ORIGINAL ARTICLE

Guillain–Barré Syndrome Associated with Zika Virus Infection in Colombia

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ABSTRACT

BACKGROUND

Zika virus (ZIKV) infection has been linked to the Guillain–Barré syndrome. From November 2015 through March 2016, clusters of cases of the Guillain–Barré syndrome were observed during the outbreak of ZIKV infection in Colombia. We characterized the clinical features of cases of Guillain–Barré syndrome in the context of this ZIKV infection outbreak and investigated their relationship with ZIKV infection.

METHODS

A total of 68 patients with the Guillain–Barré syndrome at six Colombian hospitals were evaluated clinically, and virologic studies were completed for 42 of the patients. We performed reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assays for ZIKV in blood, cerebrospinal fluid, and urine, as well as antiflavivirus antibody assays.

RESULTS

A total of 66 patients (97%) had symptoms compatible with ZIKV infection before the onset of the Guillain–Barré syndrome. The median period between the onset of symptoms of ZIKV infection and symptoms of the Guillain–Barré syndrome was 7 days (interquartile range, 3 to 10). Among the 68 patients with the Guillain–Barré syndrome, 50% were found to have bilateral facial paralysis on examination. Among 46 patients in whom nerve-conduction studies and electromyography were performed, the results in 36 patients (78%) were consistent with the acute inflammatory demyelinating polyneuropathy subtype of the Guillain–Barré syndrome. Among the 42 patients who had samples tested for ZIKV by RT-PCR, the results were positive in 17 patients (40%). Most of the positive RT-PCR results were in urine samples (in 16 of the 17 patients with positive RT-PCR results), although 3 samples of cerebrospinal fluid were also positive. In 18 of 42 patients (43%) with the Guillain–Barré syndrome who underwent laboratory testing, the presence of ZIKV infection was supported by clinical and immunologic findings. In 20 of these 42 patients (48%), the Guillain–Barré syndrome had a parainfectious onset. All patients tested were negative for dengue virus infection as assessed by RT-PCR.

CONCLUSIONS

The evidence of ZIKV infection documented by RT-PCR among patients with the Guillain–Barré syndrome during the outbreak of ZIKV infection in Colombia lends support to the role of the infection in the development of the Guillain–Barré syndrome. (Funded by the Bart McLean Fund for Neuroimmunology Research and others.)

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This article was published on October 5, 2016, at NEJM.org.

DOI: 10.1056/NEJMoa1605564

Zika virus (ZIKV), a mosquito-borne RNA flavivirus, has caused a major outbreak in the Americas that began in 2014.[1] ZIKV infection manifests as a self-limited febrile syndrome associated with rash, conjunctivitis, and arthralgias.[2-4] In 2013 and 2014, an increase in the number of cases of the Guillain–Barré syndrome was observed during an outbreak of ZIKV infection in French Polynesia.[5,6] Recently, clusters of the Guillain–Barré syndrome and microcephaly have been spatially and temporally related to the current outbreak of ZIKV infection in the Americas.[7] In Colombia, the government reported the first autochthonous case of ZIKV infection in October 2015.[8] In December 2015, the Colombian Instituto Nacional de Salud (INS) documented an unusual number of cases of the Guillain–Barré syndrome in the Caribbean and the northeastern regions of Colombia. By January 2016, the outbreak of ZIKV infection had spread to most regions of Colombia. Concomitantly, an increase in the number of neuroinflammatory disorders was reported.[7] Here, we describe an observational clinical and virologic study of the Guillain–Barré syndrome cases that were evaluated in the context of the ZIKV outbreak in Colombia, which further supports the association between ZIKV infection and the Guillain–Barré syndrome — in particular, the acute inflammatory demyelinating polyneuropathy (AIDP) form of the syndrome.

METHODS

STUDY POPULATION AND DESIGN

During the outbreak of ZIKV infection in Colombia, all patients in whom the Guillain–Barré syndrome was diagnosed at six university-based centers from January through March of 2016 were evaluated prospectively as part of the Neuroviruses Emerging in the Americas Study (NEAS) (see the Supplementary Appendix, available with the full text of this article at NEJM.org). Patients underwent clinical and neurologic evaluation by internal medicine and neurology specialists. Nerve-conduction studies and electromyography were performed as part of the standard of care, and the results were classified in accordance with previously established criteria.[9,10] Samples of blood and cerebrospinal fluid (CSF) were obtained as part of the standard of care and, when available, aliquots of these samples along with urine were used for virologic testing for ZIKV infection. The clinical and laboratory information was documented with the use of standardized questionnaires (NEAS forms), as well as the Spanish version of the evaluation form of the International GBS Outcome Study (IGOS) (see the Supplementary Appendix). The diagnosis of the Guillain–Barré syndrome was based on the Brighton Collaboration GBS Working Group criteria.[11] Brighton criteria levels indicate the certainty of a diagnosis of the Guillain–Barré syndrome. Level 1, in which the diagnosis is supported by nerve-conduction studies and the presence of albuminocytologic dissociation in CSF, indicates the highest degree of certainty. A level 2 diagnosis is supported by either a CSF white-cell count of less than 50 cells per cubic millimeter (with or without an elevated protein level) or nerve-conduction studies consistent with the Guillain–Barré syndrome (if the CSF white-cell count is unavailable). A level 3 diagnosis is based on clinical features without support from nerve-conduction or CSF studies.

