

West Nile Virus Central Nervous System Infection in Patients Treated With Rituximab: Implications for Diagnosis and Prognosis, With a Review of Literature

Sejal Morjaria,¹ Esther Arguello,¹ Ying Taur,¹ Kent Sepkowitz,¹ Vaios Hatzoglou,² Ajay Nemade,² Marc Rosenblum,³ Marcela S. Cavalcanti,³ M. Lia Palomba,⁴ and Anna Kaltsas¹

¹Infectious Disease Service, Department of Medicine, ²Neuroradiology Service, Department of Radiology, Departments of ³Pathology, and ⁴Immunology and Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York

The spectrum of West Nile virus (WNV) infection continues to be elucidated. Many cases of WNV are asymptomatic; however, in immunocompromised patients, symptoms are more likely to be severe. We describe fatal WNV central nervous system disease in lymphoma patients who received rituximab, blunting the inflammatory response and complicating diagnosis.

Keywords. immunocompromised host; lymphoma; molecular testing; rituximab; West Nile virus.

CASE 1

A 37-year-old male from Manhattan, New York (NY) carried a diagnosis of diffuse large cell lymphoma (DLBCL). His residence in NY was not close to a wooded area. He had received 3 cycles of rituximab, cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone (R-CHOP); the last cycle was given in early August. Additional history revealed that he spent weekends in rural New Jersey where he spent a lot of time outdoors; however, he did not recall having mosquito bites. Last, he had not received blood transfusions in the 3 months prior.

Received 23 July 2015; accepted 16 September 2015.

Correspondence: Sejal Morjaria, MD, Memorial Sloan Kettering Cancer Center, 1275 York Avenue Box 9, New York, NY 10065 (morjaris@mskcc.org).

Open Forum Infectious Diseases

© The Author 2015. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com. DOI: 10.1093/ofid/ofv136

At the end of August, he presented to his dermatologist with a diffuse nonpruritic rash thought to be consistent with tinea versicolor; treatment with oral fluconazole was initiated. In early September, he developed fever to 39.2°C and severe right eye pain. His absolute neutrophil count (ANC) was 0.4 K/mcL (normal: 1.5–8.8 K/mcL) and his absolute lymphocyte count (ALC) was 0.9 K/mcL (normal: 0.5–5.3 K/mcL). He was admitted to the hospital for treatment of febrile neutropenia with broad-spectrum antibiotic therapy. Blood and urine cultures collected on admission were negative.

On hospital day 1, magnetic resonance imaging (MRI) of the orbits was performed, which showed normal anatomical structures including the thalami. Over the next 2 days, he developed lethargy and involuntary jerking movements of the extremities.

On hospital day 3, a lumbar puncture (LP) revealed an opening pressure (OP) of 24 cm H₂O. Analysis of the cerebrospinal fluid (CSF) from tube number 1 showed a red blood cell count of 325/mcL and 204/mcL from tube number 4 (normal: <1/mcL); no xanthochromia was reported. Further analysis showed a leukocyte count of 6/mcL (normal: 0–5/mcL) (73% neutrophils, 19% lymphocytes, and 1% reactive lymphocytes). The CSF protein level was 75 mg/dL (normal: 21–38 mg/dL), serum protein was 5.7 g/dL (normal: 6.8–8.1 g/dL), and CSF glucose was within normal limits. The Gram stain and bacterial/fungal cultures were negative. Anti-infectives including intravenous acyclovir for the treatment of encephalitis and meningitis were administered until routine cultures and testing for fungi, bacteria, parasites, and viruses were reported negative.

On hospital day 4, the patient had progressive neurologic deficits including slurred speech, hallucinations, and nystagmus leading to intubation and transfer to the intensive care unit. A repeat brain MRI demonstrated new abnormal fluid-attenuated inversion recovery (FLAIR) hyperintense signal in the thalami.

On hospital day 7, serum and CSF WNV reverse transcription-polymerase chain reaction (RT-PCR) resulted as positive. West Nile virus serum immunoglobulin (Ig)G and IgM antibody were negative on initial testing and were not repeated; antibody testing for WNV from CSF was not obtained. Despite supportive measures, including therapy with intravenous Ig (IVIG), the patient's condition worsened. Eventually, the decision to withdraw care was made; he died on hospital day 17.

Permission was granted for an autopsy limited to only the brain and rostral cervical spinal cord. Gross examination of the brain revealed bilateral softening and hemorrhagic discoloration of the thalami principally involving the dorsomedian regions with additional small foci of necrosis and hemorrhage in

the midbrain and basis pontis, a pathologic correlate of brain MRI findings. Histologic and immunohistochemical examination showed these areas to exhibit perivascular and interstitial infiltration by T lymphocytes represented mainly by CD8⁺/CD4⁻ cytotoxic/suppressor T cells (Figure 1A). Special stains for lymphocytes expressing B-cell markers (CD20, CD79a, PAX 5) failed to identify such cells in the tissue (Figure 1B).

The affected regions also exhibited foci of rarefaction with intense histiocytic infiltration and microcavitation, extensive neuronal loss, as well as thrombotic occlusion of small blood vessels.

