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Neurologic Complications Associated With the Zika Virus in Brazilian Adults

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IMPORTANCE There are no prospective cohort studies assessing the incidence and spectrum of neurologic manifestations secondary to Zika virus (ZIKV) infection in adults.

OBJECTIVE To evaluate the rates of acute ZIKV infection among patients hospitalized with Guillain-Barré syndrome (GBS), meningoencephalitis, or transverse myelitis.

DESIGN, SETTING, AND PARTICIPANTS A prospective, observational cohort study was conducted at a tertiary referral center for neurological diseases in Rio de Janeiro, Brazil, between December 5, 2015, and May 10, 2016, among consecutive hospitalized adults (>18 years of age) with new-onset acute parainfectious or neuroinflammatory disease. All participants were tested for a series of arbovirosis. Three-month functional outcome was assessed.

INTERVENTIONS Samples of serum and cerebrospinal fluid were tested for ZIKV using real-time reverse-transcriptase–polymerase chain reaction and an IgM antibody-capture enzyme-linked immunosorbent assay. Clinical, radiographic (magnetic resonance imaging), electrophysiological, and 3-month functional outcome data were collected.

MAIN OUTCOMES AND MEASURES The detection of neurologic complications secondary to ZIKV infection.

RESULTS Forty patients (15 women and 25 men; median age, 44 years [range, 22-72 years]) were enrolled, including 29 patients (73%) with GBS (90% Brighton level 1 certainty), 7 (18%) with encephalitis, 3 (8%) with transverse myelitis, and 1 (3%) with newly diagnosed chronic inflammatory demyelinating polyneuropathy. Of these, 35 patients (88%) had molecular and/or serologic evidence of recent ZIKV infection in the serum and/or cerebrospinal fluid. Of the patients positive for ZIKV infection, 27 had GBS (18 demyelinating, 8 axonal, and 1 Miller Fisher syndrome), 5 had encephalitis (3 with concomitant acute neuromuscular disease), 2 had transverse myelitis, and 1 had chronic inflammatory demyelinating polyneuropathy. Admission to the intensive care unit was required for 9 patients positive for ZIKV infection (26%), and 5 (14%) required mechanical ventilation. Compared with admission during the period from December 5, 2013, to May 10, 2014 (before the Brazilian outbreak of ZIKV), admissions for GBS increased from a mean of 1.0 per month to 5.6 per month, admissions for encephalitis increased from 0.4 per month to 1.4 per month, and admissions for transverse myelitis remained constant at 0.6 per month. At 3 months, 2 patients positive for ZIKV infection (6%) died (1 with GBS and 1 with encephalitis), 18 (51%) had chronic pain, and the median modified Rankin score among survivors was 2 (range, 0-5).

CONCLUSIONS AND RELEVANCE In this single-center Brazilian cohort, ZIKV infection was associated with an increase in the incidence of a diverse spectrum of serious neurologic syndromes. The data also suggest that serologic and molecular testing using blood and cerebrospinal fluid samples can serve as a less expensive, alternative diagnostic strategy in developing countries, where plaque reduction neutralization testing is impractical.

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Editorial

Supplemental content

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Zika virus (ZIKV) is a flavivirus related to the dengue, yellow fever, and West Nile viruses and is transmitted by the *Aedes* mosquito species. In July 2015, 76 patients with neurologic syndromes and recent symptoms suggestive of ZIKV infection were identified in the state of Bahia, Brazil, of which 42 (55%) had confirmed Guillain-Barré syndrome (GBS).[1] By July 2016, a total of 165 932 suspected cases of ZIKV infection were reported to the Brazilian Ministry of Health, with 66 180 confirmed cases in 1850 cities and 22 of 26 states (incidence rate, 81.2 cases per 100 000 inhabitants).[2] It is speculated that 500 000 to 1.5 million people might have been infected with ZIKV in Brazil since the beginning of the outbreak.[3] The first cases of mosquito-transmitted ZIKV in the United States were reported in July 2016.[4] Despite concomitant increases in the incidence of both GBS[5] and ZIKV in Brazil, the links to neurologic complications in adults have been limited to case series on GBS,[6,7] meningoencephalitis,[8] transverse myelitis,[9] and ophthalmologic disease.[10]

We aimed to prospectively evaluate adult patients with neurologic syndromes consistent with GBS, transverse myelitis, or meningoencephalitis for molecular and serologic evidence of recent ZIKV infection. We also compared radiographic and electrophysiological findings and functional outcomes between patients positive for ZIKV infection and those negative for ZIKV infection who were admitted with each syndrome.