Because all the patients with the Guillain–Barré syndrome we studied were residing in areas that were endemic for mosquito-borne virus transmission, their illnesses were suspected to be associated with ZIKV disease as defined by the Pan American Health Organization (PAHO).[12] In patients with a diagnosis of the Guillain–Barré syndrome fitting level 1, 2, or 3 of the Brighton criteria, the diagnosis of ZIKV infection was defined as definite, probable, or suspected. Definite cases of ZIKV infection were those that were confirmed by a positive real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay for ZIKV RNA in blood, CSF, or urine. Probable cases were those that were characterized by positive results of enzyme-linked immunosorbent assays (ELISAs) for antiflavivirus antibodies in the CSF, serum, or both but negative results of RT-PCR for ZIKV and for the four dengue virus (DENV) serotypes. Suspected cases were characterized by a clinical syndrome compatible with ZIKV infection with two or more features of the PAHO case definition[12] (rash, fever, nonpurulent conjunctivitis, arthralgia, myalgia, and periarticular edema) without laboratory confirmation. To characterize the temporal profile of the disorder, the onset of suspected ZIKV infection was defined as the day of onset of systemic symptoms outlined in the case definition. The onset of neurologic symptoms was defined as the first day of

onset of limb weakness, sensory symptoms, facial paralysis, or other neurologic symptoms.

LABORATORY TESTING

Virologic testing was performed at the Virology Laboratory, Universidad del Valle, Cali, Colombia. The TaqMan RT-PCR assay used for the diagnosis of ZIKV infection was based on a protocol from Lanciotti and colleagues.[13] Serum, CSF, and urine were considered to be positive for ZIKV if the two distinct genomic regions targeted by the RT-PCR were amplified. Serum and CSF samples were also tested for the four DENV serotypes by means of nested RT-PCR.[14,15] DENV IgM-capture and IgG-capture ELISAs (Panbio Diagnostics) were performed to detect the presence of flavivirus cross-reactive antibodies. A patient was defined as having had a recent flavivirus infection if an ELISA for IgM or IgG was positive in any of the examined fluids.[16] To determine the presence of ZIKV infectious particles, viral isolates were obtained from ZIKV RT-PCR–positive serum and urine samples from four patients and cultured in C6/36 *Aedes albopictus* cell and Vero cell lines. Inoculated cells were cultured for at least 14 days, with imaging performed once daily by microscopy to assess cytopathic changes,[17] and the culture supernatants were tested for ZIKV by RT-PCR (details are provided in the Supplementary Appendix).

STUDY OVERSIGHT

The study protocol was approved by the institutional review board at the Johns Hopkins University School of Medicine and by the ethics committee at each participating center. The ethics committee of each participating center provided research guidelines, and either oral or written consent was obtained from all patients.

RESULTS

EPIDEMIOLOGIC SURVEILLANCE OF ZIKV INFECTION AND THE GUILLAIN–BARRÉ SYNDROME IN COLOMBIA

From October 2015 through March 2016, there were 2603 laboratory-confirmed ZIKV infections in Colombia and more than 58,790 suspected cases. In addition, there were 401 patients with a neurologic syndrome who had a history of ZIKV infection; 270 of the cases (67%) corresponded to the Guillain–Barré syndrome[18] (Fig. 1). On the basis of data from the registry of individual records of health care services, the INS estimated that approximately 250 cases of the Guillain–Barré syndrome per year occurred in the whole country between 2009 and 2015, for a mean of approximately 20 cases per month (unpublished data). The frequency was increased relative to that baseline rate during the ZIKV outbreak, during which more than 270 cases of the Guillain–Barré syndrome were registered up to epidemiologic week 12 of 2016, for a mean of approximately 90 cases per month.[19] (In a given year, epidemiologic week 1 ends on the first Saturday in January, as long as it falls at least 4 days into the month.) According to surveillance data from the INS, DENV had circulated in Colombia during the last decade and caused periodic outbreaks. A chikungunya virus outbreak occurred in the region during most of 2015 (see the Supplementary Appendix). However, it was not until the end of 2015 and the beginning of 2016 that ZIKV was first introduced to the region, and this period coincided with the first documented increase in the incidence of the Guillain–Barré syndrome (Fig. 1).

