Flavivirus cross-reactivity in serological tests and Guillain-Barré syndrome in
a hematopoietic stem cell transplant patient: A case report

Sonia M. Raboni¹, Carmem Bonfim², Bernardo M. Almeida³, Camila Zanluca⁴, Andrea C. Koishi⁴, Paula
R.V.P. Rodrigues⁵, Claudia K. Kay⁵, Lisandro L. Ribeiro², Rosana H. Scola⁵, Claudia N. Duarte dos
Santos⁴

¹Laboratory of Virology, Universidade Federal do Paraná, Paraná, Brazil

²Bone Marrow Transplant Division, Universidade Federal do Paraná, Paraná, Brazil

³Hospital Epidemiology Division, Universidade Federal do Paraná, Paraná, Brazil

⁴Molecular Virology Laboratory, Instituto Carlos Chagas, Fundação Oswaldo Cruz, Rio de Janeiro,
Brazil

⁵Neuromuscular Disease Service, Neurology Division, Universidade Federal do Paraná, Paraná, Brazil

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Abstract

Serological diagnosis of flavivirus infection is a challenge, particularly in the context of a disease associated with immune response enhancement in a transplant patient, where aspects such as previous flavivirus infections may be involved with the outcome. We report a case of a pediatric patient who developed Guillain-Barré syndrome (GBS) after matched-unrelated hematopoietic stem cell transplantation (HSCT). The patient lives in a Brazilian region that is experiencing an epidemic of Zika virus (ZIKV) and dengue virus (DENV). Because an increasing number of cases of GBS, likely triggered by ZIKV infection, are being reported in Brazil, samples from the patient were tested for both ZIKV and DENV infection. Serological assays strongly suggested a recent ZIKV infection, although infection by DENV or co-infection with both viruses cannot be ruled out. The presence of anti-DENV immunoglobulin-G in donor serum led to the hypothesis that antibodies from the donor could have enhanced the severity of the ZIKV infection. This hypothesis is in agreement with the recent findings that DENV sero-cross-reactivity drives antibody-dependent...
enhancement of ZIKV infection. These findings highlight the need for discussion of the indication to perform previous flavivirus tests in HSCT donors, especially in areas where ZIKV and other flaviviruses co-circulate.

**Keywords:**
Dengue virus, Guillain-Barré syndrome, hematopoietic stem cell transplantation, Zika virus.

1 INTRODUCTION

Guillain-Barré syndrome (GBS) is an acute, immune-mediated polyradiculoneuropathy typically occurring after respiratory and gastrointestinal infections and is usually caused by viral and bacterial infections. GBS is a rare complication related to hematopoietic stem cell transplantation (HSCT).

Dengue is a highly prevalent arboviral disease in the tropics caused by viruses belonging to the Flaviviridae family. The dengue virus (DENV) has four serotypes, DENV 1-4. In Brazil, since its re-introduction, dengue epidemics have been reported annually, with co-circulation of different serotypes over time. Currently, the World Health Organization estimates that approximately 2.5 billion people are living in endemic areas, and 390 million dengue cases occur per year. The incidence of neurological complications after DENV infection ranges from 1%-25%, with the most common presentations including encephalopathy, GBS, acute motor weakness, seizures, neuritis, hypokalemic paralysis, and pyramidal tract signs.

Zika virus (ZIKV) was first detected in Africa in the late 1940s and was initially considered a relatively mild disease. However, during the 2013 outbreak in French Polynesia, ZIKV infection was found to result in severe clinical presentations, including
neurological complications.\(^7\) In March 2015, Brazil reported autochthonous transmission of ZIKV,\(^8\) and an epidemic was subsequently declared, with thousands of cases identified, mainly in northeastern and southeastern Brazil. Since then, serious complications associated with ZIKV infection, including GBS and the congenital syndrome, which may lead to microcephaly and maculopathy, have been reported throughout Brazil and other territories of South America.\(^1,9\)

Here, we report a case of a 9-year-old girl who developed GBS 92 days after a matched-unrelated HSCT, with positive immunoglobulin-M (IgM) responses for DENV and ZIKV in serological tests. The Institutional Review Board approved this study (CAAE 53344515.3.0000.5248), and the patient’s legal guardian provided written consent.

