infect Dis. 2001; 7: 654-658.
82. Crichlow R, Bailey J, Gardner C. Cerebrospinal fluid neutrophilic pleocytosis in hospitalized West Nile virus patients. J Am Board Fam Pract. 2004; 17: 470-472.

83. Davis LE, DeBiasi R, Goade DE, Haaland KY, Harrington JA, Harnar JB, et al. West Nile virus neuroinvasive disease. Ann Neurol. 2006; 60: 286-300.
84. Petropoulou KA, Gordon SM, Prayson RA, Ruggierri PM. West Nile virus meningoencephalitis: MR imaging findings. Am J Neuroradiol. 2005; 26: 1986-1995.
85. Asnis DS, Conetta R, Waldman G, Teixeira AA. The West Nile virus encephalitis outbreak in the United States (1999-2000): from Flushing, New York, to beyond its borders. Ann NY Acad Sci. 2001; 951: 161-171.
86. ECDC. West Nile virus infection (WNV). Off J Eur Union. 2018: L 170/150-153.
87. Chung WM, Buseman CM, Joyner SN, Hughes SM, Fomby TB, Luby JP, et al. The 2012 West Nile encephalitis epidemic in Dallas, Texas. Jama. 2013;310(3):297-307.
88. Freifeld AG, Meza J, Schweitzer B, Shafer L, Kalil AC, Sambol AR. Seroprevalence of West Nile virus infection in solid organ transplant recipients. Transpl Infect Dis. 2010; 12: 120-126.
89. Barzon L, Pacenti M, Franchin E, Pagni S, Martello T, Cattai M, et al. Excretion of West Nile virus in urine during acute infection. J Infect Dis. 2013; 208: 1086-1092.
90. Barzon L, Pacenti M, Franchin E, Squarzon L, Sinigaglia A, Ulbert S, et al. Isolation of West Nile virus from urine samples of patients with acute infection. J Clin Microbiol. 2014; 52: 3411-3413.
91. Levi ME, Quan D, Ho JT, Kleinschmidt-Demasters BK, Tyler KL, Grazia TJ. Impact of rituximab-associated B-cell defects on West Nile virus meningoencephalitis in solid organ transplant recipients. Clin Transplant. 2010; 24: 223-228.
92. Busch MP, Kleinman SH, Tobler LH, Kamel HT, Norris PJ, Walsh I, et al. Virus and antibody dynamics in acute west nile virus infection. J Infect Dis. 2008; 198: 984-993.
93. Prince HE, Tobler LH, Yeh C, Gefter N, Custer B, Busch MP. Persistence of West Nile virus-specific antibodies in viremic blood donors. Clin Vaccine Immunol. 2007; 14: 1228-1230.
94. Morelli MC, Sambri V, Grazi GL, Gaibani P, Pierro A, Cescon M, et al. Absence of neuroinvasive disease in a liver transplant recipient who acquired West Nile virus (WNV) infection from the organ donor and who received WNV antibodies prophylactically. Clin Infect Dis. 2010; 51: e34-e37.
95. Levi ME. West Nile Virus Infection in the Immunocompromised Patient. Curr Infect Dis Rep. 2013; 15: 478-485.
96. Prince HE, Calma J, Pham T, Seaton BL. Frequency of missed cases of probable acute West Nile virus (WNV) infection when testing for WNV RNA alone or WNV immunoglobulin M alone. Clin Vaccine Immunol. 2009; 16: 587-588.
97. Sambri V, Capobianchi MR, Cavrini F, Charrel R, Donoso-Mantke O, Escadafal C, et al. Diagnosis of west nile virus human infections: overview and proposal of diagnostic protocols considering the results of external quality assessment studies. Viruses. 2013; 5: 2329-2348.
98. Sanchini A, Donoso-Mantke O, Papa A, Sambri V, Teichmann A, Niedrig M. Second international diagnostic accuracy study for the serological detection of West Nile virus infection. PLoS Negl Trop Dis. 2013; 7: e2184.
