Fatal Transplant-Associated West Nile Virus Encephalitis and Public Health Investigation—California, 2010

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Background. In December 2010, a case of West Nile virus (WNV) encephalitis occurring in a kidney recipient shortly after organ transplantation was identified.

Methods. A public health investigation was initiated to determine the likely route of transmission, detect potential WNV infections among recipients from the same organ donor, and remove any potentially infected blood products or tissues. Available serum, cerebrospinal fluid, and urine samples from the organ donor and recipients were tested for WNV infection by nucleic acid testing and serology.

Results. Two additional recipients from the same organ donor were identified, their clinical and exposure histories were reviewed, and samples were obtained. WNV RNA was retrospectively detected in the organ donor's serum. After transplantation, the left kidney recipient had serologic and molecular evidence of WNV infection and the right kidney recipient had prolonged but clinically inapparent WNV viremia. The liver recipient showed no clinical signs of infection but had flavivirus IgG antibodies; however, insufficient samples were available to determine the timing of infection. No remaining infectious products or tissues were identified.

Conclusions. Clinicians should suspect WNV as a cause of encephalitis in organ transplant recipients and report cases to public health departments for prompt investigation of the source of infection. Increased use of molecular testing and retaining pretransplantation sera may improve the ability to detect and diagnose transplant-associated WNV infection in organ transplant recipients.

Keywords: West Nile virus, Transplant-associated transmission, Encephalitis.

Since it was first detected in North America in 1999, West Nile virus (WNV) has become endemic to the continent and is responsible for focal seasonal outbreaks throughout the United States (*1*). Approximately 80% of human WNV infections are asymptomatic. Most symptomatic persons experience an acute systemic febrile illness; less than 1% of infected persons develop neuroinvasive disease, which typically manifests as meningitis, encephalitis, or acute flaccid paralysis (*2–4*). Although most WNV infections are acquired through the bite of an infected mosquito, the virus can also be transmitted through transfusion of infected blood products or solid-organ transplantation (SOT) (*5–7*).

WNV infection acquired through SOT can result in severe disease (*8, 9*). In five clusters of SOT-associated WNV infections previously reported to public health agencies in the United States, 10 of 13 (77%) organ recipients were infected (*10, 11*). Seven of the 10 (70%) infected organ recipients developed encephalitis and three of these patients died. SOT-transmitted WNV infection is difficult to prevent because, unlike blood donors, organ donors are not routinely screened for WNV infection and, even with screening, some infections in donors may not be detected (*5, 12*).

In December 2010, a case of WNV encephalitis that occurred in a kidney recipient shortly after organ transplantation was identified. After recognition in this patient, a public health investigation was initiated to determine the likely route of transmission, detect any WNV infections among recipients from the same organ donor, and remove any potentially infected blood products or tissues. We report the findings of the investigation.

RESULTS

Three organs, a liver and two kidneys, were recovered from a single deceased donor and were transplanted into three recipients from northern California on the same day in October 2010 (Table 1). No other organs or tissues from this donor were transplanted or stored. The liver recipient and left kidney recipient were transplanted at the same center, while the right kidney transplantation took place in another center.

Organ Donor

The organ donor was a 55-year-old male who had suffered blunt head trauma. He had a history of type 2 diabetes mellitus, hypertension, and drug use, and he had a coronary artery bypass graft in 2009. Routine organ donor screening showed evidence of prior cytomegalovirus and Epstein-Barr virus infection. The family reported that the donor had not had a recent history of a febrile illness and had no past history of meningitis or encephalitis. He had previously lived in Mexico for several years. Within 2 weeks before his death, mosquitoes collected in his county of residence in California had tested positive for WNV. The donor was not screened for WNV infection before organ recovery; however, a retained serum specimen collected 3 days before organ procurement was retrospectively tested as part of this investigation and was positive for WNV RNA by individual (nonpooled) nucleic acid amplification testing (NAT). The sample also tested positive for WNV IgG but was negative for WNV IgM antibodies. Although he received one unit of packed red blood cells 2 days before organ recovery, the NAT-positive serum was collected before blood transfusion. Fixed brain, lung, thyroid, and heart tissue samples tested negative for WNV antigens.

