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Guillain-Barré Syndrome outbreak caused by ZIKA virus infection in French Polynesia

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ABSTRACT

Background

From October 2013 to April 2014, French Polynesia experienced the largest Zika virus (ZIKV) outbreak ever described at that time. During the same period, an increase in Guillain-Barré syndrome (GBS) was reported, suggesting a possible association between ZIKV and GBS.

Patients and Methods

A case-control study was performed to identify the role of ZIKV and dengue virus (DENV) infection in developing GBS. Cases were GBS patients diagnosed at the Centre Hospitalier de Polynésie Française during the outbreak period. Controls were age-, gender-, and residence-matched patients who presented at the hospital with a non-febrile illness (Control group 1 [CTR1]; n=98), and age-matched patients with acute ZIKV disease and no neurological symptoms (Control group 2 [CTR2]; n=70). Virological investigations included RT-PCR for ZIKV, and both microsphere immunofluorescent and seroneutralization assays for ZIKV and DENV. Anti-glycolipid reactivity was studied in GBS patients using both ELISA and combinatorial microarrays.

Results

Forty-two patients were diagnosed with GBS during the study period. Ninety-eight percent of GBS patients had anti-ZIKV IgM or IgG, and all had neutralizing antibodies against ZIKV compared to 55.7% with neutralizing antibodies in the CTR1 group (P<0.0001). Ninety-three percent of GBS patients had ZIKV IgM and 88% had experienced a transient illness in median six days before the onset of neurological symptoms, suggesting recent ZIKV infection. GBS patients had electrophysiological findings compatible with the acute motor axonal neuropathy (AMAN) type, and had rapid evolution of disease (median duration of the installation and plateau phases was 6 and 4 days, respectively). Twelve (29%) patients required respiratory assistance. No patients died. Anti-glycolipid antibody activity, notably against GA1, was found in 13 (31%) patients by ELISA and 19/41 (46%) by glycoarray at admission. The typical AMAN-associated anti-ganglioside antibodies were rarely present. There was no significant difference in past dengue history between GBS patients and the two control groups.

Conclusion

This is the first study providing evidence for ZIKV infection causing GBS. As ZIKV is spreading rapidly across the Americas, at risk countries need to prepare for adequate intensive care beds capacity for managing GBS patients.

Background

Zika virus (ZIKV) is an arthropod-borne virus (arbovirus) in the genus *Flavivirus,* family *Flaviviridae.*[1] ZIKV was first isolated from a Rhesus monkey in 1947 in the Zika forest of Uganda.[2] The first human infection was reported in Nigeria in 1954.[3] Like dengue (DENV) and chikungunya (CHIKV) viruses, ZIKV 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 widely distributed *Aedes (Stegomyia)* mosquitoes as vectors.[4]

From the 1950s, ZIKV was only reported as circulating sporadically in Africa and South-East Asia.[5] In 2007, ZIKV was isolated for the first time in the Pacific, on the Micronesian island of Yap.[6] From October 2013 to April 2014, French Polynesia experienced the largest Zika outbreak ever reported at that time.[7] It was estimated that more than 32,000 patients consulted for suspected ZIKV infection, with a weekly incidence peaking on week 9 of the outbreak.[8] From 2014, ZIKV spread to other Pacific islands, notably Easter island (Chile). In March 2015, Brazil reported autochthonous transmission of ZIKV,[9] and an outbreak was declared 6 months later.[10] As of February 1, 2016, ZIKV had emerged in 25 countries and territories in South/Central America, with alarming reports of microcephaly cases among neonates in Brazil.[11]

Previously to the French Polynesian outbreak, ZIKV infection used to be described as a mild febrile illness with clinical symptoms including maculopapular rash, joints and muscles pain, headache and non-purulent conjunctivitis.[6] Between November 2013 and February 2014 in French Polynesia, 42 patients presented at hospital with Guillain-Barré syndrome (GBS), an autoimmune disease causing acute or subacute flaccid paralysis, contrasting with reports of 5, 10, 3 and 3 in 2009, 2010, 2011, and 2012, respectively.[12] Other arboviral diseases like West Nile, Japanese Encephalitis, chikungunya and dengue had already been reported to sometimes cause GBS,[13-16] but only during the outbreak in French Polynesia was this severe neurological complication first described associated with ZIKV infection.[17] The temporal coincidence between the peaks in incidence of ZIKV and GBS cases, and also the concurrent circulation of DENV serotypes 1 and 3[18] suggested possible causal relationship between the three events. Using two control series, we addressed the hypothesis that ZIKV infection with or without DENV concurrent or sequential infection may be a risk factor for the development of GBS.

Methods

Study design and participants

A case-control study was performed to identify the role of ZIKV and dengue virus (DENV) infection in developing GBS. Cases were GBS patients diagnosed at the Centre Hospitalier de Polynésie Française in Papeete, Tahiti, during the outbreak period. As a routine, all patients with suspicion of GBS in French Polynesia are referred to the CHPF for diagnosis confirmation. All of the patients included in this study were diagnosed as developing a GBS by neurologists or staff in intensive care units according to international criteria.[19] Clinical and demographic data were collected from medical records obtained during patients' hospitalisation. The data recorded for all patients included: patient's age, gender, island of residence, medical history and co-morbidities, clinical signs and symptoms, illness duration and severity. Electrophysiological assessment was performed for all patients using standard electromyography [EMG] 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 ZIKV infections in the general population, to be further compared with the GBS series, a first control group (CTR1, n=98) was recruited among patients hospitalised or consulting for non-febrile illness at the CHPF. Patients from the CTR1 group were matched for age (±10 years), gender and island of residence with patients in the GBS group. Each patient in CTR1 group had a blood sample taken ±7 days from the admission date of the matching GBS case.

To investigate a possible role of past DENV infection(s) in developing a GBS in ZIKV infected patients, a second control group (CTR2, n=70) was recruited among age-matched (± 10 years) patients with RT-PCR-confirmed ZIKV infection, but who did not develop any neurological complication.

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

Zika and dengue virus infection diagnosis

For the GBS group, a first blood sample was collected at hospital admission and one to three additional blood samples were collected three weeks, two and/or three months later. For the CTR1 group, the blood sample was collected within a 7-day period from the indexed GBS case for 59 (60.2%) patients, and with a median (IQR) period of 13 (9-16) days for the remaining controls.

Diagnosis of ZIKV acute infection in patients from GBS and CTR2 groups was performed using a ZIKV specific RT-PCR protocol adapted from Lanciotti et al.[20] Serum was considered positive for ZIKV if the two distinct genomic regions targeted by the RT-PCR were amplified.

Detection of IgM against ZIKV and DENV in blood samples from patients in GBS and CTR1 groups was performed using indirect immunofluorescent assay (IFA) on Vero cells (African Green Monkey kidney cells) infected with either ZIKV[PF13-251013-18] or DENV[D1-Hawaii 1944].

Detection of IgG against ZIKV and each of the four DENV serotypes was performed on blood samples from patients in GBS, CTR1 and CTR2 groups using a recombinant-antigen based microsphere immunoassay (MIA) adapted from Beck et al.[21] (see details in Supplementary material).

Detection of neutralizing antibodies against ZIKV and each of the four DENV serotypes was performed for patients in the GBS and CTR1 groups using a microseroneutralization assay performed on Vero cells inoculated with serial dilutions of each serum previously incubated with titrated ZIKV[PF13-251013-18] or DENV serotype 1 to 4 strains that were isolated during previous outbreaks in French Polynesia (see details in Supplementary material).

