West Nile virus infection transmitted by blood transfusion

Theresa Harrington, Matthew J. Kuehnert, Hany Kamel, Robert S. Lanciotti, Sheryl Hand, Mary Currier, Mary E. Chamberland, Lyle R. Petersen, and Anthony A. Marfin

BACKGROUND: A patient with transfusion-transmitted West Nile virus (WNV) infection confirmed by viral culture of a blood component is described. A 24-year-old female with severe postpartum hemorrhage developed fever, chills, headache, and generalized malaise after transfusion of 18 units of blood components; a serum sample and the cerebrospinal fluid tested positive for the presence of WNV IgM antibodies. An investigation was initiated to determine a possible association between transfusion and WNV infection.
STUDY DESIGN AND METHODS: Blood donors were assessed for recent infection through questionnaires and WNV testing of serum samples. Whole-blood retention segments and untransfused blood components were sent to the CDC to test for the presence of WNV through PCR (TaqMan, Applied Biosystems), IgM ELISA, plaque reduction neutralization testing, and viral culture.
RESULTS: Three of 15 available donor retention segments were WNV PCR-positive. WNV was recovered from one associated blood component. The implicated donor was symptomatic near the time of donation; serology confirmed WNV IgM seroconversion.
CONCLUSION: Seroconversion of a symptomatic donor, the presence of viral genetic material in an associated whole-blood retention segment, and recovery of WNV from an associated component provides compelling evidence for transfusion-acquired infection. This report has important implications for blood safety.

Approximately 4 million persons receive blood components from over 20 million donations annually in the US.[1,2] Although donor screening and testing have nearly eliminated the risk of transfusion-acquired infections attributable to HIV and hepatitis viruses, the potential emergence and spread of other pathogens, particularly those associated with asymptomatic illness, could result in unrecognized transmission through blood transfusion.[3,4]

West Nile virus (WNV), a mosquito-borne flavivirus endemic to Africa, Asia, and Europe, was first detected in the US in 1999.[5,6] Although birds are the primary vertebrate host, humans and other mammals can become infected. Most WNV infections are asymptomatic in humans; only 20 percent develop a mild febrile illness, and fewer than 1 percent develop encephalitis or meningitis.[7-12] Transmission of WNV infections through blood transfusion has been considered as biologically plausible, given that infection, including subclinical illness, is associated with a transient viremia.[13] However, transfusion-

ABBREVIATIONS: CSF = cerebrospinal fluid; MSDH = Mississippi State Department of Health; P/N ratio = OD of the patient's specimen divided by the OD of the negative control; RPL = recovered plasma; WNV = West Nile virus.

From the Epidemic Intelligence Service Branch, Division of Applied Public Health Training, Epidemiology Program Office, Centers for Disease Control and Prevention, Atlanta, Georgia; the Division of Healthcare Quality Promotion, the Division of Vector-Borne Infectious Diseases, and the Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; Blood Systems, Scottsdale, Arizona; and the Mississippi State Department of Health, Jackson, Mississippi.

Address reprint requests to: Theresa Harrington, MD, MPHTM, Mississippi State Department of Health, 570 East Woodrow Wilson, PO Box 1700, Jackson, MS 39215-1700; e-mail: tharrington@cdc.gov.

Received for publication January 31, 2003; revision received March 20, 2003, and accepted April 7, 2003.

TRANSFUSION 2003;43:1018-1022.

associated transmission of WNV or related Japanese encephalitis serogroup viruses was not documented before 2002. We describe a patient with transfusion-transmitted WNV meningitis.

CASE REPORT

In July 2002, a previously healthy 24-year-old woman was received transfusion of 6 units of RBCs and 2 units of FFP for postpartum hemorrhage. After transfer to a tertiary care hospital, she underwent a hysterectomy and received an additional 10 units of blood components (6 units of RBCs and 4 units of FFP) on postpartum Day 1 for persistent bleeding. A total of 18 units of blood components were transfused over a 30-hour period. Fever was noted on postpartum Day 2; no bacterial pathogens were isolated from urine or blood. Other than fever, the patient experienced only expected postoperative abdominal and surgical incision tenderness while hospitalized. She was discharged on postpartum Day 5 in stable condition and without fever. Antimicrobial therapy, which had been started preoperatively, was discontinued the morning of discharge.

