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Guillain-Barré syndrome during the Zika virus outbreak in Northeast Brazil: An observational cohort study

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ABSTRACT

Objective: To determine the clinical phenotype of Guillain-Barré syndrome (GBS) after Zika virus (ZIKV) infection, the anti-glycolipid antibody signature, and the role of other circulating arthropod-borne viruses, we describe a cohort of GBS patients identified during ZIKV and chikungunya virus (CHIKV) outbreaks in Northeast Brazil.

Methods: We prospectively recruited GBS patients from a regional neurology center in Northeast Brazil between December 2014 and February 2017. Serum and CSF were tested for ZIKV, CHIKV, and dengue virus (DENV), by RT-PCR and antibodies, and serum was tested for GBS-associated antibodies to glycolipids.

Results: Seventy-one patients were identified. Forty-eight (68%) had laboratory evidence of a recent arbovirus infection; 25 (52%) ZIKV, 8 (17%) CHIKV, 1 (2%) DENV, and 14 (29%) ZIKV and CHIKV. Most patients with a recent arbovirus infection had motor and sensory symptoms (72%), a demyelinating electrophysiological subtype (67%) and a facial palsy (58%). Patients with a recent infection with ZIKV and CHIKV had a longer hospital admission and more frequent mechanical ventilation compared to the other patients. No specific anti-glycolipid antibody signature was identified in association with arbovirus infection, although significant antibody titres to GM1, GalC, LM1, and GalNAc-GD1a were found infrequently.

Conclusion: A large proportion of cases had laboratory evidence of a recent infection with ZIKV or CHIKV, and recent infection with both viruses was found in almost one third of patients. Most patients with a recent arbovirus

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infection had a sensorimotor, demyelinating GBS. We did not find a specific anti-glycolipid antibody signature in association with arbovirus-related GBS.

1. Introduction

Zika virus (ZIKV), a positive sense single stranded RNA flavivirus transmitted by the *Aedes aegypti* mosquito, has caused major outbreaks in the Americas between 2015 and 2017. Brazil was severely affected by the epidemic and the incidence was especially high in the Northeast region of the country [1]. Over the last decades, Brazil also faced outbreaks of dengue virus (DENV) and chikungunya virus (CHIKV), that are transmitted by the same mosquito and, like ZIKV, can cause febrile illness with myalgia, arthralgia, and rash [2–4]. And although most infections with ZIKV are asymptomatic, or cause mild disease, in some patients severe neurological complications occur, and the most frequently reported neurological complication in adults is the Guillain-Barré syndrome (GBS) [5–9]. In patients with DENV and CHIKV infection neurological complications, including GBS, have also been reported in smaller studies [10–13].

GBS is an immune-mediated polyradiculoneuropathy that is triggered by preceding infections. Some types of infections have been shown to be associated with a specific clinical phenotype of GBS and presence of specific anti-glycolipid antibodies directed against gangliosides (a type of sialylated glycolipid) on the nerve axon [14,15].

However, a uniform description of the clinical phenotype or the anti-ganglioside antibody signature of ZIKV-related GBS has not emerged in previous studies [5,8,16–20]. Furthermore, little is known about the role of other circulating arboviruses, such as DENV and CHIKV, as potential triggers for GBS [10].

To study the relation between GBS and circulating arbovirus infections, we describe a large, well-defined, and unselected cohort of GBS patients with evidence of a preceding arbovirus infection from a single center in Northeast Brazil that was tested for arboviruses and a broad spectrum of anti-ganglioside antibodies. The area of the study hospital is endemic for DENV and cases were collected during a ZIKV and a CHIKV outbreak.

2. Methods

2.1. Study setting, population, design and ethics

All patients with a suspected preceding arbovirus infection and an acute neurological disease identified between December 2014 and December 2016 at Hospital da Restauração, a public hospital with a tertiary neurology service in Northeast Brazil, were consecutively recruited. In total, 201 neurological disease cases were identified, as we have previously described [21]. The most frequent neurological diagnoses were GBS, myelitis, and (meningo)encephalitis. For the current study, the 65 patients diagnosed with GBS from this cohort were selected and analyzed. Additionally, all GBS patients with a history of arbovirus symptoms identified between December 2016 and February 2017 were included in this study ($n = 6$). (Supplementary Fig. 1) A suspected arbovirus infection was defined as fever, arthralgia or rash within 12 months before the onset of neurological symptoms. We chose a 12 month window because we did not want to make presumptions about the latency between infection and neurological disease onset. We did a separate analysis of the cases presenting within 3 months after onset of infectious symptoms, recognizing that most GBS cases occur within this time window. Diagnosis of GBS was classified according to the Brighton Collaboration criteria, and GBS variants other than Miller Fisher syndrome were defined according to other published criteria [22,23]. To enhance diagnostic accuracy, the clinical history of all patients was reviewed by MLBF, SEL and SBL, and in case of disagreement arbitrated by BCJ. All patients signed informed consent forms. The study protocol was reviewed and approved by the Oswaldo Cruz Foundation - FIOCRUZ, Instituto Aggeu Magalhães Ethics Committee (CAAE #511.06115.80005190).