Immunochemical reaction with glycolipids

The sera from both GBS patients (n=42 at admission, n=31 at 3 months) and healthy blood donors (collected prior to April 2013; n=20) were tested by ELISA (Bühlmann-Gangliocombi®) for IgG/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 >50%, 30-50%, and <30% binding, respectively. Sera (n=41 at admission, n=27 at three months) were also tested by a combinatorial microarray screening method based on a refinement and miniaturization of previous published combinatorial glycoarray assay[22] (see details in Supplementary material).

Immunosuppression test for exploration of molecular mimicry mechanisms

The sera from six patients showing high reactivity towards GA1 were tested against ZIKV viral proteins by Western blot (see details in Supplementary material). Molecular mimicry was evaluated using the method by Neil.[23] (see details in Supplementary material).

Sample size

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

Statistical analysis

The risk of developing GBS per ZIKV infection was calculated by dividing the total estimated number of GBS cases reported in French Polynesia (n=42) by the total number of people infected by ZIKV during the epidemic period. This latter number was calculated by multiplying the attack rate (66%) estimated during a post epidemic population-based serological survey[24] by the total population of French Polynesia (268 270 inhabitants; 2012 census). The association between ZIKV positive serology, DENV positive serology, and GBS was analysed using exact conditional logistic regression. As the humoral response elicited by acute ZIKV infection may trigger production of anti-DENV IgG related to past DENV infections, we adjusted the odds-ratio (OR) describing the association between anti-DENV IgG and GBS for the presence of anti-ZIKV IgG. All ORs are given with their 95% confidence intervals. Motor nerve conduction parameters values were compared to reference values using one-sample *t* tests, and 1st week values were compared to 4th month values using Wilcoxon matched pairs signed rank sum 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 test. Data were collected using EpiData 3.1 software and all statistical analyses were performed using STATA 14, StataCorp LP.

Ethical considerations

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.

Role of the funding source

The funders had no role in the design of the study, 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 (HSK & HW), and had final responsibility for the decision to submit the publication.

Results

Epidemiological dynamics of the outbreak

Cases of ZIKV infection were reported weekly from October 2013 until March 2014 (See Figure 1). The first GBS case was reported on week 5 of the outbreak, while the peaks of the Zika epidemic and GBS cases were reached on week 9 and 12, respectively. In total, 42 cases of GBS were recorded during the ZIKV outbreak. Based on a 66% attack rate of ZIKV infection in the general population, the risk of GBS was estimated at 0.24 per 1000 ZIKV infections.

Patients characteristics

The median (IQR) age of GBS patients was 42 (36-56) years, 31 (74%) were males and 38 (90%) were born in French Polynesia .

The clinical characteristics of the patients in the GBS group are shown in Table 1. Most (88%) had a recent history of viral syndrome, in median 6 days prior to the onset of neurological manifestations. Rash (81%), arthralgia (74%), fever (58%) were the most commonly reported symptoms.

The main characteristics of the GBS were the rapid progression to nadir (median of 6 days between the onset of neurological symptoms to the nadir), and the short plateau phase (median of 4 days). Clinical presentation at admission was manifested by generalised weakness (74%), with incapacity to walk (44%). Facial palsy was common (64%). Ninety three percent of the patients had increased (> 0.52 mg/dL) protein concentration in the cerebrospinal fluid obtained by lumbar puncture. Sixteen (38%) patients were admitted to intensive care unit, and 12 (29%) required respiratory assistance. All GBS cases received treatment by immunoglobulins, and one had plasmapheresis. The median duration of hospitalisation was 11 days for all patients, and 51 days for the 16 patients who were admitted into intensive care. No patients died. Three months after discharge, 24 (43%) patients were able to walk without assistance.

ZIKV and DENV infection diagnosis

Acute ZIKV infection, as confirmed by a positive RT-PCR result, was observed for all patients in the CTR2 group, but for none of the 41 patients tested in the GBS group (Table 2A); thus corroborating clinical observations, notably the absence of fever, suggesting that the patients in the GBS group were no longer viremic at admission.

Recent infection by ZIKV was supported by the detection of anti-ZIKV IgM antibodies in 92.9% and 17.3% of the patients in GBS and CTR1 groups, respectively (Table 2A). As possible cross-reactivity between anti-DENV and anti-ZIKV IgM responses had previously been described, IFA was performed using the two viruses. In the GBS group, 73.8% of the patients had IgM against ZIKV but not against DENV. All 19.1% patients with anti-DENV IgM also had IgM against ZIKV, suggesting that the anti-DENV IgM response could result from cross-reactivity.

When combining the results of both anti-ZIKV IgM and IgG, previous occurrence of a ZIKV infection was suggested for 97.6% of the patients in the GBS group and 35.7% in the CTR1 group (OR [95% CI] = 59.7 [10.4 - +∞]; P<0.0001; Table 2C). Moreover, a neutralizing response against ZIKV was observed for 100% of the patients in the GBS group and 55.7% in the CTR1 group (OR [95% CI] = 34.1 [5.8 - +∞]; P<0.0001).

The interpretation of anti-DENV IgM is difficult due to possible cross-reactivity with anti-ZIKV IgM. Still, there was no indication of increased recent infection with DENV among GBS patients when compared to the CTR1 group (Table 2A; P>0.05). Past history of dengue was common among GBS patients (95.2% on the last sample available, away from the immunological boost associated with recent ZIKV infection). It was non significantly different from that of the CTR1 group (88.8%; OR [95% CI] = 2.0 [0.4 – 19.9]; P = 0.62) and the CTR2 group (82.9%; OR [95% CI] = 6.04 [0.81 – 269.5]; P = 0.10) (Table 2C and Suppl. Table 1). These non significant differences were further attenuated after stratifying by the presence of anti-ZIKV IgG, suggesting that the humoral response elicited by ZIKV infection also triggered production of anti-DENV IgG (Table 2B & 2C). This is corroborated by the examination of ZIKV and DENV IgG responses in the blood samples serially collected from the GBS patients. Indeed, the number of patients with anti-ZIKV IgG increased from the earliest to the intermediate and then to the latest sample, while the reverse occurred for anti-DENV IgG (Suppl. Table 1 & Suppl. Table 2). A possible explanation would be that an anamnestic anti-DENV IgG response in GBS patients might have been transiently boosted by the ZIKV 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.

Neurophysiological assessment

A total of 37 patients underwent electrophysiological examination during the first week after GBS onset (Table 3). 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 significant conduction slowing or block in intermediate motor nerve segments (throughout forearm and legs). Amplitude and conduction velocity of sensitive potentials were not significantly altered in radial and sural nerves.

A second nerve conduction study was performed four months later including 19 GBS patients for whom a baseline assessment was available (there was no difference in baseline values of the 19 patients with follow-up compared the 18 without follow-up). By comparison to the first study, results showed a clear improvement of the distal conduction abnormalities (P<0.001), with reduction of the prolonged distal latencies and near normalization of CMAP amplitudes (Table 3 and Suppl. Figure 1). Altogether, these findings are suggestive of an acute motor axonal neuropathy (AMAN).

Detection of reactivity towards glycolipids by ELISA

By ELISA, at admission, sera from 13 (31%) GBS patients showed a positive reactivity (% of binding >50%) against different glycolipids (Table 4 and Suppl. Table 2). Ten (24%) had an equivocal percentage of binding (between 30 and 50%). Among these 23 patients, 17 had a reactivity directed toward glycolipid GA1 (8 positive, 9 equivocal) and it was either isolated or shared with other glycolipids. At three 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.

Test of reactivity of GBS patient's sera with ZIKV viral proteins by Western blot

Western blot was used to test the reactivity against ZIKV viral proteins in the serum from 6 GBS patients, 4 with high reactivity against GA1 (patients 6, 11, 20 and 29 of Suppl. Table 2), and 2 patients with no reactivity against GA1 (patients 13 and 27 of Suppl. Table 2). All sera showed intense reactivity with viral proteins regardless of their reactivity towards GA1 (Suppl. Figure 2).