On postpartum Day 6, the patient developed fever, chills, headache, fatigue, and generalized weakness; fever and headache persisted despite frequent use of analgesic and antipyretic medication. Twenty-six days postpartum, the patient sought medical care for these unrelenting symptoms. Upon evaluation in the emergency department, oral temperature was 38.4°C. The patient exhibited no meningismus, photophobia, rash, motor weakness, or neurologic impairment. Laboratory studies were unremarkable except for a urinalysis showing mild pyuria without bacteriuria. A computed tomography scan of the brain was normal. The patient was treated empirically for a urinary tract infection and discharged, but returned the following day with persistent headache and fever. Lumbar puncture revealed elevated protein and mild pleocytosis. Bacterial cultures of the cerebrospinal fluid (CSF) were negative. WNV-specific IgM was detected in serum and CSF (Table 1) and was confirmed with plaque reduction neutralization technique testing to WNV on patient serum. Serum and CSF IgM antibodies to St. Louis encephalitis virus, a cross-reacting flavivirus, were also detected, but SLE infection was excluded by plaque reduction neutralization technique confirmatory testing. The patient was hospitalized for 2 days with WNV meningitis and has subsequently fully recovered.

As part of routine state-based surveillance,[14,15] the Mississippi State Department of Health (MSDH) was notified of suspected WNV illness in this patient. Upon confirmation of infection by WNV testing, the MSDH learned

TABLE 1. Diagnostic test results performed at the time of hospital admission of patient with transfusion-transmitted WNV infection, Mississippi, 2002

Laboratory study	Patient result	Normal values
Hb (g/100 mL)	10.1	12.0-16.0
WBC count (thousand cells/mm^3)	5.4	4.8-10.8
Differential (%)		
Segmented neutrophils	53	4.8-10.8
Lymphocytes	37	42.2-75.5
Monocytes	8	1.7-9.3
Eosinophils	1	0-3
Basophils	1	0-2
Serum		
Glucose (mg/dL)	85	70-105
Protein (g/dL)	6.0	6.0-8.3
CSF		
Glucose (mg/dL)	57	70-105
Protein (mg/dL)	76	15-45
WBC count (cells/mm^3)	18	0-5
Differential (%)		
Mononuclear		
Segmented neutrophils	96	
Arbovirus studies	4	
WNV IgM, P/N ratio (serum)	17.90	<2.00
SLE* virus IgM, P/N ratio (serum)	7.15	
WNV plaque reduction neutralizing antibody titer (serum)†	1 in 640	
SLE virus plaque reduction neutralizing antibody titer (serum)	1 in 10	
WNV IgM, P/N ratio (CSF)	14.31	<2.00
SLE virus IgM, P/N ratio (CSF)	3.73	<2.00

* SLE = St. Louis encephalitis.
† Plaque reduction neutralizing antibody titers are performed to distinguish between cross-reacting antibodies among flaviviridae, i.e., WNV and SLE.

that the patient had been the recipient of numerous transfusions. An investigation was begun to determine a possible association between transfusion and WNV infection.

MATERIALS AND METHODS

MSDH reviewed the transfusion recipient's hospital records, and in collaboration with hospital transfusion services, identified all associated blood donation numbers (i.e., unique identification numbers assigned to each donated blood unit and any derived components) of the units transfused to the patient. The blood collection agency responsible for the blood donations contacted all donors and requested their participation in the investigation. In addition, the blood collection agency compiled a list of all components derived from each donation. Each consenting donor was asked to complete a questionnaire and submit a blood sample for WNV serology. All available retention segments were recovered for testing. Untransfused units of blood components also were recovered for testing.