CASE 2

A 68-year-old male from Keyport, New Jersey, located on the coastline, with relapsed stage IV DLBCL diagnosed in August received 4 cycles of R-CHOP followed by therapy with ifosfamide, carboplatin, etoposide (ICE), and rituximab. He had not received any blood transfusions and denied any travel in the 3 months prior.

On October 19th, he received rituximab, and 6 days later he presented to an outside hospital for fever and neutropenia. At this admission, he reported a mosquito bite and was found to have an overlying cellulitis treated empirically with parental antibiotics.

A few weeks after this admission, in November, he received another cycle of chemotherapy. In early December, he was admitted with fever and chills. Physical exam and initial laboratory work including his ANC and ALC were unremarkable. His hospital course was notable for intermittent low-grade fevers despite empiric antibiotics. Blood and urine cultures were negative.

On hospital day 7, the patient developed bilateral lower extremity weakness, an intention tremor of the left hand, and confusion. A brain MRI was performed on this same day, which was unremarkable. An LP showed an elevated OP of 32 cm H₂O. Analysis of the CSF (tube 1) showed a red blood cell count of

286/mcL (no xanthochromia reported) with a leukocyte count of 24/mcL (27% neutrophils and 56% lymphocytes). The CSF protein level was 68 mg/dL, serum protein was 3.8 g/dL, and CSF glucose level was normal. The Gram stain, India ink stain, and bacterial/fungal cultures of the CSF were all negative. No other tubes were sent for analysis. West Nile virus RT-PCR was positive in both CSF and serum, but antibody testing for WNV obtained from CSF and serum were negative, and repeat serologic testing was not obtained. Magnetic resonance imaging of the spine was performed on hospital day 9, and axial T1 weighted pre- and postcontrast images demonstrated abnormal enhancement of the cauda equina nerve roots.

On hospital day 11, he displayed involuntary flexion of his arms and legs. He was treated with IVIG for 7 days without any improvement in his neurologic status. Thus, his family opted for comfort measures only; the patient expired 36 days after admission. Permission to perform autopsy was not granted.

DISCUSSION

Most patients with WNV infection are asymptomatic, and only a small percentage of them develop severe neuroinvasive disease and/or death [1]. We report 2 cases of fatal neuroinvasive WNV disease in patients with lymphoma who had received rituximab treatment in addition to other oncologic treatments. Both patients had abnormal skin findings that could have potentially marked the time of infection and preceded worsening illness and hospitalization.

Although there are few human studies directly linking immunosuppression to more severe WNV disease in humans, there are many case reports offering supportive evidence to this effect. One of the earliest reported cases of WNV with encephalitis in the United States was in a 70-year-old with a diagnosis of B-cell non-Hodgkin lymphoma who, 7 days after being treated R-CHOP, was admitted to the hospital with fever, joint pain, and lethargy. She developed confusion on hospital day 3 with

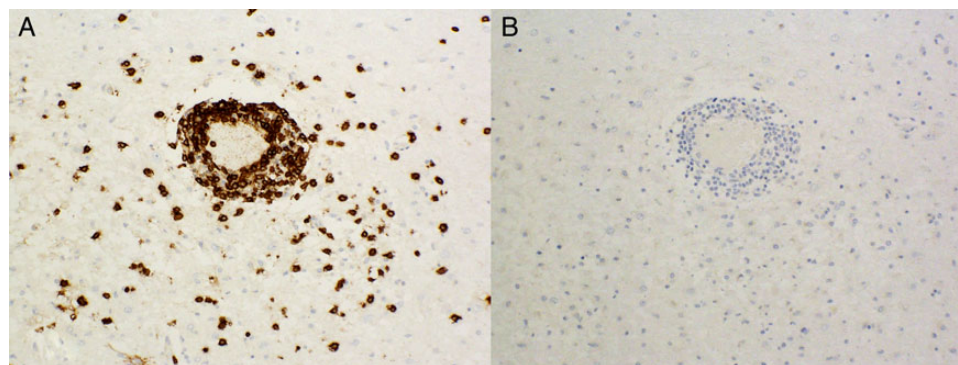


Figure 1. (A) T-cell infiltration is demonstrated in this immunohistochemical preparation for CD3 (hematoxylin counterstain, 40 \times). (B) Immunohistochemical preparation for CD79a of a contiguous section to that shown in the previous figure demonstrates complete absence of B cells in the same area (hematoxylin counterstain, 40 \times).

further mental status deterioration and death by day 35. Similar to our cases, the diagnosis was made by RT-PCR testing of the serum and CSF serologic testing was negative [2].

In addition to the cases cited above [2], 2 case reports of deadly WNV encephalitis after rituximab treatment have been described in a lung transplant and a Hodgkin's lymphoma patient. In the first case, a lung transplant patient received rituximab therapy for recurrent graft rejection. Six months after her last dose of rituximab, the patient developed rapid, fulminant WNV meningoencephalitis [3]. The non-Hodgkin's lymphoma patient had been treated with 5 monthly cycles of fludarabine/rituximab. Approximately 3 months after his last cycle of chemotherapy, the patient had evidence of encephalitis that progressed to coma [4]. Serologic testing for WNV was negative in both cases, and the diagnosis was made by CSF PCR testing alone [3, 4]. Both patients died despite best supportive measures.

