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West Nile virus blood transfusion-related infection despite nucleic acid testing

Alexandre Macedo de Oliveira, Brady D. Beecham, Susan P. Montgomery, Robert S. Lanciotti, Jeffrey M. Linnen, Cristina Giachetti, Larry A. Pietrelli, Susan L. Stramer, and Thomas J. Safranek

BACKGROUND: A case of West Nile virus (WNV) encephalitis associated with transfusion of blood that did not react when tested for WNV by minipool (MP) nucleic acid testing (NAT) is described. A Nebraska man developed clinical encephalitis 13 days after surgery and transfusion of 26 blood components. Antibody testing confirmed WNV infection. An investigation was initiated to determine the source of this infection.

STUDY DESIGN AND METHODS: The patient’s family members were interviewed to identify risk factors for WNV infection. Residual samples were retested for WNV RNA using transcription-mediated amplification (TMA) assay and two polymerase chain reaction (PCR) assays. Blood donors’ follow-up serum samples were collected. All samples were tested for WNV-specific immunoglobulin M antibodies.

RESULTS: The patient’s family denied recent mosquito exposure. The 20 blood components collected after July 2003 did not react when tested for WNV in a six-member MP-NAT at the time of donation. Retrospective individual testing identified one sample as WNV-reactive by the TMA assay and one of the PCR assays. Seroconversion was demonstrated in the donor associated with this sample.

CONCLUSION: WNV RNA detection by individual donation NAT demonstrates viremic blood escaping MP-NAT and supports transfusion-related WNV transmission. MP-NAT may not detect all WNV-infected blood donors, allowing WNV transmission to continue at low levels. WNV NAT assays might vary in sensitivity and pooling donations could further impact test performance. Understanding MP NAT limitations can improve strategies to maintain safety of the blood supply in the United States.

ABBREVIATIONS: IDT = individual donation testing; IND = investigational new drug; MP(s) = minipool(s); PRNT = plaque reduction neutralization test; SLE = St Louis encephalitis; WNV = West Nile virus.

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Molecular Systems Inc., Pleasanton, CA) and the Procleix
WNV assay (Gen-Probe Inc., San Diego, CA).17,8 (Use of
trade names is for identification purposes only and does
not constitute endorsement by the US Public Health Ser-
vice or the Centers for Disease Control and Prevention
[CDC].) The tests were implemented between June and
August 2003.9,10 Because of automation constraints, the
tests are performed with minipools (MPs) of either six
(TaqScreen assay) or 16 (Procleix assay) donations. A lim-
ited number of blood centers have the capacity to rou-
tinely screen by individual donation testing (IDT); in
addition, some programs converted to IDT for limited
periods during the 2003 epidemic. Donated blood from
reactive pools is individually retested to identify the WNV-
reactive donation. Donors associated with WNV-reactive
individual tests are deferred from donation for at least
4 weeks. The WNV-reactive donation and any of that
donor's unexpired blood components obtained in the
28 days before the reactive tests are discarded. In this
report, we describe the investigation of a patient who
developed WNV encephalitis after receiving blood that
had did not react with NAT for WNV in a six-member MP.

CASE REPORT

On August 4, 2003, an 80-year-old Nebraska man under-
went open-heart surgery. During surgery, the patient
received 26 blood components because of massive bleed-
ing: 8 units of red blood cells, 6 units of fresh frozen
plasma (FFP), 2 units of single-donor platelets (PLTs), and
10 units of random-donor PLTs. The patient recovered and
was discharged to home 10 days later.

On August 19, 2003, 15 days after trans-
fusion, the patient was rehospitalized
after experiencing mental confusion
and fever for 2 days. The patient’s con-
dition worsened, and he was placed on
ventilatory support for 3 weeks. His
condition gradually improved, and he
was subsequently transferred to a reha-
bilitation facility.

WNV encephalitis was suspected, and
sample and cerebrospinal fluid were
collected during the second hospitaliza-
tion. These tested positive for the pres-
ence of WNV-specific immunoglobulin
M (IgM) antibodies at the Nebraska
Public Health Laboratory (Table 1).