Key Points

Question What is the spectrum of neurologic manifestations in adults with Zika virus infection?

Findings This cohort study evaluated adult patients with new-onset parainfectious or neuroinflammatory disease for the presence of acute Zika virus infection in both serum and cerebrospinal fluid samples using molecular and serological testing. An increase in the incidence of Guillain-Barré syndrome and encephalitis was observed compared with the period before the outbreak of the Zika virus.

Meaning The Zika virus may be associated with a rapid increase in the incidence of life-threatening neurologic syndromes; serologic and molecular testing using blood and cerebrospinal fluid samples can serve as an alternative diagnostic strategy.

Methods

Study Design and Participants

We conducted a prospective, observational cohort study at a tertiary care, academic hospital (Antonio Pedro University Hospital of Universidade Federal Fluminense) in the state of Rio de Janeiro, Brazil. This hospital specializes in the care of patients with neuromuscular and neuroinfectious diseases and receives referrals from the Rio de Janeiro metropolitan area.[11] This study was approved by the Hospital Universitario Antonio Pedro-Universidade Federal Fluminense institutional review board. All patients or their surrogates provided written consent to participate in this study.

Consecutive patients who presented between December 5, 2015, and May 10, 2016, were approached for enrollment (eAppendix in the Supplement for inclusion and exclusion criteria). Data on demographics, clinical presentation, results of neurologic examination, hospital course, and 3-month outcomes were prospectively collected (eAppendix in the Supplement).[12]

To assess changes in the incidence of each neurologic syndrome, we compared admission rates during a similar period prior to the outbreak of ZIKV in Brazil (December 5, 2013, to May 10, 2014). We chose to evaluate admissions during a similar summer-fall time frame to control for seasonal variations in mosquito-borne illnesses.

Laboratory Testing

Testing for ZIKV infection followed the algorithm established by the Pan American Health Organization and the World Health Organization (WHO).[13] First, blood and cerebrospinal fluid (CSF) samples collected at admission were tested in duplicate using real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) for ZIKV following published methods.[14] All instances of rRT-PCR were performed using the same primers and cycle times at the Oswaldo Cruz Foundation Flavivirus Laboratory, which is the regional flavivirus reference laboratory. All positive and equivocal samples (cycle threshold, >38-40) were repeated with a second set of primer and probes for confirmation.

If the results of the rRT-PCR studies were negative, a ZIKV IgM antibody-capture enzyme-linked immunosorbent assay (Zika MAC-ELISA) was performed for both blood and CSF samples (when adequate CSF samples were available). A ratio of patient optic density to negative control of greater than 3.0 was considered positive, 2.0 to 3.0 considered indeterminate, and less than 2.0 considered negative for both the serum and CSF samples. If results were positive for ZIKV IgM, serum dengue IgM for serotypes 1 to 4 was performed to check for cross-reactivity (negative dengue IgM result defined by optical density ratio <9.0; indeterminate result, 9.0-11.0; and positive result, >11.0). If serum ZIKV and dengue IgM results were positive, then CSF IgM for dengue virus was tested as well. The ZIKV in-house MAC-ELISA was performed according to recommendations from the Centers for Disease Control and Prevention (CDC), with the use of antigens provided by the CDC.

A recent ZIKV infection was diagnosed if serum and/or CSF ZIKV rRT-PCR results were positive, or if the serum and/or CSF ZIKV IgM optical density ratio was greater than 3.0 and serum dengue IgM results were negative (optical density ratio, <9.0). If serum dengue virus IgM results were positive, acute ZIKV could be diagnosed only if both serum and CSF ZIKV IgM results were positive and CSF dengue IgM results were negative. This algorithm incorporating CSF serologic findings is based on the concept that the IgM pentamer does not cross the blood-brain barrier owing to its size, and, hence, only primary intrathecal infection would result in CSF IgM positivity.[15,16] Since December 2016, the CDC no longer recommends plaque reduction neutralization testing (PRNT) to rule out cross-reactivity among flaviviruses in countries with a high prevalence of concurrent flavivirosis owing to its low

accuracy in this setting.[17] Also, the CDC now considers MAC-ELISA serologic findings a valid method of ZIKV detection in CSF.[17] Dengue serologic studies were performed using kits approved by the WHO and the CDC.

