Zika virus detection in urine from patients with Guillain-Barré syndrome on Martinique, January 2016

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We report two cases of Guillain–Barré syndrome who had concomitant Zika virus viruria. This viruria persisted for longer than 15 days after symptom onset. The cases occurred on Martinique in January 2016, at the beginning of the Zika virus outbreak. Awareness of this possible neurological complication of ZikV infection is needed.

Two cases of Guillain–Barré syndrome (GBS) were diagnosed in January 2016 on Martinique, a French West Indies island of 390,000 inhabitants. Both patients were found to have ZikV in their urine at hospital admission. An outbreak of Zika virus (ZikV) infections has been ongoing on the island since December 2015 [1] and spread rapidly, with more than 1,000 estimated cases per week in 2016 [2].

ZikV infection is usually benign, when symptomatic. The disease is a dengue-like syndrome, characterised by fever, headache, retro-orbital pain, non-purulent conjunctivitis, maculopapular rash, arthralgia, and myalgia. The symptoms last for four to seven days and are self-limiting. Recent ZikV epidemics in French Polynesia, Brazil and Central America have been associated with Guillain–Barré syndrome (GBS) outbreaks, the probable link between these two diseases was made based on serological and anamnestic data [3-6].

Case description

Case 1

In the first week of January, four weeks after the detection of the first ZikV-positive cases on Martinique, a young adult complaining of gait disturbance was admitted to the University Hospital of Martinique. In the week before admission, the patient had felt numbness in their four extremities followed by constipation. There was no history of infectious respiratory symptoms, diarrhoea or recent arboviral infection. At first clinical evaluation, the patient had a flaccid tetraparesis, with asymmetric peripheral facial palsy and deglutition disorders (without oculomotor disturbance or ataxia). Intravenous polyvalent immunoglobulin (IVIg) (0.4 g/kg/day for five days) was administered, starting on the first day of admission. However, the neurological disorders worsened and two days later, due to paralysis of the respiratory musculature leading to respiratory failure, the patient needed mechanical ventilation. The patient recovered autonomous ventilation after seven days of intensive care, where they were hospitalised for a total of 10 days. The patient is currently in the rehabilitation unit of the University Hospital.

Electromyography (EMG) and nerve conduction studies were performed on day 15 after onset of neurological symptoms. There were abnormal sensory nerve action potentials with sural sparing pattern. Motor abnormalities were consistent with demyelination (delayed distal latencies, slowed nerve conduction velocity, temporal dispersion of waveforms, some conduction blocks and absent F waves) and the spontaneous needle EMG was normal. Total spinal cord magnetic resonance imaging (MRI) was normal. We did not perform any brain MRI. Blood, cerebrospinal fluid (CSF) and urine samples were taken before the administration of IVIg. Blood analysis showed no biochemical disorders. Blood count was normal except for a white blood cell count of 11,440 cells/mL (norm: 4–10). The analysis of CSF

Table

Microbiological data for two patients with Guillain-Barré syndrome, Martinique, January 2016