99. Chabierski S, Makert GR, Kerzhner A, Barzon L, Fiebig P, Liebert UG, et al. Antibody responses in humans infected with newly emerging strains of West Nile Virus in Europe. PLoS One. 2013; 8: e66507.
100. Brien JD, Lazear HM, Diamond MS. Propagation, quantification, detection, and storage of West Nile virus. Curr Protoc Microbiol. 2013; 31: 15d.13.11-15d.13.18.

101. Akey DL, Brown WC, Dutta S, Konwerski J, Jose J, Jurkiw TJ, et al. Flavivirus NS1 structures reveal surfaces for associations with membranes and the immune system. Science. 2014; 343: 881-885.
102. Alcon S, Talarmin A, Debruyne M, Falconar A, Deubel V, Flamand M. Enzyme-linked immunosorbent assay specific to Dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. J Clin Microbiol. 2002; 40: 376-381.
103. Ding XX, Li XF, Deng YQ, Guo YH, Hao W, Che XY, et al. Development of a double antibody sandwich ELISA for West Nile virus detection using monoclonal antibodies against non-structural protein 1. PLoS One. 2014; 9: e108623.
104. Saxena D, Kumar JS, Parida M, Sivakumar RR, Patro IK. Development and evaluation of NS1 specific monoclonal antibody based antigen capture ELISA and its implications in clinical diagnosis of West Nile virus infection. J Clin Virol. 2013; 58: 528-534.
105. Hygino da Cruz LC, Jr., Nascimento OJM, Lopes F, da Silva IRF. Neuroimaging findings of Zika virus-associated neurologic complications in adults. Am J Neuroradiol. 2018; 39: 1967-1974
106. Zare Mehrjardi M, Carteaux G, Poretti A, Sanei Taheri M, Bermudez S, Werner H, et al. Neuroimaging findings of postnatally acquired Zika virus infection: a pictorial essay. Jap J Radiol. 2017; 35: 341-349.
107. Atkinson B, Thorburn F, Petridou C, Bailey D, Hewson R, Simpson AJ, et al. Presence and Persistence of Zika Virus RNA in Semen, United Kingdom, 2016. Emerg Infect Dis. 2017; 23: 611-615.
108. FDA. Guidance for industry: revised recommendations for reducing the risk of Zika virus transmission by blood and blood components. CBER Office of Communication, Outreach, and Development. 2016.
109. Rabe IB, Staples JE, Villanueva J, Hummel KB, Johnson JA, Rose L, et al. Interim Guidance for Interpretation of Zika Virus Antibody Test Results. MMWR Morb Mortal Wkly Rep. 2016; 65: 543-546.
110. Levi ME. Zika virus: a cause of concern in transplantation? Curr Opin Infect Dis. 2017; 30: 340-345.
111. Waggoner JJ, Gresh L, Mohamed-Hadley A, Ballesteros G, Davila MJ, Tellez Y, et al. Single-Reaction Multiplex Reverse Transcription PCR for Detection of Zika, Chikungunya, and Dengue Viruses. Emerg Infect Dis. 2016; 22: 1295-1297.
112. Galel SA, Williamson PC, Busch MP, Stanek D, Bakkour S, Stone M, et al. First Zika-positive donations in the continental United States. Transfusion. 2017; 57: 762-769.
113. Calvet GA, Kara EO, Giozza SP, Botto-Menezes CHA, Gaillard P, de Oliveira Franca RF, et al. Study on the persistence of Zika virus (ZIKV) in body fluids of patients with ZIKV infection in Brazil. BMC Infect Dis. 2018; 18: 49.
114. Paz-Bailey G, Rosenberg ES, Doyle K, Munoz-Jordan J, Santiago GA, Klein L, et al. Persistence of Zika Virus in Body Fluids - Final Report. N Engl J Med. 2018; 379: 1234-1243.
115. WHO. Laboratory testing for Zika virus infection: Interim guidance. World Health Organization. 2016:http://www.who.int/csr/resources/publications/zika/laboratory-testing/en/.