Determination of specific interaction to GA1 by serum absorption with GA1

The reactivity of serum n°20 towards ZIKV proteins was not inhibited even at the highest GA1 amount (600 µg; Suppl. Figure 3). We further tested serum n°6 and did not observe any competition with a GA1 amount of 300 µg (data not shown).

Detection of reactivity towards glycolipids by combinatorial microarray

Combinatorial microarrays were used for screening glycolipid complexes as antigens. The majority of serum samples tested was negative or had low level binding to some single and or heteromeric glycolipid complex. Notably, antibodies against GA1:sulphatide complex were frequently observed (19/41; 46.3%) in patient sera, with intermediate binding intensities, above the threshold of positivity ($p=0.001$). In addition, a significant number of patient sera had antibodies raised against GA1 in complex with cholesterol and/or phosphatidylserine, although most were of low binding intensity (see Supplementary material and Suppl. Figure 4).

Discussion

This is the first study to evaluate the role of ZIKV infection in a large number of GBS patients diagnosed during a ZIKV outbreak. The serological investigations conducted on the blood samples from the 42 patients who developed a GBS during the French Polynesian ZIKV outbreak confirm that all these patients have experienced ZIKV infection. Moreover, the presence of IgM (92.9%) and the information that most (88%) patients reported a transient viral syndrome compatible with ZIKV disease in median 6 days prior to the onset of neurological symptoms, suggested a recent ZIKV infection. GBS patients were no longer viremic for ZIKV at the time of admission, consistent with previous data showing that ZIKV viremia rarely exceeds five days after disease onset.[25] However, detection of virus in urine by RT-PCR may be a valuable alternative.[26] As DENV 1 and 3 serotypes were co-circulating at the time of the ZIKV epidemic,[18] we investigated whether DENV infection might have contributed to the occurrence of GBS. Analysis of dengue serology (IFA, MIA, seroneutralization) did not support recent DENV infection. Most (95.2%) of the GBS patients had pre-existing dengue immunity, but this did not appear as being significantly different from the control groups.

GBS is an acute, immune-mediated polyradiculoneuropathy typically arising after minor viral and bacterial infections. Motor function is usually affected, beginning distally and progressing proximally over up to a 4-week period.[27] Patients suffer from generalised weakness, areflexia and varying degree of sensory disturbances and involvement of cranial nerves.[28] The risk of GBS increases with age and men are more commonly affected than women.[29] The pathophysiology is incompletely understood, but is known to mostly occur 2-8 weeks after an infection. GBS 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 GBS include upper respiratory infections, notably influenza and pseudo-influenza, digestive tract infections, notably *Campylobacter jejuni,* as well as cytomegalovirus and Epstein-Barr virus infections.[30-32] The incidence rate of GBS cases during the French Polynesian outbreak was estimated at 0.24 per 1,000 ZIKV infections, at the lower range of the 0.25 to 0.65 per 1,000 observed following *C. jejuni* infections.[33] It is unlikely that GBS cases were missed during the study period, since routine procedures for systematic confirmation of GBS diagnosis pre-existed the ZIKV epidemic, and all cases were systematically referred to the CPHF for diagnosis confirmation. While it is unknown whether attack rates of ZIKV epidemics will be as high in Latin America affected regions as compared to Pacific islands (73% in Micronesia[6] and 66% in French Polynesia[24]), high number of GBS cases may be expected in the coming months as the result of this association. The results of our study support that ZIKV should be added to the list of infectious pathogens susceptible to cause GBS.

GBS patients in the present series had electrophysiological findings compatible with the AMAN type. EMG assessments performed during the first week of the disease showed marked distal motor nerve conduction alterations, which explain the neuromuscular weakness. Prolonged distal latencies and reduced distal CMAP amplitude may have been as first interpreted as demyelinating conduction slowing and block, leading to classify the GBS 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.[34,35] In GBS patients, such nodal/paranodal dysfunctions would be rather strictly localized in distal motor nerve endings.[36] The clinical outcome of these ZIKV GBS patients 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 GBS.[37] Three months after discharge, 43% of the patients were able to walk without assistance.

Among the molecular mechanisms contributing to the pathogenesis of GBS, a broad range of anti-glycolipid IgG antibodies, notably directed to gangliosides, has been previously described, particularly in axonal variants of the disease.[38,39] Results in this current 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 and/or glycolipid complexes (see supplementary material). This low detection rate for the AMAN clinical subtype, may be a reflection of the unique nature of the preceding infection and study population, in contrast with more typical post-Campylobacter GBS/AMAN clinical cohorts. These findings suggest that there may be autoantibodies in this post-ZIKV GBS cohort that cannot be fully identified by current methods. Moreover, complementary analysis of sera with reactivity against GA1 did not reveal any competition between GA1 and ZIKV proteins, thus suggesting the lack of antigenic mimicry between ZIKV antigens and GA1 in these GBS patients and casting doubt on the relevance of the anti-GA1 antibodies to neuropathy pathogenesis. The disease may not be anti-glycolipid antibody mediated, rather be mediated by other autoantibody specificities or unknown neurotoxic factors. Alternatively viral neurotoxicity may contribute a more direct but as yet unexplained role.

Because almost all of the GBS patients were of Polynesian origin and as distribution of HLA alleles had been previously described as being involved in certain forms of GBS,[40] a possible role of ethnicity in triggering GBS was hypothesized. However, the high incidence of GBS recently reported in Brazil, El Salvador and Columbia during local ZIKV outbreaks[11,41] suggests that, whenever involved, such host factors may 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 GBS following ZIKV infection, a virus that previously used to be considered as causing only mild disease. Most (88%) of the GBS patients reported symptomatic ZIKV infection that preceded by a median of 6 days the occurrence of neurological symptoms. All GBS 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 GBS. As ZIKV is spreading rapidly across the Americas, at risk countries need to prepare for adequate intensive care beds capacity for managing GBS patients.

Contributors

VMC-L, AB, VC, HPM, DM, AF, JN and FG conceived and designed the study; VMC-L, SL, CR, JV, AT, JCM 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; ALV, CD, AB and HPM designed the case report forms and collected the epidemiological data; FG and EF performed the electrophysiological assessments; AB, TD, HPM, and AF performed the statistical analyses; VMC-L, AB, VC, HJW, EF, AF and JN wrote the first version of the manuscript. All authors critically reviewed and approved the final version of the manuscript.

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517

518

519 **FIGURE LEGENDS**

520

521 Figure 1: Epidemic curve of ZIKV virus suspected cases and Guillain-Barré syndromes in French
522 Polynesia 2013-2014. ZIKV cases are shown in orange and GBS cases in black

523

524 Suppl. Figure 1

525 Changes in motor conduction study in one of the patients. Stimulation of right ulnar nerve at wrist
526 and elbow, recording of the abductor digiti minimi. A : 5 days after paralysis onset : CMAP amplitude
527 : 1.5 mV, distal motor latency (DML) : 4.1 ms, motor conduction velocity (MCV) : 50 m/s. Calibration
528 2 mV/div., 5 ms/div. B : 3 months later : CMAP amplitude : 8.1 mV, DML : 3.4 ms, MCV : 49 m/s.
529 Calibration 5 mV/div., 5 ms/div.

530

531 Suppl. Figure 2. Test of reactivity of ZIKV positive patients' sera towards ZIKV viral proteins by
532 Western Blot
533 Lanes 1 and 2: non heated and heated control cell extracts; lanes 3 and 4: non heated and heated
534 ZIKV positive cell extracts.
535 A: Coomassie stained SDS PAGE
536 B: Western Blot revealed by using anti-ZIKV 4G2 monoclonal antibody
537 Sera 6, 11,20, 29: GA1+ patients
538 Sera 13, 27: GA1- patients

539

540 Suppl. Figure 3: Competition experiments using GA1
541 Patient's serum was incubated in presence of various amounts of GA1: 100 µg (B); 300 µg (C), 600 µg
542 (D). A: control without GA1.

