Supplementary Online Content

da Silva IRF, Frontera JA, Bispo de Filippis AM, Nascimento OJMd; RIO-GBS-ZIKV Research Group. Neurologic complications associated with the Zika virus in Brazilian adults. *JAMA Neurol.* Published online August 14, 2017. doi:10.1001/jamaneurol.2017.1703

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix. Methods

Inclusion and Exclusion Criteria

Inclusion criteria were: age ≥18 years, neurological manifestations suggestive of GBS, transverse myelitis, and/or meningoencephalitis, presentation within 60 days of neurological symptoms onset, and informed consent by the patient or legally authorized surrogate. Exclusion criteria were: history of a prior motor neuropathy or spinal cord disease, refusal to consent or participate in diagnostic testing and/or follow-up clinic visits.

Patient Evaluation

Standard patient evaluation included magnetic resonance imaging (MRI) of the brain, cervical, thoracic and lumbar spine with gadolinium, except in cases where MRI was contraindicated due to incompatible patient hardware or patient instability that precluded transport to MRI. All patients had routine serum chemistry, electrolyte, blood count and liver function analysis and HIV1, 2 testing. Routine cerebrospinal fluid (CSF) analysis was conducted in all patients (including cell count, differential, glucose, protein and culture). Suspected encephalitis patients also had CSF Herpes simplex virus PCR, syphilis, varicella, Epstein Barr Virus and cytomegalovirus testing in addition to serum hepatitis, and rheumatological studies (ANA, ENA, rheumatoid factor, ANCA and complement levels). Patients with suspected transverse myelitis underwent testing for CSF HTLV 1, 2 and serum neuromyelitis optica antibody. Serum and urine protein electrophoresis, vitamin B12, thyroid function, glycosylated hemoglobin, hepatitis, syphilis and rheumatological studies were performed in suspected GBS patients.

Electrophysiological testing

All nerve conduction studies involved at least three limbs and at least 3 sensory and 3 motor nerves with multi-site stimulation of F waves. Facial nerve testing and/or blink reflex were also performed when indicated. Electromyography (EMG) was conducted using a monopolar needle in the following muscles: deltoid, biceps, triceps, pronator, first interosseus, quadriceps, tibialis anterior, gastrocnemius and extensor hallicus. Electrophysiological and clinical criteria for GBS and chronic inflammatory demyelinating polyneuropathy (CIDP) followed published guidelines[1-4] and reference values recommended by the American Association of Electrodiagnostic Medicine[5,6] were utilized. Studies were conducted at a skin temperature between 33-37.5°C using three Nihon-Kohden MEB-9400K machines.

ZIKV MAC-ELISA testing

Antigens were provided by the CDC (Fort Collins, CO) including: Normal Vero E6 Antigen, Zika Vero E6 tissue Culture Antigen, Flavivirus IgM positive control, and chimeric monoclonal antibody specific for Flavivirus. Standardization was achieved via titration, comparing the optical densities of the reagents when reacted on viral and Normal Vero E6 antigen.

Three-month Outcomes

The Modified Rankin scale (mRS)[7] was assessed at 3-months in all patients and the Medical Research Council (MRC) sum score of six bilateral muscles in arms and legs, ranging from 0 (quadraplegic) to 60 (normal strength)[8], and Hughes scale[9] were additionally recorded in suspected GBS patients at admission and 3-month follow-up.

eTable 1. Cases of Guillain-Barré Syndrome, Encephalitis, and Transverse Myelitis Admitted During Pre-Zika Virus Control Period 12/5/2013-5/10/2014

Diagnosis	**N**	**Etiology**
GBS	5	2 cases preceded by diarrhea 3 idiopathic
Encephalitis	2	1 Herpes Simplex Virus PCR positive 1 anti-GAD antibody positive
Transverse Myelitis	3	1 HTLV positive 1 Sjogren's antibody positive 1 Neuromyelitis optica antibody positive

GBS= Guillain-Barré Syndrome

eTable 2. Studies Performed by Diagnosis Group in Entire Cohort (n = 40)