CLINICAL FEATURES

A total of 68 patients who fulfilled the Brighton criteria for the Guillain–Barré syndrome and related variants and presented to the participating centers were included: 56 patients (82%) fulfilled level 1 or 2 criteria on the basis of evidence from CSF analysis, neurophysiological studies, or both. Four patients (6%) had the Miller Fisher syndrome, and 2 patients (3%) had other Guillain–Barré syndrome variants (bilateral facial palsy with areflexia and a pure sensory syndrome). The median age of the patients was 47 years (interquartile range, 35 to 57), 38 patients (56%) were male, and 61 patients (90%) were of mixed race. A total of 66 patients (97%) had symptoms of ZIKV infection in the 4 weeks preceding the onset of neurologic symptoms (Table 1, and Table S1 in the Supplementary Appendix). Two patients did not report having had any systemic symptoms before the onset of the Guillain–Barré syndrome but were residents of a region affected by the ZIKV infection outbreak. The median duration of symptoms of ZIKV infection was 4 days; the condition manifested mainly with fever (in 69% of the patients), rash (59%), headaches (34%), myalgias (34%), nonpurulent conjunctivitis (25%), and arthralgias (22%). The median time between

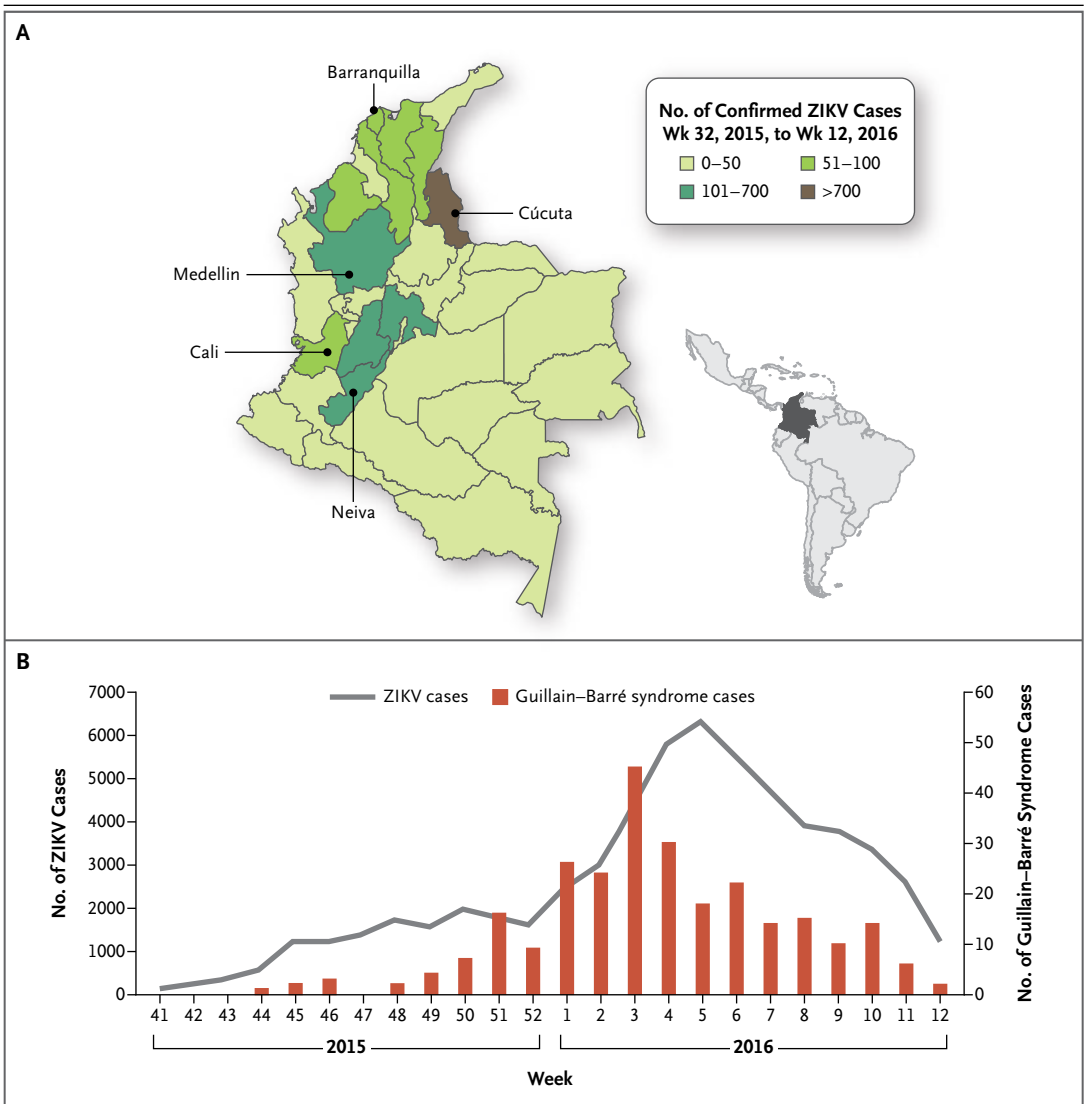


Figure 1. Cases of ZIKV Infection and the Guillain–Barré Syndrome in Colombia.

