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Neurological disease in adults with Zika and chikungunya virus infection in Northeast Brazil: a prospective observational study

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For a Portuguese translation of the abstract see **Online** for appendix 1

For a Spanish translation of the abstract see **Online** for appendix 2

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Summary

Background Since 2015, the arthropod-borne viruses (arboviruses) Zika and chikungunya have spread across the Americas causing outbreaks, accompanied by increases in immune-mediated and infectious neurological disease. The spectrum of neurological manifestations linked to these viruses, and the importance of dual infection, are not known fully. We aimed to investigate whether neurological presentations differed according to the infecting arbovirus, and whether patients with dual infection had a different disease spectrum or severity.

Methods We report a prospective observational study done during epidemics of Zika and chikungunya viruses in Recife, Pernambuco, a dengue-endemic area of Brazil. We recruited adults aged 18 years or older referred to Hospital da Restauração, a secondary-level and tertiary-level hospital, with suspected acute neurological disease and a history of suspected arboviral infection. We looked for evidence of Zika, chikungunya, or dengue infection by viral RNA or specific IgM antibodies in serum or CSF. We grouped patients according to their arbovirus laboratory diagnosis and then compared demographic and clinical characteristics.

Findings Between Dec 4, 2014, and Dec 4, 2016, 1410 patients were admitted to the hospital neurology service; 201 (14%) had symptoms consistent with arbovirus infection and sufficient samples for diagnostic testing and were included in the study. The median age was 48 years (IQR 34–60), and 106 (53%) were women. 148 (74%) of 201 patients had laboratory evidence of arboviral infection. 98 (49%) of them had a single viral infection (41 [20%] had Zika, 55 [27%] had chikungunya, and two [1%] had dengue infection), whereas 50 (25%) had evidence of dual infection, mostly with Zika and chikungunya viruses (46 [23%] patients). Patients positive for arbovirus infection presented with a broad range of CNS and peripheral nervous system (PNS) disease. Chikungunya infection was more often associated with CNS disease (26 [47%] of 55 patients with chikungunya infection *vs* six [15%] of 41 with Zika infection; p=0·0008), especially myelitis (12 [22%] patients). Zika infection was more often associated with PNS disease (26 [63%] of 41 patients with Zika infection *vs* nine [16%] of 55 with chikungunya infection; p≤0·0001), particularly Guillain-Barré syndrome (25 [61%] patients). Patients with Guillain-Barré syndrome who had Zika and chikungunya dual infection had more aggressive disease, requiring intensive care support and longer hospital stays, than those with mono-infection (median 24 days [IQR 20–30] *vs* 17 days [10–20]; p=0·0028). Eight (17%) of 46 patients with Zika and chikungunya dual infection had a stroke or transient ischaemic attack, compared with five (6%) of 96 patients with Zika or chikungunya mono-infection (p=0·047).

Interpretation There is a wide and overlapping spectrum of neurological manifestations caused by Zika or chikungunya mono-infection and by dual infections. The possible increased risk of acute cerebrovascular disease in patients with dual infection merits further investigation.

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Introduction

Over the past 5 years, Brazil has been affected by unprecedented outbreaks of arthropod-borne virus (arbovirus) infections, including Zika and chikungunya viruses, on a background of about 40 years of dengue circulation.[1–4] All three viruses are transmitted by *Aedes aegypti* mosquitoes and cause febrile illnesses with arthralgia and rash; dengue virus can also cause haemorrhagic disease and vascular leak, and it is occasionally linked to neurological disease.[1] The role of chikungunya and Zika viruses in causing neurological disease, which can result in disability and death, is being recognised increasingly by clinicians, particularly in endemic areas.[5–7]