Retention segments and untransfused components were tested at the CDC with dynamic, quantitative PCR (TaqMan, Applied Biosystems, Foster City, CA) and WNV IgM antibody-capture ELISA (MAC-ELISA).[16-19] A positive TaqMan assay was defined as a test that resulted in significant inflection and increase of fluorescent signal (in 37 cycles or less) for each of two different primer-probe sets. All positive TaqMan assays were repeated. Samples positive by TaqMan were cultured on Vero cell monolayers. A positive IgM ELISA result was defined as a P/N ratio (OD of the patient's specimen divided by the OD of the negative control) of at least 3, an equivocal result was P/N ratio of 2.0 to 2.99, and a negative result as a P/N ratio of less than 2. Positive WNV MAC-ELISAs were confirmed by plaque reduction neutralization tests with WNV and St. Louis encephalitis viruses.

RESULTS

Blood component identification

The index patient received blood components from 18 donations; these donations were from 17 different donors and produced 42 blood components (i.e., 20 units of RBCs, 7 units of FFP, 9 units of recovered plasma [RPL], and 6 units of PLTs). Nine of 42 units were transfused to other recipients (8 units of RBCs and 1 unit of PLT), 1 unit of RPL and 5 units of PLTs were discarded, and 8 units of RPL and 1 of FFP were retrieved for testing.

Blood component testing results

Fifteen retention segments were available for testing. Three (from Donors A, B, and C) were WNV TaqMan-positive but IgM- and culture-negative (Table 2). All other segments were TaqMan-negative. WNV was isolated from a TaqMan-positive, IgM-negative unit of FFP associated with the TaqMan-positive retention segment from Donor A (Table 2).

Donor identification and follow-up

Follow-up serum samples were obtained for all 17 donors; only the sample from Donor A was WNV IgM-positive, demonstrating seroconversion (Table 2). Donor A, a previously healthy 44-year-old woman, had nasal congestion, retroorbital pain, earache, and fatigue approximately 1 week before donation. However, on the day of donation, she reported no complaints necessitating donation deferral from responses given on the symptom screening questionnaire administered by the blood collection facility; oral temperature was normal.

Donor A visited her primary care physician 4 days after donation because of frontal headache, fatigue, and upper respiratory symptoms. Vital signs were within normal limits. Physical examination was notable only for sinus tenderness; she did not exhibit muscle weakness, meningismus, rash, nausea, emesis, lymphadenopathy, or mental confusion. She was prescribed empiric antimicrobial treatment for a diagnosis of sinus infection, and her symptoms subsequently resolved.

Recipients of blood components associated with TaqMan-positive retention segments

Seven blood components were made from the three donations (Donors A, B, and C) associated with TaqMan-positive retention segments. The case patient received transfusions of RBCs from Donor A, RBCs from Donor B, and FFP from Donor C. One unit of RBCs from Donor C

TABLE 2. Diagnostic test results performed on retention segments and untransfused blood components associated with transfusion-transmitted WNV infection, Mississippi, 2002

	Retention segment			Untransfused component				Donor follow-up
Donor	TaqMan	Culture	WNV IgM	Type	TaqMan	Culture	WNV IgM	WNV IgM
A	Positive	Negative	Negative	Plasma	Positive	Positive	Negative	Positive
B	Positive	Negative	Negative	Plasma	Negative	Negative	Negative	Negative
C	Positive	Negative	Negative	None				Negative

was transfused to another patient, but the patient died before serum could be tested for evidence of WNV infection. Review of the medical record showed no clinical evidence of WNV illness. No other components associated with TaqMan-positive retention segments were transfused.

DISCUSSION

We describe transmission of WNV through blood transfusion. This case report specifically demonstrates WNV transmission from transfusion of RBCs; however, the finding of culture-positive plasma suggests that FFP may also be capable of transmitting WNV infection. The association of a donor with symptoms suggestive of clinical illness around the time of blood donation, viral genetic material present in the whole-blood donor segment, confirmation of donor seroconversion after donation, and culture of WNV from a component from the implicated donation provides compelling evidence to support this hypothesis.