Panel A shows a map of Colombia with Neuroviruses Emerging in the Americas Study (NEAS) participating sites in the context of the Zika virus (ZIKV) infection outbreak. Numbers of confirmed cases of ZIKV infection from epidemiologic week 32 of 2015 through week 12 of 2016 are shown. Panel B shows the cases of the Guillain–Barré syndrome that were diagnosed in parallel with ZIKV cases reported to the Colombian National Institute of Health and epidemiological surveillance system in Colombia from October 2015 through March 2016.

the onset of the ZIKV infection symptoms and the onset of the Guillain–Barré syndrome was 7 days (interquartile range, 3 to 10).

The clinical and laboratory features of the patients with the Guillain–Barré syndrome are summarized in Table 2, and in Table S2 in the Supplementary Appendix. The symptoms at presentation included limb weakness (97%), paresthesias (76%), and facial palsy (32%). A total of 56 patients (82%) reported an ascending pattern of weakness. On neurologic examination, the median Medical Research Council (MRC) sum score (which indicates muscle strength in 12 different muscle groups and ranges from 0 to 60, with higher scores indicating more preserved muscle strength) was 40 (interquartile range, 26 to 47).[20] Cranial neuropathies were present in 43 patients, with bilateral facial palsy being the most common (in 50% of the 68 patients). Autonomic dysfunction was present in 21 patients (31%). A total of 40 pa-

Table 1. Clinical and Demographic Characteristics of the 68 Patients with the Guillain–Barré Syndrome.

Characteristic	Value (N = 68)
Median age (interquartile range) — yr	47 (35–57)
Male sex — no. (%)	38 (56)
General symptoms before the onset of the Guillain–Barré syndrome — no. (%)	66 (97)
Fever	47 (69)
Rash	40 (59)
Headache	23 (34)
Myalgia	23 (34)
Conjunctivitis	17 (25)
Arthralgia	15 (22)
Diarrhea	6 (9)
Median duration of ZIKV infection symptoms (interquartile range) — days	4 (3–5)
Median time from onset of ZIKV infection symptoms to onset of the Guillain–Barré syndrome (interquartile range) — days	7 (3–10)
ZIKV infection diagnostic category — no. (%)*	
Definite	17 (25)
Probable	18 (26)
Suspected	33 (49)
Neurologic diagnosis — no. (%)†	
Guillain–Barré syndrome Brighton criteria level 1	30 (44)
Guillain–Barré syndrome Brighton criteria level 2	26 (38)
Guillain–Barré syndrome Brighton criteria level 3	6 (9)
Miller Fisher syndrome	4 (6)
Other Guillain–Barré syndrome variant	2 (3)

* The diagnosis of Zika virus (ZIKV) infection was defined as definite (positive real-time reverse-transcriptase–polymerase-chain-reaction [RT-PCR] assay for ZIKV RNA in blood, CSF, or urine), probable (positive enzyme-linked immunosorbent assays [ELISAs] for antiflavivirus antibodies in the cerebrospinal fluid [CSF], serum, or both but negative results of RT-PCR for ZIKV and for the four dengue virus [DENV] serotypes), or suspected (clinical syndrome compatible with ZIKV infection with two or more features of the Pan American Health Organization case definition[12] without laboratory confirmation).

† Brighton criteria levels indicate the certainty of a diagnosis of the Guillain–Barré syndrome. A level 1 diagnosis is supported by nerve-conduction studies and the presence of albuminocytologic dissociation in CSF and indicates the highest degree of certainty. A level 2 diagnosis is supported by either a CSF white-cell count of less than 50 cells per cubic millimeter (with or without an elevated protein level) or nerve-conduction studies consistent with the Guillain–Barré syndrome (if the CSF white-cell count is unavailable). A level 3 diagnosis is based on clinical features without support from nerve-conduction or CSF studies.

tients (59%) were admitted to intensive care units, and 31% of all patients required mechanical ventilation. Treatment was administered to 46 patients (68%); intravenous immune globulin was the most commonly used treatment (62% of the 68 patients). Three patients (4%) died after respiratory failure and sepsis. The median modified Rankin score (which indicates the severity of disability and ranges from 0 to 6, with 0 indicating no symptoms and 6 indicating death) at nadir was 4 (interquartile range, 3 to 5).