2 CASE REPORT

A 9-year-old girl with Fanconi anemia underwent a matched-unrelated HSCT in June 2015 in Curitiba, Southern Brazil. She engrafted promptly after transplantation and had no infectious complications. She was discharged on post-transplant day +23, and chimerism analysis on day +30 showed 100% donor cells. The patient had no complications during the immediate transplant period and returned to her hometown in the state of Maranhão (northeast Brazil) on day +75. The patient had no prior diagnosis of flavivirus infection, but received vaccine for yellow fever 9 years ago.

On day +92, the patient returned to Curitiba (i.e., a 17-day interval) complaining of lower extremity paresthesia, muscle weakness, and inability to walk for 2 days. Fever, diarrhea, skin rash, and other symptoms were absent. Neurophysiological studies showed mixed sensory-motor demyelinating neuropathy with an axonal damage pattern, consistent with acute inflammatory demyelinating polyneuropathy. The F-wave response was absent in
both the upper and lower limbs, and the left ulnar sensory nerve and left superficial peroneal nerve showed no response, indicating GBS.

Cerebrospinal fluid (CSF) analysis revealed leukocytosis with lymphocyte predominance and an elevated total protein level. CSF and blood cultures for common bacteria, *Mycobacterium tuberculosis*, and fungi were negative. Polymerase chain reactions (PCRs) for *Toxoplasma gondii*, Epstein-Barr virus, cytomegalovirus, adenovirus, human herpesvirus type-6, enterovirus, and herpes simplex virus were also negative. Computed tomography of the brain, magnetic resonance imaging findings, and ophthalmic evaluation were normal. She had normal blood counts and renal and hepatic functions; chimerism showed 100% donor cells on day +95 (Figure 1A).

Following a diagnosis of GBS, the patient received 2 g/kg intravenous immunoglobulin (IVIg) (Tegeline®; LFB Biomedicaments, Lille, France), and her neurological symptoms consequently improved. She was discharged after 1 week, with partial recovery of strength, sensitivity, and reflexes, and total recovery of paresthesia. A week later, because of worsening of the neurological symptoms and respiratory insufficiency that required mechanical ventilation, she underwent plasmapheresis and received another cycle of IVIg (total, 3 g/kg). The patient slowly recovered and, 4 months after the initial diagnosis, the patient did not present any neurological sequelae.

Blood and CSF samples previously collected on post-transplant days +92, +100, and +296 were submitted for laboratory diagnosis of DENV and ZIKV infections, as the patient’s hometown was experiencing a double epidemic of ZIKV and DENV (Figure 1A). The serum sample from day +92 post HSCT was negative for the presence of DENV NS1 antigen (PanBio®, Alere, USA), and reverse transcription-PCR was negative for DENV and ZIKV nucleic acids. Molecular generic flavivirus detection was also negative. Regarding flavivirus
serology, the serum samples taken on days +92 and +100 were positive for anti-DENV and anti-ZIKV IgM; however, on day +296, only anti-ZIKV IgM persisted positive (Table 1). The anti-DENV immunoglobulin-G (IgG) tests using the three blood samples revealed high titers, and anti-DENV IgG was also detected in the CSF samples. All serum samples were positive for DENV and ZIKV antibodies in a neutralization assay (plaque reduction neutralization test) (Figure 1B), and negative for yellow fever virus (YFV). In addition, a serum sample collected from the unrelated Brazilian donor was negative for both IgM anti-DENV and anti-ZIKV antibodies, and positive for anti-DENV IgG (Table 1), and a neutralization assay for DENV, ZIKV, and YFV yielded non-neutralizing antibodies.