2.2. Clinical data procedures

Clinical information was recorded on standardized case report forms and included demographics, history of suspected arbovirus infection and neurological examination, ancillary investigations and disease progression that were collected until 12 months after onset of neurological symptoms.(See Supplementary Material) The online registry for mortality of the Brazilian Ministry of Health was consulted to document mortality following hospital discharge within the study period. For Fig. 1, the number of GBS cases was based on hospital records reviewed by MLBF, and the outbreak periods of ZIKV, DENV and CHIKV were based on reported epidemiological data from the Instituto Aggeu Magalhães, Fiocruz Pernambuco (2000–2006), and the Brazilian Ministry of Health (Ministério de Saúde, Secretaria de Vigilância em Saúde, 2006–2018) [24,25]. As these numbers were defined around routine surveillance they should be interpreted with caution.

2.3. Diagnostic virology

Serum and cerebrospinal fluid (CSF) samples were collected and sent to the Flavivirus Reference Laboratory, Oswaldo Cruz Foundation, Recife, Brazil for arbovirus diagnostic testing. Viral RNA was extracted from serum samples using the QIAamp Viral RNA kit (Qiagen, Hilden - Germany). ZIKV, CHIKV and DENV real time RT-PCR (rRT-PCR) reactions were performed from purified RNA serum samples [26–28]. Anti-DENV and anti-CHIKV IgM and IgG antibodies were detected using commercially available capture enzyme-linked immunosorbent assay (ELISA) kits (dengue- Panbio, Alere - USA; chikungunya - EuroImmun AG, Luebeck - Germany). ZIKV specific IgM antibodies were detected by IgM-Capture ELISA (MAC-ELISA), which uses ZIKV and DENV antigens in parallel [29]. Serotype-specific anti-dengue antibodies and anti-Zika antibodies were assessed by 50% plaque reduction neutralization tests (PRNT), following a previously described protocol. The cut-off for positivity was defined based on a 50% reduction in plaque count ($PRNT_{50}$) [30].

We considered there to be evidence of recent ZIKV, CHIKV or DENV infection if there was viral RNA or specific IgM antibodies in patient serum or CSF, as defined previously [4,27–29]. Presence of ZIKV neutralizing antibodies on PRNT and negative IgM was considered as insufficient evidence of a recent ZIKV infection. In samples IgM-positive for both ZIKV and DENV, the PRNT assay was used to quantify neutralizing antibody titers to ZIKV and DENV serotypes 1–4 and determine viral diagnosis. If patients had neutralizing antibodies against both viruses without a PCR positive test confirming infection with one or the other, we deemed this an indeterminate flavivirus infection and, given the epidemiological linkage, presumed it to be Zika as others have previously [7,30].

2.4. Anti-glycolipid serology

Glycolipid microarray analysis of serum samples was performed at the University of Glasgow, United Kingdom, to detect IgM and IgG antibodies against 16 commonly studied glycolipids in GBS: GM1, GM2, phosphatidylserine, GM4, GA1, GD1a, GD1b, GT1a, GT1b, GQ1b, GD3, SGPG, LM1, GalNAc-GD1a, GalC and sulfatide, plus their possible heterodimeric complexes as previously described [31]. Matrixes were scanned using Genepix 4300A (Molecular Devices, California, USA) and

heat maps were created using MeV software. Due to the heterogeneous pattern of anti-glycolipid antibodies found in GBS, the small sample size, the known presence of naturally occurring anti-carbohydrate antibodies in the normal population and the lack of baseline control sera, statistical comparison of the array results was limited. Therefore, for the purpose of assay standardization, the anti-glycolipid antibody profile in patients with GBS were compared to the profile obtained from the sera of patients with other neurological diseases seen during the same study period at the same hospital, either with or without evidence of a recent arbovirus infection.

2.5. Statistical analysis

We used IBM SPSS Statistics 25® for data analysis, comparing clinical features between the different arbovirus diagnostic groups with the Mann-Whitney *U* test or the Kruskal-Wallis test for continuous data, and the Chi square or Fisher's exact test for proportions.