Nerve-conduction studies and electromyography were performed with the use of standard techniques in 46 patients (68%). In accordance with published criteria,[9,10] 36 patients (78% of the 46 patients) were determined to have the AIDP subtype of the Guillain–Barré syndrome, 1 patient (2%) had the acute motor axonal neuropathy (AMAN) subtype, and 4 patients (9%) had equivocal studies that did not allow a subtype classification (Table 2). No abnormalities were noted in hematologic testing performed at admission. CSF

Table 2. Clinical and Laboratory Findings in the 68 Patients with the Guillain–Barré Syndrome.*

Finding	Value (N=68)
Neurologic symptoms on admission — no. (%)	
Limb weakness	66 (97)
Ascending paralysis	56 (82)
Paresthesias	52 (76)
Facial palsy	22 (32)
Results of neurologic examination	
Cranial neuropathy — no. (%)	
Any	43 (63)
Bilateral facial nerve	34 (50)
Bulbar cranial nerves	15 (22)
Cranial nerves III, IV, and VI	7 (10)
Median MRC sum score at admission (interquartile range)†	40 (26–47)
Areflexia or hyporeflexia — no. (%)	64 (94)
Sensory deficit — no. (%)	17 (25)
Severity of illness	
Admitted to the ICU — no. (%)	40 (59)
Required mechanical ventilation — no. (%)	21 (31)
Had any autonomic dysfunction — no. (%)	21 (31)
Median modified Rankin score at nadir (interquartile range)‡	4 (3–5)
Died — no. (%)	3 (4)
Results of CSF analysis	
Increased protein level — no./total no. (%)§	45/55 (82)
Median white-cell count (interquartile range) — cells/mm^3	0 (0–2.5)
Results of nerve-conduction studies and EMG — no./total no. (%)	
AIDP	36/46 (78)
Equivocal	4/46 (9)
Normal	2/46 (4)
Inexcitable	3/46 (7)
AMAN	1/46 (2)

* AIDP denotes acute inflammatory demyelinating polyneuropathy, AMAN acute motor axonal neuropathy, EMG electromyography, and ICU intensive care unit.
† The Medical Research Council (MRC) sum score indicates muscle strength in 12 different muscle groups; scores range from 0 to 60, with higher scores indicating more preserved muscle strength.
‡ The modified Rankin score is a measure of the severity of disability and ranges from 0 to 6, with 0 indicating no symptoms and 6 indicating death.
§ A protein level higher than 52 mg per deciliter was considered to be increased.

analysis was performed in 55 patients (81%); the median white-cell count was 0 cells per cubic millimeter (interquartile range, 0 to 2.5), and the median protein concentration was 116 mg per deciliter (interquartile range, 67 to 171). A total of 45 patients (82%) had albuminocytologic dissociation in CSF, indicated by increased protein levels (>52 mg per deciliter) in the absence of pleocytosis (<10 cells per cubic millimeter).

LABORATORY TESTS FOR ZIKV INFECTION

Of the 68 patients, 42 (62%) underwent testing for ZIKV by RT-PCR in at least one of three biologic samples: urine (24 patients), serum (31 pa-

tients), and CSF (30 patients) (Fig. 2 and Table 3, and Fig. S1 in the Supplementary Appendix). A total of 17 patients (40%) tested positive for ZIKV by RT-PCR; most of the positive results were in urine samples (16 patients). Three patients had positive ZIKV RT-PCR results in CSF (Fig. S2 in the Supplementary Appendix); only 1 patient had a positive result in serum, and this patient's serum remained positive at 31 days after the onset of ZIKV infection (Patient 29 in Fig. 2B). The median time from the onset of the symptoms of viral illness to the collection of the first ZIKV-positive urine sample was 16.5 days (interquartile range, 11.5 to 19.7), with 1 patient remaining positive at 48 days after onset (Patient 29 in Fig. 2B). The results of RT-PCR for all four DENV serotypes were negative in the 39 patients tested.

ZIKV was cultured from the serum and urine of Patient 29 and from the urine of Patients 32 and 36 (Fig. 2B) in C6/36 and Vero cell lines. The presence of ZIKV in the culture supernatants was confirmed by RT-PCR. Light microscopic imaging showed cytopathic changes consistent with flavivirus infection (Fig. S3 in the Supplementary Appendix). The profile of antiflavivirus antibodies is shown in Table 3 and Figure 2A, and in Table S3 in the Supplementary Appendix. A total of 32 of the 37 patients with the Guillain–Barré syndrome who were tested (86%) had evidence of a recent flavivirus infection, as indicated by the presence of cross-reactive IgM or IgG antiflavivirus antibodies. The pattern of expression of antiflavivirus antibodies stratified according to the results of the ZIKV RT-PCR is shown in Table S3 in the Supplementary Appendix. On the basis of clinical profiles and laboratory testing, the diagnosis of ZIKV infection was classified as definite in 17 patients, probable in 18 patients, and suspected in 33 patients (Tables 1 and 3).

TEMPORAL PROFILE OF THE GUILLAIN–BARRÉ SYNDROME CASES WITH LABORATORY TESTING

Of the 68 patients with the Guillain–Barré syndrome, 42 underwent laboratory testing for the identification of ZIKV infection. Figure 2 shows the laboratory and clinical temporal profiles of the infection in these 42 patients. The period from the onset of symptoms of ZIKV infection to the onset of neurologic symptoms and the time to nadir is outlined for each case. Two patients did not have any symptoms of ZIKV infection preceding the neurologic symptoms, and 2 patients had simultaneous onset of ZIKV infection and neurologic symptoms. A total of 20 patients (48%) in this group had a rapid onset of neurologic symptoms without an asymptomatic period after ZIKV infection symptoms (parainfectious onset), whereas the other patients had a variable asymptomatic period between ZIKV infection and the onset of neurologic symptoms (postinfectious onset).