Electrophysiological Testing

Electromyography and nerve conduction studies were performed for all patients with suspected GBS and for patients with other neurologic syndromes as clinically indicated. All studies were performed by a group of 3 board-certified neurologists with specialty training in neuromuscular disease using electrophysiological criteria endorsed by the European Federation of Neurological Societies/Peripheral Nerve Society and the American Association of Neuromuscular and Electrodiagnostic Medicine.[12,18-22]

Diagnostic Definitions

Guillain-Barré syndrome was diagnosed according to international clinical, laboratory, and electrophysiological criteria.[21,22] Following WHO recommendations, the Brighton criteria[12] were applied to delineate levels of diagnostic certainty for GBS. Clinical subcategories of GBS were diagnosed according to established criteria.[23] Transverse myelitis, encephalitis, and chronic inflammatory demyelinating polyneuropathy (CIDP) were diagnosed according to published guidelines.[24-26]

Table 1. Final Diagnoses During Enrollment Period

Diagnosis	Patients, No. (%) (n = 40)	
	Zika Virus Positive	Zika Virus Negative
Guillain-Barré syndrome	27 (68)[a]	2 (5)
Encephalitis	5 (13)	2 (5)
Transverse myelitis	2 (5)	1 (3)
Chronic inflammatory demyelinating polyneuropathy	1 (3)	0

[a] A total of 24 patients (89%) were at Brighton Level 1 diagnostic certainty.

Statistical Analysis

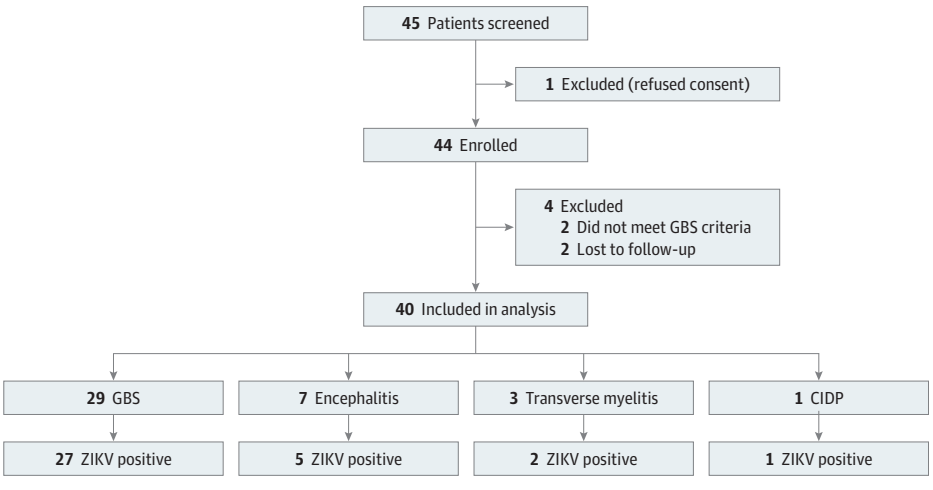
The 2-sided Fisher exact test was used to compare dichotomous variables, and logistic regression was used to compare 3-month modified Rankin scores between patients who were positive and patients who were negative for ZIKV infection, adjusting for diagnosis type. All analyses were performed using commercially available SPSS, version 21, statistical software (SPSS Inc). Significance was set at $P < .05$.

Results

A total of 40 patients were enrolled during the 5.2-month enrollment period (**Table 1**). The median age was 44 years (range, 22-72 years), and 15 (38%) were women. **Figure 1** shows a flowchart of case enrollments. All patients lived in the Rio de Janeiro metropolitan area (which includes the cities of Rio de Janeiro, Niteroi, and Sao Gonçalo, which are all within a 16-km [10-mile] radius). Overall, 29 patients were diagnosed with GBS (90% Brighton level 1 certainty), 7 with encephalitis, 3 with transverse myelitis, and 1 with new-onset CIDP (Table 1). Of the patients with GBS or CIDP, 24 of 30 (80%) underwent electromyography in addition to nerve conduction studies; the median time between onset of neurologic symptoms and undergoing electromyography and nerve conduction studies was 18 days (range, 10-35 days). Compared with a seasonally similar time frame prior to the first documented ZIKV index case in Brazil (December 5, 2013, to May 10, 2014; eTable 1 in the Supplement), admissions to our institution for GBS, transverse myelitis, and encephalitis increased 4-fold. Admissions for GBS increased from a mean of 1.0 per month to 5.6 per month, admissions for encephalitis increased from 0.4 per month to 1.4 per month, and admissions for transverse myelitis remained constant at 0.6 per month.

