

## RESEARCH ARTICLE

**Zika virus and Guillain–Barré syndrome in Bangladesh**

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**Introduction**

Major outbreaks of Zika virus (ZIKV), a mosquito-borne neurotropic flavivirus, have been reported in the island of Yap (2007), French Polynesia (2013–2014), and several Latin-American countries (2014).<sup>1–4</sup> During the ZIKV outbreak in French Polynesia, a profound 20-fold increase in the number of Guillain–Barré syndrome (GBS) was

**Abstract**

**Objective:** Previous studies have associated Guillain–Barré syndrome (GBS) with Zika virus (ZIKV) outbreaks in South America and Oceania. In Asia, ZIKV is known to circulate widely, but the association with Guillain–Barré syndrome is unclear. We investigated whether endemic ZIKV infection is associated with the development of GBS. **Methods:** A prospective study was conducted from 2011 to 2015 in Bangladesh. A total of 418 patients and 418 healthy family controls were included in the study. Patients were diagnosed with GBS prior to inclusion according to established criteria. Detailed information on the epidemiology, clinical presentation, electrophysiology, diagnosis, disease severity, and clinical course were obtained during a follow-up of 1 year using a predefined protocol. **Results:** ZIKV-neutralizing antibodies were detected in our study from 2013 onwards. The prevalence of ZIKV-neutralizing antibodies was not significantly higher in patients with GBS compared to healthy controls (OR 2.23,  $P = 0.14$ , 95% CI 0.77–6.53). Serological evidence for prior ZIKV infection in patients with GBS was associated with more frequent cranial, sensory, and autonomic nerve involvement compared to GBS patients with *Campylobacter jejuni*, the predominant preceding infection in GBS worldwide. Nerve-conduction studies revealed that ZIKV antibodies were associated with a demyelinating subtype of GBS, while *C. jejuni* infections were related to an axonal subtype. **Interpretation:** No significant association was found between ZIKV infection and GBS in Bangladesh, but GBS following ZIKV infection was characterized by a distinct clinical and electrophysiological subtype compared to *C. jejuni* infection. These findings indicate that ZIKV may precede a specific GBS subtype but the risk is low.

reported.<sup>5</sup> GBS is an acute polyradiculoneuropathy causing a rapidly progressive limb weakness and is triggered by various types of preceding infection.<sup>6</sup> Recently, the association between ZIKV and GBS has also been reported in various Latin-American countries following outbreaks of ZIKV.<sup>4,7–9</sup> In Asia, where ZIKV has been endemic for several decades,<sup>10–12</sup> the occurrence of GBS and other neurological complications after ZIKV infection

have thus far not been reported. The frequency of ZIKV infections in endemic areas is lower than during outbreaks, but considering the size and continuity of the exposed population, a considerable number of people in Asia are expected to be at risk to develop GBS.

GBS is a heterogeneous disorder of which the correct clinical diagnosis and classification may be challenging.<sup>13</sup> The disease diversity is associated with the variety in preceding infections. *Campylobacter jejuni* is the predominant infection triggering GBS worldwide,<sup>14</sup> and is associated with severe acute motor axonal neuropathy (AMAN)-type of GBS with a poor clinical outcome.<sup>15</sup> Cytomegalovirus in contrast can cause severe senso-motoric disorders and a GBS subtype described as acute inflammatory demyelinating polyneuropathy (AIDP).<sup>16</sup> The frequency of these GBS subtypes differs between geographical regions, which is in part explained by the local endemic infections.

In our study, we assessed whether endemic circulation of ZIKV in Bangladesh is associated with the development of GBS in a well-defined prospective case-control study. We compared the clinical phenotype and electrophysiological classification of GBS cases with detected ZIKV-neutralizing antibodies versus GBS cases with a preceding *C. jejuni* infection.

## Materials and Methods

### Study design

Four hundred and eighteen patients with GBS were prospectively included at Dhaka Medical College and Hospital (DMCH) or the National Institute of Neuroscience (NINS) in Dhaka, Bangladesh. The first 250 patients were included between January 2011 and June 2013. The remaining 168 patients were included as part of the ongoing International GBS Outcome Study (IGOS) between November 2013 and December 2015.<sup>17</sup>

A clinical neurologist examined all eligible patients within 2 days of admission.