DISCUSSION

The identification of the ZIKV genome by RT-PCR in biologic samples from 17 patients with the Guillain–Barré syndrome, together with the presence of immune responses (IgG, IgM, or both) to flaviviruses in the CSF in most of the patients tested, supports the involvement of ZIKV in these cases of the Guillain–Barré syndrome during the outbreak of ZIKV infection in Colombia. In addition, the clinical features of a preceding viral illness consistent with ZIKV infection and the evidence indicating that DENV infection was not present (i.e., negative RT-PCR results for the four DENV serotypes and the absence of laboratory features typical of DENV infections) are also supportive of such a relationship. However, the fact that there are cross-reactive antiflavivirus antibodies between DENV and ZIKV complicates the serologic assessment. The increase in cases of the Guillain–Barré syndrome during the time of the ZIKV outbreak in Colombia and the absence of such an increase while DENV and chikungunya virus were circulating within the region in previous years[21-23] provides epidemiologic evidence of the link between ZIKV infection and the Guillain–Barré syndrome. Before our study, the most compelling evidence of an association between the Guillain–Barré syndrome and ZIKV infection came from a case–control study conducted during the 2013–2014 ZIKV outbreak in French Polynesia. In that study, 42 patients with the Guillain–Barré syndrome had serologic evidence of recent flavivirus infection.[5]

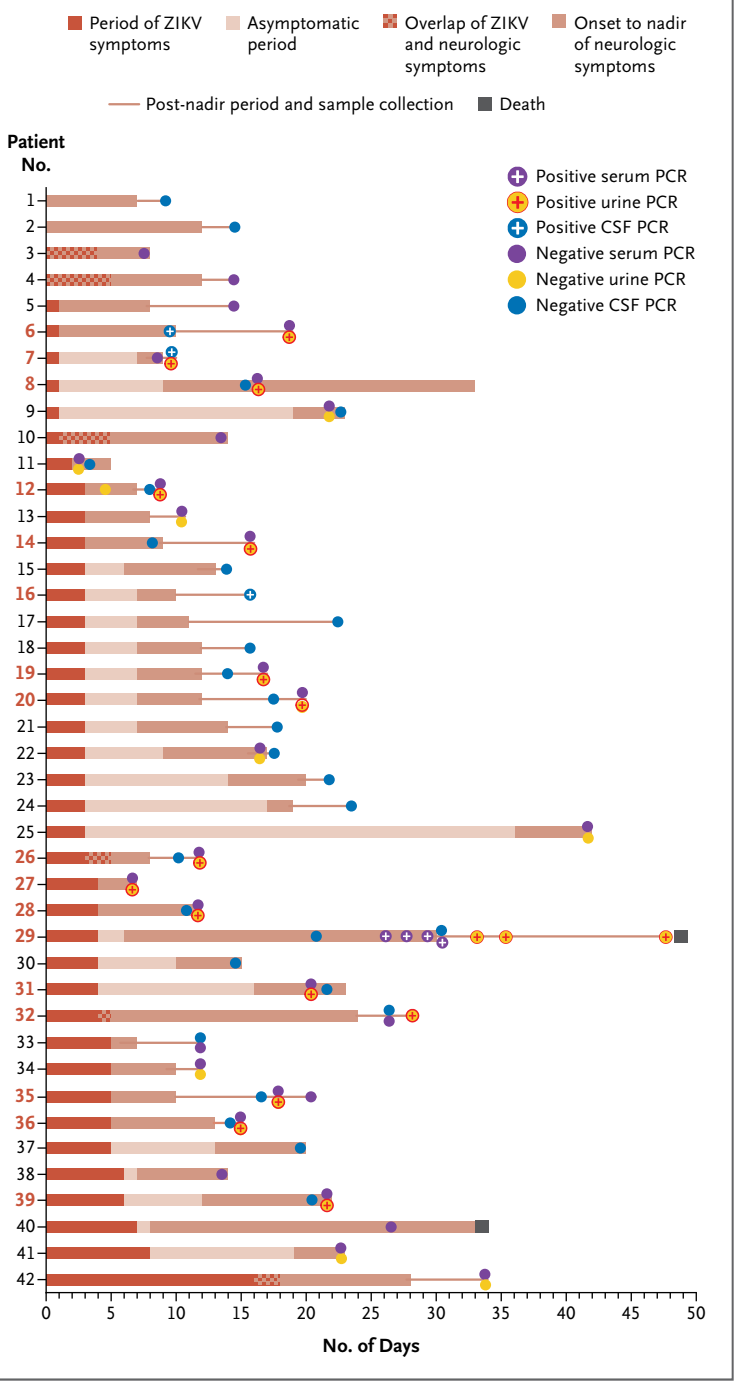
The clinical features of the Guillain–Barré syndrome that were observed during the Colombian outbreak of ZIKV infection, including a preceding viral illness of short duration (median, 4 days) in 97% of the patients, are similar to those described in French Polynesia.[5] Similar to the symptoms seen in patients who had the Guillain–Barré syndrome during the ZIKV infec-

