

Zika Virus Infection Associated with Severe Thrombocytopenia

Tyler M. Sharp^{1,*}, Jorge Muñoz-Jordán¹, Janice Perez-Padilla¹, Melissa I. Bello-Pagán², Aidsa Rivera¹, Daniel M. Pastula^{3,4}, Jorge L. Salinas⁵, Jose H. Martínez Mendez⁶, Mónica Méndez⁷, Ann M. Powers⁸, Stephen Waterman¹, Brenda Rivera-García²

¹Centers for Disease Control and Prevention, Dengue Branch

²Puerto Rico Department of Health

³University of Colorado Denver

⁴Centers for Disease Control and Prevention, Division of Vector-Borne Diseases

⁵Centers for Disease Control and Prevention, Epidemic Intelligence Service

⁶San Juan City Hospital

⁷Hospital HIMA San Pablo – Bayamón

⁸Centers for Disease Control and Prevention, Arboviral Diseases Branch

*corresponding author: 1324 Calle Cañada, San Juan, PR 00920, Email: tsharp@cdc.gov

Alternative corresponding author: Brenda Rivera-Garcia, brenda.rivera@salud.pr.gov,

787.274.6831, Phone: 787.706.2245

Abstract

We report two patients who developed severe thrombocytopenia after Zika virus infection. The first had 1,000 platelets/mm³, and died following multiple hemorrhages. The second had 2,000 platelets/mm³, melena, ecchymoses, and recovered after receiving intravenous immunoglobulin. Physicians should be aware that Zika virus may be associated with immune-mediated severe thrombocytopenia.

Introduction

In severe cases, dengue and select other flaviviruses (e.g., yellow fever and Omsk hemorrhagic fever viruses) are associated with life-threatening hemorrhagic manifestations [1-3]. Zika virus (ZIKV) is a mosquito-transmitted flavivirus closely related to dengue virus [4]. After it was first detected in Brazil in 2015 [5], ZIKV rapidly spread throughout the Americas [6]. During November 1, 2015 – April 14, 2016, a total of 683 laboratory-positive ZIKV disease cases were identified in Puerto Rico [7].

Most ZIKV infections are asymptomatic or result in only mild disease [4]; however, Guillain-Barré Syndrome (GBS) and congenital infection leading to severe birth defects including microcephaly may result in fatal outcome [4]. Severe thrombocytopenia has recently been reported to be an uncommon manifestation in patients with ZIKV infection [7-11] (Supplemental Table 1). We report two cases from February 2016 with life-threatening severe thrombocytopenia that developed soon after resolution of ZIKV disease.

Case 1

A 72-year-old male with a medical history of untreated hypertension and hyperlipidemia treated with simvastatin presented to an urgent care clinic in San Juan, Puerto Rico with bloody buccal mucosa that had developed 1 day prior. He reported 3 days of fever, malaise, and generalized myalgia that had resolved 2 days prior to presentation. Initial physical examination revealed a blood pressure of 181/90 mmHg and multiple blood clots in the oral cavity. He was diagnosed with hypertensive crisis, prescribed oral clonidine, and discharged home. Soon after, he lost consciousness at home and fell backwards, hitting the posterior aspect of his head. He was transported to the same urgent care clinic, where he had a blood pressure of 211/111 mmHg, was pale and diaphoretic, and had active bleeding from the oral mucosa. He was comatose with dilated and unreactive pupils; Glasgow Coma Scale was 4. Laboratory results included a hemoglobin of 12.8 mg/dL, white blood cell count of $8.5/\text{mm}^3$, and platelet count of $1,000/\text{mm}^3$. Serum glucose was 165 mg/dL. Serum liver enzymes and creatinine were within normal ranges. He was transferred to a local hospital with a presumptive diagnosis of a cerebral vascular accident.