Evidence of acute ZIKV infection was found in 35 patients (88%), and the median time from onset of viral symptoms to testing for ZIKV was 14 days (range, 2-32 days). Re-

Figure 1. Flowchart of Enrollment



CIDP indicates chronic inflammatory demyelinating polyneuropathy; GBS, Guillain-Barré syndrome; and ZIKV, Zika virus.

sults of ZIKV rRT-PCR were positive for 3 patients. Of these, results of serum ZIKV rRT-PCR were positive for 2 patients with encephalitis and 1 patient with GBS; the median time from viral symptoms to testing for these patients was 6 days (range, 4-7 days). Results of CSF ZIKV rRT-PCR were positive for 2 patients (1 patient with encephalitis who also tested positive by serum rRT-PCR and 1 patient with GBS who tested negative by serum rRT-PCR); the median time from viral symptoms to testing was 12 days (range, 6-18 days). Results of serum and CSF ZIKV IgM were both positive for 29 of the 35 patients with ZIKV infection (83%), and 4 patients (11%) had positive results only for serum ZIKV IgM (eTable 2 in the Supplement). Cerebrospinal fluid ZIKV IgM was not tested in 3 patients owing to insufficient quantities of CSF; however, no patient had positive CSF ZIKV IgM results or negative serum ZIKV IgM results. Among patients who had both serum and CSF ZIKV IgM tested, the median CSF IgM optic density ratio was higher than that for serum (5.0 vs 4.7; $P < .001$). All patients underwent serum dengue IgM testing. Two patients with classic demyelinating GBS had positive results for serum dengue IgM; however, both patients had positive ZIKV IgM results in both serum and CSF samples and had negative dengue IgM results in CSF samples. No patient's condition was diagnosed based on serum or CSF ZIKV IgM results alone, without negative serum and/or CSF dengue virus IgM results. All patients had negative results for human immunodeficiency virus 1 and 2, and results of infectious and inflammatory studies were negative for other etiologic causes of disease.

Five patients tested negative for ZIKV, including 2 with classic demyelinating GBS (1 following a yellow fever vaccine), 2 with encephalitis associated with chikungunya virus infection, and 1 with idiopathic transverse myelitis. No patient in the study had a history of yellow fever, Japanese B encephalitis, or West Nile virus (none of which are endemic in the study area) and only the aforementioned patient with GBS who was negative for ZIKV infection had a history of flavivirus vaccination.

Among the 35 patients positive for ZIKV infection, a viral prodrome occurred in 32 patient (91%), compared with 2 of the 5 patients negative for ZIKV infection (40%), with the most common symptoms being fever and rash ($P = .02$; **Table 2**). The median time from viral symptoms to onset of neurologic symptoms was 10 days (range, 2-22 days), but the time varied based on diagnosis (Table 2). Although fever and rash were significantly more common among patients who were positive for ZIKV infection (fever, 31 of 35 patients; and rash, 30 of 35 patients) compared with patients negative for ZIKV infection (fever, 2 of 5 patients; and rash, 0 of 5 patients), and the positive predictive value of these symptoms alone or in combination ranged from 94% to 100%, the negative predictive values were low (43%-50%). Three patients with GBS without a viral prodrome had positive results for ZIKV IgM in their serum and/or CSF samples. Of the 27 patients with GBS who were positive for ZIKV infection, classic acute inflammatory demyelinating polyneuropathy was the most common type in 15 patients (56%); however, axonal variants were diagnosed for 8 patients (30%; **Table 3**).[18,23] Although 24 patients with GBS who were positive for ZIKV infection (89%) met Brighton level 1 diagnostic certainty, 3 patients diagnosed with the acute motor and sensory axonal neuropathy variant of GBS (based on results of examination and electromyography and nerve conduction studies and with normal magnetic resonance imaging [MRI] findings) had increased reflexes, which precludes Brighton level diagnostic certainty but has been described in the literature.[27] In addition to the 27 patients with GBS who were positive for ZIKV infection, 3 of 5 patients with encephalitis who were positive for ZIKV infection also had findings from electromyography and nerve conduction studies suggestive of acute neuromuscular disease (1 with acute inflammatory demyelinating polyneuropathy, 1 with acute motor axonal neuropathy, and 1 with lower motor neuron disease; **Figure 2**A), and none met Bickerstaff encephalitis criteria.[23] One patient was diagnosed with CIDP 8 weeks after initial presentation, when he had recurrent symptoms and underwent repeated electrophysiological studies consistent with published CIDP criteria.[26,28] One of the 7 patients initially diagnosed with encephalitis was later found to have primary central nervous system (CNS) lymphoma by brain biopsy (diffuse, large B cell). This 26-year-old patient was positive for ZIKV IgM in both the serum and CSF samples (optic density ratios of 8.8 and 12.5, respectively) and was negative for human immunodeficiency virus and Epstein-Barr virus. His serum and CSF ZIKV PCR results were both negative; however, the very elevated CSF and serum ZIKV IgM optic density ratios are consistent with primary CNS ZIKV infection. Cranial nerve, cauda equina, and/or nerve root enhancement on results of MRI was only observed among patients with GBS who were positive for ZIKV infection (4 of 21 who underwent MRI [19%]), while none of the patients with GBS who were negative for ZIKV exhibited this enhancement on MRI scans (Figure 2B and C).