	No. (%)			
	GBS (n = 29)	**Transverse Myelitis (n = 3)**	**Encephalitis (n = 7)**	**CIDP (n = 1)**
Laboratory testing				
Time from viral sympotm onset to ZIKV testing, median (range), d	14 (4-25)	8 (8)	5 (2-18)	32 (32)
Serum ZIKV PCR positive (% of those tested)	1/29 (3)	0/3 (0)	2/7 (29)	0
Serum ZIKV IgM positive (% of those tested)	26/28 (93)[a]	2/3 (67)	4/6 (67)[a]	1 (100)
Serum dengue IgM positive (% of those tested)	2/29 (7)	0/3 (0)	0/7 (0)	0
Routine CSF Analysis performed	29 (100)	3 (100)	7 (100)	1 (100)
WBC, median (range), cells/mm^3	3 (0-55)	6 (1-46)	15 (0-150)[b]	1
Glucose, median (range), mg/dL	67 (50-109)	63 (62-67)	67 (48-70)	58
Protein, median (range), mg/dL	93 (44-279)	65 (64-115)	72 (47-111)	48
Albuminocytological dissociation	28 (97)	Not available	Not available	1 (100)
CSF ZIKV PCR positive (% of those tested)	1/29 (3)	0/3 (0)	1/7 (14)	0
CSF ZIKV IgM positive (% of those tested)	23/26 (88)	2/3 (67)	4/6 (67)[a]	0
CSF dengue IgM positive (% of those tested)	0/2 (0)	Not tested	Not tested	Not tested
Serum and CSF ZIKV IgM positive (% of those tested for both)	23/26 (88)	2/3 (67)	4/6 (67)[a]	0
Positive for recent ZIKV infection (% of diagnosis group)	27/29 (93)	2/3 (67)	5/7 (71)	1 (100)
Ancillary testing				
Brain MRI performed (% of diagnosis group)	21 (72)	3 (100)	6 (86)[c]	1 (100)
Normal (% of those tested)[d]	17 (80)	3 (100)	2 (33)	1 (100)
Parenchymal T2 hyperintense lesions	0	0	4 (67)	0
Cranial nerve enhancement	4 (19)[e]	0	0	0
Spine MRI performed (% of diagnosis group)	21 (72)	3 (100)	6 (86)[c]	1 (100)
Normal (% of those tested)[d]	17 (80)	0	5 (83)	1 (100)

Intraaxial T2 hyperintensity	0	3 (100)	1 (17)[b]	0
Cauda equina or nerve root enhancement	4 (19)[e]	0	1 (17)[e]	0
EMG/NCS performed (% of diagnosis group)	29 (100)	1 (33)	4 (57)	1 (100)
Normal (% of those tested)	0	1 (100)	1 (25)	0
GBS or CIDP findings	29 (100)	0	2 (50)	1 (100)
Lower motor neuron pattern	0	0	1 (25)	0

Abbreviations: CIDP, chronic inflammatory demyelinating polyneuropathy; CSF, cerebrospinal fluid; EMG/NCS, electromyography and nerve conduction studies; GBS, Guillain-Barré Syndrome; MRI, magnetic resonance imaging; OD, optic density; PCR, real time reverse-transcriptase polymerase chain reaction; WBC, white blood cells; ZIKV, Zika virus.

[a]1 patient who was ZIKV PCR positive did not undergo serum ZIKV IgM testing.

[b]Patient with 0 cells in CSF had MRI T2 hyperintensities in the brainstem and anterior horn of the thoracic spine as well as lumbar/Sacral nerve root enhancement.

[c]1 patient with encephalitis had a head CT only revealing diffuse cerebral edema.

[d]Normal defined as no intraaxial or extra-axial abnormalities, including cranial nerve, nerve root or cauda equina abnormalities.

[e]Abnormalities only seen in ZIKV+ patients.

Positive ZIKV IgM defined as OD ratio >3.0, 2.0-3.0 indeterminate and <2.0 negative.

Positive Dengue IgM defined as OD ratio >11.0, 9.0-11.0 indeterminate and <9.0 negative.

Median values among those who tested positive.

eReferences

1. Hadden RD, Cornblath DR, Hughes RA, et al. Electrophysiological classification of Guillain-Barre syndrome: clinical associations and outcome. Plasma Exchange/Sandoglobulin Guillain-Barre Syndrome Trial Group. Annals of neurology 1998;44:780-8.
2. Hughes RA, Cornblath DR. Guillain-Barre syndrome. Lancet (London, England) 2005;366:1653-66.
3. Sejvar JJ, Kohl KS, Gidudu J, et al. Guillain-Barre syndrome and Fisher syndrome: case definitions and guidelines for collection, analysis, and presentation of immunization safety data. Vaccine 2011;29:599-612.
4. Wakerley BR, Uncini A, Yuki N, Group GBSC, Group GBSC. Guillain-Barre and Miller Fisher syndromes--new diagnostic classification. Nature reviews Neurology 2014;10:537-44.
5. Guidelines in electrodiagnostic medicine. Recommended policy for electrodiagnostic medicine. Muscle & nerve Supplement 1999;8:S91-105.
6. Guidelines in electrodiagnostic medicine. American Association of Electrodiagnostic Medicine. Muscle & nerve 1992;15:229-53.
7. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. Stroke; a journal of cerebral circulation 1988;19:604-7.
8. Kleyweg RP, van der Meche FG, Schmitz PI. Interobserver agreement in the assessment of muscle strength and functional abilities in Guillain-Barre syndrome. Muscle & nerve 1991;14:1103-9.
9. Hughes RA, Newsom-Davis JM, Perkin GD, Pierce JM. Controlled trial prednisolone in acute polyneuropathy. Lancet (London, England) 1978;2:750-3.