The patients were included in the study after the validation of the clinical diagnosis using the criteria defined by NINDS.<sup>18</sup> Detailed, standardized information on demographic and clinical data were collected, including age, sex, place of residence (district of Bangladesh); clinical symptoms of preceding infections or other events; time and degree of maximum weakness; cranial, sensory, and autonomic nerve involvement; respiratory failure; and requirement for mechanical ventilation. Disease severity was evaluated using the GBS disability score,<sup>19</sup> a widely accepted scoring system used to assess functional status. It is scored as 0: normal; 1: minor symptoms and capable of running; 2: can walk 10 m or more without assistance but unable to run; 3: can walk 10 m across an

open space with help; 4: bedridden or chair-bound; 5: requiring assisted ventilation for at least part of the day; 6: death. The diagnosis in all patients was classified according to the GBS criteria of the Brighton Collaboration, ranging from level 1 (highest level of diagnostic certainty) to level 4 (reported as Guillain-Barré syndrome, possibly due to insufficient data for further classification).

Blood and CSF were collected upon admission following local laboratory standards and prior to any possible treatment; a protein level  $\leq 0.45$  g/L and a cell count  $\leq 5/\mu\text{L}$  was categorized as normal. NCS was performed by a trained clinical electrophysiologist, usually within 10–14 days of onset of weakness, and classified as AIDP, AMAN, motor and sensory axonal (AMSAN), unclassified, or normal.<sup>20</sup> Patients were frequently re-examined and followed up for 1 year to exclude the possibility of alternative diagnoses.

For each GBS patient, a household healthy control (HC) was identified and included. A HC was defined as a healthy family member older than 15 years and living in the same household. Blood samples of the HC were collected upon inclusion of the GBS patient.

### Ethical consideration

All project protocols were reviewed and approved by the institutional review board and ethical committees at ICDDR,B and Dhaka Medical College and Hospital, Bangladesh (PR-13061). The IGOS protocol was also reviewed and approved by the institutional review board of Erasmus MC (MEC-2011-477). Written informed consent was obtained from participants or their legal representatives.

### Serology

Presence of ZIKV-reactive IgM and IgG antibodies was assessed by the NS1 ELISA assay (Euroimmun<sup>TM</sup>, Lübeck, Germany)<sup>21</sup> for all patient and HC sera following manufacturers' instructions at the Department of Virology, Erasmus MC, Rotterdam, the Netherlands. All sera with borderline or detectable ZIKV NS1-IgM and/or NS1-IgG antibodies were confirmed by in-house ZIKV micro-VNT (Virus Neutralisation Test; Erasmus MC). For ZIKV micro-VNT test, twofold serum dilutions were incubated with 100 TCID<sub>50</sub> of ZIKV Suriname strain 2016 102 (Genbank reference KU937936, EVAg Ref-SKU: 011V-01621) at 37°C, and used to inoculate Vero cells for 5 days at 37°C. ZIKV infection was determined by cytopathic effect. A reciprocal VNT ZIKV titer of  $\geq 1/32$  was considered positive. DENV NS1 IgG ELISAs (Euroimmun<sup>TM</sup>) were performed for all patient sera and all ZIKV NS1 IgG-positive HC sera. Antibodies against *C. jejuni* were determined for all patient sera using an indirect IgG

ELISA and antibody class capture ELISAs for IgM and IgA antibodies at the Department of Medical Microbiology, Reinier de Graaf Gasthuis, Delft, The Netherlands, as previously described.<sup>22</sup>

### ZIKV quantitative real-time polymerase chain reaction

Viral loads of all patient samples, and all HC sera with equivocal or positive ZIKV IgM, were tested by quantitative real-time polymerase chain reaction (qRT-PCR) targeting both the Asian and African ZIKV lineage (ZIKV\_1086\_fwd, ZIKV\_1107\_probe and ZIKV\_1162c).<sup>23</sup> The MagnaPureLC system (Roche Diagnostics, Almere, The Netherlands) was used to extract total nucleic acid from 50  $\mu$ L serum.