A

● Positive ● Negative ● Assay not done

Patient No.	ZIKV Infection	ZIKV PCR	CSF IgG	CSF IgM	Serum IgG	Serum IgM
1	Probable	●	●	●	●	●
2	Probable	●	●	●	●	●
3	Probable	●	●	●	●	●
4	Suspected	●	●	●	●	●
5	Suspected	●	●	●	●	●
6	Definite	●	●	●	●	●
7	Definite	●	●	●	●	●
8	Definite	●	●	●	●	●
9	Probable	●	●	●	●	●
10	Probable	●	●	●	●	●
11	Suspected	●	●	●	●	●
12	Definite	●	●	●	●	●
13	Probable	●	●	●	●	●
14	Definite	●	●	●	●	●
15	Probable	●	●	●	●	●
16	Definite	●	●	●	●	●
17	Probable	●	●	●	●	●
18	Probable	●	●	●	●	●
19	Definite	●	●	●	●	●
20	Definite	●	●	●	●	●
21	Probable	●	●	●	●	●
22	Probable	●	●	●	●	●
23	Probable	●	●	●	●	●
24	Probable	●	●	●	●	●
25	Suspected	●	●	●	●	●
26	Definite	●	●	●	●	●
27	Definite	●	●	●	●	●
28	Definite	●	●	●	●	●
29	Definite	●	●	●	●	●
30	Suspected	●	●	●	●	●
31	Definite	●	●	●	●	●
32	Definite	●	●	●	●	●
33	Probable	●	●	●	●	●
34	Probable	●	●	●	●	●
35	Definite	●	●	●	●	●
36	Definite	●	●	●	●	●
37	Suspected	●	●	●	●	●
38	Probable	●	●	●	●	●
39	Definite	●	●	●	●	●
40	Suspected	●	●	●	●	●
41	Probable	●	●	●	●	●
42	Probable	●	●	●	●	●

B



tion outbreak in French Polynesia, the neurologic symptoms at presentation in the patients in our series consisted of ascending limb weakness (82%), paresthesias (76%), and facial palsy (32%). In our study, 46 patients (68%) underwent electrophysiological studies, and the results of these studies were consistent with the AIDP form of the Guillain–Barré syndrome in 78% of these patients. This observation is consistent with the more classical presentation of the Guillain–Barré syndrome and contrasts with the AMAN form described from French Polynesia,[5] a

Figure 2 (facing page). Laboratory Testing and Temporal Profiles of Illness in 42 Patients with the Guillain–Barré Syndrome during the ZIKV Infection Outbreak in Colombia.

Panel A shows the results of testing for flavivirus infection in various biologic samples from the 42 patients who underwent laboratory testing. Results of TaqMan reverse-transcriptase (RT)–polymerase-chain-reaction (PCR) assays for ZIKV and of IgM and IgG enzyme-linked immunosorbent assays for antiflavivirus antibodies are shown for each patient. The diagnosis of ZIKV infection was defined as definite (positive real-time RT-PCR assay for ZIKV RNA in blood, CSF, or urine), probable (positive enzyme-linked immunosorbent assays [ELISAs] for antiflavivirus antibodies in the cerebrospinal fluid [CSF], serum, or both but negative results of RT-PCR for ZIKV and for the four dengue virus serotypes), or suspected (clinical syndrome compatible with ZIKV infection with two or more features of the Pan American Health Organization case definition[12] without laboratory confirmation). Panel B shows the temporal profile of clinical symptoms and results of ZIKV RT-PCR testing in patients with the Guillain–Barré syndrome. The bars represent the duration in days of the various periods of the clinical profile and the timeline of illness for each of the 42 patients who underwent virologic testing. The period of systemic symptoms of ZIKV infection in some patients overlaps with or is immediately followed by the onset of neurologic symptoms; in other patients, there is an asymptomatic period preceding the onset of neurologic symptoms. Two patients (Patients 29 and 40) died from respiratory and infection complications. Numbers of patients with definite ZIKV infection are shown in red.

finding that may reflect a variable clinical phenotype of ZIKV-associated Guillain–Barré syndrome, evolutionary changes of the virus, or host-dependent factors in the two countries.