Upon arrival at the hospital he was intubated, with bright red blood noted in the endotracheal tube. Physical examination revealed petechiae on the extremities, lips, and upper palate, and bilateral inspiratory rales and rhonchi. Repeat laboratory results demonstrated a platelet count of $1,000/\text{mm}^3$; platelets were markedly decreased on smear. Prothrombin (PT) and partial thromboplastin time (PTT) results were within normal limits. Urinalysis showed 8–10 red blood cells/high powered field. Computed tomography (CT) of the head without contrast revealed a large right subdural hematoma, right to left shift of approximately 5 millimeters,

diffuse subarachnoid blood, and a right posterior subgaleal hematoma (Figure 1A). CT scan of the chest without contrast revealed dense patchy pulmonary infiltrates throughout the lungs, most consistent with diffuse alveolar hemorrhage (Figure 1B). Dengue hemorrhagic fever was suspected to be the cause of severe thrombocytopenia, and dengue diagnostic testing was ordered. The patient died 7 hours after admission from the sequelae of diffuse alveolar and multiple intracranial hemorrhages.

An autopsy was declined by the patient's family. Pre-mortem serum sent to the Puerto Rico Department of Health was positive for ZIKV, and negative for dengue virus (DENV) and chikungunya virus (CHIKV) by RT-PCR (Supplemental Table 2). Anti-ZIKV and anti-DENV IgM and IgG antibodies were detected by ELISA.

Case 2

A 38-year old morbidly obese (body mass index = 43.6) male presented to an urgent care clinic after two days of rash, myalgia, malaise, headache, nausea, and fever. Physical exam revealed a blood pressure of 146/85 mmHg and a generalized macular rash. The complete blood count was normal, including a platelet count of 200,000/mm³ (Supplemental Figure 1). The rash was ascribed to viral infection, he received acetaminophen (500 mg PO) and diphenhydramine (50 mg IM), and was discharged home with a recommendation for diphenhydramine 25 mg PO q 8 hours. His symptoms resolved over the following 3 days. One day after the resolution of his illness he developed petechiae and bloody ulcers on the tongue and buccal mucosa (Supplemental Figure 2). One day later he presented to a hospital emergency room. Physical examination was unremarkable, with the exception of a blood pressure of 159/98 mmHg and

generalized petechiae. He was diagnosed with dengue-like syndrome, given intravenous fluids, and admitted for care. Chest x-ray was unremarkable. His platelet count was $2,000/\text{mm}^3$; platelets were markedly decreased on smear. He was given 2 units of platelets, acetaminophen 500 mg PO, methylprednisone 60 mg IV q 6 hours, and ceftriaxone 2 g IV q day. Dengue diagnostic testing was ordered.

The following morning he developed melena and ecchymoses on his arms and abdomen. He was given an additional unit of platelets, and was transferred to the intensive care unit (ICU). That afternoon, his platelet count was $6,000/\text{mm}^3$. Since he was not responding to platelet transfusions, his severe thrombocytopenia was ascribed to immune thrombocytopenic purpura. The dose of methylprednisone was increased to 80 g IV q 8 hours, and intravenous immunoglobulin was initiated at 40 g IV q day for 5 days. Over the following 3 days his vital signs remained stable, and he had no evidence of plasma leakage, shock, or additional hemorrhagic manifestations. His platelet count rose steadily, and he was transferred from the ICU to the in-patient ward on day 5 of hospitalization. On day 6 he was discharged home in good condition with a prescription for oral prednisone tablets (20 mg 3 times daily for 42 days).

Blood chemistries were obtained only on day 4 of hospitalization, and were normal except for mildly elevated alanine transaminase (64 units/L). HIV-1/2 titers as well as blood and urine bacterial cultures were negative. Serum collected upon hospital admission was positive for anti-ZIKV IgM antibody, negative for anti-DENV IgM antibody, and negative for ZIKV, DENV, and CHIKV by RT-PCR (Supplemental Table 2). Convalescent serum, saliva, and urine specimens collected 46 days after illness onset were negative for detection of ZIKV nucleic acid. The same convalescent serum specimen was positive for detection of anti-ZIKV IgM antibody and negative for detection of anti-DENV IgM. Plaque reduction neutralization test performed on

both serum specimens demonstrated 4-fold rise in anti-ZIKV antibody titer, and prior DENV infection (Supplemental Table).