543

544 Suppl. Figure 4: Combinatorial microarray heatmaps

Each patient and healthy control serum was screened against 78 single and heteromeric glycolipid targets on a microarray assay. For ease of comparison, IgG (A) and IgM (B) data was visually displayed as heat maps, in which the rainbow scale was used to assign a colour to each interaction, which indicated the intensity of the antibody binding for that target.

550 *Table 1:* Clinical characteristics of patients with GBS (n=42) in French Polynesia 2013-2014

	n (%) or median	IQR
Age (years)	42	36-56
Male sex	31 (74)	
Obesity	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)	
Arthalgia (n=31)	23 (74)	
Edema 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 (43)	
Areflexia or decreased reflexes	26 (62)	
Facial palsy	27 (64)	
Bilateral facial palsy	14 (33)	
Unilateral facial palsy	13 (31)	
Trouble swallowing	10 (24)	
Paresthesia	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 hospitalisation (days)	11	7-20
Duration of hospitalisation for patients admitted to intensive care (days)	51	16- 70
Lumbar puncture results		
Proteins (mg/dL)	1.47	0.92-2.21
Increased CSF protein concentration (cut-off : 0.52 mg/dL)	39 (93)	
Cells (/mm3)	4	1-7

- Table 2. Results of molecular and immunological analyses of GBS and control patients in French Polynesia 2013-2014

	Zika									**IgM Zika/ IgM Dengue n (%)**			
	vRNA	IgM	IgG	IgM/IgG					Neut.				
	n (%)	n (%)	n (%)	+/+	+/-	-/+	-/-	ZIKV^{+} n (%)	n (%)	+/+	+/-	-/+	-/-
GBS (N=42[a])	0 (0)	39 (92.9)	29 (69.0)	27	12	2	1	41 (97.6)	42 (100)	8 (19.1)	31 (73.8)	0 (0)	3 (7.1)
CTR1 (N=98)	ND	17 (17.3)	25 (25.5)	7	10	18	63	35 (35.7)	54 (55.7)	6 (6.1)	11 (11.2)	8 (8.2)	73 (74.5)
CTR2 (N=70)	70 (100)	ND	5 (7.1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 2A. Detection of Zika RNA (RT-PCR), Zika & Dengue IgM (IFA), Zika IgG (MIA) and neutralizing antibodies (Neut).

[a]RT-PCR was only performed for 41 GBS patients; tested samples for GBS patients are late samples (±3 months after admission), except for the RT-PCR (admission sample)

	IgG Dengue n *(%)*																**Dengue Neut. n (%)**	
	GBS[a]						CTR1						CTR2					
	IgG Z^{-}[b]		IgG Z+[b]		Total		IgG Z^{-}		IgG Z^{+}		Total		IgG Z^{-}		IgG Z^{+}		Total		GBS	
≥ 2 serotypes	11	(84.6)	25	(86.2)	***36***	***(85.7)***	42	(57.5)	23	(92.0)	***65***	***(66.3)***	42	(64.6)	4	(80.0)	***46***	***(65.8)***	**41**	***(100)***
1 serotype	0	-	4	(13.8)	***4***	***(9.5)***	20	(27.4)	2	(8.0)	***22***	***(22.5)***	11	(16.9)	1	(20.0)	***12***	***(17.1)***	**0**	**-**
No infection	2	(15.4)	0	-	***2***	***(4.8)***	11	(15.1)	0	-	***11***	***(11.2)***	12	(18.5)	0	-	***12***	***(17.1)***	**0**	**-**
	13		29		***42***		73		25		***98***		65		5		***70***		**41**	

Table 2B. Dengue IgG (MIA) and neutralizing responses (Neut)

[a]tested samples for GBS patients are late samples (±3 months after admission); [b]IgG Z- and IgG Z+ mean samples IgG (MIA) negative and positive for ZIKV, respectively.

	GBS[a] (n=42) n (%)	CTR1 (n=98) n (%)	OR [95% CI]	OR[a] [95% CI]	CTR2 (n=70) n (%)	OR [95% CI]	OR[a] [95% CI]
ZIKV IgM and/or IgG positivity	41 (97.6)	35 (35.7)	59.7 [10.4 - +∞]				
Positive ZIKV seroneutralization	42 (100.0)	54 (55.7)	34.1 [5.8 - +∞]				
DENV IgG positivity	40 (95.2)	87 (88.8)	2.0 [0.4 - 19.9]	1.0 [0.2 - 11.5]	58 (82.9)	6.0 [0.8 - 269.5]	4.0 [0.5-184.7]

Table 2C: ZIKV and DENV serological patterns associated with GBS

[a]Tested samples for GBS patients are late samples (±3 months after admission); [b]Adjusted for ZIKV IgG positivity

Table 3: Evolution of motor nerve conduction parameters (mean values) after GBS onset.

	Median			Ulnar			Fibular		
	DML (ms)	Ampli (mV)	MCV (m/s)	DML (ms)	Ampli (mV)	MCV (m/s)	DML (ms)	Ampli (mV)	MCV (m/s)
	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[a]	2.9[a]	47	5.8[a]	3.9[a]	52	9.9[a]	2.4[c]	41
1st week subgroup n = 19	14.1[a]	2.4[a]	45	6.1[a]	3.7[b]	49	10.1[c]	2.1[c]	39
4th month n = 19	7.1[c]	6.0[c]	47	4.4[c]	5.6[c]	46	6.0[c]	3.8[c]	46

DML: distal motor latency. Ampli : amplitude of the distal compound muscle action potential. MCV**: m**otor conduction velocity.

1st week values are compared to reference values; 4th month values are compared to 1st week values of the same subgroup (n=19)