Microorganism	Detection	Case 1	Case 2
Campylobacter jejuni	Serology	Ratio < 8 (N < 128)	Ratio < 8 (N < 128)
Campylobacter fetus	Serology	Ratio < 8 (N < 128)	Ratio < 8 (N < 128)
Mycoplasma pneumoniae	Serology	IgM < 0.8 (N < 0.8) IgG < 10 (N < 10)	IgM < 0.8 (N < 0.8) IgG < 10 (N < 10)
Epstein–Barr virus	Serology	IgM anti-VCA: 0.14 IU/mL (N < 0.9 UI/ml) IgG anti-VCA: 2.42 IU/mL (N < 0.9 UI/ml) IgG anti-EBNA: 2.42 IU/mL (N < 0.9 UI/ml)	IgM anti-VCA: 0.12 IU/mL (N < 0.9 UI/ml) IgG anti-VCA: 2.66 IU/mL (N < 0.9 UI/ml) IgG anti-EBNA: 2.42 IU/mL (N < 0.9 UI/ml)
Human immunodeficiency virus	Serology	Ratio: 0.23 (N < 0.9)	Ratio: 0.15 (N < 0.9)
Herpes simplex virus	CSF (PCR)	Negative	Negative
Cytomegalovirus	Serology	Ratio IgM: 0.22 (N < 0.7) Ratio IgG: 270 (N < 0.5)	Ratio IgM: 0.19 (N < 0.7) Ratio IgG: 233 (N < 0.5)
	CSF (PCR)	Negative	Negative
Varicella zoster virus	CSF (PCR)	Negative	Negative
Enterovirus, incl poliovirus	CSF (RT-PCR)	Negative	Negative
Dengue virus	Serology	Ratio IgM: 0.63 (N < 0.9) Ratio IgG: 6.28 (N < 1.8)	Ratio IgM: 1.24 (N < 0.9) Ratio IgG: 3.74 (N < 1.8)
	Plasma (RT-PCR)	Negative	Negative
	CSF (RT-PCR)	Negative	Negative
Chikungunya virus	Serology	Ratio IgM: 0.079 (N < 0.8) Ratio IgG: 0.136 (N < 0.8)	Ratio IgM: 0.304 (N < 0.8) Ratio IgG: 2.669 (N < 0.8)
	Plasma (RT-PCR)	Negative	Negative
	CSF (RT-PCR)	Negative	Negative
Zika virus	Plasma (RT-PCR)	Negative	Negative
	CSF (RT-PCR)	Negative	Negative
	Urine D[a]5 (RT-PCR)	Not tested	Positive
	Urine D15 (RT-PCR)	Positive	Positive
	Urine D21 (RT-PCR)	Negative	Positive

CSF: cerebrospinal fluid; EBNA: Eppstein–Barr nuclear antigen; IgG: immunoglobuline G; IgM: immunoglobuline M; IU: international unit; N: normal value; PCR: polymerase chain reaction; RT-PCR: reverse transcription polymerase chain reaction; VCA: viral capsid antigen;

[a] Day after the onset of the neurological symptoms.

showed an albuminocytological dissociation with 1.50 g/L proteins (norm: 0.15–0.40) and a white blood cell count of 4/mL (norm < 10). The glycorachia/glycaemia ratio was normal (norm > 0.5).

The patient was screened for the common aetiologies of GBS: serological tests for *Campylobacter jejuni, C. fetus, Mycoplasma pneumoniae* and human immunodeficiency virus (HIV) were negative. Direct detection in CSF of herpes simplex virus, varicella zoster virus and cytomegalovirus by PCR were negative. Direct detection of ZikV by RT-PCR in urine gave a positive result on day 15 after onset of neurological symptoms (Table) but was negative in plasma and CSF.

Case 2

In the third week of January 2016, a person in their 50s was admitted to the University Hospital of Martinique complaining of gait disturbance. Numbness of the four extremities and constipation had started three days before admission. The patient had no history of infectious respiratory symptoms, diarrhoea or recent arboviral infection. At first clinical evaluation, flaccid tetraparesis with bilateral asymmetric peripheral facial palsy and signs of respiratory distress were present. IVIg (0.4 g/kg/day for five days) was promptly administered. On the day after admission, the patient was tetraplegic and paralysis of the respiratory musculature, leading to respiratory failure, necessitated support by mechanical ventilation. The patient was hospitalised in intensive care unit for one month.