### Statistical analyses

Quantitative variables are presented as number (percentage), mean, and standard deviation or median. Differences in sex and age categories between GBS patients and healthy controls were examined using the McNemar test. To compare the differences in (virus neutralizing) antibodies between the different years, we used a chi-square test with a categorical outcome variable. Differences in the proportion of individuals with ZIKV neutralizing antibodies in GBS patients versus healthy controls were tested using an univariate conditional logistic regression analysis, adjusted for age as a categorical variable. Clinical characteristics between three groups of GBS patients were compared: group A (only ZIKV neutralizing antibodies), group B (only evidence of recent *C. jejuni* infection) and group C (no antibodies detected against ZIKV and *C. jejuni*). A Chi-square was used, and a Fischer's exact test if appropriate. All statistical tests were performed using IBM SPSS version 22.0 (Armonk, NY, USA).

## Results

### GBS and HC cohort description

Four hundred and eighteen patients with GBS and 418 HC were prospectively included from 2011 to 2015. Their characteristics are provided in Table 1. GBS patients were predominantly young adult males (64%) with a median age of 27 years (IQR, 16–41). They did not differ from HC with respect to sex and time of blood sampling, but HC were older as children younger than 15 years old were not included in the control group. Among GBS patients, diarrhea (44%) was the most commonly preceding event, followed by respiratory symptoms (18%) and diverse clinical signs like fever and

rash (8%); 21% of patients did not report any clinical signs prior to neurological symptoms. The severity of neurological symptoms upon hospital admission was assessed using the GBS disability score:<sup>19</sup> 341/418 (82%) of patients were bedbound (score of 4 or 5), of whom 80/341 (19%) required mechanical ventilation (score of 5). Fifty-six patients (14%) died within 1 year after the diagnosis (score 6). NCS was conducted on 306/418 patients; 183/306 (60%) of all cases were classified as AMAN or AMSAN and 84/306 (28%) as AIDP. The patients were also classified according to the Brighton diagnostic criteria for GBS. Brighton level 1 was met in 246 (59%) patients, level 2 in 136 (32%) patients, level 3 in 23 (6%) patients, and level 4 in 8 (2%) patients (data not shown). Five patients could not be classified as they presented a variant of GBS with exaggerated deep tendon reflexes in weak limbs. In these five patients, other diagnosis were excluded, all had albumin-cytological dissociation and the three cases who had undergone NCS showed motor axonal neuropathy.

### ZIKV infection in GBS versus HC

Serological analyses for all 418 patients are presented in Figure 1. The first GBS patient with detectable ZIKV-neutralizing antibodies was included in the study in December 2013. In 2014, 16 of 92 (17%) patients had detectable ZIKV IgG antibodies of which 12/16 (75%) were confirmed by virus neutralization. In 2015, ZIKV IgG antibodies were detected in 15 of 52 (28%) of GBS patients and were confirmed by virus neutralization in 5 of 15 (33%). The seroprevalence of anti-DENV IgG in GBS patients increased significantly from 35% in 2011 to 55% in 2012 ( $P = 0.01$ ), but stabilized between 2013 and 2015 (Fig. 1).

Table 2 depicts an increased detection rate of ZIKV-neutralizing antibodies in GBS patients (18/418), but this difference was not significant by conditional logistic regression analysis when compared to HC (13/418) (OR 2.23, 95% CI 0.77–6.53,  $P = 0.14$ ). Of the 18 GBS patients with ZIKV-neutralizing antibodies, one patient had IgM antibodies against ZIKV (indicative of a recent infection) versus three of the HC (data not shown). We did not detect ZIKV genome in the serum of any of the GBS patients (Table 3).