Research in context

Evidence before this study

We searched PubMed for articles investigating the link between neurological disease in adults and infection with arboviruses, from database inception to Nov 1, 2019, with no language restrictions. Search terms included: "Zika", OR "chikungunya"; "meningitis", "encephalitis", "meningoencephalitis", "myelitis", "myelopathy", "Guillain-Barré syndrome", "radiculitis", "mononeuropathy", "cranial neuropathy", "stroke", "transient ischaemic attack", "central nervous system", "peripheral nervous system", and "neurological". We found 98 case reports and case series, four cohort studies, and two case-control studies describing neurological complications of chikungunya infection. Encephalopathy was the most common presentation and studies were mainly done in India, Reunion Island, the French West Indies, and Latin America. Zika has been linked to Guillain-Barré syndrome in case-control studies in French Polynesia, Brazil, Mexico, and Puerto Rico, and to other forms of neurological disease in case reports and series, mainly from Latin America, but the full range of neurological complications and their relative importance has not been described. For both chikungunya and Zika, most reports are for a single virus infection, although evidence exists that arboviruses increasingly co-circulate. More detailed information is needed on the neurological features of patients infected with different arboviruses and on the effect of dual infection.

Added value of this study

To our knowledge, this study is the largest of its kind to describe the neurological features of infection for several arboviruses circulating at the same time, including a thorough investigation of patients with dual infection. This approach differentiates our study from previous ones, which usually describe specific disease syndromes linked to Zika, chikungunya, or dengue virus alone. Our study shows a high frequency of dual chikungunya and Zika virus infections in patients with neurological disease, underscoring the need for clinicians to investigate for evidence of both pathogens. Although both chikungunya and Zika viruses caused a large range of CNS and peripheral nervous system (PNS) manifestations, chikungunya was more often associated with CNS disease, especially myelitis, whereas Zika was more often linked to PNS disease, especially Guillain-Barré syndrome.

Implications of all the available evidence

An increase in acute neurological disease might be a sentinel for new Zika or chikungunya virus outbreaks, as was seen in 2015 across the Americas. Epidemiological studies indicate that Zika and chikungunya seroprevelance is not so high as to preclude such outbreaks in the future. Our study will help clinicans to recognise and appropriately investigate patients with arbovirus infections, which will allow them to provide accurate diagnosis and plan management accordingly. Policy makers should consider the burden of arbovirus-associated neurological complications when developing public health programmes. Future studies should investigate how often arbovirus-associated neurological disease occurs in children, an understudied population, and further characterise the contribution of arbovirus infections to stroke.

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An association between Zika virus and Guillain-Barré syndrome was first described in French Polynesia in 2013,[8] and subsequently described elsewhere.[9,10] Large Zika outbreaks affected Brazil from 2015 onwards, when other forms of acute neurological and congenital Zika disease were first reported.[11,12] The role of Zika virus in causing CNS disease after the neonatal period is still not well defined, with the literature comprising mostly case reports and small case series.[5,12–14] Chikungunya virus infection has been linked to both CNS and peripheral nervous system (PNS) disease, though there have been few large studies[6] describing its importance.

The diagnosis of infection with these arboviruses is based on detection of virus genome by PCR testing or measurement of specific IgM antibodies by ELISA. Because Zika and dengue are both flaviviruses (family Flaviviridae), serological cross-reactivity between them can complicate antibody-based diagnostics;[15] this is not an issue with chikungunya virus because it belongs to a different genus and family (Alphavirus, family Togaviridae). After infection with an arbovirus, life-long immunity typically develops. For dengue, sequential infection by different serotypes can cause more severe disease.[16] Although laboratory studies suggest that Zika virus entry into cells can be enhanced by dengue antibodies,[17,18] epidemiological data indicate that previous exposure to dengue might protect against symptomatic Zika infection.[19] Infection with more than one pathogen is thought to contribute to more severe disease in some brain infections.[20,21] The effect of previous dengue or chikungunya infection on the risk of developing neurological Zika disease is not known, although arbovirus co-infection has been reported in some patients with neurological disease.[13,22]

To better understand the spectrum of infectious and post-infectious immune-mediated neurological disease caused by arboviruses, and the effect of dual infection, we studied adults with suspected arboviral neurological disease in Pernambuco, Northeast Brazil, during the 2015–16 Zika and chikungunya outbreaks. We were especially interested in whether the pattern of disease differed according to the infecting arbovirus, and whether patients with more than one virus infection had a different disease spectrum or severity.