Left Kidney Recipient

The left kidney recipient was a 73-year-old male with type 2 diabetes mellitus and end-stage renal disease. His medical history included cerebrovascular disease, chronic obstructive pulmonary disease, and prior heavy alcohol use. He was unsensitized, a zero human leukocyte antigen mismatch with his donor, and received basiliximab for induction therapy. Because of his age and history of diabetes, his maintenance regimen consisted of cyclosporine and mycophenolate mofetil (MMF). On posttransplantation day (PTD) 4, he received one unit of packed red blood cells. He was discharged home on PTD 6. He developed confusion on PTD 8, presented to another hospital emergency room on PTD 10, where his cyclosporine levels were markedly elevated and he was discharged home. He was rehospitalized at the transplant center on PTD 13 because of further deterioration of his mental status. At the time of readmission, he was

hyponatremic (sodium 127 mmol/L) and had a possible infiltrate on chest radiograph. His symptoms were attributed to a combination of these findings. However, correction of his electrolytes, broad-spectrum antibiotics, and reduction of his cyclosporine dose did not yield improvement and further evaluation was performed to exclude other infectious etiologies. His mental status subsequently deteriorated rapidly, progressing to coma with limited brainstem reflexes and quadriplegia. An electroencephalogram showed diffuse slowing and a magnetic resonance imaging of the brain without contrast showed a chronic left posterior cerebellar artery infarct without other significant abnormalities. Electromyography and nerve conduction studies were normal. Cerebrospinal fluid (CSF) collected on PTD 18 revealed 32 white blood cells/mm^3 (79% lymphocytes, 16% monocytes, and 5% neutrophils), two red blood cells/mm^3, elevated protein (142 mg/dL), elevated glucose (105 mg/dL), negative bacterial culture, and negative cryptococcal antigen testing. During the third week of illness, samples were submitted to the California Encephalitis Project to test for viral etiologic agents of encephalitis, including WNV. Sera tested positive for WNV IgM and IgG antibodies that were confirmed by plaque reduction neutralization testing (PRNT). CSF collected on PTD 26 and 48 tested positive for WNV IgM antibodies, but no WNV RNA was detected. Retained pretransplantation serum collected 1 day before transplantation tested negative for WNV RNA and for both IgM and IgG antibodies. The patient's immunosuppressive regimen was further reduced and ultimately withdrawn completely. He experienced transient improvement in responsiveness around PTD 67, but his condition subsequently declined and he died on PTD 113. An autopsy was conducted and postmortem brain tissues tested positive for WNV RNA.

The patient had no known travel history, and there were no recent reports of WNV infections among humans or collected mosquitoes in his county of residence. The packed red blood cells that were received before his onset of encephalitis had tested negative for WNV during routine screening by minipool NAT at donation 5 weeks earlier. Follow-up testing of the blood donor at 114 days after the donation was negative for WNV RNA by individual (nonpooled) NAT, negative for WNV IgM antibodies, and positive for WNV IgG antibodies.

Right Kidney Recipient

The right kidney was transplanted into a 52-year-old female with polycystic kidney disease. Her medical history included migraine headaches and hypercoagulable state due to heparin-induced thrombocytopenia. Her immunosuppressive regimen consisted of induction with rabbit antithymocyte globulin followed by maintenance with tacrolimus, MMF, and prednisone. Valganciclovir was given for cytomegalovirus prophylaxis. Despite postoperative slow graft function, no hemodialysis was required. To prevent acute antibody-mediated injury, she received five plasmapheresis treatments from PTD 1 to 13 followed by 120 g of intravenous immunoglobulin over PTD 16 to 19. She experienced gastrointestinal bleeding and received transfusions of several units of packed red blood cells and fresh frozen plasma on PTD 4 to 20. On PTD 16, she complained of headache and backache. Head computed tomography scan without contrast revealed no abnormality. The headache improved by PTD 19 with

TABLE 1. Donor and recipient characteristics of solid organ transplant-associated WNV transmission cluster—California, 2010

Patient	Age	Sex	Underlying medical conditions	Immunosuppression	Clinical course	WNV laboratory results		WNV infection and disease status	Outcome
						Pretransplantation specimens	Posttransplantation specimens		
Deceased-organ donor	55	M	Diabetes mellitus, intravenous drug use, coronary artery disease, hypertension	None	Brain death due to blunt head trauma	Serum: RNA positive, IgG positive, IgM negative; brain tissue: IHC negative	Not applicable	WNV infection/ clinically inapparent	Died
Left kidney recipient	73	M	Diabetes mellitus, renal failure	Basiliximab, cyclosporine, MMF	Encephalitis to progressive obtundation	Serum: IgM and IgG negative	CSF: IgM positive; serum: IgM positive and neutralizing antibodies positive; brain tissue: RNA positive	WNV infection/ encephalitis	Died (PTD 113)
Right kidney recipient	52	F	Polycystic kidney disease, renal failure, migraine, headache, hypercoagulable state	Thymoglobulin, MMF, tacrolimus, prednisone	Afebrile with intermittent headache; no other clinical symptoms	Serum: IgM and IgG negative	Serum: RNA positive, IgM positive, and neutralizing antibodies positive; urine: RNA positive	WNV infection/ clinically inapparent	Survived
Liver recipient	47	M	Hypertension, chronic active hepatitis, hepatocellular carcinoma	MMF, tacrolimus, prednisone	No clinical symptoms	None available	Serum: IgM negative, IgG positive	Prior flavivirus infection/ no disease	Survived