### ZIKV-associated GBS subtype

An in-depth analysis was performed on the 18 patients with GBS who presented with ZIKV-neutralizing antibodies during 2013–2015. IgA and/or IgM antibodies against *C. jejuni* were identified in 9/18 patients (Table 3), suggesting recent (co-)infection.<sup>22</sup> All patients with serological evidence of a recent *C. jejuni* (co-)infection

**Table 1.** Characteristics of 418 GBS patients and 418 healthy family controls.

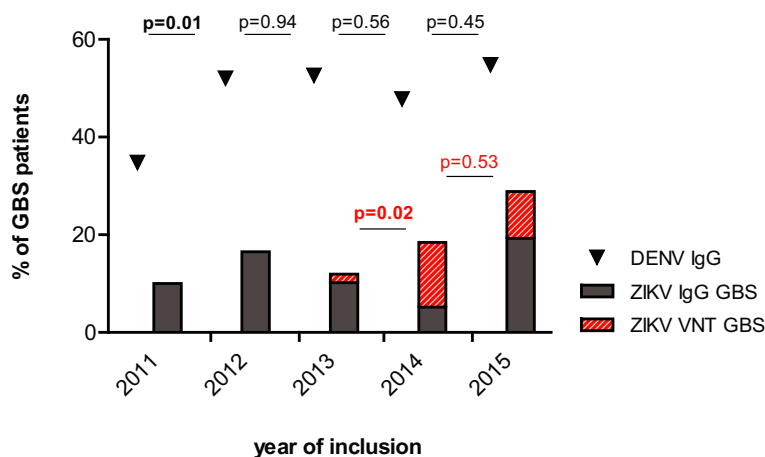
	GBS	Healthy controls	P-value
Total number	418	418	
Sex			0.11
Male	266 (63.6%)	231 (58.2%)	
Female	152 (36.4%)	164 (41.3%)	
Median age (range)	27 (0–75)	34 (17–75)	
Age category (years)			<0.001
<15	101 (24.2%)	0 (0.0%)	
16–30	146 (34.9%)	156 (41.5%)	
31–45	97 (23.2%)	169 (44.9%)	
> 45	74 (17.7%)	51 (13.6%)	
Antecedent symptoms			
Diarrhea	184 (44.0%)		
Respiratory	76 (18.2%)		
Others <sup>1</sup>	34 (8.1%)		
None	86 (20.6%)		
Unknown	35 (8.4%)		
Neurological symptoms			
Cranial nerve impairment	273 (65.3%)		
Sensory deficits	124 (29.7%)		
Ataxia	59 (14.2%)		
Autonomic dysfunction	96 (23.0%)		
Days from onset symptoms to inclusion	18.8 (10.1)		
Days from onset weakness to inclusion	10.6 (7.9)		
GBS score at entry			
0–1	2 (0.4%)		
2	24 (5.7%)		
3	51 (12.2%)		
4	261 (62.4%)		
5	80 (19.1%)		
Last known GBS score (1 year after diagnosis)			
0	122 (29.8%)		
1	97 (23.7%)		
2	94 (23.0%)		
3	27 (6.6%)		
4	13 (3.2%)		
5	-		
6	56 (13.7%)		
Electrophysiology			
AMAN	157 (51.3%)		
AMSAN	26 (8.5%)		
AIDP	84 (27.5%)		
Unclassified	35 (11.4%)		
Normal	4 (1.3%)		

Data are presented as numbers (proportions) or mean (SD).

<sup>1</sup>Others: other mentioned clinical symptoms included fever, rash, dysuria.

clinically presented with a pure motor subtype of GBS, in line with previous reports from Bangladesh.<sup>15</sup> In contrast, 6/9 patients with ZIKV-neutralizing antibodies but no evidence of recent *C. jejuni* infection clinically presented with the sensory-motor subtype, with cranial nerve involvement (8/9) and autonomic dysfunction (5/9). By electrophysiology, 4/9 GBS cases with recent *C. jejuni* (co-)infection were classified as AMAN, whereas 5/7 GBS

cases with ZIKV-neutralizing antibodies were classified as AIDP. All 18 patients with ZIKV-neutralizing antibodies presented with the classical tetraparesis (data not shown); 14 were severely affected with a nadir disability score of 4 or 5; however, 13 recovered well and could walk independently at 3 months follow-up. Of these 13 patients, eight did not receive specific therapy (intravenous immunoglobulin [IVIG] or plasmapheresis) but only