Of the 35 patients positive for ZIKV infection, 9 (26%) required admission to the intensive care unit and 5 (14%) required mechanical ventilation (Table 2). At 3 months, 2 patients positive for ZIKV infection (1 with encephalitis and 1 with GBS) and 1 patient with GBS who was negative for ZIKV infection died. The patient with ZIKV and encephalitis experienced catastrophic cerebral edema and brainstem herniation and was placed on palliative care, and the 2 patients with GBS died of complications secondary to hospital-associated pneumonia. The median 3-month modified Rankin scale score was 2 (range, 0-5) among surviving patients who were positive for ZIKV infection and 0 (range, 0-1) among surviving patients negative for ZIKV infection ($P = .05$ after adjusting for diagnosis type). At 3 months, 19 of the 27 patients with GBS (70%) were ambulatory, with 17 (63%) able to walk without assistance. Chronic pain was reported in 18 of the 35 patients who were positive for ZIKV infection.

Discussion

This is the first prospective study, to our knowledge, that enrolled consecutive hospitalized adult patients with new-onset acute neuroinflammatory disease and evaluated them for the presence of acute ZIKV infection in both serum and CSF samples using molecular and serologic testing. We observed a trend for an increase in the incidence of hospital admis-

Table 2. Demographic, Clinical, Radiographic, Laboratory Features, and 3-Month Clinical Outcomes

