

# Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study



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## Summary

**Background** Between October, 2013, and April, 2014, French Polynesia experienced the largest Zika virus outbreak ever described at that time. During the same period, an increase in Guillain-Barré syndrome was reported, suggesting a possible association between Zika virus and Guillain-Barré syndrome. We aimed to assess the role of Zika virus and dengue virus infection in developing Guillain-Barré syndrome.

**Methods** In this case-control study, cases were patients with Guillain-Barré syndrome diagnosed at the Centre Hospitalier de Polynésie Française (Papeete, Tahiti, French Polynesia) during the outbreak period. Controls were age-matched, sex-matched, and residence-matched patients who presented at the hospital with a non-febrile illness (control group 1; n=98) and age-matched patients with acute Zika virus disease and no neurological symptoms (control group 2; n=70). Virological investigations included RT-PCR for Zika virus, and both microsphere immunofluorescent and seroneutralisation assays for Zika virus and dengue virus. Anti-glycolipid reactivity was studied in patients with Guillain-Barré syndrome using both ELISA and combinatorial microarrays.

**Findings** 42 patients were diagnosed with Guillain-Barré syndrome during the study period. 41 (98%) patients with Guillain-Barré syndrome had anti-Zika virus IgM or IgG, and all (100%) had neutralising antibodies against Zika virus compared with 54 (56%) of 98 in control group 1 ( $p < 0.0001$ ). 39 (93%) patients with Guillain-Barré syndrome had Zika virus IgM and 37 (88%) had experienced a transient illness in a median of 6 days (IQR 4–10) before the onset of neurological symptoms, suggesting recent Zika virus infection. Patients with Guillain-Barré syndrome had electrophysiological findings compatible with acute motor axonal neuropathy (AMAN) type, and had rapid evolution of disease (median duration of the installation and plateau phases was 6 [IQR 4–9] and 4 days [3–10], respectively). 12 (29%) patients required respiratory assistance. No patients died. Anti-glycolipid antibody activity was found in 13 (31%) patients, and notably against GA1 in eight (19%) patients, by ELISA and 19 (46%) of 41 by glycoarray at admission. The typical AMAN-associated anti-ganglioside antibodies were rarely present. Past dengue virus history did not differ significantly between patients with Guillain-Barré syndrome and those in the two control groups (95%, 89%, and 83%, respectively).

**Interpretation** This is the first study providing evidence for Zika virus infection causing Guillain-Barré syndrome. Because Zika virus is spreading rapidly across the Americas, at risk countries need to prepare for adequate intensive care beds capacity to manage patients with Guillain-Barré syndrome.

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## Introduction

Zika virus is an arthropod-borne virus (arbovirus) in the genus *Flavivirus*, family *Flaviviridae*.<sup>1</sup> Zika virus was first isolated from a Rhesus macaque in 1947 in the Zika forest of Uganda;<sup>2</sup> the monkey had been brought in the country by researchers as an experimental animal. The first human infection was reported in Nigeria in 1954.<sup>3</sup> Like dengue virus and chikungunya viruses, Zika virus adapted from an ancestral transmission cycle involving non-human primates and a broad spectrum of canopy dwelling mosquito species as vectors to an urban–periurban cycle involving humans as reservoirs and the widely distributed *Aedes (Stegomyia)* mosquitoes as vectors.<sup>4</sup>

Since the 1950s, Zika virus has only been reported as circulating sporadically in Africa and southeast Asia.<sup>5</sup> In

2007, Zika virus was isolated for the first time in the Pacific, on the Micronesian island of Yap.<sup>6</sup> Between October, 2013, and April, 2014, French Polynesia experienced the largest Zika outbreak ever reported at that time.<sup>7</sup> More than 32 000 patients were assessed for suspected Zika virus infection, with a weekly incidence peaking on week 9 of the outbreak.<sup>8</sup> Since 2014, Zika virus has spread to other Pacific islands, notably Easter Island (Chile). In March, 2015, Brazil reported autochthonous transmission of Zika virus,<sup>9</sup> and an outbreak was declared 6 months later.<sup>10</sup> As of Feb 1, 2016, Zika virus had emerged in 25 countries and territories in South or Central America, with alarming reports of microcephaly cases among neonates in Brazil.<sup>11</sup>

Before the French Polynesian outbreak, Zika virus infection used to be described as a mild febrile illness with

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## Research in context

### Evidence before this study

The ongoing Zika virus disease epidemic in Latin America is the largest epidemic ever recorded. On Feb 1, 2016, the WHO declared the suspected link between Zika virus and neurological disorders and neonatal malformations a Public Health Emergency of International Concern. The WHO Secretariat briefed the Emergency Committee convened by the Director General on the clusters of microcephaly and Guillain-Barré syndrome that have been temporally associated with Zika virus transmission in some settings, including French Polynesia, and urged further research to be conducted to confirm the link between Zika virus and these complications. We searched MEDLINE from Jan 1, 1990, to Feb 14, 2016, for evidence linking Zika virus and Guillain-Barré syndrome. The simultaneous occurrence of Zika virus and Guillain-Barré syndrome outbreaks has been reported in few studies in French Polynesia and Brazil, but only one case report provided serological evidence linking one patient with Guillain-Barré syndrome to recent Zika virus infection in French Polynesia in November, 2013. We provide here a complete description of a series of 42 cases of Guillain-Barré syndrome in French Polynesia, and serological evidence linking these cases to recent Zika virus infection.