116. Lustig Y, Zelena H, Venturi G, Van Esbroeck M, Rothe C, Perret C, et al. Sensitivity and Kinetics of an NS1-Based Zika Virus Enzyme-Linked Immunosorbent Assay in Zika Virus-Infected

Travelers from Israel, the Czech Republic, Italy, Belgium, Germany, and Chile. J Clin Microbiol. 2017; 55: 1894-1901.
117. Tsai WY, Youn HH, Tyson J, Brites C, Tsai JJ, Pedroso C, et al. Use of Urea Wash ELISA to Distinguish Zika and Dengue Virus Infections. Emerg Infect Dis. 2018; 24: 1355-1359.
118. Sardi SI SS, Naccache SN, Bandeira AC, Tauro LB, Campos GS, Chiu CY. Coinfections of Zika and Chikungunya viruses in Bahia, Brazil, identified by Metagenomic next-generation sequencing. J Clin Microbiol. 2016; 54: 2348-2353.
119. Wilson MR, Zimmermann LL, Crawford ED, Sample HA, Soni PR, Baker AN, et al. Acute West Nile virus meningoencephalitis diagnosed via metagenomic deep sequencing of cerebrospinal fluid in a renal transplant patient. Am J Transplant. 2017; 17: 803-808.
120. Kattika Kaarj PAJ-YY. Simpler, faster, and sensitive Zika virus assay using smartphone detection of loop-mediated isothermal amplification on paper microfluidic chips. Sci Rep. 2018; 8: 12438.
121. Theel ES, Hata DJ. Diagnostic testing for Zika virus: A postoutbreak update. J Clin Microbiol. 2018; 56. doi: 10.1128/JCM.01972-17.
122. Diamond MS. Progress on the development of therapeutics against West Nile virus. Antiviral Res. 2009; 83: 214-227.
123. Srivastava R, Ramakrishna C, Cantin E. Anti-inflammatory activity of intravenous immunoglobulins protects against West Nile virus encephalitis. J Gen Virol. 2015; 96: 1347-1357.
124. Planitzer CB, Modrof J, Kreil TR. West Nile virus neutralization by US plasma-derived immunoglobulin products. J Infect Dis. 2007; 196: 435-440.
125. Hamdan A, Green P, Mendelson E, Kramer MR, Pitlik S, Weinberger M. Possible benefit of intravenous immunoglobulin therapy in a lung transplant recipient with West Nile virus encephalitis. Transpl Infect Dis. 2002; 4: 160-162.
126. Saquib R, Randall H, Chandrakantan A, Spak CW, Barri YM. West Nile virus encephalitis in a renal transplant recipient: the role of intravenous immunoglobulin. Am J Kidney Dis. 2008; 52: e19-e21.
127. Rhee C, Eaton EF, Concepcion W, Blackburn BG. West Nile virus encephalitis acquired via liver transplantation and clinical response to intravenous immunoglobulin: case report and review of the literature. Transpl Infect Dis. 2011; 13: 312-317.
128. Haley M, Retter AS, Fowler D, Gea-Banacloche J, O'Grady NP. The role for intravenous immunoglobulin in the treatment of West Nile virus encephalitis. Clin Infect Dis. 2003; 37: e88-e90.
129. Agrawal AG, Petersen LR. Human immunoglobulin as a treatment for West Nile virus infection. J Infect Dis. 2003; 188: 1-4.
130. Ben-Nathan D, Lustig S, Tam G, Robinzon S, Segal S, Rager-Zisman B. Prophylactic and therapeutic efficacy of human intravenous immunoglobulin in treating West Nile virus infection in mice. J Infect Dis. 2003; 188: 5-12.
131. CDC. West Nile virus infections in organ transplant recipients--New York and Pennsylvania, August-September, 2005. MMWR Morb Mortal Wkly Rep. 2005; 54: 1021-1023.
132. Makhoul B, Braun E, Herskovitz M, Ramadan R, Hadad S, Norberto K. Hyperimmune gammaglobulin for the treatment of West Nile virus encephalitis. Israel Med Assoc J. 2009; 11: 151-153.