Characteristic	Patients, No. (%)						
	GBS		Transverse Myelitis		Encephalitis		CIDP
	ZIKV Positive (n = 27)	ZIKV Negative (n = 2)	ZIKV Positive (n = 2)	ZIKV Negative (n = 1)	ZIKV Positive (n = 5)	ZIKV Negative (n = 2)	ZIKV Positive[a] (n = 1)
Demographics							
Age, median (range), y	42 (22-67)	46 (45-47)	33 (23-43)	39	47 (26-52)	57 (42-72)	61
Female sex	9 (33)	0	1 (50)	1 (100)	2 (40)	1 (50)	1 (100)
Preceding prodrome							
Any viral symptoms	24 (89)	0	2 (100)	0	5 (100)	2 (100)	1 (100)
Rash	23 (85)	0	2 (100)	0	4 (80)	0	1 (100)
Conjunctivitis	8 (30)	0	1 (50)	0	1 (20)	0	0
Fever	23 (85)	0	2 (100)	0	5 (100)	2 (100)	1 (100)
Arthralgias	12 (44)	0	1 (50)	0	1 (20)	2 (100)	1 (100)
Peripheral edema	3 (11)	0	0	0	1 (20)	2 (100)	0
Headache	7 (26)	0	1 (50)	0	3 (60)	0	0
>1 Viral symptom	24 (89)	0	2 (100)	0	5 (100)	0	1 (100)
Time from onset of viral symptoms to neurologic symptoms, median (range), d	10 (4-22)	No viral symptoms	7 (7)	No viral symptoms	10 (2-14)	2.5 (2-3)	15 (15)
Clinical signs and symptoms							
Back or lower extremity pain	17 (63)	1 (50)	1 (50)	0	1 (20)	0	1 (100)
Dysautonomia	6 (22)	1 (50)	0	0	0	0	1 (100)
Encephalopathy	0	0	0	0	4 (100)	2 (100)	0
Facial weakness	11 (41)	1 (50)	0	0	1 (20)	0	0
Bifacial weakness	5 (19)	1 (50)	0	0	0	0	0
Facial numbness	3 (11)	0	0	0	0	0	0
Dysphagia	3 (11)	1 (50)	0	0	1 (20)	0	0
Multiple cranial neuropathic symptoms	9 (33)	1 (50)	0	0	0	0	0
Symmetric upper and lower extremity muscle weakness	5 (19)	0	0	0	2 (40)	0	0
Lower extremity weakness predominant	22 (82)	2 (100)	2 (100)	1 (100)	2 (40)	0	1 (100)
Sensory deficits	20 (74)	0	2 (100)[b]	1 (100)[b]	3 (60)	0	1 (100)
Areflexia or reduced reflexes	24 (89)	2 (100)	0	0	2 (40)	0	1 (100)
Ataxia	19 (70)	0	1 (50)	1 (100)	2 (40)	0	1 (100)
Treatment in hospital							
Corticosteroids	0	0	2 (100)	1 (100)	3 (60)	0	0
IVIG	26 (96)	2 (100)	0	0	3 (60)	0	1 (100)
Plasmapheresis	0	0	1 (50)	0	0	0	0
Mechanical ventilation	2 (7)	1 (50)	0	0	3 (60)	0	0
Required ICU stay	4 (15)	2 (100)	2 (100)	0	3 (60)	2 (100)	0
ICU LOS, median (range), d	0 (0-14)	14.5 (14-15)	5 (5)	0	7 (0-90)	2.5 (2-3)	0
Hospital LOS, median (range), d	8 (6-20)	18 (14-22)	10.5 (10-11)	5	20 (7-180)	6.5 (6-7)	0
3-mo Outcomes							
Death	1 (4)	1 (50)	0	0	1 (20)	0	0
Modified Rankin Scale score, median (range)	2 (1-6)	3.5 (1-6)	2 (2)	1	2 (0-6)	0	2
MRC score, median (range)	56 (44-58)	54	NA	NA	NA	NA	56
Change in MRC score from nadir to 3 mo, median (range)	7 (2-16)	12	NA	NA	NA	NA	0
Hughes GBS Disability Scale score, median (range)	1 (0-4)	1	NA	NA	NA	NA	3
Chronic pain	15 (56)	1 (50)	0	0	2 (40)	0	1 (100)

Abbreviations: CIDP, chronic inflammatory demyelinating polyneuropathy; GBS, Guillain-Barré syndrome; ICU, intensive care unit; IVIG, intravenous immunoglobulin; LOS, length of stay; MRC, Medical Research Council; NA, not applicable; ZIKV, Zika virus.

[a] No patients with CIDP who were negative for ZIKV infection presented during the enrollment period.

[b] Sensory level.

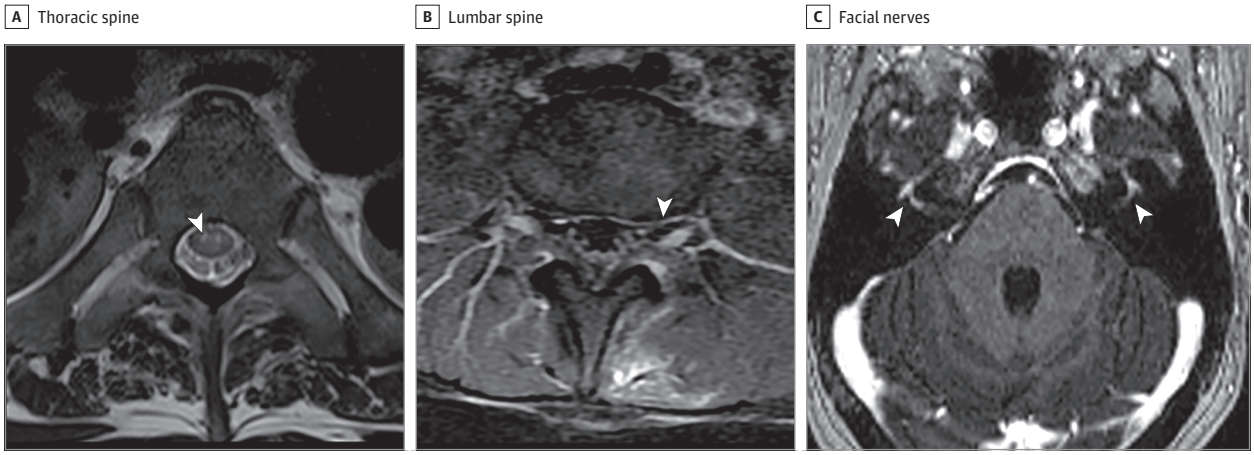
Table 3. Clinical and Electrophysiologic Patterns in Patients with Zika Virus–Associated Guillain-Barré Syndrome