### Added value of this study

This is the first study to document a large series of patients who developed a Guillain-Barré syndrome following Zika virus

infection, a virus that was previously considered to cause only mild disease. This study not only confirms the link between Zika virus infection and Guillain-Barré syndrome, but also provides useful findings regarding the clinical characteristics of the Guillain-Barré syndrome cases: most had electrophysiological findings compatible with the acute motor axonal neuropathy (AMAN) type of the syndrome, and had rapid evolution of the disease. The clinical outcome of these patients with Zika virus and Guillain-Barré syndrome was generally favourable, despite a rapid onset and short plateau phase, as may be seen in other patient groups suffering from the AMAN type of Guillain-Barré syndrome. No clear pathophysiological mechanism for the Guillain-Barré syndrome could be identified, because the typical AMAN-associated anti-ganglioside antibodies were rarely present. We also speculated whether past dengue history might have contributed to the development of Guillain-Barré syndrome, but could not find any evidence for it.

### Implications of all the available evidence

The results of our study support that Zika virus should be added to the list of infectious pathogens susceptible to cause Guillain-Barré syndrome. As Zika virus is spreading rapidly across the Americas, at risk countries need to be prepared to have adequate intensive care beds capacity to manage patients with Guillain-Barré syndrome.

clinical symptoms including maculopapular rash, joint and muscle pain, headache, and non-purulent conjunctivitis.<sup>6</sup> Between November, 2013, and February, 2014, in French Polynesia, 42 patients presented at hospital with Guillain-Barré syndrome, an autoimmune disease causing acute, or subacute flaccid paralysis, contrasting with reports of five, ten, three, and three, in 2009, 2010, 2011, and 2012, respectively.<sup>12</sup> Other arboviral diseases like West Nile, Japanese encephalitis, chikungunya, and dengue had already been reported to sometimes cause Guillain-Barré syndrome,<sup>13–16</sup> but only during the outbreak in French Polynesia was this severe neurological complication first described to be associated with Zika virus infection.<sup>17</sup> The temporal coincidence between the peaks in incidence of Zika virus and cases of Guillain-Barré syndrome, and also the concurrent circulation of dengue serotypes 1 and 3<sup>18</sup> suggested a possible causal relation between the three events. Using two control series, we address the hypothesis that Zika virus infection with or without dengue concurrent or sequential infection could be a risk factor for the development of Guillain-Barré syndrome.

## Methods

### Study design and participants

In this case-control study, cases were patients with Guillain-Barré syndrome who were diagnosed at the Centre Hospitalier de Polynésie Française (CHPF) in Papeete, Tahiti, French Polynesia, during the outbreak

period. As routine, all patients with suspicion of Guillain-Barré syndrome in French Polynesia are referred to the CHPF for diagnosis confirmation. All patients included in this study were diagnosed as developing a Guillain-Barré syndrome by neurologists or staff in intensive care units according to international criteria.<sup>19</sup> Clinical and demographic data were collected from medical records obtained during patients' hospital stay. The data recorded for all patients included patient's age, sex, island of residence, medical history and comorbidities, clinical signs and symptoms, and illness duration and severity. Electrophysiological assessment was done for all patients by standard electromyography techniques including motor nerve conduction studies of the median nerve (recording of the abductor pollicis brevis), the ulnar nerve (recording of the abductor digiti minimi), and the peroneal nerve (recording of the extensor digitorum brevis), as well as sensory nerve conduction studies in radial and sural nerves.

To estimate the proportion of Zika virus infections in the general population, to be further compared with the series of Guillain-Barré syndrome, a first control group (control group 1; n=98) was recruited among patients in hospital or consulting for, non-febrile illness at the CHPF. Patients from the control group 1 were matched for age (within a 10-year margin), sex, and island of residence with patients in the Guillain-Barré syndrome group. Each patient in control group 1 had a blood

sample taken about 7 days from the admission date of the matching Guillain-Barré syndrome case.

To investigate a possible role of past dengue infection in developing Guillain-Barré syndrome in a Zika virus infected patient, a second control group (control group 2; n=70) was recruited among age-matched (within a 10-year margin) patients with RT-PCR-confirmed Zika virus infection, but who did not develop any neurological complication.

The epidemic curve of Zika virus in French Polynesia was obtained by extrapolating data from a sentinel network of clinicians who have been reporting the number of suspected Zika virus cases on a weekly basis from October, 2013, until April, 2014, to the Bureau de Veille Sanitaire–Direction de la Santé de Polynésie Française (Papeete, Polynésie Française).

The study protocol was approved by the Comité d’Ethique de la Polynésie Française (N°69/CEPF 2014), and all patients provided informed consent for their participation in the study.

## Procedures

In the Guillain-Barré syndrome group, a first blood sample was collected at hospital admission and one to three additional blood samples were collected 3 weeks, 2 months, and 3 months later. In control group 1, the blood sample was collected within a 7-day period from the

indexed Guillain-Barré syndrome case for 59 (60%) patients, and with a median period of 13 (IQR 9–16) days for the remaining controls.