Proportions were described as number of patients with the variable present divided by the number of patients with the variable reported, excluding those with missing values. A two-sided *P*-value <0.05 was considered significant.

3. Results

A total of 71 patients with GBS were identified for the study between December 2014 and February 2017 (Supplementary Fig. 1). During the recruitment period, at the time of the ZIKV and CHIKV outbreak, a peak in GBS admissions was seen in the study hospital compared with the previous years (Fig. 1) [30,32].

3.1. Demographic, clinical and diagnostic features

Demographic and clinical features are shown in Table 1. The median age was 46 (interquartile range (IQR) 32–56) years. Thirty-six patients (51%) were female. One child, aged 9, was included in the study.

Rash (92%), arthralgia (57%), and myalgia (56%) were the most frequently reported symptoms of a preceding infection. The median time between infectious and neurological symptoms was 8 days (IQR) 4–24), two patients developed infectious and neurological symptoms on the same day, and 35 (49%) developed neurological symptoms within 1 week. (Supplementary Fig. 2).

The median time between onset of neurological symptoms and hospital admission was 5 days (IQR 2–11). Limb weakness and absent or diminished reflexes were found in the vast majority of patients. Sixty-one (86%) patients had either sensory symptoms or sensory loss identified in neurological examination. Cranial neuropathy was found in 39 (56%) patients, and facial and bulbar palsy were most frequently reported. Twelve patients (17%) had a clinical variant form of GBS: paraparetic ($n = 7$), pure sensory ($n = 1$), Miller Fisher syndrome (MFS) (n = 1), MF-GBS-overlap syndrome (n = 1), and bilateral facial paralysis with sensory signs ($n = 2$).

CSF was examined for cell count and protein level in all patients. A combination of a normal cell count and increased (>45 mg/dL) protein level (albumino-cytological dissociation) was found in 89%. Sixty-four (90%) patients had a cell count of ≤5 cells/uL and none had a cell count of >20. Electrophysiological studies were performed in 21 (30%) patients, ten (62%) had features of a demyelinating, and six (28%) of an axonal motor or axonal motor and sensory neuropathy (Table 2). The date of electrophysiological studies was available in 15 (71%) cases, and studies were performed at a median of 24 days (IQR 13–47) after onset of neurological symptoms. Cranial or spinal computed tomography or magnetic resonance imaging was done to exclude alternative diagnoses in 35 (47%) patients.

Thirteen (18%) patients fulfilled Brighton criteria level 1, 45 (63%) level 2, and 13 (18%) level 4 [22]. Of the patients with Brighton Level 4, three had a variant form of GBS, eight had normal or increased tendon reflexes, in one data on reflexes was missing, and one reached their nadir after 28 days. Twelve (92%) of these patients had either albumino-cytological dissociation in the CSF or electrophysiological studies compatible with GBS.

3.2. Arbovirus diagnostics

In total, 112 serum samples and 19 CSF samples were available for arbovirus testing and in 28 patients serial serum samples were available. Forty-eight (68%) had evidence of a recent arbovirus infection of which 25 (52%) had a recent ZIKV, 8 (17%) CHIKV, one (2%) DENV, and 14 (29%) had evidence of both a recent ZIKV and CHIKV infection. (Table 3, Fig. 2) Serum or CSF was IgM positive for both ZIKV and DENV in eight patients, six of these were ZIKV PCR positive, in one the neutralizing titer for ZIKV was higher than DENV, and in one no PRNT was done and this case was classified as a recent ZIKV infection on epidemiological grounds.[7, 34](Supplementary Figs. 2 and 3).

Of the patients with samples collected within the first 2 months after onset of neurological symptoms, 77% had evidence of a recent arbovirus infection, whereas after 2 months 52% did. In the 29 cases with late