**Figure 1.** Seroprevalence of DENV and ZIKV antibodies in 418 GBS patients 2011–2015. The bars represent the percentage of GBS patients with IgG antibodies against ZIKV (gray), in red the percentage of patients with antibodies confirmed by virus neutralization. *P*-values in red above the bars are related to the differences in virus-neutralizing antibody titers. Triangles represent the percentage of GBS patients with IgG antibodies against DENV. *P*-values in black above the triangles are related to the differences in DENV IgG. Bold numbers represent  $P < 0.05$

**Table 2.** Frequency of ZIKV-neutralizing antibodies in 418 GBS patients and 418 case-matched healthy family controls over time.

	2011	2012	2013	2014	2015	2011–2015
No of patients with GBS	112	104	58	92	52	418
GBS (%)	0	0	1 (1.7%)	12 (13.2%)	5 (9.6%)	18 (4.3%)
Healthy controls (%)	0	0	0 (0%)	7 (7.7%)	6 (11.5%)	13 (3.1%)
Odds ratio	–	–	–	–	–	2.23
95% CI	–	–	–	–	–	0.77–6.53
<i>P</i> -value	–	–	–	–	–	0.14

supportive care. One patient was treated with small volume plasma exchange and four received IVIG.

To test whether GBS patients with a putative antecedent ZIKV infection presented with distinct clinical and electrophysiological features, we compared the clinical parameters of the 18 GBS patients with ZIKV-neutralizing antibodies to those of all patients with serological evidence of *Campylobacter* infection. One hundred and forty-one consecutive patients included from 2013 onward (the year of ZIKV introduction to the cohort) were eligible. Table 4 depicts the clinical and electrophysiological characteristics of three subgroups: (1) patients with ZIKV-neutralizing antibodies and no detectable IgA/IgM antibodies against *Campylobacter* (9/141; 6%), (2) patients without neutralizing antibodies against ZIKV but with IgA/IgM antibodies against *Campylobacter* (74/141; 52%), and (3) patients in whom neither ZIKV nor *C. jejuni* antibodies were detected (58/141; 41%). Patients with ZIKV-neutralizing antibodies were significantly older than patients with evidence of recent *Campylobacter* infection ( $P = 0.002$ ). Cranial nerves were impaired in all three subgroups of patients; however, sensory deficits and

autonomic dysfunction were reported significantly more often in ZIKV-related GBS than *Campylobacter*-related GBS ( $P = 0.02$ ). Electrophysiological patterns also differed: 36/49 (74%) of *Campylobacter*-related cases were classified as AMAN versus 1/6 (17%) of ZIKV-related cases (Table 4;  $P = 0.01$ ). In contrast, 3/6 (50%) ZIKV-related cases were classified as AIDP versus 6/49 (12%) of *Campylobacter*-related cases. The outcome of ZIKV-related GBS appeared more favorable than *Campylobacter*-related GBS (GBS disability scores of 0–2 in 88% vs. 66%, respectively; Table 4).

## Discussion

This is the first prospective and systematic study from a country with endemic ZIKV circulation, to investigate the association between ZIKV infection and GBS. Our findings indicate that ZIKV is circulating in Bangladesh since 2013 and that ZIKV-neutralizing antibodies can be detected in up to 10% of the study population. We observed that ZIKV-neutralizing antibodies did not appear more frequently in GBS patients than in HC (OR