Rituximab inhibits the humoral immune response by targeting the surface protein CD20, leading to B-cell death [5]. Induced B-cell death occurs within 24–48 hours of administration. With rituximab therapy, pre-B cells and mature B cells remain at low or undetectable levels for 2–6 months before returning to pretreatment levels, generally taking at least 12 months to resume normal levels. A review of 64 cases of patients who experienced severe viral infections with hepatitis B, cytomegalovirus infection, and varicella-zoster virus after rituximab treatment demonstrated that the median time period from start of rituximab to diagnosis of viral infection was 5 months (range: 1–20 months); our cases fell within this range [6].

In rodent models, a blunted innate and adaptive immunity to WNV increases the risk of dissemination and central nervous system (CNS) disease [7, 8]. Diamond et al addressed this mechanism by infecting B cell-deficient mice (μ MT mice), who then developed increased CNS viral burdens and were vulnerable to lethal infection at much lower doses of WNV compared with wild-type mice. Moreover, μ MT mice had an ~500-fold increase in serum viral load at day 4 after infection, a time point when low levels of neutralizing Igs were already detected in wild-type mice. All B and T cell-deficient mice rapidly succumbed to infection after receiving injections even with a low inoculum (10^2 plaque-forming units) of virus [9]. In addition to possibly inviting more severe disease, rituximab likely complicates routine diagnosis of WNV infection through its effect on humoral immunity. In recent years, IgM and IgG capture enzyme-linked immunosorbent assays from serum or CSF have become the most useful and widely used tests for the diagnosis of arboviral encephalitis [10]. In our 2 patients, these tests were negative [11]. Pathologic findings from Case 1—particularly the lack of B cells on staining of inflamed brain tissue (Figure 1B)—support the notion that reduced humoral immunity in both cases predisposed them to more severe disease leading to death. Although both of our patients received additional

chemotherapy and prednisone, the lack of B cells on staining of brain tissue and the known effect of rituximab on humoral immunity suggest that rituximab may have played a larger role than other immunosuppressive agents.

CONCLUSIONS

In conclusion, we report 2 fatal cases of WNV infection in lymphoma patients. We posit that the blunted humoral response caused by rituximab both compromised inflammatory response and complicated diagnosis. Clinicians should be aware that a patient receiving rituximab may have negative serologic tests, including for WNV disease. Therefore, WNV disease must be considered in immunocompromised patients who present with a compatible syndrome, because there is wide variability in clinical presentation and imaging findings. In such patients, PCR testing is essential for diagnosis.

Acknowledgments

Financial support. This research was funded in part through the NIH/NCI Cancer Center Support (grant P30 CA008748).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

References

1. Winston DJ, Vikram HR, Rabe IB, 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**; 97:881–9.
2. Huang C, Slater B, Rudd R, et al. First isolation of West Nile virus from a patient with encephalitis in the United States. *Emerg Infect Dis* **2002**; 8:1367–71.
3. Levi ME, Quan D, Ho JT, et al. Impact of rituximab-associated B-cell defects on West Nile virus meningoencephalitis in solid organ transplant recipients. *Clin Transplant* **2010**; 24:223–8.
4. Mawhorter SD, Sierk A, Staugaitis SM, et al. Fatal West Nile virus infection after rituximab/fludarabine-induced remission for non-Hodgkin's lymphoma. *Clin Lymphoma Myeloma* **2005**; 6:248–50.
5. Gea-Banacloche JC. Rituximab-associated infections. *Semin Hematol* **2010**; 47:187–98.
6. Aksoy S, Harputluoglu H, Kilickap S, et al. Rituximab-related viral infections in lymphoma patients. *Leuk Lymphoma* **2007**; 48:1307–12.
7. Samuel MA, Diamond MS. Pathogenesis of West Nile virus infection: a balance between virulence, innate and adaptive immunity, and viral evasion. *J Virol* **2006**; 80:9349–60.
8. Diamond MS, Sitati EM, Friend LD, et al. A critical role for induced IgM in the protection against West Nile virus infection. *J Exp Med* **2003**; 198:1853–62.
9. Diamond MS, Shrestha B, Marri A, et al. B cells and antibody play critical roles in the immediate defense of disseminated infection by West Nile encephalitis virus. *J Virol* **2003**; 77:2578–86.
10. Centers for Disease Control and Prevention. West Nile Virus. Available at: <http://www.cdc.gov/ncidod/dvbid/westnile/surv@control.htm>. Accessed 18 December 2014.
11. Martin DA, Muth DA, Brown T, et al. Standardization of immunoglobulin M capture enzyme-linked immunosorbent assays for routine diagnosis of arboviral infections. *J Clin Microbiol* **2000**; 38:1823–6.