MMF, Mycophenolate mofetil; CSF, cerebrospinal fluid; RNA, Ribonucleic acid; WNV, West Nile virus.

sumatriptan. Lumbar puncture was not performed. Kidney biopsy on PTD 20 demonstrated acute tubular necrosis with no evidence of acute rejection or C4d complement fragment deposition. She was discharged in stable condition on PTD 27. Arboviral testing on stored serum collected pretransplantation showed no evidence of WNV RNA or antibodies. After transplantation, she had evidence of WNV RNA by NAT on serum samples collected from PTD 96 to 103 and urine specimens collected from PTD 110 to 181. WNV IgM and IgG antibodies were detected on the earliest available posttransplantation serum sample collected on PTD 53. There was no immunohistochemical or molecular evidence of WNV infection on renal transplant tissue that was biopsied on PTD 20 and 178. The blood products that this patient received were not investigated further as a potential source of WNV infection because of the epidemiologic linkage to the WNV-positive organ donor and left kidney recipient as well as the effectiveness of blood donor screening. She remained in stable condition at more than 1 year after transplantation.

Liver Recipient

The liver was transplanted into a 47-year-old male with a history of chronic active hepatitis B virus infection resulting in liver cirrhosis and hepatocellular carcinoma. His exposure history was notable for extensive and frequent travel within the United States and to Southeast Asia. His postoperative course was uneventful and his immunosuppressive regimen consisted of MMF, tacrolimus, and prednisone; he remained on lamivudine and adefovir for the hepatitis B virus infection. On outpatient follow-up, he reported no symptoms suggestive of WNV infection after transplantation. Serum collected on PTD 52 showed no detectable WNV RNA or IgM antibodies. WNV IgG antibodies were present but PRNT revealed fourfold greater neutralizing antibody titers against dengue viruses compared with WNV and St. Louis encephalitis virus. He remains in stable condition at more than 1 year after transplantation.

DISCUSSION

This is the sixth cluster of confirmed SOT-transmitted WNV infections reported to public health agencies in the United States. Although the diagnosis of WNV encephalitis in the left kidney recipient was delayed until after 3 weeks of severe neuroinvasive disease, the ensuing public health investigation retrospectively confirmed organ donor viremia and excluded other routes of WNV transmission. Fortunately, no potentially infectious donor products or tissues remained that could have been transplanted in the interval between transplantation and the public health response.

Diagnosis of WNV in organ transplant recipients may be confounded by the initial attribution of symptoms to immunosuppressive drug toxicity or other infectious etiologies (14). Establishing the diagnosis of WNV infection in a transplant recipient may be challenging, as WNV viremia may be prolonged and antibody development may be delayed due to immunosuppressive medications (15, 16). This prolonged viremia and delayed antibody production was demonstrated in the right kidney recipient; however, her lack of severe symptoms is noteworthy and dissimilar to previous reports of prolonged symptomatic viremia in

immunosuppressed patients (16). Overall, these findings suggest that molecular diagnostics may be of value for a longer time period after the initial infection and should be considered, in addition to antibody testing, to diagnose WNV infections in organ recipients.

The route of WNV transmission in recent organ recipients can only be deduced by establishing the timing of infection and investigating other possible exposures. To determine the timing and source of infection, paired sera collected immediately before and for several weeks after transplantation should be tested for WNV, and information on blood products administered and mosquito exposures should be obtained. The lack of archived pretransplantation recipient samples from recipients is a commonly cited limitation of donor-derived transmission investigations. Although donor sera are banked for 10 years by organ procurement organizations, sera and tissue biopsies from organ recipients before transplant are not routinely stored (17). In this investigation, the absence of pretransplantation serum from the liver recipient precluded a definitive determination of whether or not transplant-associated WNV infection had occurred. The presence of IgG antibodies and higher neutralizing titers against dengue in this recipient's posttransplantation serum without detectable IgM antibodies suggests prior flavivirus infection. The prior flavivirus infection may have been acquired during travel to Asia or within the United States and might have conferred some protection against subsequent infection in this recipient (18–20). In terms of alternate routes of transmission, public health WNV surveillance data did not support exposure to infected mosquitoes in the recipients' counties of residence. One potential alternate source of WNV infection was investigated, namely, the packed red blood cells transfused to the left kidney recipient before symptom onset; however, this unit tested negative for WNV by minipool NAT at the time of donation. Furthermore, the blood donor of the unit was WNV IgM negative and IgG positive within 4 months after donation. Although this does not definitively rule out recent WNV infection, the probability of IgM dropping to undetectable levels within that interval is low and the positive WNV IgG likely represents distant WNV infection in the blood donor (21). This, together with the epidemiologic link of the organ donor and both kidney recipients, makes the blood transfusion an unlikely source of infection in the left kidney recipient.