**Table 3.** Clinical characteristics and laboratory findings in 18 ZIKA VNT-positive GBS patients.

	DENV		C. jejuni		Neurological manifestations				CSF			Treatment	Outcome	
	No	IgG	IgA	IgM	Clinical subtype	Cranial nerve involvement	Autonomic dysfunction	GBS disability score	MRC score	Cell n/mL	Prot mg/d			EMG
ZIKV and C. jejuni pos	1	pos	neg	pos	PM	–	–	3	35	2	107	AMAN (Motor)	Supportive	Independent walking at 1 week
	2	pos	pos	pos	PM	Facial & Bulbar	Tachycardia	5	0	n.a.	–	Inexcitable	Supportive	Died at 20th day from septic shock
	3	pos	pos	neg	PM	–	Hypertension	4	20	0	89	Normal	Plasmapheresis	Independent walking at 1 week
	4	pos	pos	neg	PM	Facial, Bulbar & extraocular	Hypertension	4	16	0	38	AMAN	Supportive	Independent walking at 3 months
	5	pos	neg	pos	PM	–	–	2	54	0	66	Normal	Supportive	Cured at 4 weeks
	6	pos	neg	pos	PM	Accessory	–	4	22	0	165	AMAN	Supportive	Independent walking at 2 months
	7	pos	neg	pos	PM	–	–	4	8	0	190	AMAN	SVPE	Independent walking at 6 months
	8	pos	pos	pos	PM	Bulbar	–	5	18	2	41	Not done	Supportive	Bed-bound at 3 months
	9	pos	pos	neg	PM	–	–	3	28	0	67	AIDP	IVIg	Independent walking at 1 month
ZIKV pos	10	pos	neg	neg	SM	Facial & Bulbar	Constipation	4	18	n.a.	–	Not done	IVIg	Independent walking at 3 months
	11	pos	neg	neg	SM	Facial & Bulbar	–	5	48	1	267	AIDP	IVIg	Independent walking at 1 month
	12	pos	neg	neg	SM	Facial & Bulbar	Tachycardia & Constipation	5	29	1	228	AIDP	SVPE	Independent walking at 2 months
	13	pos	neg	neg	PM	Facial, Bulbar & Accessory	–	5	0	2	64	Not done	Supportive	Died at 15th day
	14	pos	neg	neg	SM	Bulbar	Constipation	3	44	0	324	Not done	Supportive	Independent walking at 2 weeks
	15	pos	neg	neg	SM	Bulbar	–	4	42	n.a.	–	AIDP	Supportive	Independent walking at 1 month
	16	pos	neg	neg	PM	–	Constipation, urinary incontinence	4	38	2	18	Unclassified	IVIg	Independent walking at 2 months
	17	pos	neg	neg	SM	Bulbar	–	4	34	0	363	AIDP	Supportive	Independent walking at 1 month
	18	pos	neg	neg	PM	Bulbar & Accessory	Hyperhydrosis, hypersalivation	5	0	0	120	(Motor) Inexcitable	SVPE	Independent walking at 6 months

VNT, virus neutralization assay; AMAN, acute motor axonal neuropathy; AIDP, acute inflammatory demyelinating polyneuropathy; PM, pure -motor; SM, sensory-motor; IVIg, intravenous immunoglobulins; SVPE, small volume plasma exchange; pos, positive; neg, negative.



**Table 4.** Comparison of the clinical characteristics of GBS patients from 2013 to 2015 ( $n = 141$ ) stratified by serological response to ZIKV and *Campylobacter jejuni*.