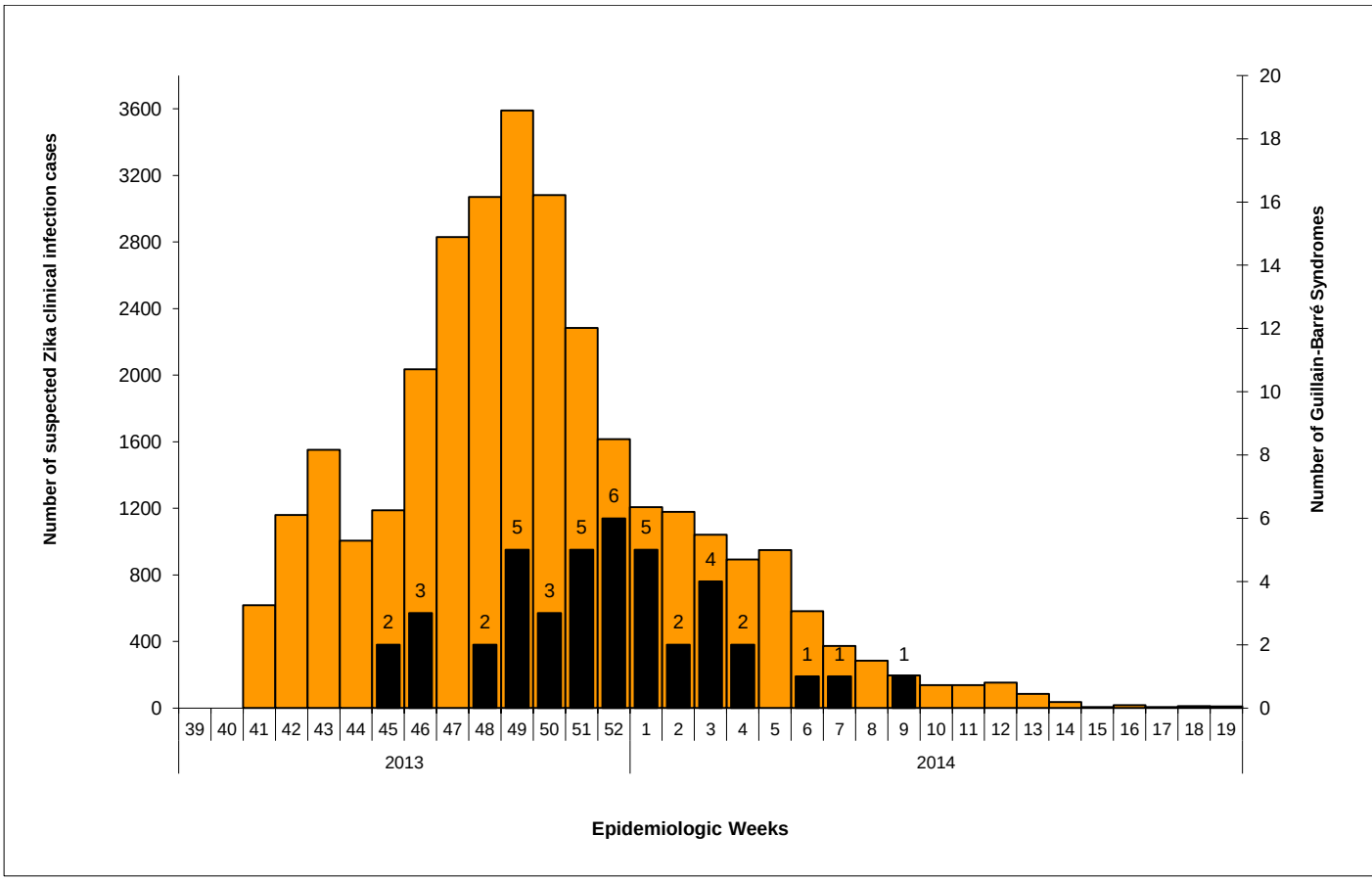
[a] P < 0.0001; P < 0.001; [c] P < 0.05.

Table 4: Positive (>50%) reactivity to glycolipids in sera of GBS patients (n=42) and controls (n=20) in French Polynesia 2013-2014

	GBS at onset (n=42) n (%)	GBS at 3 months (n=31) n (%)	Controls[a] (n=20) n (%)
Glycolipid			
GM1	0 (0)	8 (26)	0 (0)
GA1	8 (19)	10 (32)	0 (0)
GM2	2 (5)	1 (3)	0 (0)
GD1a	5 (12)	9 (29)	0 (0)
GD1b	3 (7)	9 (29)	0 (0)
GQ1b	0 (0)	0 (0)	0 (0)
Any	13 (31)	15 (48)	0 (0)

[a]Blood donors

Figure 1: Weekly cases of suspected Zika virus infections and Guillain-Barré syndromes in French Polynesia from October 2013 until April 2014



Supplementary material

ZIKV and DENV infection diagnosis

Detection of IgM against ZIKV and DENV was performed using indirect immunofluorescent assay (IFA) on the blood samples from patients in both GBS and CTR1 groups. Briefly, ZIKV [PF13-251013-18] isolated during the epidemic in French Polynesia[1] and DENV [D1-Hawaii 1944 reference strain] were independently inoculated onto Vero cells (African Green Monkey kidney cells). One week later, ZIKV- or DENV-infected cells were fixed on microscope slides and exposed to sera, and the presence of anti-ZIKV or anti-DENV IgM was revealed by incubation with goat anti-Human IgM antibody, FITC conjugate (Novex, Life technologies).

Detection of IgG against ZIKV and each the four DENV serotypes was performed on blood samples from patients in GBS, CTR1 and CTR2 groups using a recombinant-antigen based SHERPAxMap microsphere immune assay (MIA) adapted from Beck et al.[2] and detailed in Vanhomwegen J.[3] Briefly, sera were diluted 1/400 and incubated with a mix of microspheres coupled with either DENV-1, -2, -3,- -4 or ZIKV recombinant antigens (E protein domain III) produced in Drosophila S2 expression system. Detection of anti-DENV-1,-2,-3,-4 or anti-ZIKV IgG was performed using a Biotin-SP-conjugated Goat Anti-Human IgG antibody (Jackson ImmunoResearch) and streptavidin, R-phycoerythrin conjugate (SAPE, Life Technologies). The median fluorescence intensity was read on a MagPix instrument (Bio-Rad Laboratories). The cut-off of the MIA was determined by ROC curve analysis for all the antigens using positive and negative control sera.

Detection of neutralizing antibodies against ZIKV and each of the four DENV serotypes was performed for patients in GBS (latest blood sample) and CTR1 groups. Briefly, Vero cells cultured on 96-well plates were inoculated with serial dilutions of each serum previously incubated with titrated ZIKV [PF13-251013-18], DENV-1 [PF15-080108-88], DENV-2 [PF96-300896-243/158], DENV-3 [PF90-300190-30/56] or DENV-4 [PF09-290509-104]. One week later, infected cells were detected by ELISA using primary mouse pan-flavivirus E mAb 4G2 which reacts with ZIKV E protein[4] and a secondary goat anti-mouse IgG HRP-conjugated antibody (Santa Cruz). The neutralizing antibody titer was defined as the inverse of the latest serum dilution that inhibited the virus.

Reactivity of sera with ZIKV by Western blot

ZIKV [PF13-251013-18][1] was used to infect BHK21 and Vero cells with a MOI of 1 for 1hr at 37°C. Fresh medium was added and after 48h of incubation, the supernatant was removed, the cells were washed three times with PBS and collected in RIPA buffer. The cell extract was centrifuged 10 min at 4000g and the supernatant was kept at -80°C.

ZIKV cell extracts were mixed with LDS buffer and was or not heated 10 min at 95°C. Non-infected BHK21 and Vero cells extracts were used as control. Viral proteins recognised by patients' antibodies were detected by Western blot after sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of ZIKV cell extracts. After transfer, the nitrocellulose strips were saturated in PBS (pH 7.2) containing 5% dried milk (PBSM) for 1 h at room temperature (RT). The GBS patient's sera were diluted 1:1000 in PBSM containing Tween 20 (0·1%). Pan-flavivirus monoclonal antibody 4G2 was used as control and was diluted 1:1000. They were incubated with the strips for 1 hr at RT. After washings, peroxidase-conjugated goat anti-human IgM and IgG (Cappel) (diluted 1:3000) or anti-mouse antibody IgG(H+L) (BioRad) were added and incubated for 1 h. After 3 washes in PBS, strips

were incubated with peroxidase chemiluminescent substrate (Immobilon®, Sigma) using standard conditions and detected according to the manufacturer's recommendations.

Immunosuppression test for exploration of molecular mimicry mechanisms

Molecular mimicry was evaluated using a previously published method[5]. The serum from the patient showing highest reactivity towards GA1 without showing reactivity towards any other glycolipid (n°20 on Suppl. Table 2) was first diluted at 1:2000 with normal saline; then 5 ml aliquots were incubated overnight with 100 µg, 300 µg, and 600 µg of purified GA1 before repeating the Western blot analysis, using an aliquot at the initial dilution as control. Immunosuppression was also tested with sera n°6 after incubation with 300 µg of GA1.