3 DISCUSSION

GBS is a rare complication in the HSCT setting, and its etiological investigation is challenging, as it involves evaluation of immune response, which in the context of an immunosuppressed patient may be complex. It generally occurs between 10 days to 12 months post transplant and is typically associated with viral infections.\textsuperscript{13}

Neurological involvement in patients with dengue is estimated to be 21% in Brazil\textsuperscript{5}, and is usually associated with direct central nervous system infection caused by the virus.\textsuperscript{15} To date, a few cases of GBS following DENV infection have been reported. Therefore, during dengue outbreaks in endemic countries and in cases where the etiology of encephalitis is uncertain, DENV infection should be considered.\textsuperscript{16,17}

Notably, an increasing incidence of GBS has been reported in countries where there is extensive co-circulation of DENV and ZIKV.\textsuperscript{18} It has been hypothesized that the association between ZIKV infection and GBS may be a result of molecular mimicry, similar to that noted in other bacterial and viral infections associated with GBS. In this scenario, antibodies
against the microorganism may react with antigens on the nerve tissues or may cause direct damage via viral neurotropism.^{19} The first hypothesis became more likely after Cao-Lormeau et al.\textsuperscript{1} demonstrated a temporal association between peaks in the incidence of ZIKV infections and GBS, and Siu et al.\textsuperscript{19} described an acute ZIKV infection concurrent with the onset of GBS in a patient experiencing a secondary flavivirus infection. Furthermore, this hypothesis is supported by the demonstration of serological cross-reactivity between infections caused by viruses of the Flaviviridae family, such as DENV, YFV, West Nile, and Japanese encephalitis viruses.\textsuperscript{20} Recently, reports have demonstrated that ZIKV infection, in individuals with a history of a previous flavivirus infection (or vaccination), such as DENV and YFV, may result in a boost in the pre-existing antibodies, which could enhance the infectivity of ZIKV and the severity of the infection.\textsuperscript{21-23} The serum sample of the bone marrow donor contained IgG antibodies against DENV, and if the patient was recently infected by ZIKV, the previous DENV antibodies from the donor would have enhanced the severity of the ZIKV infection, leading to GBS. In one previous study, patients with GBS after ZIKV infection had atypically low levels of anti-ganglioside antibodies compared to patients with GBS caused by other etiologies, suggesting that ZIKV may induce GBS by different mechanisms.\textsuperscript{1}

In the case reported here, all molecular tests for identifying acute flavivirus infection were negative. However, we could not exclude a recent ZIKV infection because the viremia during the acute phase of the disease is usually low and transient.\textsuperscript{19,24} Notably, the patient had no classical symptoms related to the acute phase of infection by both viruses. Regarding ZIKV infection cases, more studies are needed to determine the dynamics of viral clearance and the kinetics of the immune response, as well as the risk of chronicity, reactivation, and reinfection by this pathogen among immunosuppressed patients.

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Concerning the serological profile, typically, in the course of flavivirus infections, IgM is detected within a few days after the onset of symptoms and may persist up to 3 months. IgG antibodies appear a few days later and can persist for years.\textsuperscript{19} Although the patient described in this study lives in a region with a high prevalence of both DENV and ZIKV, she had no known history of previous DENV infection, but a YFV vaccination in 2006.

It is well documented that serological assays to detect DENV and ZIKV infection may not be able to discriminate between these infections, owing to the extended cross-reactivity. This cross-reactivity is probably caused by the high amino acid sequence homology of the envelope E protein with flaviviruses belonging to the same serocomplex.\textsuperscript{25} To circumvent this problem and discriminate ZIKV and DENV infections, we performed neutralization assays for both viruses (Figure 1B).