	A ZIKV VNT-positive ( $n = 9$ )	B <i>C. jejuni</i> IgM- and/or IgA-positive ( $n = 74$ )	C ZIKV- & <i>C. jejuni</i> - negative ( $n = 58$ )	P-value	
				A versus B	A versus C
Sex				0.07	0.43
Male	8 (88.9%)	40 (54.1%)	42 (72.4%)		
Female	1 (11.1%)	34 (45.9%)	16 (27.6%)		
Median age (range)	50.00 (27–59)	23.00 (0–72)	30.00 (0–60)		
Age category (years)				<b>0.002</b>	<b>0.02</b>
<15	0 (0.0%)	24 (32.4%)	12 (20.7%)		
16–30	1 (11.1%)	28 (37.8%)	18 (31.0%)		
31–45	2 (22.2%)	10 (13.5%)	17 (29.3%)		
> 45	6 (66.7%)	12 (16.2%)	11 (19.0%)		
Antecedent infection or event				0.24	0.62
Diarrhea	2 (22.2%)	33 (44.6%)	12 (20.7%)		
Respiratory symptoms	0 (0.0%)	6 (8.1%)	11 (19.0%)		
Other	0 (0.0%)	3 (4.1%)	2 (3.4%)		
None	4 (44.4%)	24 (32.4%)	20 (34.5%)		
Unknown	3 (33.3%)	8 (10.8%)	13 (22.4%)		
Neurological symptoms					
Cranial nerve impairment	8 (88.9%)	41 (55.4%)	40 (69.0%)	0.08	0.43
Sensory deficits	4 (44.4%)	8 (10.8%)	26 (44.8%)	<b>0.02</b>	0.92
Ataxia	0 (0.0%)	1 (1.4%)	10 (17.2%)	0.32	<b>0.02</b>
Autonomic dysfunction	5 (55.6%)	13 (17.6%)	12 (20.7%)	<b>0.02</b>	0.04
Mean number of days between the onset of preceding symptoms and signs, and study inclusion (SD)	16.80 (9.63)	16.86 (8.23)	21.84 (11.40)	0.99	0.35
Mean number of days between the onset of weakness and study inclusion (SD)	7.33 (3.28)	8.43 (4.16)	10.90 (6.40)	0.45	0.11
GBS score at entry				0.41	0.43
0	0 (0.0%)	0 (0.0%)	0 (0.0%)		
1	0 (0.0%)	0 (0.0%)	1 (1.7%)		
2	0 (0.0%)	7 (9.5%)	5 (8.6%)		
3	2 (22.2%)	9 (12.2%)	5 (8.6%)		
4	4 (44.4%)	45 (60.8%)	37 (63.8%)		
5	3 (33.3%)	13 (17.6%)	10 (17.2%)		
Last known GBS score (within 1 year)				0.42	0.96
0	3 (33.3%)	7 (9.5%)	14 (24.1%)		
1	3 (33.3%)	21 (28.4%)	21 (36.2%)		
2	2 (22.2%)	21 (28.4%)	11 (19.0%)		
3	0 (0.0%)	14 (18.9%)	1 (1.7%)		
4	0 (0.0%)	2 (2.7%)	4 (6.9%)		
5	0 (0.0%)	1 (1.4%)	2 (3.4%)		
6	1 (11.1%)	8 (10.8%)	5 (8.6%)		
EMG type				<b>0.01</b>	0.71
AMAN	1 (16.7%)	36 (73.5%)	9 (29.0%)		
AMSAN	2 (33.3%)	3 (6.1%)	4 (12.9%)		
AIDP	3 (50.0%)	6 (12.2%)	15 (48.4%)		
Unclassified	0 (0.0%)	4 (8.2%)	2 (6.5%)		
Normal	0 (0.0%)	0 (0.0%)	1 (3.2%)		

The P-values depicted in bold represent  $P < 0.05$

2.23, 95% CI 0.77–6.53,  $P = 0.14$ ). GBS patients with ZIKV-neutralizing antibodies mostly presented with a clinical and electrophysiological phenotype that is distinct from the predominant phenotype worldwide associated with *C. jejuni*. These findings indicate that ZIKV may precede a specific GBS subtype but that the risk is low.

Up to present, studies describing the role of ZIKV in GBS have focused on outbreak areas and symptomatic ZIKV patients.<sup>4,5,8,9,24–27</sup> Interestingly, ZIKV infections are symptomatic in only an estimated 20% of cases and ZIKV will probably soon be endemic in most affected areas. In addition, not all previous studies were originally set-up to study the association between ZIKV and GBS and therefore have several limitations. Most studies were retrospective, restricting the accuracy of GBS diagnosis. Only few studies used an adequate case-control design and specific data on the clinical and electrophysiological subtype of GBS and on other preceding infections are often lacking. In our study, we certified the accuracy of GBS diagnosis by applying the Brighton case definitions criteria and an extensive standardized follow-up period.