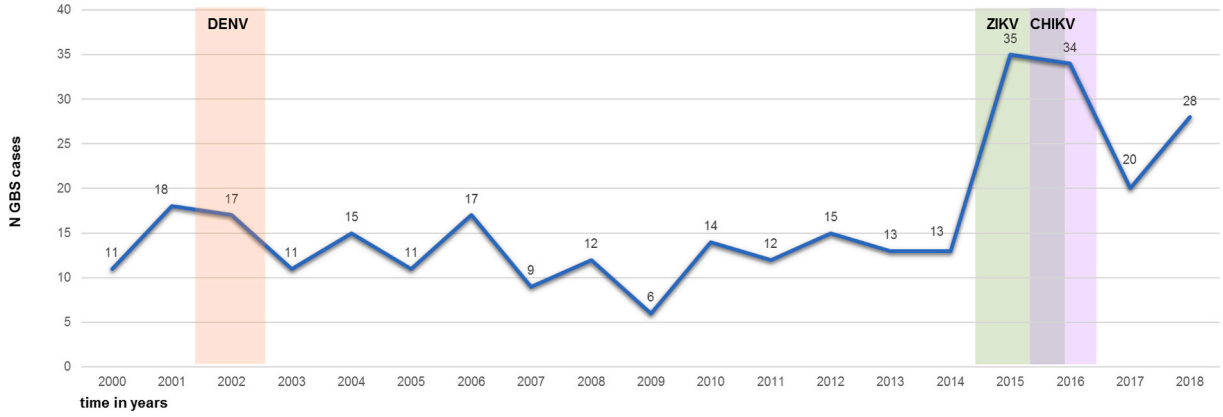

Fig. 1. Number of GBS cases in study hospital in relation to outbreak periods of Dengue, Zika and Chikungunya virus.
GBS cases in the study hospital in Recife, Pernambuco, Brazil between 2000 and 2018 in relation to periods of outbreaks of dengue virus (DENV, orange), Zika virus (ZIKV, green) and chikungunya virus (CHIKV, purple). The numbers in the line graph indicate the number of new GBS patients identified at the hospital per year. Outbreak periods were defined based on epidemiological data of the Pernambuco state from the Brazilian Ministry of Health. The number of notified DENV cases in 2002(±116,000) and the number of notified CHIKV cases in 2016 (±50,000) were 5–10 times higher compared to previous and following years. The ZIKV outbreak in 2014–2016 was based on the high number of suspected DENV cases (±110,000 in 2015) that were in later studies determined as probable ZIKV cases [33].

Table 1
Demographic, infectious and neurological symptoms.

	All cases (n = 71)	No lab evidence of recent arbovirus (n = 23)	ZIKV (n = 25)	CHIKV (n = 8)	ZIKV+CHIKV (n = 14)	*p value*
Age	46 (32–56)	45 (34–57)	39 (30–50)	51 (37–58)	50 (32–57)	*p = 0.59*
Male: Female (ratio)	35:36 (0.97)	9:14 (0.64)	14:11 (1.27)	3:5 (0.6)	8:6 (1.33)	
Infectious symptoms						
Rash	65 (92)	18 (78)	25 (100)	8 (100)	13 (93)	***p = 0.01***
Arthralgia	40/70 (57)	13/22 (59)	13 (52)	6 (75)	8 (57)	*p = 0.77*
Myalgia	39/70 (56)	16/22 (73)	9 (36)	6 (75)	7 (50)	*p = 0.05*
Fever	38/70(54)	11 (48)	10 (40)	5 (63)	12 (86)	***p = 0.04***
Headache	38/70 (54)	12/22 (55)	11 (44)	4 (50)	10 (71)	*p = 0.44*
Infectious- neurological symptoms (days)*	8 (4–24)	6 (4–15)	7 (3−12)	29 (18–111)	9 (6–31)	***p = 0.007***
Neurological symptoms						
Facial weakness	36 (51)	11 (48)	14 (56)	5 (63)	5 (36)	*p = 0.58*
Bulbar symptoms	25 (35)	10 (44)	8 (32)	3 (38)	4 (29)	*p = 0.80*
Limb weakness	69 (97)	22 (96)	24 (96)	8 (100)	14 (100)	*p = 1.0*
Sensory symptoms	61 (86)	17 (74)	23 (92)	8 (100)	12 (86)	*p = 0.25*
Neurological examination						
Cranial neuropathy	39/70 (56)	12/23 (52)	16 (67)	5 (63)	5 (36)	*p = 0.31*
Oculomotor weakness	2 (3)	1 (4)	1 (4)	0 (0)	0 (0)	*p = 1.00*
Facial palsy	38/70 (54)	10/22 (46)	16 (64)	5 (63)	6 (43)	*p = 0.48*
Bulbar palsy	17 (24)	7 (30)	5 (20)	3 (38)	2 (14)	*p = 0.52*
Limb weakness	67 (94)	22 (96)	23 (92)	8 (100)	13 (93)	*p = 1.00*
Tetraparesis	60 (85)	17 (74)	21 (74)	8 (100)	13 (93)	*p = 0.34*
Paraparesis	7 (10)	5 (22)	2 (8)	0 (0)	0 (0)	*p = 0.17*
Reflexes absent or low	61/70 (86)	19 (83)	22 (92)	6 (75)	14 (100)	*p = 0.18*
Sensory deficits	28 (39)	10 (44)	16 (64)	6 (75)	7 (50)	*p = 0.67*
Ataxia	8/68 (12)	1/22 (5)	5 (22)	1 (13)	1 (7)	*p = 0.34*
Unable to walk	36 (52)	14 (61)	9 (39)	4 (50)	9 (64)	*p = 0.39*
Dysautonomia†	18/68 (27)	7/21 (33)	7 (28)	2 (25)	2 (15)	*p = 0.66*