Combinatorial microarray

A combinatorial microarray screening method that is based on a refinement and miniaturisation of our previous published combinatorial glycoarray assay[6] was used to assess ZIKV GBS sera for anti-complex antibodies. Lipid microarrays were fabricated in-house using a non-contact, piezoelectric dispensing system (S3 flexarrayer, Scienion, Germany). All lipids (GM1, GM2, GA1, GD1a, GD1b, Phospatidylserine (PS), Cholesterol (Chol), Sulphatide (Sulph) and Galactocerebroside (GalC)) were obtained from Sigma (Poole, UK), with the exception of GQ1b and CTH (Matreya, Pleasant Gap, USA), and SGPG and LM1, which were kindly gifted by Prof. R. Yu (Georgia Regents University, Augusta, USA). Working solutions of each lipid was prepared at a concentration of 200µg/ml in methanol and heteromeric lipids were prepared by mixing equal volumes of working solution, with a total concentration of 200µg/ml. Microarray platforms were made by adhesion of low fluorescence PVDF membrane (Millipore, Nottingham, UK) on to glass microscope slides. Each slide was printed with 16 individual arrays. For each array, approximately 500pl (100pg/spot) of lipid solution was applied at predetermined locations, in a grid-like pattern. As all lipids were printed in duplicate, arrays were designed with a line of symmetry, diagonally from top left to bottom right, with all single lipids printed in the first row and column. The location of all heteromeric complexes are identified in the array grid, as a 1:1 ratio of the two different single lipids printed in the first position of each row and column. Each array contained 91 different lipid targets (13 single lipids and 78 heteromeric complexes) in duplicate.

Sera Testing

Due to the limited availability of patient samples, only 41 sera at the initial time point and 27 of the 42 patients sera at 3 months were screened by combinatorial array. Microarray slides were blocked in 2% Bovine Serum Albumin (BSA, Europa Bioproducts, Ely, UK) in PBS on a rocker platform, for 1 hour at room temperature. After which, microarray slides were placed in a FAST frame device containing a 16-well incubation chamber (Maine Manufacturing, Sanford, USA), thereby enabling the isolation of each of the 16 subarrays and corresponding samples, per slide. All patient and control sera were diluted 1:50 in 1% BSA/PBS and 100µl was added per sub-array for 1 hour at 4˚C. Serum samples were aspirated from each well and washed twice with 1% BSA/PBS prior to removal from the FAST frame, after which microscope slides were washed twice, *en masse* in 1% BSA/PBS for 15 mins, on a rocker platform. Antibody-lipid interactions were identified using Alexafluor-647 conjugated anti- human IgG (Jackson ImmunoResearch Laboratories, West Grove USA) and Alexafluor-555 conjugated anti-human IgM (Life Technologies, Eugene, USA) isotype specific antibodies. Array slides were place in the FAST frame device once more and 100µl of secondary detection antibodies were applied at 4µg/ml in 1% BSA/PBS to each well, allowing identification of

both isotypes binding events, in each array. Slides were incubated for 1hour at 4˚C, followed by washing twice *en masse* in 1%BSA/PBS for 30mins followed by 5 mins in PBS and a further 5 mins in deionised water, before air drying.

Array imaging and quantification

Arrays were visualised with a FLAIR scanner (Sensovation, Radolfzell, Germany) and quantified using ProScanArray Express software (Perkin Elmer, Seer Green, UK). Median fluorescent intensity signals were calculated for each lipid target, with the local background signal subtracted. Mean intensity values were calculated for duplicate spots. Samples were tested on 3 separate occasions and the mean was calculated. Each of the 88 sera samples, were tested against 91 different targets, resulting in the measurement of 8008 individual interactions for each antibody isotype. For ease of comparison, data was visually displayed as heat maps (TM4-MeV MultiExperiment Viewer v4.66 software), in which the rainbow scale was used to assign a colour to each interaction, which indicated the intensity of the antibody binding for that target. Hierarchical clustering was performed with Pearson's correlation, to group sera with similar binding profiles. In order to establish the normal range of naturally occurring anti-glycolipid antibodies in this discrete population, the 95th percentile of the healthy controls was calculated for each target. These values were used as the threshold of positivity when screening the GBS cohort. As a result 5 % of all controls were identified as positive for each target (specificity=95%). Fisher's exact test was used for comparison of proportionality.

Results

Amongst the anti-glycolipid IgG antibody repertoire, complex dependent GM2:LM1 binding was observed in 4 sera (3 patients and 1 control; sensitivity=7.3%, specificity= 95%), in which no binding was observed with the single component lipids (Suppl Figure 4). Whilst not significant, it is interesting to note that the one control sample had a GM2:LM1 binding intensity approximately one-third of the average intensity observed in the patient's serum (1205 IU vs 3820 IU). Eight patient samples had strong IgG binding to GA1, as a single glycolipid (sensitivity= 19.5%, specificity 95%). Whilst not a significant target alone, when GA1 was in complex with sulphatide, antibody binding was enhanced, reaching a sensitivity of 46.3% and specificity of 95% (p=0.001), of which, 13 samples were complex dependent, in which antibody binding intensities were below the positivity threshold for the individual components (GA1 and sulphatide). In addition, a significant number of patient sera were positive for GA1 in complex with cholesterol and/or phosphatidylserine (48.8%), however many were of low binding intensity, despite being above the threshold. One patient sample had strong GM1 single (2867IU) and complex reactivity which also reacted with GA1. One patient sample contained antibodies strongly reactive against single GD1a (1278 IU), but only in the 3 month sample. This interaction was attenuated in complexes containing other large glycolipid molecules (namely GD1b, GQ1b, SGPG, LM1 and CTH). The binding profile of IgM antibodies in patient and control sera was very similar, in which binding to single and complexes of GA1 was frequently observed. Three of the 41 patient samples taken at the initial time point, demonstrated IgM binding to single and complexes of GM1 (sensitivity=7.3%, specificity= 95%), one of which also had weak binding to GD1b. While heteromeric GA1:sulphatide, GA1:cholesterol and GA1:phosphatidylserine were all significant targets in this screen, it is interesting to note the absence of single and complex targets (for example GM1, GD1a, GD1a:GD1b) frequently associated with the AMAN variant of GBS in other studies.

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Suppl Table 1. Dengue IgG (MIA) and neutralizing (Neut.) responses by serotype.