Methods

Study design and patients

This prospective observational study was done at Hospital da Restauração, Recife, a secondary-level and tertiary-level hospital that sees an estimated 70% of the patients

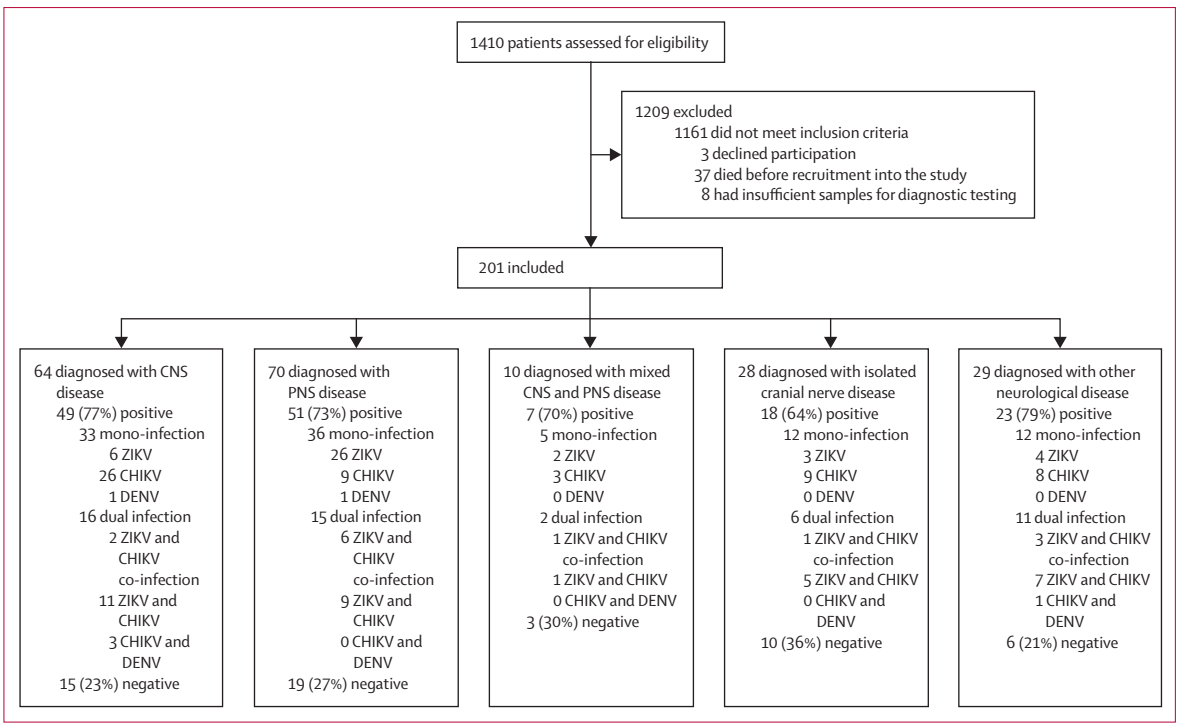

Figure 1: **Study population**
Eligible patients presented with neurological disease to the hospital neurology service in Recife between Dec 4, 2014, and Dec 4, 2016. CHIKV=chikungunya virus. DENV=dengue virus. PNS=peripheral nervous system. ZIKV=Zika virus.

with neurological conditions in the state of Pernambuco, Northeast Brazil. We consecutively recruited patients aged 18 years or older who presented to neurology inpatient services with symptoms of a suspected arboviral-associated neurological disease: history of fever, arthralgia, or rash within the preceding 12 months, followed by paralysis, encephalopathy, a seizure, cranial neuropathy, or other focal deficit. We chose a 12-month window because we did not want to make presumptions about the latency between infection and neurological disease onset. However, we also did an analysis for presentations within 3 months, recognising that most infection-related neurological disease occurs within this time window.[23,24] Written informed consent was provided by patients, or relatives if they did not have capacity. The protocol was approved by the Oswaldo Cruz Foundation, Instituto Aggeu Magalhães Ethics Committee (CAAE #511·06115·8 000 5190).