Guillain-Barré Clinical Pattern[23]	EMG/NCS Pattern,[18] Patients, No. (%) (n = 27)			
	AIDP	AMAN	AMSAN	Other Abnormality
Classic	15 (75)	1 (5)	4 (20)	0
Paraparetic	3 (50)	1 (17)	2 (33)	0
Miller Fisher syndrome (classic)	0	0	0	1[a]

Abbreviations: AIDP, acute inflammatory demyelinating polyneuropathy; AMAN, acute motor axonal neuropathy; AMSAN, acute motor and sensory axonal neuropathy; EMG/NCS, electromyography and nerve conduction study.

[a] Absent blink reflex bilaterally.

Figure 2. Magnetic Resonance Imaging (MRI) Findings in Patients With Zika Virus (ZIKV)–Associated Neurologic Complications



A, Axial T2-weighted MRI scan of the thoracic spine of a patient positive for ZIKV infection with encephalitis and lower motor neuron signs. The white arrowhead indicates signal hyperintensity in the area of the anterior horn cells. B, Axial, postcontrast, fat-suppressed T1-weighted MRI scan shows gadolinium enhancement of both facial nerves in a patient with Guillain-Barré syndrome positive for ZIKV infection (white arrowhead). C, Axial, postcontrast, fat-suppressed T1-weighted MRI scan of the lumbar spine demonstrates contrast enhancement of the nerve roots and cauda equine in a patient with Guillain-Barré syndrome positive for ZIKV infection (white arrowheads).

sions for acute neurologic syndromes including GBS, encephalitis, and transverse myelitis compared with the pre-ZIKV era in Brazil, and 88% of these patients (35 of 40) tested positive for recent ZIKV infection (eTable 1 in the Supplement).

One strength of our study is the concomitant testing of ZIKV in serum and CSF samples. The fact that IgM serologic findings were positive in the CSF samples of many patients suggests recent intrathecal antibody synthesis and direct ZIKV CNS penetration because the IgM pentamer is considered too large to cross the blood-brain barrier.[16] The ZIKV IgM levels were significantly higher in the CSF samples than in the serum samples in this cohort. The detection of ZIKV by rRT-PCR in the CSF samples of 2 patients further supports this assertion. The low overall rates of ZIKV rRT-PCR positivity in our study are likely because ZIKV rRT-PCR remains positive in the CSF and blood for around 5 to 7 days[29] and most of our patients developed neurologic complications after the first week of viral prodrome. There is currently literature suggesting that rRT-PCR for ZIKV can remain positive for 2 to 4 weeks in the urine[7,29]; however, we did not test urine samples.

Diagnosing acute ZIKV infection is complicated in regions with endemic dengue virus because cross-reactivity among flavivirus serologic findings is known to occur.[30] Although 2 patients in this cohort tested positive for dengue IgM in the serum samples, neither had detectable dengue IgM in the CSF samples and both had positive results for ZIKV IgM in the serum and CSF samples. Although virus-specific neutralizing antibody testing using PRNT has been suggested as a more definitive tool to distinguish between flaviviruses,[31] this testing is expensive and requires cell culture and specialized, experienced laboratories because no commercial kit is available. There is, additionally, significant cross-reactivity among flaviviruses with PRNT, and ZIKV titers must be 4-fold higher than titers of any other flavivirus to be considered indicative of ZIKV infection.[13] Since December 2016, the CDC no longer recommends PRNT to rule out cross-reactivity among flaviviruses in countries with a high prevalence of concurrent flavivirosis owing to its low accuracy in this setting.[17] We believe that our method of matching CSF and blood serologic findings offers a substantial aid to minimize cross-reactivity in countries with a high prevalence of other concurrent flavivirosis. Also, the WHO considers that evidence of recent exposure to ZIKV can be inferred in patients with positive IgM serologic findings for ZIKV, with negative IgM serologic findings for dengue and chikungunya in the midst of a ZIKV outbreak, and with symptoms highly suggestive of ZIKV.[13] Recently, a study from Brazil using serum and CSF samples of neonates with ZIKV infection has shown a 100% correlation between positive IgM in the CSF samples and PRNT.[32] A major limitation of another cohort study in French Polynesia of ZIKV in patients with GBS was that 95% of the patients with GBS tested positive