The conclusions drawn by our investigation have two main limitations. First, since Mississippi was endemic for WNV in summer 2002, mosquito-borne transmission cannot definitively be excluded. Samples obtained before transfusion from the infected transfusion recipient were unavailable.

Second, viral RNA was detected in retention segments associated with whole-blood donations from three different donors, raising the possibility that there were multiple components that could have transmitted infection to the transfusion recipient. However, only one donation was associated with a WNV culture-positive component and seroconversion in the donor. The detection of viral RNA in retention segments by TaqMan PCR in the absence of donor seroconversion after donation might reflect the presence of noninfectious viral particles in donor serum that do not initiate immunogenic response, false-positive TaqMan results, or true donor viremia and false-negative IgM antibody test after donation, which would imply infection in 3 of 15 (20%) donors tested. Although the TaqMan assay used in this investigation can detect as few as 0.1 plaque-forming units per 100 µL, the specificity and positive-predictive value had not been evaluated for use in blood components at the time of testing (Lanciotti R, personal communication, 2002). Despite these limitations, the existence of a WNV culture-positive component strongly implicates the associated whole-blood donation as the most probable source of infection.

Despite the theoretical risk of transfusion-associated transmission from WNV and related flaviviruses, infections from transfusions of blood or blood components have not been reported before the 2002 WNV epidemic. During 2002, 190 of more than 3800 laboratory-positive human cases of WNV illness reported in the US occurred in Mississippi; several infections were suspected to be transmitted through blood transfusion or organ transplantation.[20-23] The risk of WNV transmission by transfusion is related to the incidence of infection and the duration of viremia in the population donating blood during an epidemic period. Mathematical models estimated a risk of 1 in 3700 to 1 in 5555 for acquiring WNV infection from a blood transfusion at the epicenter of the 1999 epidemic in Queens, New York, during the time period when mosquito-borne infections to humans were known to have occurred.[24]

Health-care providers should recognize that there is a risk of WNV transmission through blood transfusion. To help identify potential transfusion-transmitted WNV infections, patients diagnosed with WNV illness with a history of blood transfusion in the 4 weeks preceding onset of symptoms, or donors who had onset of symptoms consistent with WNV illness within 2 weeks of blood donation, should be reported to local public health authorities.[25] Rapid recognition of possible infection, through effective partnership between state and federal governmental agencies, health-care transfusion services, clinicians, and blood collection agencies, could lead to prompt initiation of an investigation, with successful quarantine of blood components containing WNV.

This report, and reports of other suspected transfusion-associated WNV cases, emphasizes the importance of effective blood donor screening for WNV. The cost-effectiveness of laboratory screening (e.g., nucleic acid-based testing) is unknown; furthermore, implementation of such screening might be challenging since the incidence of WNV varies, both temporally and geographically. Because an estimated 20 percent of persons infected with WNV become symptomatic, donor exclusion based on health screening might have limited effectiveness.[25] In addition to implementation of methods for effective blood bank screening of WNV, it is imperative that health-care providers rapidly recognize and report transfusion-associated illness, to allow for identification of emerging transfusion-transmitted infectious agents, and to ensure the continued safety of the nation's blood supply.

ACKNOWLEDGMENTS

We are indebted to the contribution of Chris Van Beneden, Daniel Jernigan, Robin Moseley, Stacy Neff, and David Withum of the Centers for Disease Control and Prevention and Janie Waggoner of Blood Systems for their assistance with collection of data and preparation of the manuscript.

REFERENCES

1. Comprehensive report on blood collection and transfusion, 1997-1999. Bethesda: National Blood Data Resource Center; 2001.