Data are presented as n/N(%) or median (IQR). Statistical analysis of categorical variables with Chi square/Fisher's exact, of continuous variables with Mann-Whitney U test or the Kruskal-Wallis. The p-value is the comparison between ZIKV, CHIKV, ZIKV-CHIKV and arbovirus-negative groups. *When excluding the 7 patients with time onset infectious – neurologic symptoms of >3 months, differences between the ZIKV, CHIKV, ZIKV-CHIKV and no recent infection groups were still significant (p = 0.02). †hypo- or hypertension (n = 10), excessive transpiration (n = 6), tachycardia (n = 4).

Table 2
Ancillary investigations, treatment and outcome.

	All cases (N = 71)	No lab evidence of recent arbovirus (N = 23)	ZIKV (n = 25)	CHIKV (n = 8)	ZIKV+CHIKV (n = 14)	*p value*
Ancillary investigations						
CSF cell count (cells/uL)	1 (0.33–2.7)	1 (0.33–2)	1 (0.33–3.33)	0.33 (0.33–1.83)	0.67 (0.33–2.33)	*p = 0.80*
<50 cells/uL	71 (100)	23 (100)				
CSF protein level (mg/dL)	95 (60–172)	72 (58–140)	102 (90–172)	124 (49–197)	66 (51–172)	*p = 0.13*
>45 mg/dL	63 (89)	20 (87)	24 (96)	7 (88)	11 (79)	*p = 0.35*
Nerve conduction studies	21 (30)	6 (26)	6 (24)	4 (50)	5 (36)	
AIDP	13/21 (62)	3/6 (50)	5/6 (83)	2/4 (50)	3/5 (60)	*p = 0.64*
AMAN	3/21 (14)	2/6 (33)	0/6 (0)	1/4 (25)	0/5 (0)	
AMSAN	3/21 (14)	1/6 (17)	0/6 (0)	1/4 (25)	1/5 (20)	
Equivocal/other	2/21 (10)	0/6 (0)	1/6 (17)	0/4 (0)	1/5 (20)	
Treatment						
Immunomodulating therapy	70 (99)	23 (100)	25 (100)	8 (100)	13 (93)	*p = 0.31*
IVIg	63 (89)	21 (91)	24 (96)	7 (88)	11 (79)	*p = 0.30*
Steroids	7 (10)	2 (9)	1 (4)	1 (13)	2 (14)	*p = 0.57*
Disease progression						
Duration of hospital admission	19 (13–24)	19 (9–25)	16 (11−20)	17 (15–20)	24 (20–29)	***p = 0.02***
Respiratory insufficiency	12 (17)	2 (9)	3 (12)	2 (25)	5 (36)	*p = 0.15*
Intensive Care Unit	14/69 (20)	7/22 (32)	1 (4)	1 (13)	5 (36)	***p = 0.031***
Duration Intensive Care Unit	16 (8–52)	17 (6–90)	73	9	14 (14–19)	*p = 0.55*
Intubated	9/66 (14)	3/20 (15)	1 (4)	0 (0)	5 (36)	***p = 0.049***
Outcome						
Died	0 (0)	0 (0)				
Sequela at discharge	64/68 (94)	21 (91)	22 (92)	7 (88)	13 (93)	*p = 0.38*
Recovered last follow-up	11/27 (41)	1/10 (10)	3/7 (43)	4/5 (80)	3/4 (75)	***p = 0.02***

Data are presented as n/N(%) or median [range], (IQR). IVIg = intravenous immunoglobulin, onset = onset of neurological symptoms. Time in days. Statistical analysis of categorical variables with Chi square/Fisher's exact, of continuous variables with Mann-Whitney U test or the Kruskal-Wallis. The p-value represents the comparison between ZIKV, CHIKV, ZIKV-CHIKV and arbovirus negative groups. When patient groups had zero patients to compare, no p-value was calculated.