Diagnosis of Zika virus acute infection in patients in the Guillain-Barré syndrome group and control group 2 was done with a Zika virus specific RT-PCR protocol adapted from Lanciotti and colleagues.<sup>20</sup> Serum was considered positive for Zika virus if the two distinct genomic regions targeted by the RT-PCR were amplified.

Detection of IgM against Zika virus and dengue virus in blood samples from patients in the Guillain-Barré syndrome group and control group 1 was done with indirect immunofluorescent assay on Vero cells (African green monkey kidney cells) infected with either Zika virus [PF13-251013-18] or dengue virus [D1-Hawaii 1944].

Detection of IgG against Zika virus and each of the four dengue serotypes was done on blood samples from patients in the Guillain-Barré syndrome group, control group 1, and control group 2 using a recombinant-antigen-based microsphere immunoassay adapted from Beck and colleagues (appendix).<sup>21</sup>

Detection of neutralising antibodies against Zika virus and each of the four dengue serotypes was done for patients in the Guillain-Barré syndrome group and control group 1 by microseroneutralisation assay done on Vero cells inoculated with serial dilutions of each serum previously incubated with titrated Zika virus

See Online for appendix

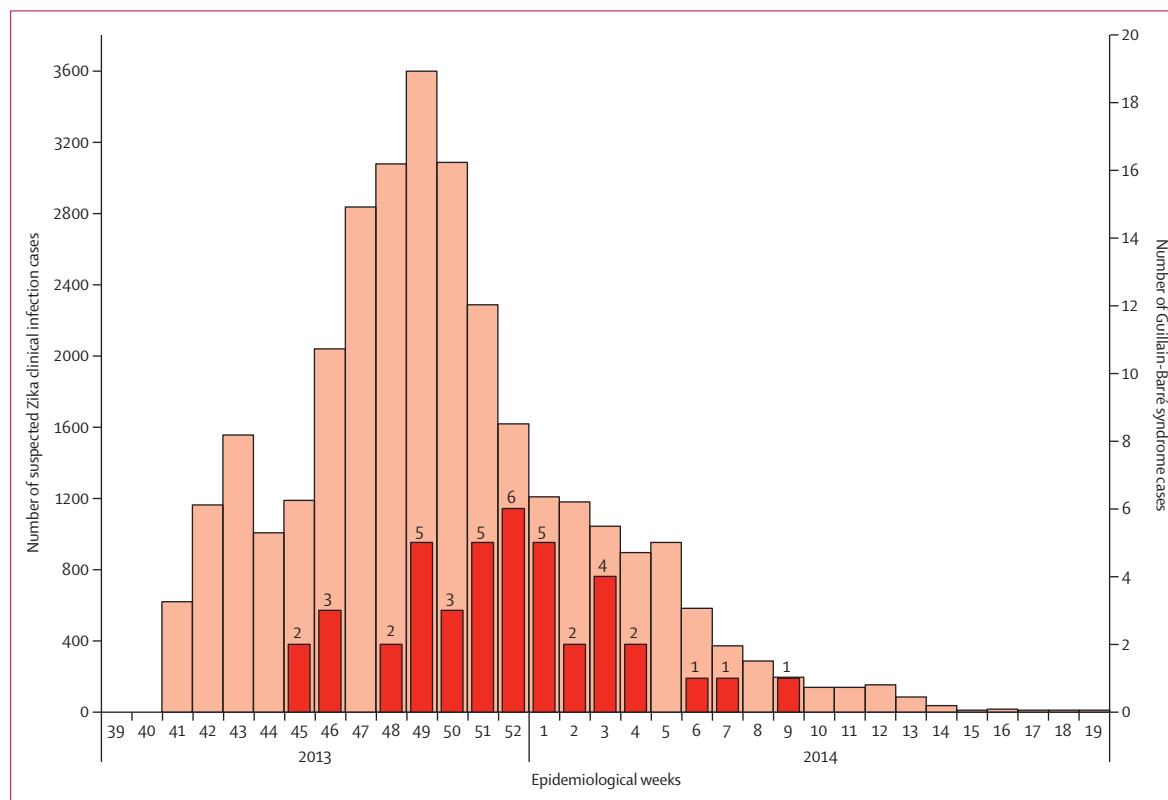


Figure: Weekly cases of suspected Zika virus infections and Guillain-Barré syndrome in French Polynesia between October, 2013, and April, 2014