Discussion

These 2 cases along with previous reports [9, 10] suggest an association between ZIKV and severe thrombocytopenia. Neither of the 2 cases described here nor 1 from Suriname [10] had evidence of involvement of the other hematologic cell lines, pre-existing bleeding disorders, or severe liver disease that would explain the patients' severe thrombocytopenia. In Case 1, a normal PT and PTT argue against disseminated intravascular coagulation as the cause of severe thrombocytopenia. In Case 2 and the Suriname case [10], interventions for immune thrombocytopenic purpura (i.e., IVIG) were associated with clinical improvement. Lack of available medical history or details of clinical course from 3 fatal cases with severe thrombocytopenia in Colombia [9] and 4 cases of ITP during the ZIKV outbreak in French Polynesia [11] preclude interpretation of the relative contribution of ZIKV infection, pre-existing medical conditions, and clinical interventions to patient outcome. Investigation of additional cases of severe thrombocytopenia in Puerto Rico is ongoing to better characterize the mechanism of association with ZIKV infection.

Both cases presented herein are noteworthy in that bloody lesions in the buccal mucosa developed 1 day after resolution of an acute febrile illness with generalized rash. The Suriname case had onset of hematomas 10 days after an acute illness with fever and rash; however, she did not reach nadir platelet count ($10,000/\text{mm}^3$) until 29 days after illness onset [10]. We therefore suspect that the mechanism of severe thrombocytopenia in these cases may be immune-

mediated, as ZIKV infection has been associated with other immune-mediated processes (e.g., GBS) [4]. Nonetheless, as diagnostic testing for evidence of other infections (e.g., HIV, hepatitis C virus) and autoimmune disease was not performed for all 3 patients with ZIKV infection and severe thrombocytopenia, other etiologies cannot be ruled out.

Although both patients from Puerto Rico had an illness consistent with ZIKV disease, neither was diagnosed with suspected ZIKV infection. Instead, as with other cases of ZIKV-associated severe thrombocytopenia [9, 10], both were suspected to have dengue due to severe hemorrhage and thrombocytopenia. Notably, no patient with ZIKV-associated severe thrombocytopenia had evidence of plasma leakage, which precedes the onset of major hemorrhagic manifestations in most patients with severe dengue [1]. This observation suggests that hemorrhage in these patients was the result of severe thrombocytopenia and not prolonged shock, as is more often the case in dengue patients [1]. Moreover, both the timing and mechanism of thrombocytopenia in patients with ZIKV infection would appear to be distinct from those of dengue patients, who most frequently develop thrombocytopenia during the febrile phase due to direct infection of progenitor cells in the bone marrow and/or destruction of mature platelets [12]. The diagnosis of immune thrombocytopenic purpura in Case 2 and the Suriname case [10] triggered treatment with IVIG and a corticosteroid; however, corticosteroids are not recommended for dengue patients since they are not associated with improved patient outcome [1]. Hence, due to the lack of available point-of-care diagnostic tests to confidently differentiate patients with ZIKV disease and dengue, administration of corticosteroids to patients with suspected dengue or ZIKV disease should be reserved for those who have evidence of immune thrombocytopenic purpura or other complications for which clinical management includes corticosteroids.

Because of known cross-reactivity of antibodies directed against DENV with ZIKV antigen [4], a limitation of this investigation includes Case 2 having only serologic diagnostic evidence of ZIKV infection. Although original antigenic sin has been observed in patients with ZIKV infection and prior DENV infection [13], lack of detection of anti-DENV IgM antibody in both acute and convalescent specimens along with four-fold increase in ZIKV PRNT titer are together highly suggestive of ZIKV infection. Moreover, although Case 2 did not have ZIKV detected in a urine specimen collected one-and-a-half months after illness onset, such testing may have improved sensitivity for diagnosis of patients with recent ZIKV infection [14]. Similarly, this investigation was benefited by the availability of the TrioPlex RT-PCR assay, which simultaneously detects nucleic acid from ZIKV, DENV, and CHIKV, and enabled diagnosis of ZIKV infection in Case 1 when dengue diagnostic testing was ordered. Therefore, ZIKV-infected patients with severe thrombocytopenia and hemorrhage may be under-recognized due to misdiagnosis as severe dengue. Clinicians seeing febrile patients who live in or have recently returned from areas where ZIKV is circulating should be aware that acute ZIKV infection may be associated with severe thrombocytopenia.