For the **study protocol** see http://hpruezi.nihr.ac.uk/publications/2020/neuro-zika-protocol

Procedures

Patient history, neurological examination findings, and results of investigations—including details of serum and CSF testing, neuroimaging, nerve conduction studies, and electromyography—were noted on case record forms. After a review by a senior neurologist (MLBF), patients were classified by use of standardised case definitions,[25–31] with levels of diagnostic certainty (appendix 3 pp 1–7), as having CNS disease, if they had well recognised arboviral-associated syndromes (encephalitis, including meningoencephalitis, rhomboencephalitis, and cerebellitis; myelitis; acute disseminated encephalomyelitis [ADEM]; or seizures); PNS disease (Guillain-Barré syndrome or a variant such as Miller Fisher syndrome, radiculopathy, or sensory polyneuropathy); or both. Patients with optic neuritis or other cranial nerve lesions, in the absence of other disease, were classified as having cranial neuropathies. The remainder were classified as having other neurological diseases.

See Online for appendix 3

All serum and CSF samples were blindly tested for Zika, chikungunya, and dengue virus at the Flavivirus Reference Laboratory (Oswaldo Cruz Foundation, Pernambuco, Brazil) by use of well established protocols (appendix 3, p 8). Zika, chikungunya, or dengue infection were diagnosed by presence of viral RNA or specific IgM antibodies in serum or CSF, as defined previously.[13] Patients with more than one virus detected on PCR or IgM antibody testing were classified as having dual infection, accepting that infections might be contemporaneous or sequential. If both viruses were detected at the same time by RT-PCR, we classified this as co-infection. When a patient's samples were positive for IgG but negative for IgM antibodies and virus detection on PCR, this was taken to indicate previous exposure to the virus.

Samples with IgM antibodies for Zika and dengue virus were also assessed for neutralising antibodies against these viruses by use of plaque reduction neutralisation testing (PRNT) to exclude false positives due to flavivirus