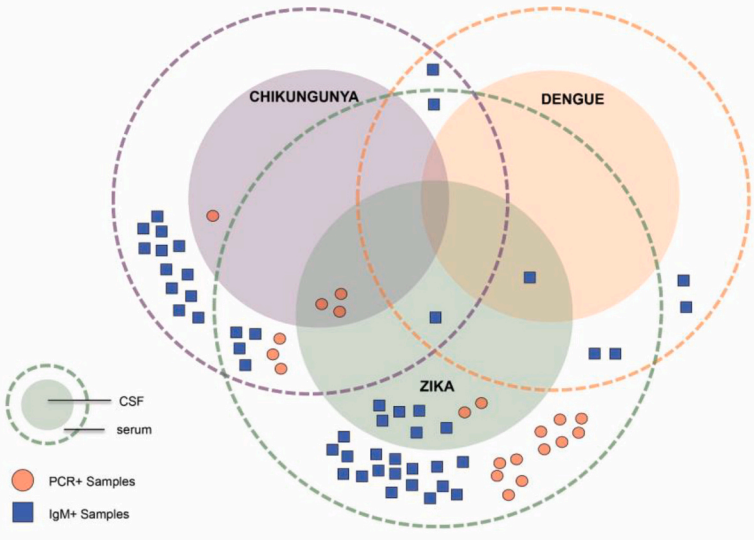

Fig. 2. Venn diagram of arbovirus diagnostic groups.
Overview of positive PCR and IgM samples for Zika virus (ZIKV), chikungunya virus (CHIKV) and dengue virus (DENV) in serum and cerebrospinal fluid (CSF).

Table 3
Arbovirus test results

Virus	Sample	Test	ZIKV	CHIKV	ZIKV-CHIKV	DENV	All cases
			n = 25	n = 8	n = 14	n = 1	*n* = 72
ZIKV	Serum	PCR only	5/25	–	5/12	–	10/66
		IgM only	13/23	–	4/14	–	17/68
		PCR & IgM	1/23	–	0/12	–	1/66
	CSF	PCR only	0/11	–	2/6	–	2/19
		IgM only	1/8	–	0/6	–	1/16
		PCR & IgM	0/8	–	1/6	–	1/15
	CSF & serum	PCR CSF, PCR serum	0/11	–	1/6	–	1/19
		IgM CSF, IgM serum	3/7	–	1/6	–	3/15
		IgM CSF, PCR & IgM serum	1/7	–	0/6	–	1/15
		PCR & IgM CSF, PCR serum	1/8	–	0/6	–	1/15
CHIKV	Serum	PCR only	–	0/8	2/13	–	2/64
		IgM only	–	8/8	7/14	–	15/71
		PCR & IgM	–	0/8	1/13	–	1/64
	CSF	PCR only	–	–	1/6	–	1/12
	CSF & serum	PCR CSF, IgM serum	–	–	3/6	–	3/12
DENV	serum	IgM only	2/25	0/7	4/14	1/1	7/71
	CSF	IgM only	0/8	–	1/6	–	1/57

Arbovirus test results stratified according to infection with Zika virus (ZIKV) chikungunya (CHIKV), dengue virus (DENV), and Zika and chikungunya virus (ZIKV-CHIKV). Number of positive tested patients is displayed in relation to total number of patients tested for each test or combination of tests (n/N) for each diagnostic category (ZIKV, CHIKV, ZIKV-CHIKV, DENV). PCR = polymerase-chain-reaction, IgM = immunoglobulin M, CSF = cerebrospinal fluid.

samples available, 14 (48%) neutralization assays were done, of which 12 (86%) were positive.

Demographic or clinical features did not differ significantly between arbovirus diagnostic groups, with some exceptions. The median time between infectious and neurological symptoms was significantly longer in patients with CHIKV, and paraparesis was found more frequently in laboratory negative- compared to the other patients. No differences were found in frequency of electrophysiological subtypes between groups.

In the post-hoc analysis, the median time between onset of infection to onset of neurologic symptoms was 7 days (IQR 4–15). The findings in this analysis did not differ from the overall analysis, with the exception that the percentage of cases with rash and fever was not significantly different across groups.

3.3. Glycolipid antibody testing

Anti-glycolipid IgG and IgM antibody testing was performed on a subset of 52 GBS cases and a group of 40 controls with other neurological diseases. Of the 52 GBS sera examined, 41 (79%) tested positive for a recent arbovirus infection and of the 40 control sera, 27 (68%) had evidence of a recent arbovirus infection. We did not detect a glycolipid antigen-specific marker for arbovirus-associated GBS. The typical antibody signature (anti-GM1, anti-GM1b, anti-GD1a, anti-GalNAc-GD1a) most frequently associated with the axonal form of GBS was not seen in this cohort. In serum samples where anti-glycolipid antibodies were detected, most antibody reactivities were of very low intensity and not significantly different between GBS cases and other neurological controls, either with or without evidence of a recent arbovirus infection (Supplementary Fig. 4). Regardless of the group analysis, rare samples contained significant antibody titres to individual or groups of nerve-enriched glycolipids including GM1 (patient #169), GalC (patient #92), LM1 (patients #92 and 97) and GalNAc-GD1a (patient #39). Whilst these never reached significance in a group analysis, they were absent from the control group at these titres, but their relevance in individual cases is unclear and notably pathophysiologically unproven. The case with MFS did not have significant antibody titres to GQ1b, which is detected in ~90% MFS patients [35]. Of the patients with significant glycolipid antibody titers, only patient #169 had nerve conduction studies done, which showed an acute motor-sensory axonal neuropathy.