	n (%) or median (IQR)
Age (years)	42 (36–56)
Men	31 (74%)
Obese	11 (26%)
Smoking (n=40)	12 (30%)
High blood pressure	7 (17%)
Heart disease	3 (7%)
Previous viral syndrome	37 (88%)
Conjunctivitis (n=31)	15 (48%)
Rash (n=36)	29 (81%)
Fever (n=31)	18 (58%)
Arthralgia (n=31)	23 (74%)
Oedema of the limbs (n=29)	9 (31%)
Time between reported viral syndrome and onset of neurological symptoms (days) (n=37)	6 (4–10)
Time between onset of neurological symptoms and admission (days)	4.5 (2–8)
Symptoms at admission	
Muscle weakness	31 (74%)
Symmetric muscle weakness	27 (64%)
Muscle weakness limited to lower limbs	18 (43%)
Incapacity to walk (n=41)	18 (44%)
Areflexia or decreased reflexes	26 (62%)
Facial palsy	27 (64%)
Bilateral facial palsy	14 (33%)
Unilateral facial palsy	13 (31%)
Trouble swallowing	10 (24%)
Paraesthesia	35 (83%)
Time between onset of neurological symptoms and peak of illness (days)	6 (4–9)
Time between admission and peak of illness (days)	1 (0–2)
Symptoms at nadir	
Muscle weakness	36 (86%)
Symmetric muscle weakness	33 (79%)
Muscle weakness limited to lower limbs	17 (40%)
Incapacity to walk	26 (62%)
Areflexia or decreased reflexes	20 (48%)
Facial palsy	33 (79%)
Bilateral facial palsy	25 (60%)
Unilateral facial palsy	8 (19%)
Trouble swallowing	19 (45%)
Trouble breathing	14 (33%)
Duration of plateau phase of illness (days)	4 (3–10)
Treatment	
Intravenous immune globulins	42 (100%)
Plasmapheresis	1 (2%)
Patients admitted to intensive care	16 (38%)
Trouble swallowing	12 (29%)
Respiratory assistance	12 (29%)
Duration of hospital stay (days)	11 (7–20)
Duration of hospital stay for patients admitted to intensive care (days)	51 (16–70)
Lumbar puncture results	
Proteins (g/L)	1.47 (0.92–2.21)
Increased CSF protein concentration (cutoff: 0.52 g/L)	39 (93%)
Cells (per mm <sup>3</sup> )	4 (1–7)

**Table 1: Clinical characteristics of patients with Guillain-Barré syndrome (n=42) in French Polynesia 2013–14**

[PF13-251013-18] or dengue serotype 1 to 4 strains that were isolated during previous outbreaks in French Polynesia (appendix).

The sera from patients in the Guillain-Barré syndrome group (n=42 at admission, n=31 at 3 months) and healthy blood donors (collected before April, 2013; n=20) were tested by ELISA (Bühlmann-Gangliocombi, Schönenbuch, Switzerland) for IgG or IgM reactivity to the glycolipids GM1, GA1, GM2, GD1a, GD1b, and GQ1b at 1:100 dilution. As per kit instructions, results were considered as positive, equivocal, and negative when showing more than 50%, 30–50%, and less than 30% binding, respectively. Sera (n=41 at admission, n=27 at 3 months) were also tested by a combinatorial microarray screening method based on a refinement and miniaturisation of previous published combinatorial glycoarray assay<sup>22</sup> (appendix).

The sera from six patients showing high reactivity towards GA1 were tested against Zika virus proteins by western blot (appendix). Molecular mimicry was assessed using the method by Neil and colleagues (appendix).<sup>23</sup>

### Statistical analysis

The primary objectives of this study were to determine the association between Guillain-Barré syndrome and Zika virus infection in French Polynesia and to determine whether possible co-infection or pre-existing immunity to dengue (and a specific dengue serotype) seem to facilitate the development of Guillain-Barré syndrome. With two controls per case, and on the assumption that 70% of patients with Guillain-Barré syndrome and 40% of controls reported a recent Zika virus infection, the statistical power to detect a difference between the Guillain-Barré syndrome group and the control group was calculated to be 86%.

The risk of developing Guillain-Barré syndrome per Zika virus infection was calculated by dividing the total estimated number of cases of Guillain-Barré syndrome reported in French Polynesia (n=42) by the total number of people infected by Zika virus during the epidemic period. This latter number was calculated by multiplying the attack rate (66%) estimated during a post-epidemic population-based serological survey<sup>24</sup> by the total population of French Polynesia (268 270 inhabitants; 2012 census). The association between Zika virus positive serology, dengue positive serology, and Guillain-Barré syndrome was analysed with exact conditional logistic regression. Because the humoral response elicited by acute Zika virus infection might trigger production of anti-dengue virus IgG related to past dengue infections, we adjusted the odds ratio (OR) describing the association between anti-dengue IgG and Guillain-Barré syndrome for the presence of anti-Zika virus IgG. All ORs are given with 95% CIs.

Motor nerve conduction parameter values were compared with reference values using one-sample *t* tests, and 1st week values were compared with 4th month values using Wilcoxon matched pairs signed rank sum

	viral RNA	IgM	IgG	Zika IgM/IgG				Zika virus positive	Neutralising antibodies	IgM Zika/IgM dengue			
				+/+	+/-	-/+	-/-			+/+	+/-	-/+	-/-
Guillain-Barré syndrome (N=42*)	0 (0)	39 (93%)	29 (69%)	27	12	2	1	41 (98%)	42 (100%)	8 (19%)	31 (74%)	0	3 (7%)
Control group 1 (N=98)	ND	17 (17%)	25 (26%)	7	10	18	63	35 (36%)	54 (56%)	6 (6%)	11 (11%)	8 (8%)	73 (75%)
Control group 2 (N=70)	70 (100%)	ND	5 (7%)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Data are n (%) or n. \*RT-PCR was only done for 41 patients with Guillain-Barré syndrome; tested samples for patients with Guillain-Barré syndrome are late samples (around 3 months after admission), except for the RT-PCR (admission sample). ND=not done. IFA=immunofluorescent assay. MIA=microsphere immunoassay.