133. Campbell GL, Marfin AA, Lanciotti RS, Gubler DJ. West Nile virus. Lancet Infect Dis. 2002; 2: 519-529.
134. Petersen LR, Marfin AA. West Nile virus: a primer for the clinician. Ann Int Med. 2002; 137: 173-179.
135. Beigel JH, Nordstrom JL, Pillemer SR, Roncal C, Goldwater DR, Li H, et al. Safety and pharmacokinetics of single intravenous dose of MGAWN1, a novel monoclonal antibody to West Nile virus. Antimicrob Agents Chemother. 2010; 54: 2431-2436.
136. Wang Q, Yan J, Gao GF. Monoclonal antibodies against Zika virus: Therapeutics and their implications for vaccine design. J Virol. 2017; 91: e01049-17.
137. Sapparapu G, Fernandez E, Kose N, Bin C, Fox JM, Bombardi RG, et al. Neutralizing human antibodies prevent Zika virus replication and fetal disease in mice. Nature. 2016; 540: 443-447.
138. Beasley DW. Vaccines and immunotherapeutics for the prevention and treatment of infections with West Nile virus. Immunotherapy. 2011; 3: 269-285.
139. Boldescu V, Behnam MAM, Vasilakis N, Klein CD. Broad-spectrum agents for flaviviral infections: dengue, Zika and beyond. Nat Rev Drug Discov. 2017; 16: 565-586.
140. Chowers MY, Lang R, Nassar F, Ben-David D, Giladi M, Rubinshtein E, et al. Clinical characteristics of the West Nile fever outbreak, Israel, 2000. Emerg Infect Dis. 2001; 7: 675-678.
141. Graci JD, Cameron CE. Quasispecies, error catastrophe, and the antiviral activity of ribavirin. Virology. 2002; 298: 175-180.
142. Tam RC, Lau JY, Hong Z. Mechanisms of action of ribavirin in antiviral therapies. Antivir Chem Chemother. 2001; 12: 261-272.
143. Sookoian S, Castano G, Flichman D, Cello J. Effects of ribavirin on cytokine production of recall antigens and phytohemaglutinin-stimulated peripheral blood mononuclear cells. (Inhibitory effects of ribavirin on cytokine production). Ann Hepatol. 2004; 3: 104-107.
144. Morrey JD, Smee DF, Sidwell RW, Tseng C. Identification of active antiviral compounds against a New York isolate of West Nile virus. Antiviral Res. 2002; 55: 107-116.
145. Jordan I, Briese T, Fischer N, Lau JY, Lipkin WI. Ribavirin inhibits West Nile virus replication and cytopathic effect in neural cells. J Infect Dis. 2000; 182: 1214-1217.
146. Morrey JD, Day CW, Julander JG, Blatt LM, Smee DF, Sidwell RW. Effect of interferon-alpha and interferon-inducers on West Nile virus in mouse and hamster animal models. Antivir Chem Chemother. 2004; 15: 101-109.
147. Chan JF, Chik KK, Yuan S, Yip CC, Zhu Z, Tee KM, et al. Novel antiviral activity and mechanism of bromocriptine as a Zika virus NS2B-NS3 protease inhibitor. Antiviral Res. 2017; 141: 29-37.
148. Xu M, Lee EM, Wen Z, Cheng Y, Huang WK, Qian X, et al. Identification of small-molecule inhibitors of Zika virus infection and induced neural cell death via a drug repurposing screen. Nat Med. 2016; 22: 1101-1107.
149. da Silva S, Oliveira Silva Martins D, Jardim ACG. A review of the ongoing research on Zika virus treatment. Viruses. 2018; 10: 255.
150. Eyer L, Nencka R, Huvarová I, Palus M, Alves MJ, Gould EA, et al. Nucleoside inhibitors of Zika virus. J Infect Dis. 2016; 214: 707-711.
151. Bullard-Feibelman KM, Govero J, Zhu Z, Salazar V, Veselinovic M, Diamond MS, et al. The FDA-approved drug sofosbuvir inhibits Zika virus infection. Antiviral Res. 2017; 137: 134-140.