The results of serological tests with samples collected on days +92, +100, and +296 showed antibodies against both DENV and ZIKV. The anti-ZIKV IgM titers had a four-fold increase in the paired samples, while anti-DENV IgM titers were constant in the first two samples and undetectable on day +296. These findings suggest that the patient had a recent ZIKV infection. Nevertheless, we cannot exclude the possibility that the patient was co-infected by DENV and ZIKV during the 17-day stay in her hometown, because both viruses co-circulate in the region. However, it is also possible that the pre-existing donor antibodies to flaviviruses such as DENV would act as an enhancement factor, leading to the severe clinical picture of GBS. Reinforcing this hypothesis, two subsequent analyses showed 100% chimerism in the patient samples, i.e., all recipient hematopoietic cells were from the donor.
Our extensive search of the medical literature revealed no related cases. In conclusion, this is the first report to our knowledge of a pediatric patient who developed GBS following a flavivirus infection post-HSCT and whose diagnostic confirmation was difficult because of the extensive cross-reactivity between antibodies against related flaviviruses. This brief report highlights some important aspects that must be considered by clinicians within the context of transplant patients, such as: (i) Could a blood transfusion or other material from a donor with asymptomatic infection be the source of transmission of this infection? (ii) If this is a possibility, what would be the impact on the pathogenesis of these cases, considering the current epidemiological picture in almost all Central and South America countries, where ZIKV and DENV co-circulate, causing huge epidemics? (iii) Could the immune response to flavivirus infection observed in an HSCT patient with 100% donor chimerism be a result of a memory immune response from the patient or from the donor living in a region with a high prevalence of DENV and ZIKV?

Moreover, this study highlights the urgent need for case-control studies concerning the impact of previous flavivirus antibodies on the severity of ZIKV infections, especially in countries such as Brazil where the vast majority of the population has DENV antibodies and a high percent of the population has been vaccinated against yellow fever.

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Author contributions: S.M.R. and C.M.B. conceived and designed the study, analyzed the data, and participated in the writing of the paper. C.N.D.S., A.C.K., and C.Z. conceived the study, performed laboratory tests, analyzed the data, and participated in the writing of the
paper. B.M.A. and L.L.R. analyzed the clinical and epidemiological data. P.R.V.P., C.S.K.K., and R.H.S. performed the neurophysiological studies and neurological follow-up. All authors drafted and approved the final version of the report.

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REFERENCES


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**Figure Legend**

FIGURE 1. (A) Timeline of the patient’ infection course. (B) Zika virus (ZIKV) and dengue virus (DENV) neutralization assays. For the neutralization assay, the serum samples were serially diluted and added to the virus. After this neutralization step, the inoculum was added to Huh7.5 cells for 1 hour and later replaced with fresh media. After 48 hours, images were taken and analyzed with using an Operetta automated microscope (Perkin Elmer). The neutralization titers (endpoint 50) are represented in the graph. The dashed line indicates
the test cutoff. HSCT, hematopoietic stem cell transplantation; GBS, Guillain-Barré syndrome; CSF, cerebrospinal fluid; RT-PCR, reverse transcription polymerase chain reaction; IgM, immunoglobulin-M.

**TABLE 1** Results and sample titers of the hematopoietic stem cell transplant (HSCT) patient and donor investigated for dengue virus (DENV) and Zika virus (ZIKV) infections by ELISA and neutralization assays.

<table>
<thead>
<tr>
<th>Source</th>
<th>Samples</th>
<th>Days Post HSCT</th>
<th>DENV</th>
<th>ZIKV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ELISA IgM&lt;sup&gt;1&lt;/sup&gt;</td>
<td>ELISA IgG&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Patient</td>
<td>Serum</td>
<td>92</td>
<td>100</td>
<td>≥6,400</td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td>92</td>
<td>Negative</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>100</td>
<td>100</td>
<td>≥6,400</td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td>100</td>
<td>Negative</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>296</td>
<td>Negative</td>
<td>≥6,400</td>
</tr>
<tr>
<td>Donor</td>
<td>Serum</td>
<td>Negative</td>
<td>400</td>
<td>Negative</td>
</tr>
</tbody>
</table>

<sup>1</sup>PanBio Dengue IgM capture ELISA and PanBio Dengue IgG Indirect ELISA.

<sup>2</sup>Neutralization assay (endpoint 50) – plaque reduction neutralization test. Cutoff value = 30.

<sup>3</sup>Zika serology: in-house IgM capture ELISA.

ELISA, enzyme-linked immunosorbent assay; IgM, immunoglobulin-M; IgG, immunoglobulin-G; CSF, cerebrospinal fluid.