On September 10, 2003, 37 days
after transfusion, a convalescent serum
sample was collected from the patient.
This sample tested positive for the pres-
ence of WNV by WNV-specific IgM
enzyme-linked immunosorbent assay
(ELISA) and WNV plaque reduction
eutralization test (PRNT) and negative for the presence
of St Louis encephalitis (SLE) by PRNT at the CDC
(Table 1).

Upon receipt of the first positive WNV IgM test, the
hospital infection-control practitioners notified the Office
of Epidemiology at the Nebraska Health and Human Ser-
vices System and an investigation was launched to deter-
mine the source of this infection.

MATERIALS AND METHODS

Family members of the infected patient were interviewed
in August 2003 to evaluate the potential for mosquito
exposure after surgery. In addition, all transfused blood
components were identified and traced to their donors.
Residual samples from the donation testing tubes and
unused co-components were located and stored frozen
(−20°C). Implicated donors were interviewed about symp-
toms consistent with WNV infection, and a follow-up
blood sample was collected in September 2003.

Quarantined residual samples were tested individu-
ally for the presence of WNV RNA with three different lab-
ory tests: the two investigational assays used for WNV
blood donor screening and an in-house assay. The tests
were a transcription-mediated amplification assay (Pro-
cleix WNV assay, Gen-Probe Inc.) and two different
dynamic quantitative polymerase chain reaction (PCR)
assays: TaqScreen WNV test (Roche Molecular Systems
Inc.) and an in-house PCR (CDC, Fort Collins, CO).11-13
Reactive specimens were further tested with the commer-
cial assays: 10 replicates with undiluted samples and 10

### TABLE 1. Laboratory tests performed on the index patient at time of readmission and convalescence, Nebraska, 2003

<table>
<thead>
<tr>
<th>Laboratory study</th>
<th>Collection date</th>
<th>Result</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/100 mL)</td>
<td>8/20/2003</td>
<td>11</td>
<td>13.5-17.0</td>
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<tr>
<td>WBC count (£10⁹ cells/mm³)</td>
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<td>9</td>
<td>4-10</td>
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<td>Differential (%)</td>
<td></td>
<td></td>
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<tr>
<td>Neutrophils</td>
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<td>57</td>
<td>40-80</td>
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<td>Lymphocytes</td>
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<td>39</td>
<td>20-45</td>
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<td>Monocytes</td>
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<td>3</td>
<td>0-10</td>
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<tr>
<td>Eosinophils</td>
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<td>1</td>
<td>0-7</td>
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<tr>
<td>Basophils</td>
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<td>0</td>
<td>0-2</td>
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<tr>
<td>Serum</td>
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<tr>
<td>Glucose (mg/dL)</td>
<td>8/20/2003</td>
<td>103</td>
<td>65-120</td>
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<tr>
<td>Protein (g/dL)</td>
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<td>6.1</td>
<td>6.0-8.3</td>
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<td>CSF*</td>
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<tr>
<td>Glucose (mg/dL)</td>
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<td>Protein (mg/dL)</td>
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<td>Segmented neutrophils (%)</td>
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<td>Arbovirus studies</td>
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<tr>
<td>WNV IgM (serum)</td>
<td>8/20/2003</td>
<td>Positive</td>
<td>Negative</td>
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<tr>
<td>WNV IgM (CSF)</td>
<td>8/20/2003</td>
<td>Positive</td>
<td>Negative</td>
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<td>WNV IgM (serum)</td>
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<td>Negative</td>
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<td>WNV PRNT</td>
<td>9/10/2003</td>
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<td>Negative</td>
</tr>
<tr>
<td>SLE PRNT</td>
<td>9/10/2003</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
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* CSF = cerebrospinal fluid.

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with MP-dilution levels (1:6 for the TaqScreen assay and 1:16 for the Procleix assay). Viral load of reactive specimens was determined by quantitative PCR (SuperQuant for WNV, National Genetics Institute, Los Angeles, CA) and through dilutional studies at CDC and Gen-Probe Inc.