152. Zmurko J, Marques RE, Schols D, Verbeken E, Kaptein SJ, Neyts J. The viral polymerase inhibitor 7-Deaza-2'-C-Methyladenosine is a potent inhibitor of in vitro Zika virus replication and delays disease progression in a robust mouse infection model. PLOS Neglect Trop Dis. 2016: 10: e0004695.
153. Best SM. The many faces of the flavivirus NS5 protein in antagonism of type I interferon signaling. J Virol. 2017; 91: e01970-16. doi: 10.1128/JVI.01970-16.
154. Rahal JJ, Anderson J, Rosenberg C, Reagan T, Thompson LL. Effect of interferon-alpha2b therapy on St. Louis viral meningoencephalitis: clinical and laboratory results of a pilot study. J Infect Dis. 2004; 190: 1084-1087.
155. Solomon T, Dung NM, Wills B, Kneen R, Gainsborough M, Diet TV, et al. Interferon alfa-2a in Japanese encephalitis: a randomised double-blind placebo-controlled trial. Lancet. 2003; 361: 821-826.
156. Kalil AC, Devetten MP, Singh S, Lesiak B, Poage DP, Bargenquast K, et al. Use of interferon-alpha in patients with West Nile encephalitis: Report of 2 cases. Clin Infect Dis. 2005; 40: 764-766.
157. Lewis M, Amsden JR. Successful treatment of West Nile virus infection after approximately 3 weeks into the disease course. Pharmacotherapy. 2007; 27: 455-458.
158. Chan-Tack KM, Forrest G. Failure of interferon alpha-2b in a patient with West Nile virus meningoencephalitis and acute flaccid paralysis. Scand J Infect Dis. 2005; 37: 944-946.
159. Savidis G, Perreira JM, Portmann JM, Meraner P, Guo Z, Green S, et al. The IFITMs inhibit Zika virus replication. Cell Rep. 2016; 15: 2323-2330.
160. Li K, Markosyan RM, Zheng YM, Golfetto O, Bungart B, Li M, et al. IFITM Proteins Restrict Viral Membrane Hemifusion. PLoS Pathog. 2013: 9: e1003124.
161. Asif A, Manzoor S, Tuz-Zahra F, Saalim M, Ashraf M, Ishtiyaq J, et al. Zika virus: Immune evasion mechanisms, currently available therapeutic regimens, and vaccines. Viral Immunol. 2017; 30: 682–690.
162. Liu SY, Aliyari R, Chikere K, Li G, Marsden MD, Smith JK, et al. Interferon-Inducible Cholesterol-25-Hydroxylase Broadly Inhibits Viral Entry by Production of 25-Hydroxycholesterol. Immunity. 2013; 38: 92-105.
163. Blanc M, Hsieh WY, Robertson KA, Kropp KA, Forster T, Shui G, et al. The transcription factor STAT-1 couples macrophage synthesis of 25-hydroxycholesterol to the interferon antiviral response. Immunity. 2013; 38: 106-118.
164. O'Grady J. The immunoreactive patient: Rejection and autoimmune disease. Liver Transplant. 2011; 17 Suppl 3: S29-S33.
165. Baid S, Tolkoff-Rubin N, Saidman S, Chung R, Williams WW, Auchincloss H, et al. Acute humoral rejection in hepatitis C-infected renal transplant recipients receiving antiviral therapy. Am J Transplant. 2003; 3: 74-78.
166. Kumar P, Lee SK, Shankar P, Manjunath N. A single siRNA suppresses fatal encephalitis induced by two different flaviviruses. PLoS Med. 2006; 3: e96.
167. Deas TS, Bennett CJ, Jones SA, Tilgner M, Ren P, Behr MJ, et al. In vitro resistance selection and in vivo efficacy of morpholino oligomers against West Nile virus. Antimicrob Agents Chemother. 2007; 51: 2470-2482.
168. Lai JH, Lin YL, Hsieh SL. Pharmacological intervention for dengue virus infection. Biochem Pharmacol. 2017; 129: 14-25.