Although proven effective treatment options for WNV disease remain elusive, clinicians caring for transplant recipients would benefit from early notification of possible SOT-transmitted WNV infections to exclude other diagnoses, consider revision of immunosuppressive therapy, or contemplate use of investigational therapies such as polyclonal intravenous immunoglobulins in a transplant recipient with WNV infection (10, 15, 22). It is unclear whether the immunoglobulin administered to the right kidney recipient in the early posttransplantation period to prevent acute antibody-mediated injury may have ameliorated the clinical course of WNV infection, especially because the patient failed to develop severe WNV disease despite a long period during which viral RNA was detectable in blood and urine.

During the investigation, retrospective WNV testing of stored serum from the organ donor demonstrated viremia

3 days before organ procurement, absence of IgM antibodies, and presence of IgG antibodies. The serologic findings may be attributable to prior non-WNV flavivirus infection with cross-reactive IgG antibodies but before developing an IgM antibody response to the acute WNV infection. WNV screening of organ donors is not a regulatory requirement and most organ procurement organizations have not employed systematic NAT-based screening for WNV among organ donors because of concerns that false-positive tests and prolonged turnaround times could compromise the availability of an already scarce supply of organs. In this case, the organ procurement organization did not perform routine WNV testing for these reasons as well as the low incidence of disease within their service area and the low level of reported WNV transmission nationally (23). Furthermore, because there have been prior reports of clusters in which organ donor viremia could not be detected, there is additional concern over the potential for missing cases by NAT testing (5, 12). Further prevention effectiveness evaluation is needed to determine if routine screening of organ donors results in a net benefit for transplant outcomes.

The conclusions drawn from this investigation are limited in two further aspects. First, the positive NAT on the donor serum, although replicated on repeat testing, was not confirmed by an alternative assay; second, the blood products transfused to the right kidney recipient were not investigated further as a potential source of infection.

Clinicians should have a high index of suspicion for WNV as a cause of systemic febrile illness or encephalitis in organ transplant recipients. Suspected cases should be reported to public health departments in a timely manner to enable a prompt investigation to identify and remove any potentially infected products and enable identification of other exposed recipients so they may be managed appropriately. Laboratory confirmation and determination of timing of WNV infection is dependent on appropriate testing, which could be improved by increased use of molecular methods in immunosuppressed patients, as well as banking of pretransplantation recipient sera. Furthermore, data from clusters of SOT-transmitted WNV infections should be collected systematically, reported, and analyzed to inform future preventive efforts.

MATERIALS AND METHODS

Case Investigation

We reviewed medical records and exposure history for the deceased-organ donor and obtained stored premortem sera as well as postmortem tissues for WNV testing. Investigators also searched for possible sources of the left kidney recipient's WNV infection by reviewing his medical records and public health reports of local WNV activity (human and ecologic) in his county of residence. His stored sera and CSF collected before and after transplantation, respectively, were obtained for WNV testing to determine the timing of infection. Other recipients from the same deceased donor were identified and assessed for WNV infection by testing remaining pretransplantation samples and solicitation of posttransplantation samples as well as prospective clinical evaluation for signs of WNV disease. Finally, blood products that had been transfused into the left kidney recipient and organ donor were traced and investigated.

Laboratory Testing

Serum, CSF, and urine samples were tested for WNV at the California Department of Public Health, the Centers for Disease Control and Prevention, and commercial laboratories. Sera were tested for WNV RNA by reverse transcription-polymerase chain reaction (24). Organ donor sera also were tested for WNV RNA by NAT using the Gen-Probe Procleix assay, which uses a larger input sample volume for testing to increase sensitivity (25). All serum and CSF samples were tested for WNV-specific IgM and IgG antibodies by enzyme-linked immunosorbent assay or microsphere-based immunoassay (26, 27). Antibody specificity for WNV was confirmed by PRNT (28). Tissue samples were tested for WNV RNA by reverse transcription-polymerase chain reaction and for WNV and pan-flavivirus antigens by immunohistochemistry (24, 29).

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