	Dengue IgG n (%)																														**Dengue Neut. n (%)**	
	GBS[a]						GBS[b]						GBS[c]						CTR1						CTR2							
					GBS[a]					***GBS[b]***					***GBS[c]***				***CTR1***						***CTR2***							
	IgG Z⁻		IgG Z⁺				IgG Z⁻		IgG Z⁺				IgG Z⁻		IgG Z⁺				IgG Z⁻		IgG Z⁺				IgG Z⁻		IgG Z⁺				GBS[c]	
D1234	6	(33.3)	18	(75.0)	***24***	***(57.1)***	4	(28.6)	17	(60,7)	***21***	***(50.0)***	5	(38.4)	9	(31.0)	**14**	***(33.3)***	23	(31.5)	16	(64.0)	***39***	***(39.8)***	10	(15.4)	2	(40.0)	***12***	***(17.1)***	35	(85.4)
D134	4	(22.2)	3	(12.5)	***7***	***(16.7)***	3	(21.5)	7	(25.0)	***10***	***(23.8)***	4	(30.8)	7	(24.2)	**11**	***(26.2)***	7	(9.6)	4	(16.0)	***11***	***(11.2)***	8	(12.3)	0	-	***8***	***(11.4)***	1	(2.4)
D124	1	(5.6)	1	(4.2)	***2***	***(4.8)***	-	-	-	-	***0***		0	-	0	-	**0**	**-**	2	(2.7)	1	(4.0)	***3***	***(3.1)***	2	(3.1)	1	(20.0)	***3***	***(4.3)***	0	-
D234	-	-	-	-	***0***	**-**	-	-	-	-	***0***		0	-	0	-	**0**	**-**	1	(1.4)	0	-	***1***	***(1.0)***	0	-	0	-	***0***	**-**	5	(12.2)
D14	2	(9.0)	1	(4.2)	***3***	***(7.1)***	2	(14.3)	2	(7.1)	***4***	***(9.5)***	2	(15.4)	5	(17.2)	**7**	***(16.7)***	5	(6.8)	2	(8.0)	***7***	***(7.1)***	13	(20.0)	0	-	***13***	***(18.6)***	0	-
D13	1	(5.6)	1	(4.2)	***2***	***(4.8)***	2	(14.3)	1	(3.6)	***3***	***(7.1)***	0	-	4	(13.8)	**4**	***(9.5)***	4	(5.5)	0	-	***4***	***(4.1)***	7	(10.8)	1	(20.0)	***8***	***(11.4)***	0	-
D12	1	(5.6)	-	-	***1***	***(2.4)***	1	(7.1)	-	-	***1***	***(2.4)***	0	-	0	-	**0**	**-**	0	-	0	-	***0***	**-**	0	-	0	-	***0***	**-**	0	-
D34	-	-	-	-	***0***	**-**	-	-	-	-	***0***		0	-	0	-	**0**	**-**	0	-	0	-	***0***	**-**	2	(3.1)	0	-	***2***	***(2.9)***	0	-
D1	3	(6.0)	-	-	***3***	***(7.1)***	1	(7.1)	1	(3.6)	***2***	***(4.8)***	0	-	4	(13.8)	**4**	***(9.5)***	15	(20.5)	2	(8.0)	***17***	***(17.3)***	8	(12.3)	1	(20.0)	***9***	***(12.9)***	0	-
D2	-	-	-	-	***0***	**-**	-	-	-	-	***0***		0	-	0	-	**0**	**-**	1	(1.4)	0	-	***1***	***(1.0)***	0	-	0	-	***0***	**-**	0	-
D3	-	-	-	-	***0***	**-**	-	-	-	-	***0***		0	-	0	-	**0**	**-**	0	-	0	-	***0***	**-**	2	(3.1)	0	-	***2***	***(2.9)***	0	-
D4	-		-		***0***	**-**	-	-	-	-	***0***		0	-	0	-	**0**	**-**	4	(5.5)	0	-	***4***	***(4.1)***	1	(1.5)	0	-	***1***	***(1.4)***	0	-
D Neg	-	-	-	-	***0***	**-**	1	(7.1)	-	-	***1***	***(2.4)***	2	(15.4)	0	-	**2**	***(4.8)***	11	(15.1)	0	-	***11***	***(11.2)***	12	(18.4)	0	-	***12***	***(17.1)***	0	-
	18		24		***42***		14		28		***42***		13		29		***42***		73		25		***98***		65		5		***70***		41	

[a]GBS **first** sample; [b]GBS **intermediate** sample; [c]GBS **last** sample.

Sensitivity and specificity of positivity thresholds for dengue and Zika IgG detection by MIA. These Specificity and Sensitivity values were calculated by GraphPad Prism 6 software based on the analysis of Receiver-Operator Characteristic (ROC) curves obtained for each antigen tested on a set of negative and positive controls.

	D1	D2	D3	D4	ZIK
Sensitivity	100%	90%	80.95%	100%	82.72%
Specificity	100%	98.8%	100%	100%	92.11%

Suppl. Table 2. Zika and Dengue IgG (MIA) and neutralizing (Neut) responses.

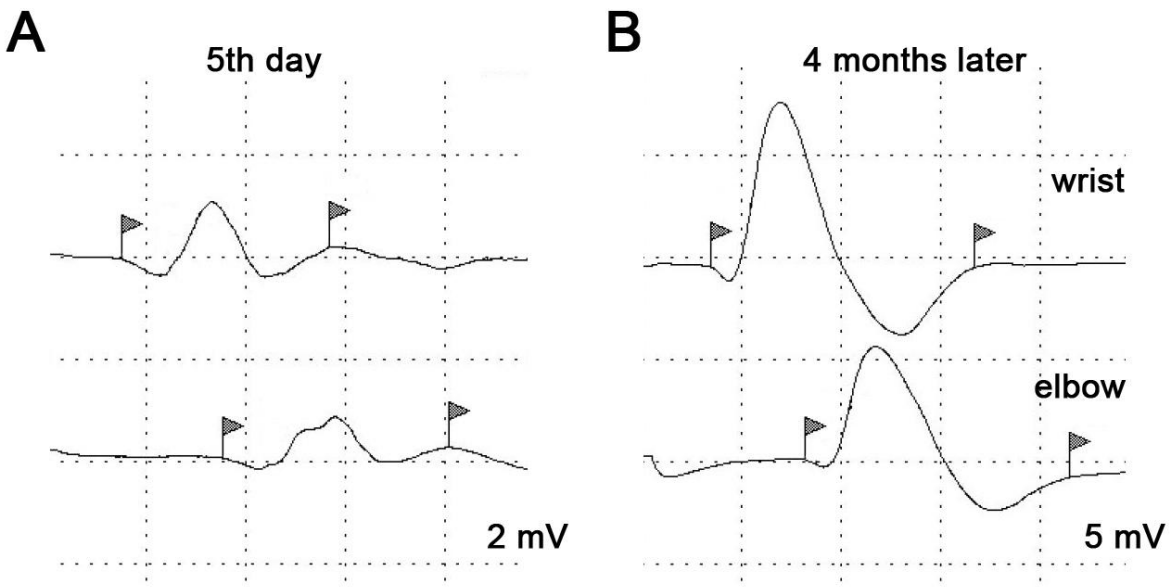
Patient	Zika and Dengue IgG															Zika and Dengue Neut[a] (Last sample)				
	First sample					Intermediate sample					Last sample									
	ZIKA	D1	D2	D3	D4	ZIKA	D1	D2	D3	D4	ZIKA	D1	D2	D3	D4	ZIKA	D1	D2	D3	D4
GB1	POS	POS	NEG	POS	POS	POS	POS	NEG	POS	POS	POS	POS	NEG	POS	NEG	320	640	40	80	20
GB2	NEG	POS	NEG	POS	POS	POS	POS	NEG	POS	NEG	POS	POS	NEG	NEG	NEG	80	320	640	80	40
GB3	NEG	POS	NEG	POS	POS	NEG	POS	NEG	POS	NEG	POS	POS	NEG	POS	NEG	320	1280+	1280+	320	160
GB4	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	POS	320	640	320	320	80
GB5	POS	POS	NEG	POS	NEG	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	1280+	ND	ND	ND	ND
GB6	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	POS	320	320	80	40	20
GB7	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	80	320	160	40	0
GB8	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	80	640	640	80	320
GB9	NEG	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	80	1280+	160	160	80
GB10	NEG	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS	POS	POS	POS	160	1280+	640	160	160
GB11	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS	160	1280+	320	80	80
GB12	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	80	160	320	80	80
GB13	POS	POS	POS	NEG	POS	POS	POS	NEG	POS	POS	NEG	POS	NEG	POS	POS	160	320	640	80	80
GB14	NEG	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	160	320	320	80	160
GB15	POS	POS	NEG	POS	POS	NEG	POS	NEG	POS	POS	NEG	POS	NEG	POS	POS	160	320	320	40	160
GB16	NEG	POS	POS	POS	POS	NEG	POS	POS	POS	POS	NEG	POS	POS	POS	POS	160	320	320	160	80
GB17	NEG	POS	NEG	POS	POS	NEG	POS	NEG	POS	POS	NEG	POS	NEG	POS	POS	320	640	640	640	320
GB18	NEG	POS	POS	NEG	NEG	NEG	POS	POS	NEG	NEG	NEG	NEG	NEG	NEG	NEG	320	160	640	80	40
GB19	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	POS	POS	POS	NEG	NEG	POS	320	640	160	80	40
GB20	NEG	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	NEG	POS	POS	160	320	640	160	80
GB21	NEG	POS	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	160	160	320	80	80
GB22	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	NEG	640	320	160	160	20
GB23	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	640	1280+	320	640	40
GB24	NEG	POS	NEG	NEG	POS	NEG	POS	POS	POS	POS	NEG	POS	POS	POS	POS	640	1280+	640	640	320
GB25	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS	160	640	160	320	320
GB26	NEG	POS	NEG	NEG	NEG	NEG	POS	NEG	NEG	NEG	POS	POS	NEG	NEG	NEG	160	640	320	320	160
GB27	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	1280+	1280+	1280+	1280+	640
GB28	NEG	POS	POS	NEG	POS	NEG	POS	NEG	NEG	POS	NEG	POS	NEG	NEG	POS	160	640	320	320	160
GB29	POS	POS	NEG	POS	POS	POS	POS	NEG	POS	POS	POS	POS	NEG	POS	NEG	640	1280+	640	1280+	20
GB30	NEG	POS	NEG	POS	NEG	NEG	POS	NEG	POS	NEG	POS	POS	NEG	POS	NEG	320	1280+	160	1280+	320
GB31	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS	160	640	640	640	80
GB32	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	NEG	POS	POS	POS	POS	160	160	320	160	80
GB33	POS	POS	NEG	NEG	POS	POS	POS	NEG	NEG	POS	POS	POS	NEG	NEG	POS	640	160	80	160	80
GB34	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	POS	320	160	10	80	160
GB35	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS	POS	640	320	40	160	40
GB36	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	NEG	POS	POS	640	1280+	320	1280+	160
GB37	NEG	POS	NEG	NEG	NEG	POS	POS	NEG	NEG	NEG	POS	POS	NEG	NEG	NEG	1280+	1280+	1280+	1280+	80
GB38	NEG	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS	320	160	160	160	320
GB39	NEG	POS	NEG	POS	POS	NEG	POS	NEG	POS	POS	NEG	POS	NEG	POS	POS	640	1280+	1280+	1280+	1280+
GB40	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	1280+	1280+	80	320	160
GB41	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	NEG	POS	POS	640	1280+	640	640	160
GB42	NEG	POS	NEG	NEG	POS	NEG	POS	NEG	NEG	POS	NEG	POS	NEG	NEG	POS	640	1280+	1280+	1280+	1280+
POS	***24***	***42***	***27***	***33***	***36***	***28***	***41***	***22***	***34***	***35***	***29***	***40***	***14***	***29***	***32***	***42***	***41***	***40***	***41***	***36***
NEG	***18***	***0***	***15***	***9***	***6***	***14***	***0***	***0***	***0***	***0***	***13***	***0***	***0***	***0***	***0***	***0***	***0***	***1***	***0***	***5***