**Table 2: Detection of Zika RNA (by RT-PCR), Zika and dengue IgM (by IFA), Zika IgG (MIA), and neutralising antibodies**

	IgG dengue						Dengue neut				
	Guillain-Barré syndrome*			Control group 1			Control group 2			Guillain-Barré syndrome	
	IgG Zika negative	IgG Zika positive	Total	IgG Zika negative	IgG Zika positive	Total	IgG Zika negative	IgG Zika positive	Total		
≥2 serotypes	11 (85%)	25 (86%)	36 (86%)	42 (56%)	23 (92%)	65 (66%)	42 (65%)	4 (80 %)	46 (66%)	41 (100%)	
1 serotype	0	4 (14%)	4 (10%)	20 (27%)	2 (8%)	22 (23%)	11 (17%)	1 (20 %)	12 (17%)	0	
No infection	2 (15%)	0	2 (5%)	11 (15%)	0	11 (11%)	12 (19%)	0	12 (17%)	0	
Total	13	29	42	73	25	98	65	5	70	41	

Data are n (%). \*Tested samples for patients with Guillain-Barré syndrome are late samples (±3 months after admission).

**Table 3: Dengue IgG (by microsphere-based immunoassay) and neutralising responses (neut)**

tests. First week values of the 19 patients with electrophysiological measurements at 4th month were compared to those of the 18 patients without follow-up using a Mann-Whitney *U* test. Data were collected with EpiData 3.1 software and were analysed with Stata 14 (StataCorp LP Lakeway, TX, USA).

### Role of the funding source

The funders had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study, except for the results of the combinatorial microarray (SKH and HJW), and had final responsibility for the decision to submit the publication.

### Results

Cases of Zika virus infection were reported weekly from October, 2013, to March, 2014 (figure). The first case of Guillain-Barré syndrome was reported on week 5 of the outbreak, while the peaks of the Zika epidemic and Guillain-Barré syndrome cases were reached on weeks 9 and 12, respectively. In total, 42 cases of Guillain-Barré syndrome were recorded during the Zika virus outbreak. Based on a 66% attack rate of Zika virus infection in the general population, the risk of Guillain-Barré syndrome was estimated to be 0.24 per 1000 Zika virus infections.

The median age of the patients with Guillain-Barré syndrome was 42 years (IQR 36–56) years, 31 (74%) were men, and 38 (90%) were born in French Polynesia. The clinical characteristics of the patients in the Guillain-Barré

syndrome group are shown in table 1. Most patients (88%) had a recent history of viral syndrome in a median of 6 days (IQR 4–10) before the onset of neurological manifestations. Rash (81%), arthralgia (74%), and fever (58%) were the most commonly reported symptoms (table 1).

The main characteristics of the Guillain-Barré syndrome were rapid progression to nadir (median of 6 days [IQR 4–9] between the onset of neurological symptoms to the nadir), and the short plateau phase (median of 4 days [IQR 3–10]). Clinical presentation at hospital admission was manifested by generalised muscle weakness (74%), with incapacity to walk (44%). Facial palsy was common (64%). 39 (93%) patients had increased (>0.52 g/L) protein concentration in the CSF obtained by lumbar puncture. 16 (38%) patients were admitted to intensive care units and 12 (29%) required respiratory assistance. All cases (100%) of Guillain-Barré syndrome received treatment by immunoglobulins, and one (2%) had plasmapheresis. The median duration of hospital stay was 11 days (IQR 7–20) for all patients, and 51 days (16–70) for the 16 patients who were admitted into intensive care. No patients died. 3 months after discharge, 24 (57%) patients were able to walk without assistance.

Tables 2 and 3 display the results of virological analyses among patients with Guillain-Barré syndrome and the two control groups. Acute Zika virus infection, as confirmed by a positive RT-PCR result, was observed for all patients in control group 2, but for none of the 41 patients tested in the Guillain-Barré syndrome group (table 2); thus corroborating clinical observations, notably



	Guillain-Barré syndrome* (n=42)	Control group 1 (n=98)	OR (95% CI)	OR† (95% CI)	Control group 2 (n=70)	OR (95% CI)	OR† (95% CI)
Zika virus IgM and/or IgG positivity	41 (98%)	35 (36%)	59.7 (10.4-∞)	..	..	..	..
Positive Zika virus seroneutralisation	42 (100%)	54 (56%)	34.1 (5.8-∞)	..	..	..	..
Dengue virus IgG positivity	40 (95%)	87 (89%)	2.0 (0.4-19.9)	1.0 (0.2-11.5)	58 (83%)	6.0 (0.8-269.5)	4.0 (0.5-184.7)

Data are n (%), unless otherwise shown. \*Tested samples for patients with Guillain-Barré syndrome are late samples (around 3 months after admission). †Adjusted for Zika virus IgG positivity. OR=odds ratio.