Residual samples from donations and follow-up samples were tested with WNV IgM antibody-capture ELISA. WNV infection was confirmed with PRNT for WNV and SLE.14

RESULTS

Exposure assessment

Family members denied recent mosquito exposure, reporting that the patient was hospitalized or bedridden at home during the 2 weeks before symptom onset. The infected patient received 26 blood components from 26 different donors. These donations generated 41 additional blood components: 18 were transfused to other patients, 17 were quarantined, and 6 were discarded before our investigation began.

Six of the 26 donations took place in February 2003 and had no residual samples available at the time of investigation. The remaining 20 donations were made in July and August 2003. Thirty of these had approximately 200 mL of quarantined FFP available for testing; the other 7 had approximately 2 mL of serum remaining.

Results from donations

The CDC PCR, the investigational TaqScreen assay, and the investigational Procleix assay were all carried out on the 13 high-volume specimens by the developers of each test. One specimen reacted by the Procleix assay, equivocal by the CDC PCR, and did not react by the TaqScreen assay. A subsequent CDC PCR assay with an increased RNA extraction volume reacted. The viral load in this sample was estimated to be 560 copies per mL by the SuperQuant assay and 40 and 30 copies per mL by CDC and Gen-Probe Inc., respectively. Replicate results with the Procleix assay showed reactivity in all 10 replicates with undiluted samples and in 3 of 10 at 1:16 dilution. The TaqScreen assay exhibited reactivity in 5 of 10 and 2 of 10 replicates at undiluted and 1:6-diluted samples, respectively. This donation generated 1 unit of PLTs, which was transfused to the index patient. The other 12 high-volume samples did not react in all NAT assays.

The seven 2-mL specimens were aliquotted and sent to CDC and the other test developers’ laboratories. They all did not react by the CDC PCR and the Procleix assay. Volume was insufficient for the TaqScreen assay.

WNV-specific IgM was negative for all 20 donations that had residual samples available for testing. Test results are shown in Table 2.

Donor follow-up

We obtained follow-up samples from 24 of the 26 donors in mid-September 2003 and tested these for WNV-specific IgM antibodies. The median interval between donation and follow-up sample collection was 44 days (range, 38-219 days). The donor associated with the NAT-positive sample (Donor A) and two other donors tested positive for IgM antibodies (Table 2). Confirmatory PRNT tests were positive for the presence of WNV and negative for the presence of SLE on these three samples. These three donors denied WNV symptoms during the 30 days before and after donation.

Blood co-components from WNV-specific IgM-positive donors

The two additional blood components derived from Donor A’s donation were not transfused. Units from the other two IgM-positive donors were transfused to two other patients. Both recipients were asymptomatic for WNV. One tested negative for the presence of WNV IgM, and the other declined testing.

| Table 2. Test results obtained on donated blood and follow-up samples for the 26 donors, Nebraska, 2003 |
|---|---|---|---|---|---|---|---|
| Donor | Original donation | Follow-up sample |
| | WNV IgM | CDC PCR | Procleix assay | TaqScreen assay | WNV IgM | WNV PRNT | SLE PRNT |
| A | Negative | Reactive* | Reactive | Did not react | Positive | Positive | Negative |
| B | Negative | Did not react | Did not react | Did not react | Positive | Positive | Negative |
| C | Negative | Did not react | Did not react | NA† | Positive | Positive | Negative |
| D-M | Negative | Did not react | Did not react | Did not react | Negative | NA | NA |
| N | Negative | Did not react | Did not react | Did not react | NA | NA | NA |
| O-T | Negative | Did not react | Did not react | NA | Negative | NA | NA |
| U-Y‡ | NA | NA | NA | NA | Negative | NA | NA |
| Z‡ | NA | NA | NA | NA | NA | NA | NA |

* Test performed using high extraction volume.
† NA = not available.
‡ Donations from February 2003.
DISCUSSION

This investigation describes a patient with WNV encephalitis acquired through transfusion of WNV viremic blood that went undetected by MP-NAT screening but reacted for WNV by IDT. Our conclusion is supported by the detection of WNV RNA in one of the donations, followed by seroconversion in the corresponding donor. The implicated donor never developed symptomatic WNV infection. WNV RNA likely was present at levels below the detection limit of the MP format.