3.4. Treatment and disease progression

The median duration of hospital admission was 19 days (IQR 13–24). The majority of patients were treated with intravenous immunoglobulin (IVIg), and seven (10%) received steroids (as monotherapy) in another hospital, prior to admission to the study hospital. Fourteen of 69 reported patients (20%) were admitted to the Intensive Care Unit (ICU) and 9 of 66 (14%) were intubated. Patients with laboratory evidence of both a recent ZIKV and CHIKV infection had a longer duration of hospitalization, were admitted to the ICU, and intubated significantly more frequently than the other patients (Table 2). PCR-positive patients more often were intubated (5/17 vs 1/29, $p = 0.02$), had respiratory insufficiency (8/19 vs 2/29, $p = 0.008$) and had a longer duration of hospitalization ($p = 0.027$) compared to those with only serological evidence of a recent arbovirus infection. In patients with evidence of both ZIKV and CHIKV infection, a larger proportion of those who were PCR-positive compared to those who were negative had respiratory insufficiency (0/4 vs 5/10), were admitted to the ICU (0/4 vs 5/10), or intubated (0/4 vs 5/10), although findings were not significant in this small subgroup.

None of the patients died during hospitalization. At discharge, 94% of patients had functional disability. Of the 27 patients followed up for 6 months or longer, 11 (41%) had recovered completely at last follow-up, six (22%) still had weakness in arms or legs, and seven (26%) had persisting facial weakness, which was still present more than 3 years after onset in five patients. Although numbers between groups were small, patients with laboratory evidence of a recent arbovirus infection were more likely than those without laboratory evidence to have recovered at last follow-up and presence of facial weakness was less common in this group (Table 2).

4. Discussion

A large proportion of GBS patients in this Brazilian cohort had laboratory evidence of a recent infection with ZIKV or CHIKV, and recent infection with both of these viruses was found in almost one third of patients. This indicates that both of these viruses may be associated with GBS, building upon evidence from previous studies [4,10,12]. A recent DENV infection was found in just one patient in this cohort. This may be because there was no outbreak of DENV during the study period, also, there have been conflicting reports in literature about the presumed association between DENV and GBS [34,36]. A larger proportion of cases with a recent infection with both ZIKV and CHIKV was admitted to the ICU and mechanically ventilated compared to the other patients, and the duration of hospital admission was longer in this group. This is important information for clinicians, as the geographic distributions of these arboviruses largely overlap and populations are therefore potentially at risk of contracting both infections. Furthermore, although the *A. aegypti* mosquito is the most prolific vector for both viruses, CHIKV is also effectively transmitted by *A. albopictus*, which populates more temperate regions, including southern Europe [37]. Therefore, clinicians working in these areas should be aware of this virus as a possible trigger for GBS.

The finding that a recent infection with both ZIKV and CHIKV could lead to more severe GBS may be due to a larger underlying pathological immune response or a higher viral load. A more severe disease progression in PCR-positive versus -negative patients further suggests that viral load may be a factor in disease severity, as has been shown previously [38]. Most patients with a recent infection with both ZIKV and CHIKV developed neurological symptoms more than 1 week after infectious disease onset, and as the acute phase of ZIKV and CHIKV infections usually lasts a week, it seems unlikely that acute infectious symptoms alone caused the severe disease progression in these patients. However, in patients with CHIKV infection polyarthralgia lasting weeks to months has been described [3].

Our cohort was younger and more often female than expected based on other studies on GBS [39]. A similar demographic profile has previously been described in GBS following other viral infections, including cytomegalovirus [40,41]. This indicates that females and a younger age group may be more prone to develop GBS after a viral infection. However, young women have also been shown to be at highest risk for ZIKV infection, and the Latin American population is younger compared to Europe and North America, where most previous GBS studies have been conducted [42–44]. The general clinical profile of GBS following a recent arbovirus infection with ZIKV and/or CHIKV in our study was a sensorimotor GBS with facial palsy. Electrophysiological studies showed demyelination in most, although not all, cases. This is again similar to what has been described in GBS after other virus infections and is in contrast to the clinical profile of GBS after a *C. jejuni* infection, that has been associated with higher frequencies of a pure motor GBS variant and an axonal electrophysiological subtype [40,41].