Table 4: Zika virus and dengue virus serological patterns associated with Guillain-Barré syndrome

	Median			Ulnar			Fibular		
	DML (ms)	Ampli (mV)	MCV (m/s)	DML (ms)	Ampli (mV)	MCV (m/s)	DML (ms)	Ampli (mV)	MCV (m/s)
Reference value*	N<3.7	N>6.0	N>48	N<3.0	N>6.0	N>48	N<5.0	N>3.0	N>42
1st week (n=37)	12.4†	2.9†	46.5	5.8†	3.9†	51.8	9.9†	2.4§	40.7
1st week subgroup (n=19)	14.1†	2.4†	44.6	6.1†	3.7‡	48.9	10.1§	2.1§	39.3
4th month (n=19)	7.1§	6.0§	46.4	4.4§	5.6§	46.5	6.0§	3.8§	45.4

1st week values are compared with reference values; 4th month values are compared with 1st week values of the same subgroup (n=19). DML=distal motor latency. Ampli=amplitude of the distal compound muscle action potential. MCV=motor conduction velocity. \*Reference values are as recommended by the American Association of Electrodiagnostic Medicine). †p<0.0001. ‡p<0.001. §p<0.05.

Table 5: Evolution of motor nerve conduction parameters (mean values) after onset of Guillain-Barré syndrome

the absence of fever, suggesting that the patients in the Guillain-Barré syndrome group were no longer viraemic at admission.

Recent infection by Zika virus was supported by the detection of anti-Zika virus IgM antibodies in 93% of patients in the Guillain-Barré syndrome group and 17% of patients in control group 1 (table 2). Because possible cross-reactivity between anti-dengue and anti-Zika virus IgM responses had previously been described, immunofluorescent assay was done using the two viruses. In the Guillain-Barré syndrome group, 74% of the patients had IgM against Zika virus but not against dengue. All 19% patients with anti-dengue IgM also had IgM against Zika virus, suggesting that the anti-dengue IgM response could result from cross-reactivity.

When combining the results of both anti-Zika virus IgM and IgG, previous occurrence of a Zika virus infection was shown for 41 (98%) patients in the Guillain-Barré syndrome group and 35 (36%) in control group 1 (OR 59.7 [95% CI 10.4-∞]; p<0.0001; table 4). Moreover, a neutralising response against Zika virus was observed for 100% of patients in the Guillain-Barré syndrome group and 56% in control group 1 (OR 34.1 [95% CI 5.8-∞]; p<0.0001).

The interpretation of anti-dengue IgM is difficult because of possible cross-reactivity with anti-Zika virus IgM. Still, there was no indication of increased recent infection with dengue among patients with Guillain-Barré syndrome when compared with the control group 1 (table 2; p>0.05). Past history of dengue was common among patients with Guillain-Barré syndrome (95% on the last sample available, away from the immunological boost associated with recent Zika virus infection) compared with the control group 1 (89%; OR 2.0 [95% CI 0.4-19.9]; p=0.62) and control

group 2 (83%; OR 6.0 [95% CI 0.8-269.5]; p=0.10; table 4; appendix p 5). These non-significant differences were further attenuated after stratification by the presence of anti-Zika virus IgG, suggesting that the humoral responses elicited by Zika virus infection also triggered production of anti-dengue IgG (tables 3 and 4). This is corroborated by the examination of Zika virus and dengue IgG responses in the blood samples serially collected from the patients with Guillain-Barré syndrome. The number of patients with anti-Zika virus IgG increased from the earliest to the intermediate and then to the latest sample, whereas the reverse occurred for anti-dengue IgG (appendix pp 5, 6). A possible explanation could be that an anamnestic anti-dengue IgG response in the patients with Guillain-Barré syndrome might have been transiently boosted by the Zika virus infection.

Serological tests for *Campylobacter jejuni* (n=41), HIV (n=42), cytomegalovirus (n=32), Epstein-Barr virus (n=32), and herpes simplex virus type 1 and 2 (n=8) were negative.

37 patients underwent electrophysiological examination during the first week of Guillain-Barré syndrome onset (table 5). Motor nerve conduction study showed the same pattern in all tested nerves, with prolonged distal latencies (p<0.0001) and marked reduction of the distal compound muscle action potential (CMAP) amplitude (p<0.0001), indicative of severe conduction alteration in the distal nerve segments. By contrast, there was no substantial conduction slowing or block in intermediate motor nerve segments (throughout forearm and legs; table 5). Amplitude and conduction velocity of sensitive potentials were not significantly altered in radial and sural nerves (sensory conduction velocity was 49 m/s and 42 m/s, and amplitude was 17.0 µV and 13.7 µV for

	Guillain-Barré syndrome at onset (n=42)	Guillain-Barré syndrome at 3 months (n=31)	Controls* (n=20)
GM1	0	8 (26%)	0
GA1	8 (19%)	10 (32%)	0
GM2	2 (5%)	1 (3%)	0
GD1a	5 (12%)	9 (29%)	0
GD1b	3 (7%)	9 (29%)	0
GQ1b	0	0	0
Any	13 (31%)	15 (48%)	0

Data are n (%). \*Blood donors.