In our study, an analysis of the manifestation of neurologic symptoms among the patients with a diagnosis of definite or probable ZIKV infection suggests that the temporal profile of neurologic symptoms does not follow the classical postinfectious profile of the Guillain–Barré syndrome that is associated with other conditions, such as *Campylobacter jejuni* infection.[10,24-26] Although the overall median time from the onset of the viral syndrome to the Guillain–Barré syndrome in our study was similar to that among cases in French Polynesia[5] (7 and 6 days, respectively), an analysis of the temporal profile of the illnesses in the 42 patients who underwent laboratory testing showed that 20 patients (48%) had neurologic symptoms during or immediately after the viral syndrome associated with ZIKV infection. These observations suggest that in cases of the Guillain–Barré syndrome associated with ZIKV infection, the Guillain–Barré syndrome may follow the pattern of a parainfectious disorder rather than the classic postinfectious profile.[24,26] The reason for this is uncertain, but possible explanations include the following: that ZIKV starts a process of immune molecular mimicry against nervous system antigens before the clinical symptoms of viral infection are manifested, that ZIKV produces immune dysregulation that leads to the Guillain–Barré syndrome through a mechanism or mechanisms not related to molecular mimicry, that ZIKV produces a hyperacute immune response, or that there are direct viral neuropathogenic mechanisms that are as yet unknown for the Guillain–Barré syndrome. Although the presence of ZIKV in the CSF and the replicating capability of the virus in three cases may suggest a ZIKV neuroinvasive process in the Guillain–Barré syndrome, more studies are needed to assess such a mechanism.

Another important observation in our study is the finding that in patients with the Guillain–Barré syndrome and definite ZIKV infection, there is a prolonged period of viruria, which persists for days after the viral syndrome is over. Although the frequency of detection of ZIKV genome in CSF and serum was low, the higher frequency of detection of ZIKV in urine makes this biologic sample one that can be considered potentially useful for the diagnosis of ZIKV infection. In our study, the median time between the onset of ZIKV infection symptoms to collection of the first urine sample that tested positive was 16.5 days, and in one of our patients, ZIKV viruria was observed up to 48 days after the onset of the viral syndrome. These observations are consistent with reported cases of prolonged ZIKV viruria in patients with the Guillain–Barré syndrome.[27]

We also found a potential relationship between the Guillain–Barré syndrome in association with ZIKV infection and previous exposure to DENV infection. A total of 32 of the 37 patients (86%) with the Guillain–Barré syndrome who were tested for antiflavivirus antibodies had evidence of a recent flavivirus infection, as indicated by positivity for antiflavivirus cross-reactive IgM antibodies, IgG antibodies, or both. The antibody titers detected by the IgG-capture

Table 3. Laboratory Studies for the Investigation of Flavivirus Infection in 42 Patients with the Guillain–Barré Syndrome.

Test	Patients Tested	Patients with Positive Result	Patients with Negative Result
	no.	*no. (%)*	
TaqMan RT-PCR for ZIKV			
Any fluid	42	17 (40)	25 (60)
Serum	31	1 (3)	30 (97)
CSF	30	3 (10)	27 (90)
Urine	24	16 (67)	8 (33)
Nested RT-PCR for DENV			
Any fluid	39	0	39 (100)
Serum	29	0	29 (100)
CSF	10	0	10 (100)
Antiflavivirus antibody ELISA			
IgM or IgG in any fluid	37	32 (86)	5 (14)
IgM in serum	28	9 (32)	19 (68)
IgG in serum	28	23 (82)	5 (18)
IgM in CSF	27	5 (19)	22 (81)
IgG in CSF	27	25 (93)	2 (7)

ELISA are consistent with an anamnestic response to DENV (see the Supplementary Appendix). These data, along with negative DENV RT-PCR results, suggest that these patients had previously been exposed to DENV and that the ZIKV infection may have been a secondary flavivirus infection. There were 5 patients with the Guillain–Barré syndrome who had no detectable flavivirus antibodies; these patients may have had a primary flavivirus infection with ZIKV,[13] as is suggested by the negative antibody profile of one patient who tested positive for ZIKV by RT-PCR in urine.

Our study provides virologic evidence of ZIKV infection in patients with the Guillain–Barré syndrome in Colombia. The onset of the Guillain–Barré syndrome can parallel the onset of systemic manifestations of ZIKV infection, indicating a so-called parainfectious onset, which suggests that factors different from the known postinfectious mechanisms may be present in ZIKV-related Guillain–Barré syndrome. Most of the patients had the AIDP form of the Guillain–Barré syndrome. Our results indicate that RT-PCR testing of urine is a valuable diagnostic tool for the identification of ZIKV infection in patients with the Guillain–Barré syndrome.

Supported by the Bart McLean Fund for Neuroimmunology Research; Johns Hopkins Project Restore; and VIREM, Virology Laboratory Fund, Department of Microbiology, Universidad del Valle.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the patients and their families, members of the Colombian Association of Neurologists, Nancy Bustos, Diana Patricia Murillo, Liliana Soto, Melissa Pelaez, Constanza Nuñez, the staff of the Laboratorio Clínico Especializado AIDA Ascencio and BioSystems for technical support, and Kiran Thakur, Avindra Nath, and Alvaro Muñoz for their valuable comments.

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