for serum dengue IgG and 19% of the patients with GBS tested positive for dengue IgM.[6] Although all patients had PRNT-neutralizing antibodies to ZIKV, only 1 patient had titers 4 or more times higher than dengue titers, making it impossible to eliminate the possibility of cross-reactivity. This study did not evaluate CSF. Another cohort study of ZIKV-associated GBS in Colombia was only able to definitively diagnose both ZIKV infection and GBS (by Brighton level 1 criteria[12]) in 14 of 68 patients (21%) initially examined.[7,33] Although others have detected ZIKV in the CSF using rRT-PCR[7,9,34,35] and MAC-ELISA,[7,36] our study is the first, to our knowledge, to use a paradigm of simultaneously testing CSF ZIKV and dengue IgM to exclude flavivirus cross-reactivity.

Another novel aspect of this study is the identification of certain unusual neurologic features among patients positive for ZIKV infection. Among those with GBS, cranial nerve involvement occurred in 13 of 27 patients (48%), and 3 of 27 patients (11%) had facial numbness and/or dysphagia. Cranial nerve enhancement was observed on MRI scans of 4 of 19 patients. In large studies of non-ZIKV-associated GBS, cranial nerve abnormalities have been described in 36% of patients,[21] and cranial nerve enhancement has primarily been described in children with GBS.[37] Although others have described the acute motor axonal neuropathy variant of GBS among patients with ZIKV,[6] we found a variety of GBS subtypes including acute inflammatory demyelinating polyneuropathy, acute motor axonal neuropathy, acute motor and sensory axonal neuropathy, and a Miller Fisher syndrome variant. Three patients diagnosed with acute motor and sensory axonal neuropathy had brisk reflexes in weak limbs. Hyperreflexia has been reported in up to 10% of patients with GBS,[27] and some reports have suggested that axonal variants of GBS more commonly have preserved reflexes.[21] Among patients with encephalitis who are positive for ZIKV infection, 2 of 4 individuals had concomitant acute neuromuscular findings, which have also been described in other flavivirus-associated encephalitis cases (West-Nile virus[38] and Japanese B encephalitis[39]) but has not been described before with ZIKV. Finally, 1 patient who was initially diagnosed with encephalitis was found to have primary CNS lymphoma after brain biopsy. Primary CNS lymphoma has been linked to both human immunodeficiency virus and Epstein-Barr virus, and it has been hypothesized that the neoplastic transformation of B cells may occur in the context of inflammatory or infectious disease.[40] Although the CNS ZIKV infection preceding the primary diagnosis of CNS lymphoma may represent a coincidence, we thought it important to include this patient in light of the known association of viruses and primary CNS lymphoma. The rates of admissions to the intensive care unit, disability at 3 months, and mortality did not differ substantially in our cohort compared with other published cohorts of ZIKV-associated GBS from Colombia[7] and French Polynesia.[6] In addition, our outcomes were similar to those observed in a large cohort of patients from Europe with GBS secondary to other causes.[21] However, our cohort had lower rates of mechanical ventilation.

Limitations

The limitations of this study bear mentioning. We selected neurologic syndromes that we hypothesized had a higher likelihood of being associated with ZIKV because they are historically associated with other viruses. The study time period included only 5 months (during the summer season) when transmission of ZIKV by mosquitoes is more pronounced; therefore, annual incidence rates of ZIKV-associated neurologic complications may be lower when factoring in winter months. In addition, this study was limited to a single, tertiary neurologic referral center in the state of Rio de Janeiro. It is possible that heightened awareness of ZIKV led to increased referrals to our center during the study period compared with the historical period. Finally, we have not performed neutralizing antibody PRNT, but our protocol of testing CSF and serum samples for ZIKV and dengue may be a more accurate, affordable, and reproducible approach in most ZIKV- and dengue-endemic countries.

Conclusions

In this single-center Brazilian cohort, ZIKV appeared to be associated with an increase in the incidence of a diverse spectrum of serious neurologic syndromes among adults. Concomitant serum and CSF ZIKV and dengue testing may constitute a practical algorithm for ZIKV diagnosis in territories with a high prevalence of flavivirosis.

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