**Table 6: Positive (>50%) reactivity to glycolipids in sera of patients with Guillain-Barré syndrome (n=42) and controls (n=20) in French Polynesia 2013–14**

radial and sural nerves, respectively;  $p > 0.05$  for comparison with reference values).

A second nerve conduction study was done 4 months later including 19 Guillain-Barré syndrome patients for whom a baseline assessment was available (there was no difference in baseline values of the 19 patients with follow-up compared with the 18 without follow-up). By comparison to the first study, results showed a clear improvement of the distal conduction abnormalities ( $p < 0.05$ ) with reduction of the prolonged distal latencies and near normalisation of CMAP amplitudes (table 5; appendix p 8). Together, these findings are suggestive of an acute motor axonal neuropathy (AMAN).

By ELISA, at admission, sera from 13 (31%) patients with Guillain-Barré syndrome showed a positive reactivity (>50% binding) against different glycolipids (table 6; appendix p 7). Ten (24%) patients had an equivocal percentage of binding (30–50%). Among these 23 patients, 17 had a reactivity directed toward glycolipid GA1 (eight positive, nine equivocal) and it was either isolated or shared with other glycolipids (appendix p 7). At 3 months, the proportion of reactive sera had slightly increased (48%). Controls were negative (n=20). These results were heterogeneous and low intensity for 50% of them.

Western blot was used to test the reactivity against Zika virus proteins in the serum from six patients with Guillain-Barré syndrome, four with high reactivity against GA1 (patients 6, 11, 20, and 29 as shown in the appendix [p 7]), and two patients with no reactivity against GA1 (patients 13 and 27 as shown in the appendix [p 7]). All sera showed intense reactivity with viral proteins regardless of their reactivity towards GA1 (appendix p 9).

The reactivity of serum number 20 towards Zika virus proteins was not inhibited even at the highest GA1 amount (600 µg; appendix p 9). We further tested serum number 6 and did not observe any competition with a GA1 amount of 300 µg (data not shown).

Combinatorial microarrays were used to screen glycolipid complexes as antigens. Most serum samples

tested were negative or had low level binding to some single or heteromeric glycolipid complex. Notably, antibodies against GA1–sulphatide complex were frequently observed (19/41; 46%) in patient sera, with intermediate binding intensities, above the threshold of positivity ( $p = 0.001$ ). Additionally, a substantial number of patient sera had antibodies raised against GA1 in complex with cholesterol or phosphatidylserine or both, although most were of low binding intensity (appendix p 10).

## Discussion

This is the first study to assess the role of Zika virus infection in a large number of patients with Guillain-Barré syndrome diagnosed during a Zika virus outbreak. The serological investigations done on the blood samples from the 42 patients who developed a Guillain-Barré syndrome during the Zika virus outbreak in French Polynesia confirm that all patients had experienced Zika virus infection. Moreover, the presence of IgM (93%) and the information that most patients (88%) reported a transient viral syndrome compatible with Zika virus disease in a median of 6 days before the onset of neurological symptoms, suggested a recent Zika virus infection. Patients with Guillain-Barré syndrome were no longer viraemic for Zika virus at the time of admission, consistent with previous data showing that Zika virus viraemia rarely exceeds 5 days after disease onset.<sup>25</sup> However, detection of virus in the urine by RT-PCR could be a valuable alternative.<sup>26</sup> Because dengue serotypes 1 and 3 were co-circulating at the time of the Zika virus epidemic,<sup>18</sup> we investigated whether dengue infection could have contributed to the occurrence of Guillain-Barré syndrome. Analysis of dengue serology (immunofluorescent assay, microsphere immunoassay, and seroneutralisation) did not support recent dengue infection. Most patients (95%) with Guillain-Barré syndrome had pre-existing dengue immunity, but this did not differ significantly from the control groups.

Guillain-Barré syndrome is an acute, immune-mediated polyradiculoneuropathy typically occurring after minor viral and bacterial infections. Motor function is usually affected, beginning distally and progressing proximally over up to a 4-week period.<sup>27</sup> Patients have generalised weakness, areflexia, and a varying degree of sensory disturbances and involvement of cranial nerves.<sup>28</sup> The risk of Guillain-Barré syndrome increases with age and men are more commonly affected than women.<sup>29</sup> The pathophysiology is incompletely understood, but is known to mostly occur 2–8 weeks after an infection. Guillain-Barré syndrome is the leading cause of non-traumatic paralysis, with a global incidence of 1–4 per 100 000 persons-years. The range of infections reported to have preceded Guillain-Barré syndrome include upper respiratory infections, notably influenza and pseudo-influenza, digestive tract infections, notably *Campylobacter jejuni*, as well as cytomegalovirus and

Epstein-Barr virus infections.<sup>30-32</sup> The incidence of Guillain-Barré syndrome cases during the French Polynesian outbreak was estimated to be 0.24 per 1000 Zika virus infections, at the lower range of the 0.25 to 0.65 per 1000 observed following *C jejuni* infections.<sup>33</sup> It is unlikely that Guillain-Barré syndrome cases were missed during the study period, because routine procedures for systematic confirmation of diagnosis of Guillain-Barré syndrome pre-existed the Zika virus epidemic, and all cases were systematically referred to the CHPF for diagnosis confirmation. Although it is unknown whether attack rates of Zika virus epidemics will be as high in affected regions in Latin America than in the Pacific Islands (73% in Micronesia<sup>6</sup> and 66% in French Polynesia<sup>29</sup>), high numbers of cases of Guillain-Barré syndrome might be expected in the coming months as the result of this association. The results of our study support that Zika virus should be added to the list of infectious pathogens susceptible to cause Guillain-Barré syndrome.