It has been suggested that ZIKV-related GBS is caused by direct infection or para-infectious nerve damage, due to the short time between onset of infectious and neurological symptoms [7]. However, although some patients developed neurological symptoms on the same day as the onset of infectious symptoms, the median time between infectious and neurologic symptoms in our cohort was 8 days, which is similar to GBS followed by other infections and is in accordance with a post-infectious pathogenesis of GBS [45]. The incubation time of ZIKV is estimated at 7–14 and of CHIKV and DENV at 2–10 days, which may in part explain the differences we found in time between infectious- and neurological symptoms [46,47].

We did not find a specific anti-ganglioside antibody signature associated with arbovirus-related GBS. There was clear variation in basal levels of antibodies to the different glycolipid targets assessed across the tested population, irrespective of arbovirus or neurological status, as can be demonstrated upon visual inspection of the heat map (Supplementary Fig. 4). Due to the absence of healthy control samples, we were unable to validate whether there was an increased frequency compared with baseline levels in the local population of anti-GA1 antibodies, which we previously observed in the smaller French Polynesian ZIKV-GBS cohort [5]. The low intensity antibodies that were observed may represent low affinity naturally occurring anti-carbohydrate antibodies in this population, or an epiphenomenon of neurological disease pathology. Our results contradict a Brazilian cohort study of patients with acute ZIKV infection without neurological disease that had elevated levels of anti-GD3 antibodies [48]. It was hypothesized that during a subsequent infection these antibodies would breach a critical threshold, resulting in neurological pathology. However, a subsequent study by the same group did not identify GD3 as a sole antibody target in patients with ZIKV-GBS, instead, they reported a universal increase in anti-glycolipid antibodies [49]. This is likely due to differences in assay methodology including the setting of background assay noise and the restricted use of control samples, thereby under-estimating the extensive variation of non-specific binding amongst individuals observed in our assay platform.

The peak in GBS cases that was observed in Recife before epidemiological surveillance for ZIKV was set up in the area, indicates the potential of GBS to act as a sentinel for the occurrence of outbreaks of arbovirus infection in areas where monitoring of such outbreaks is difficult. However, careful exclusion of other potential causes is crucial, as was seen in a recent outbreak of GBS in Peru, that was thought to be linked to ZIKV but later associated with *C. jejuni* and the typical anti-ganglioside antibody profile associated with this bacterium [50].

Our study has several limitations. Clinical data and biological material could not always be collected in the acute phase of the disease, and we were unable to collect healthy controls for a case-control analysis. This study was therefore not designed to determine causality and evidence of a recent infection does not necessarily mean that this was indeed the infection triggering the onset of GBS, especially as we were unable to test for other infections associated with GBS. The late collection of samples may have led to falsely classifying patients as negative that may no longer have had virus RNA or IgM antibodies detectable,

suggested by the lower frequency of positive results by PCR and IgM in patients with samples collected >2 months after start of neurological symptoms, but the high percentage (86%) of positive neutralization tests in these later samples. Furthermore, EMG examination was performed infrequently owing to a paucity of equipment and expertise in this study setting and was not classified on a uniform basis. The Brighton criteria were helpful in showing the diagnostic certainty based on the information available for all reported patients. These limitations are naturally inherent to studies conducted in an outbreak setting, in a low income region of Brazil.

In conclusion, our study indicates that besides ZIKV, CHIKV, may be associated with GBS. No specific anti-glycolipid antibody signature was identified in our cohort in connection to arbovirus-related GBS. The severity of disease in patients with GBS and evidence of both a recent ZIKV and CHIKV infection emphasizes the impact of arbovirus infections on patients and healthcare services. As threats of emerging infectious diseases persist it is important to advance our response to future outbreaks of GBS [51].

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Author contributions

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Suzannah B. Lant	data cleaning, analysis and interpretation, revision of the manuscript for intellectual content
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Carlos Alexandre Antunes de Brito	data collection and analysis, revision of the manuscript for intellectual content
Lívia Brito Bezerra de Albuquerque	data collection, revision of the manuscript for intellectual content
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Bart C. Jacobs	funding acquisition, data analysis, revision of the manuscript for intellectual content
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Declaration of Competing Interest

Tom Solomon is an adviser to the GlaxoSmithKline Ebola Vaccine programme, chairs a Siemens Diagnostics clinical advisory board and has a test for bacterial meningitis based on a blood test, filed for patent (No. GB 1606537.7 14th April 2016), approval pending.

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