[a]Neutralization titres were considered as positive if ≥ 40

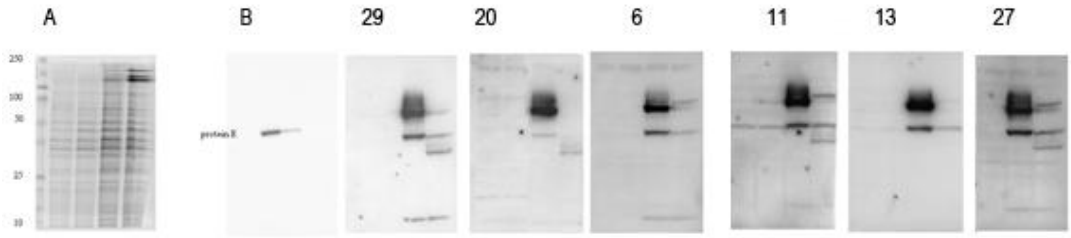
Suppl. Table 3. Percentage of binding against glycolipids in serum of patients at admission (n=42) and at 3 months (n31).

Patient	At admission						At 3 months					
	GM1	GA1	GM2	GD1a	GD1b	GQ1b	GM1	GA1	GM2	GD1a	GD1b	GQ1b
1	NEG	72%	38%	NEG	NEG	NEG						
2	NEG	35%	NEG	NEG	NEG	NEG	NEG	54%	NEG	NEG	NEG	NEG
3	NEG	48%	NEG	NEG	NEG	NEG	51%	56%	NEG	82%	53%	NEG
4	NEG	71%	59%	119%	107%	NEG	141%	46%	54%	136%	162%	NEG
5	NEG	NEG	NEG	NEG	NEG	NEG						
6	NEG	79%	NEG	NEG	NEG	NEG						
7	NEG	72%	44%	NEG	NEG	NEG	78%	55%	NEG	83%	90%	NEG
8	NEG	NEG	NEG	NEG	NEG	NEG						
9	NEG	NEG	NEG	44%	NEG	NEG	46%	NEG	NEG	58%	60%	NEG
10	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
11	NEG	45%	NEG	NEG	NEG	NEG	NEG	70%	NEG	NEG	NEG	NEG
12	NEG	NEG	NEG	NEG	NEG	NEG	57%	41%	42%	67%	77%	NEG
13	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
14	NEG	NEG	48%	NEG	57%	NEG	NEG	NEG	NEG	NEG	NEG	NEG
15	NEG	45%	58%	83%	51%	NEG	52%	60%	NEG	66%	60%	NEG
16	NEG	NEG	NEG	49%	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
17	NEG	NEG	NEG	55%	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
18	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
19	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
20	NEG	232%	NEG	NEG	NEG	NEG	NEG	321%	NEG	NEG	NEG	NEG
21	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
22	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
23	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
24	NEG	NEG	NEG	NEG	NEG	NEG						
25	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
26	NEG	35%	NEG	NEG	NEG	NEG						
27	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
28	NEG	NEG	NEG	163%	NEG	NEG	NEG	NEG	NEG	133%	NEG	NEG
29	NEG	78%	NEG	NEG	37%	44%	NEG	81%	NEG	NEG	NEG	NEG
30	38%	91%	NEG	NEG	NEG	NEG	51%	77%	NEG	NEG	NEG	NEG
31	37%	34%	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
32	NEG	NEG	NEG	NEG	NEG	NEG						
33	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
34	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
35	NEG	NEG	NEG	NEG	NEG	NEG						
36	NEG	56%	NEG	132%	39%	NEG	93%	92%	NEG	138%	98%	NEG
37	48%	47%	36%	NEG	NEG	NEG						
38	EC	EC	EC	EC	EC	EC						
39	NEG	NEG	NEG	NEG	63%	41%	NEG	NEG	NEG	NEG	73%	NEG
40	NEG	NEG	NEG	NEG	NEG	NEG						
41	NEG	41%	NEG	NEG	NEG	NEG	60%	60%	NEG	63%	68%	NEG
42	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG

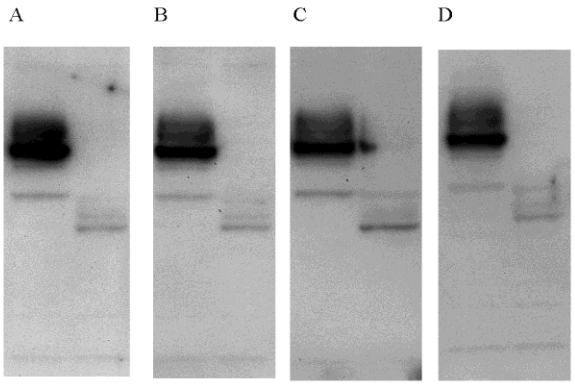
Suppl. Figure 1



Suppl. Figure 2



Suppl Figure 3



Suppl Figure 4