Patients with Guillain-Barré syndrome in our study had electrophysiological findings compatible with the AMAN type. Electromyography assessments done during the first week of the disease showed substantial distal motor nerve conduction alterations, which explain the neuromuscular weakness. Prolonged distal latencies and reduced distal CMAP at admission could have been interpreted as demyelinating conduction slowing and block, leading to the classification of the Guillain-Barré syndrome pattern as acute inflammatory demyelinating neuropathy (AIDP) with possible axonal degeneration. However, the disappearance of the distal motor conduction alterations during the follow-up in a subset of patients, without development of abnormal temporal dispersion or conduction slowing in intermediate nerve segments, was consistent with “reversible conduction failure” already described in AMAN.<sup>34,35</sup> In patients with Guillain-Barré syndrome, such nodal or paranodal dysfunctions would be rather strictly localised in distal motor nerve endings.<sup>36</sup> The clinical outcome of these patients with Zika virus and Guillain-Barré syndrome was generally favourable, despite a rapid onset and short plateau phase, as has been seen in other patient groups with the AMAN type of Guillain-Barré syndrome.<sup>37</sup> 3 months after discharge, 24 (57%) patients were able to walk without assistance.

Among the molecular mechanisms contributing to the pathogenesis of Guillain-Barré syndrome, a broad range of anti-glycolipid IgG antibodies, notably directed to gangliosides, has been previously described, particularly in axonal variants of the disease.<sup>38,39</sup> Results in this study, using both ELISA and combinatorial microarray techniques, found less than 50% of sera at admission with a significant autoimmune response against glycolipids, including gangliosides or glycolipid complexes (appendix p 10). This low detection rate for the AMAN clinical subtype could be a reflection of the

unique nature of the preceding infection and study population, by contrast with more typical post *Campylobacter* Guillain-Barré syndrome AMAN clinical cohorts. These findings suggest that there might be autoantibodies in this post-Zika virus Guillain-Barré syndrome cohort that cannot be fully identified by current methods. Moreover, complementary analysis of sera with reactivity against GA1 did not show any competition between GA1 and Zika virus proteins, thus suggesting the absence of antigenic mimicry between Zika virus antigens and GA1 in these patients with Guillain-Barré syndrome and casting doubt on the relevance of the anti-GA1 antibodies to neuropathy pathogenesis. The disease might not be anti-glycolipid antibody mediated, but rather be mediated by other autoantibody specificities or unknown neurotoxic factors. Alternatively, viral neurotoxicity might contribute a more direct but as yet unexplained role.

Because almost all of the patients with Guillain-Barré syndrome were of Polynesian origin and because distribution of HLA alleles has been previously described as being involved in certain forms of Guillain-Barré syndrome,<sup>40</sup> a possible role of ethnicity in triggering Guillain-Barré syndrome was hypothesised. However, the high incidence of Guillain-Barré syndrome recently reported in Brazil, El Salvador, and Colombia during local Zika virus outbreaks<sup>11,41</sup> suggests that, whenever involved, such host factors might not be specific to the ethnic groups living in French Polynesia.

In conclusion, this is the first study to document a large series of patients who developed a Guillain-Barré syndrome following Zika virus infection, a virus that previously used to be considered as causing only mild disease. Most (88%) of the patients with Guillain-Barré syndrome reported symptomatic Zika virus infection that preceded the occurrence of neurological symptoms by a median of 6 days. All patients with Guillain-Barré syndrome were of the AMAN type, characterised by distal motor nerve involvement, the absence of typical patterns and levels of anti-glycolipid antibodies, and faster recovery than usually observed in typical Guillain-Barré syndrome. Because Zika virus is spreading rapidly across the Americas, at risk countries need to be prepared to have adequate intensive care beds capacity to manage patients with Guillain-Barré syndrome.

#### Contributors

V-MC-L, AB, VC, H-PM, DM, AF, JN, and FG conceived and designed the study. V-MC-L, SL, CR, JV, AT, J-CM, and PD developed, performed, and interpreted the virological analyses. VC, HJW, SKH, LM, and JN developed, performed, and interpreted the immunological analyses. SM, LB, PL, and FG provided care to the patients and designed the clinical report forms. A-LV, CD, AB, and H-PM designed the case report forms and collected the epidemiological data. FG and EF performed the electrophysiological assessments. AB, TD, H-PM, and AF performed the statistical analyses. V-MC-L, AB, VC, HJW, EF, AF, and JN wrote the first version of the report. All authors critically reviewed and approved the final version of the report.

#### Declaration of interests

We declare no competing interests.



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