Acknowledgements

The findings and conclusions of this report are those of the authors and do not necessarily reflect the official position of the Centers for Disease Control and Prevention.

We thank Dr. Jennifer Read for helpful manuscript review.

All authors declare no conflicts of interest. This investigation was supported by the Centers for Disease Control and Prevention and Puerto Rico Department of Health.

REFERENCES

1. Simmons CP, Farrar JJ, Nguyen v V, Wills B. Dengue. *N Engl J Med* **2012**; 366(15): 1423-32.
2. Ruzek D, Yakimenko VV, Karan LS, Tkachev SE. Omsk haemorrhagic fever. *Lancet* **2010**; 376(9758): 2104-13.
3. Monath TP, Vasconcelos PF. Yellow fever. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology* **2015**; 64: 160-73.
4. Petersen LR, Jamieson DJ, Powers AM, Honein MA. Zika Virus. *N Engl J Med* **2016**; 374(16): 1552-63.
5. Campos GS, Bandeira AC, Sardi SI. Zika Virus Outbreak, Bahia, Brazil. *Emerging infectious diseases* **2015**; 21(10): 1885-6.
6. Organization PAH. Countries and territories with autochthonous transmission in the Americas reported in 2015-2016. Available at: http://www.paho.org/hq/index.php?option=com_content&view=article&id=11603&Itemid=41696&lang=en. Accessed April 1.
7. Dirlikov E, Ryff KR, Torres-Aponte J, et al. Update: Ongoing Zika Virus Transmission - Puerto Rico, November 1, 2015-April 14, 2016. *MMWR Morbidity and mortality weekly report* **2016**; 65(17): 451-5.
8. Zammarchi L, Stella G, Mantella A, et al. Zika virus infections imported to Italy: clinical, immunological and virological findings, and public health implications. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology* **2015**; 63: 32-5.
9. Sarmiento-Ospina A, Vasquez-Serna H, Jimenez-Canizales CE, Villamil-Gomez WE, Rodriguez-Morales AJ. Zika virus associated deaths in Colombia. *The Lancet infectious diseases* **2016**.

10. Karimi O, Goorhuis A, Schinkel J, et al. Thrombocytopenia and subcutaneous bleedings in a patient with Zika virus infection. *Lancet* **2016**; 387(10022): 939-40.
11. Iosifidis S, Mallet HP, Leparac Goffart I, Gauthier V, Cardoso T, Herida M. Current Zika virus epidemiology and recent epidemics. *Medecine et maladies infectieuses* **2014**; 44(7): 302-7.
12. de Azeredo EL, Monteiro RQ, de-Oliveira Pinto LM. Thrombocytopenia in Dengue: Interrelationship between Virus and the Imbalance between Coagulation and Fibrinolysis and Inflammatory Mediators. *Mediators of inflammation* **2015**; 2015: 313842.
13. Lanciotti RS, Kosoy OL, Laven JJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerging infectious diseases* **2008**; 14(8): 1232-9.
14. Interim Guidance for Zika Virus Testing of Urine - United States, 2016. *MMWR Morbidity and mortality weekly report* **2016**; 65(18): 474.

Accepted Manuscript

Figure Hemorrhagic manifestations in Case 1. (A) Computed tomography (CT) of the head without contrast demonstrating right-sided subdural hemorrhage (black arc), diffuse subarachnoid hemorrhage (arrows), subgaleal hematoma (white arc), and a 5 millimeter right to left midline shift. (B) CT of the chest without contrast demonstrating diffuse alveolar hemorrhage.