2. Wallace EL, Churchill WH, Surgenor DM, Cho G, McGurk S. Collection and transfusion of blood and blood components in the United States, 1994. Transfusion 1998;38: 625-35.
3. AuBuchon JP, Birkmeyer JD, Busch MP. Safety of the blood supply in the United States: opportunities and controversies. Ann Intern Med 1997;127:904-9.
4. Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ. The risk of transfusion-transmitted viral infections. The Retrovirus Epidemiology Donor Study. N Engl J Med 1996;334: 1685-90.
5. Nash D, Mostashari F, Fine A, et al. The outbreak of West Nile virus infection in the New York City area in 1999. N Engl J Med 2001;344:1807-14.
6. Outbreak of West Nile-like viral encephalitis—New York 1999. MMWR Morb Mortal Wkly Rep 1999;48:845-59.
7. Tsai TF, Popovici F, Cernescu C, Campbell GL, Nedelcu NI. West Nile encephalitis epidemic in southeastern Romania. Lancet 1998;352:767-71.
8. Serosurveys for West Nile virus infection—New York and Connecticut Counties 2000. MMWR Morb Mortal Wkly Rep 2001;50:37-9.
9. Mostashari F, Bunning M, Kitsutani PT, et al. Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey. Lancet 2001; 358:261-4.
10. Petersen LR, Marfin AA. West Nile virus: a primer for the clinician. Ann Intern Med 2002;137:173-9.
11. Weiss D, Carr D, Kellachan J, et al. Clinical findings of West Nile virus infection in hospitalized patients, New York and New Jersey, 2000. Emerg Infect Dis 2001;7:654-8.
12. Chowers MY, Lang R, Nassar F, et al. Clinical characteristics of the West Nile fever outbreak, Israel, 2000. Emerg Infect Dis 2001;7:675-8.
13. Southam CM, Moore AE. Induced virus infections in man by the Egypt isolates of West Nile virus. Am J Trop Med Hyg 1954;3:19-50.
14. Case definitions for infectious conditions under public health surveillance. MMWR Morb Mortal Wkly Rep 1997;46(RR10);1-55.
15. Epidemic/epizootic West Nile virus in the United States: revised guidelines for surveillance, prevention, and control. Workshop, 2001 Jan 31-Feb 4, Charlotte, NC. Atlanta: Centers for Disease Control and Prevention [accessed 2003 May 1]. Available from: http://www.cdc.gov/ncidod/dvbid/westnile/publications.htm.
16. Lanciotti RS, Kerst AJ, Nasci RS, et al. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan® reverse transcriptase-PCR assay. J Clin Microbiol 2000;38:4066-71.
17. Martin DA, Muth DA, Brown T, et al. Standardization of IgM capture enzyme-linked immunosorbent assays for routine diagnosis of arboviral infections. J Clin Microbiol 2000;38:1823-6.
18. Martin DA, Biggerstaff BJ, Allen B, et al. Use of immunoglobulin M cross-reactions in differential diagnosis of human flaviviral encephalitis infections in the United States. Clin Diagn Lab Immunol 2002;9:544-9.
19. Tardei G, Ruta S, Chitu V, et al. Evaluation of immunoglobulin IgM and IgG enzyme immunoassays in serologic diagnosis of West Nile infection. J Clin Microbiol 2000;38: 2232-9.
20. Update: investigations of West Nile virus infection in recipients of organ transplantation and blood transfusion. MMWR Morb Mortal Wkly Rep 2002;51:833-6.
21. West Nile virus activity—United States September 26-October 2, 2002, and investigations of West Nile virus infections in recipients of blood transfusion and organ transplantation. MMWR Morb Mortal Wkly Rep 2002; 51:884, 895.
22. Investigations of West Nile virus infections in recipients of blood transfusions. MMWR Morb Mortal Wkly Rep 2002;51:973-4.
23. West Nile virus activity—United States, October 17-23, 2002. MMWR Morb Mortal Wkly Rep 2002;51:951-2.
24. Biggerstaff BJ, Petersen LR. Estimated risk of West Nile virus transmission through blood transfusion during an epidemic in Queens, New York City. Transfusion 2002; 42:1019-26.
25. Guidance for industry: recommendations for the assessment of donor suitability and blood and blood product safety in cases of known or suspected West Nile virus infection (Internet). Rockville (MD): Center for Biologics Evaluation and Research [accessed 2003 May 1]. Available from: http://www.fda.gov/cber/gdlns/wnvguid.htm