In accordance with our earlier report from Bangladesh,<sup>15</sup> there was a considerable delay before the GBS patients reached the hospital (an average of 11 days after onset of weakness). This delay resulted in a large mean interval between a possible antecedent infection and specimen collection (19 days), which is important when interpreting the results of the diagnostic assays. It is a plausible explanation for not detecting ZIKV genome by PCR in serum. The assessment of ZIKV in urine or whole blood would have been a valuable addition to the study protocol and should be considered in future studies.<sup>28</sup> The lack of detected IgM responses may be due to the limited sensitivity of the serological method used for IgM detection (Euroimmun ZIKV ELISA).<sup>29,30</sup> Furthermore, IgM responses can be attenuated in infected individuals with flavivirus infections in the past<sup>31–33</sup> and ZIKV serology is further complicated by extensive cross-reactivity with other endemic flaviviruses.<sup>21,34</sup> As virus-specific neutralizing antibody testing has been suggested the most definitive tool to confirm the presence of ZIKV-specific antibodies, we performed ZIKV neutralization assays on all sera with detectable ZIKV IgG antibodies. The specificity of the detected ZIKV-neutralizing antibodies is supported by the kinetics of the DENV IgG antibodies in our study.

The first study reporting on the association between ZIKV and GBS indicated that ZIKV infections were exclusively associated with axonal GBS, whereas recent reports from Brazil and Colombia show that AIDP is the subtype of GBS associated with ZIKV infection.<sup>5,8,25–27</sup> Some of this variety may be attributed to retrospective analysis of nonstandardized clinical and electrophysiological data to describe subtypes of GBS. In our study, all included GBS

patients fulfilled the NINDS-criteria for the diagnosis of GBS.<sup>18</sup> We performed subgroup analysis of our GBS cohort to compare the clinical and electrophysiological characteristics of patients with evidence of ZIKV infection versus recent *C. jejuni* infection, although the subgroups are small. Patients with *C. jejuni*-associated GBS without ZIKV-neutralizing antibodies developed the pure motor form of GBS more often than patients with evidence of ZIKV infection without evidence of a recent *C. jejuni* infection. The latter patients predominantly developed a sensory-motor form of GBS. In addition, we demonstrated that patients with both ZIKV-neutralizing antibodies and recent *C. jejuni* infection all developed a pure motor type of GBS and usually the axonal type, emphasizing the need for *C. jejuni* testing in patients who develop GBS following ZIKV infection.

This study has several limitations. First, confirmation of a preceding ZIKV infection in GBS patients is generally complicated by the delay between infection and the first neurological manifestations of GBS as mentioned in previous studies.<sup>4,35</sup> In this study, there was an additional delay between this neurological onset and hospital admission that further reduced the chances of demonstrating the viral genome in serum. Therefore, serological tests were used for the analyses in this study which demonstrated ZIKV-specific antibodies by the gold standard method – virus-specific neutralization. Second, we choose to use case-matched HC from the same family and household, as they live in the same geographic area and are likely to have the same socio-economic status. It is thus expected that they were equally exposed to mosquitoes which might have decreased the OR. Third, HC were significantly older than the GBS patients, but correcting for age in the analyses did not affect the statistical outcome. Finally, the performed study is an observational study. Although a first report on circulation of ZIKV in Bangladesh was recently published,<sup>36</sup> there are no peer-reviewed studies on the seroprevalence of antibodies against ZIKV in Bangladesh. Studies from surrounding areas indicate that the seroprevalence of ZIKV will not exceed 20%.<sup>11</sup> A larger study population might thus have been required to increase the power of the study.

In conclusion, this study in a well-defined cohort of patients with GBS from Bangladesh provides evidence that ZIKV infections in an endemic area may trigger a distinct clinical and electrophysiological subtype of GBS although the lack of association between ZIKV and GBS indicates that the risk is low.

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## Author Contributions

CG, ZI, MK, BJ, HE, and CR contributed to the conception and design of the study; CG, ZI, MI, IS, IJ, QM, SK, NP, CR, RM, DV, FP, QM, and RP contributed to the acquisition and analysis of the data; CG, SK, ZI, MI, BJ, and HE contributed to drafting a significant proportion of the manuscript or figures.

## Conflict of Interest

No conflicts of interest.

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