Although this investigation suggests WNV blood transfusion-related infection, it has three main limitations. First, the infected patient could have contracted WNV before surgery. Because no presurgical samples from the infected patient were available for testing, excluding existing infection was impossible. Second, it was not possible to rule out mosquito exposure as the mode of transmission. The patient denied symptoms before surgery, however, and his convalescent condition kept him hospitalized or indoors at home, making this other source of infection unlikely. Finally, because two other seroconversions were documented among these donors, we cannot exclude the possibility of an alternate-source donor. Recipients of co-components, however, from donated blood from the seroconverters did not experience WNV symptoms, nor was WNV RNA identified in residual samples from their donations. The high incidence of WNV infection in Nebraska during summer 2003 makes it possible that these persons were infected after donating blood.

Although the six donations from February 2003 were not available for NAT, these donations occurred before WNV was circulating in Nebraska. In addition, follow-up samples from five of these donors were negative for the presence of WNV-specific IgM antibodies, making them an unlikely source of infection.

WNV blood transfusion-related infections were first described during the 2002 epidemic. To minimize this risk, the Food and Drug Administration (FDA) recommended deferring donors reporting fever with headache in the week before donation. In addition to the clinical screening, beginning June 2003, NAT was introduced as a blood-screening tool with protocols conducted under FDA’s investigational new drug (IND) mechanisms. Recognizing technology and resource constraints, the IND protocols allowed for blood donation screening in MPs of 6 (TaqScreen assay) or 16 (Procleix assay) members. Pooling donations may reduce assay sensitivity and increase the possibility of missing donors with low-level viremia.

In this report, the six-member MP TaqScreen assay performed on the donations did not react, and the blood products were released for use in accordance with the IND protocol. A patient developed WNV encephalitis 13 days after transfusion with these products, a time frame compatible with WNV’s incubation period. Residual samples from these donations were subsequently retested at three laboratories. For one donation, samples tested with replicate tests inconsistently reacted by the TaqScreen assay but reacted by the Procleix assay. Viral quantitation of that sample detected low levels of WNV RNA, although variability among quantitative results was observed. Dilutional testing showed that the MP format compromised test performance compared with IDT. The replicate testing also indicated that differences in sensitivity might exist between the investigational NAT assays. Further evaluation is necessary to confirm these conclusions.

The timely development of WNV NAT and its implementation under the IND mechanism are the result of an extraordinary effort by commercial laboratories, CDC, FDA, and the blood-collection community to enhance the safety of the blood supply in the US. In 2003, a total of 818 US blood donations were identified as “presumed viremic,” which is defined as repeat reactivity on the same or on a different NAT assay. The high number of viremic donors with asymptomatic WNV infection highlights the need for highly sensitive WNV-specific NAT to ensure blood supply safety. In addition, strategies such as the implementation of IDT in regions with high WNV infection rates should be considered.

NAT for human immunodeficiency virus-1 and hepatitis C virus has improved the identification of infected donors and is universally performed by the US blood industry, although it has been demonstrated to be cost-ineffective by traditional expenditure versus benefit estimates for medical interventions. NAT could be cost-effective for WNV screening of the blood supply, because seasonal incidence is high in certain geographic areas, infection is asymptomatic in more than 80 percent of cases, and long periods of viremia can occur.

WNV can be effectively transmitted through blood transfusions. The burden of WNV blood transfusion-related disease is probably underestimated owing to the low rate of symptomatic disease among recipients and the difficulty in establishing blood transfusion as the source of WNV infection. The presence of infectious WNV in donated blood necessitates the application of viral detection testing such as NAT. Additional studies are needed to define the sensitivity of current NAT assays, their ability to detect low-level viremic donations, and their cost-effectiveness. This would allow optimization of testing protocols.

Emerging infectious diseases pose risks to blood safety. Health-care workers and public-health authorities should maintain surveillance for the possibility of WNV and other arboviral transmission through blood transfusions. The development and implementation of WNV NAT for blood screening has contributed to a decreased risk of blood-borne transmission in the US. Nevertheless, low-viremic donors may go undetected by MP-NAT.
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