EMG and nerve conduction studies were performed on day 10 after onset of neurological symptoms. The results were similar as for Case 1. There were abnormal sensory nerve action potentials with sural sparing pattern. Motor abnormalities were consistent with demyelination (delayed distal latencies, slowed nerve conduction velocities, temporal dispersion of waveforms, some conduction blocks and absent F waves) and the spontaneous needle EMG was normal. Total spinal cord MRI was normal. We did not perform any

brain MRI. Blood, CSF and urine samples were taken before the administration of intravenous immunoglobulin. The analysis of CSF showed an albuminocytological dissociation with 0.79 g/L protein (norm: 0.15–0.40) and a white blood cell count of 1/mL (norm < 10). The glycorachia/glycaemia ratio (norm > 0.5) was normal. The patient was screened for the common aetiologies of GBS: serological tests for *Campylobacter jejuni, C. fetus, Mycoplasma pneumonia* and HIV were negative. Direct detection of herpes simplex virus, varicella zoster virus and cytomegalovirus by PCR in CSF were negative. Direct detection of ZikV by RT-PCR in urine was positive on days 5, 15 and 21 after onset of neurological symptoms. Detailed microbiological results are shown in Table.

Discussion

We present two typical cases of GBS according to clinical, electrophysiological and lab findings. Laboratory confirmation of ZikV infections is based on the detection of viral RNA in serum by RT-PCR and of IgM against ZikV by enzyme-linked immunosorbent assay (ELISA). This is challenging because viraemia in ZikV-infected patients is short. Furthermore, there is cross-reactivity between ZikV antibodies and antibodies against other flaviviruses (including dengue virus (DENV)) [7]. ZikV antibody specificity can be determined by plaque reduction neutralisation tests [3]; these tests are done by the French National Reference Laboratory for arboviruses in Marseille, France, which is 7,800 km away from Martinique. All ZikV serology for the University Hospital of Martinique is done in that reference laboratory. The serological results for the two patients are currently pending, which limits this report in that ZikV infection has not yet been confirmed, although we consider the diagnosis to be likely.

Case 2 had a DENV IgM ratio of 1.24 (normal < 0.9). DENV-specific IgM ELISA is an appropriate test for serum specimens collected from day 5 of illness. However, its positive predictive value is limited by potential cross-reactivity with other flaviviruses and false positivity due to other pathogens causing acute febrile illness such as leptospirosis or due to past DENV infection [8]. Martinique has experienced several outbreaks of dengue fever since 2001 and, in 2011, a prospective study in adult blood donors reported a 93% seroprevalence for DENV antibodies [9]. The IgM result for Case 2 could be explained by persistent seropositivity from an older DENV infection or a cross-reaction to a recent ZikV infection.

For dengue, Zika and West Nile virus infections, several authors have demonstrated that RNA is detectable in urine at higher load and for a longer time than in plasma, and proposed that detection of RNA in urine could be used for the diagnosis of these infections [7,10,11]. For these reasons, direct RNA identification by RT-PCR in plasma and urine may be a good way to confirm flavivirus infections in populations exposed to *Aedes* spp. mosquitos.

Conclusion

This report has introduced two patients with GBS who had concomitant ZikV viruria. However, the detected asymptomatic prolonged excretion may not be related to the neurological symptoms. The average annual incidence of GBS on Martinique is close to eight cases per 390,000 inhabitants (data not shown). An association with ZikV infection has to be confirmed on further cases. Potential physiopathological mechanisms of ZikV-related GBS should be explored.

Viral excretion in urine was longer than 15 days in our patients, whereas RNA detection in blood was negative. We think that ZikV viruria needs to be investigated for a period longer than 15 days after onset of the neurological symptoms in GBS cases. We have to investigate if the genito-urinary compartment is a sanctuary for persistent replication.

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Conflict of interest

None declared.

Authors' contributions

BR, FN, PH, AC Wrote the manuscript. AS, JF, KA, JJ, SJ, MS, YB, HM, RV and the Zika Working Group took part in the clinical management of the patients. FN, RC Collaborated in molecular biology techniques. LF, RC, FN collaborated on the serological techniques. All authors participated in the outbreak investigation. All authors read and approved the final manuscript.

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