169. CDC. https://www.cdc.gov/zika/vector/integrated_mosquito_management.html. 2018.
170. Jimenez A, Shaz BH, Bloch EM. Zika virus and the blood supply: What do we know? Transfus Med Rev. 2017; 31: 1-10.
171. FDA. https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM189464.pdf. 2009.
172. FDA. https://wwwfdagov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM518213pdf. 2018.
173. Saa P, Proctor M, Foster G, Krysztof D, Winton C, Linnen JM, et al. Investigational testing for Zika virus among U.S. blood donors. N Engl J Med. 2018; 378: 1778-1788.
174. OPTN. https://optn.transplant.hrsa.gov/news/special-announcement-from-hrsa-regarding-west-nile-virus/ 2004.
175. Nett RJ, Kuehnert MJ, Ison MG, Orlowski JP, Fischer M, Staples JE. Current practices and evaluation of screening solid organ donors for West Nile virus. Transpl Infect Dis. 2012; 14: 268-277.
176. OPTN. https://optn.transplant.hrsa.gov/resources/guidance/identifying-risk-factors-for-west-nile-virus-wnv-during-evaluation-of-potential-living-donors/. 2012.
177. Singh N, Levi ME. Arenavirus and West Nile virus in solid organ transplantation. Am J Transplant. 2013; 13 Suppl 4: 361-371.
178. OPTN. https://optn.transplant.hrsa.gov/news/recorded-zika-virus-webinar-is-available/#faq. 2016.
179. FDA. https://www.fda.gov/downloads/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/tissue/ucm488582.pdf. 2018.
180. Saigal S, Choudhary NS, Saraf N, Kataria S, Mohanka R, Soin AS. Transmission of dengue virus from a donor to a recipient after living donor liver transplantation. Liver Transplant. 2013; 19: 1413-1414.
181. Tan FL, Loh DL, Prabhakaran K, Tambyah PA, Yap HK. Dengue haemorrhagic fever after living donor renal transplantation. Nephrol Dial Transplant. 2005; 20: 447-448.
182. Iwamoto M, Jernigan DB, Guasch A, Trepka MJ, Blackmore CG, Hellinger WC, et al. Transmission of West Nile virus from an organ donor to four transplant recipients. N Engl J Med. 2003; 348: 2196-2203.
183. CDC. West Nile virus transmission via organ transplantation and blood transfusion - Louisiana, 2008. MMWR Morb Mortal Wkly Rep. 2009; 58: 1263-1267.
184. Costa AN, Capobianchi MR, Ippolito G, Palu G, Barzon L, Piccolo G, et al. West Nile virus: the Italian national transplant network reaction to an alert in the north-eastern region, Italy 2011. Euro Surveillance. 2011; 16.
185. Richner JM, Diamond MS. Zika virus vaccines: immune response, current status, and future challenges. Curr Opin Immunol. 2018; 53: 130-136.
186. Collins MH, Metz SW. Progress and works in progress: Update on flavivirus vaccine development. Clin Ther. 2017; 39: 1519-1536.
187. Amanna IJ, Slifka MK. Current trends in West Nile virus vaccine development. Expert Rev Vaccines. 2014; 13: 589-608.

188. Shankar MB, Staples JE, Meltzer MI, Fischer M. Cost effectiveness of a targeted age-based West Nile virus vaccination program. Vaccine. 2017; 35: 3143-3151.
189. Rosen J. Travel medicine and the solid-organ transplant recipient. Infect Dis Clin N Am. 2013; 27: 429-457.
190. Trubiano JA, Johnson D, Sohail A, Torresi J. Travel vaccination recommendations and endemic infection risks in solid organ transplantation recipients. J Travel Med. 2016; 23.
191. Rubin LG, Levin MJ, Ljungman P, Davies EG, Avery R, Tomblyn M, et al. 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. Clin Infect Dis. 2014; 58: 309-318.
192. CDC. https://wwwnc.cdc.gov/travel/destinations/list. 2018.
193. Fauci AS, Morens DM. Zika virus in the Americas--Yet another arbovirus threat. N Engl J Med. 2016; 374: 601-604.