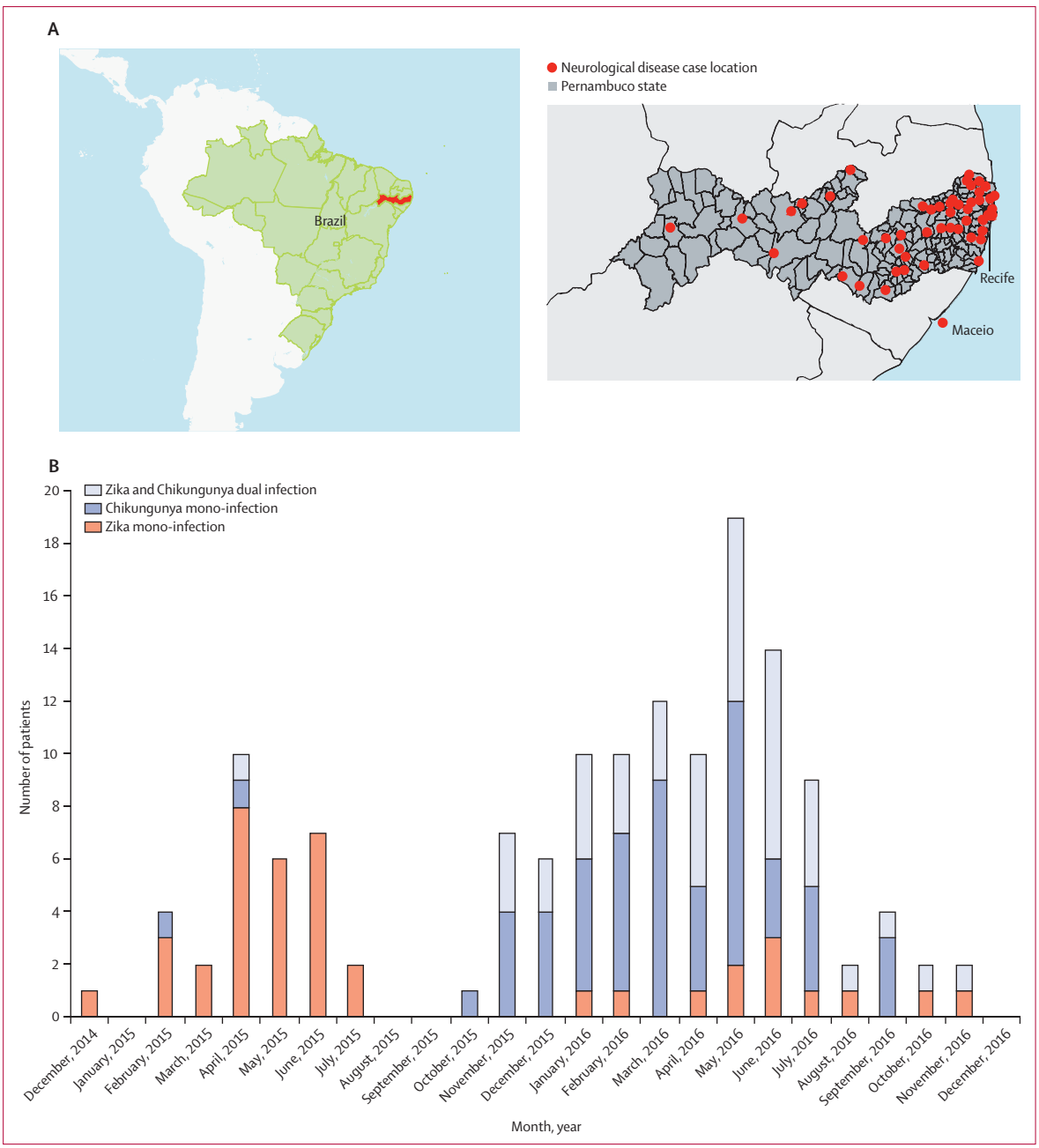

***Figure 2*: Epidemiological data**
Map of neurological disease case locations in Pernambuco state, Brazil (A), and patient recruitment rate per month over the study period, grouped according to arbovirus laboratory diagnosis (B). Mono-infection was defined as laboratory test evidence of infection with a single virus; dual infection was defined as evidence of infection with more than one virus on the basis of a positive PCR or IgM test, in whom infection might be concurrent or sequential. Additionally, there were two patients with dengue mono-infection in 2015; four patients with chikungunya and dengue dual infection between November, 2015, and April, 2016; and 53 patients without laboratory evidence of arbovirus infection recruited over the study period.

cross-reactivity. If patients had neutralising antibodies against both viruses without a positive PCR test confirming infection with one or the other, we deemed this to be an indeterminate flavivirus infection and, given the epidemiological linkage,[4] presumed it to be Zika virus infection, as have others previously.[9] We also repeated our analysis excluding these patients.

We collected data about admission to intensive care, the need for mechanical ventilation, ongoing disability at discharge, and death.

Statistical analysis

Our primary aim was to describe and compare demographic and clinical features (including neurological

	Mono-infection (n=98)				Dual infection (n=50)				All cases (n=201)		
	ZIKV (n=41)	CHIKV (n=55)	DENV (n=2)	p value*	ZIKV + CHIKV co-infection (n=13)	ZIKV + CHIKV (n=33)	CHIKV + DENV (n=4)	p value†	Laboratory evidence of acute arbovirus infection (n=148)	No laboratory evidence of acute arbovirus infection (n=53)	p value‡
Demographics§											
Age (years)	41 (30–50)	53 (34–68)	38 (35–41)	0·059	47 (28–54)	57 (42–67)	51·5 (42–71)	0·17	48 (34–63)	44 (34–53)	0·23
Sex	..	..	..	0·33	..	..	..	0·23	..	..	0·052
Men	22 (54%)	24 (44%)	1 (50%)	..	9 (69%)	18 (55%)	2 (50%)	..	76 (51%)	19 (36%)	..
Women	19 (46%)	31 (56%)	1 (50%)	..	4 (31%)	15 (45%)	2 (50%)	..	72 (49%)	34 (64%)	..
Area of residence	..	..	..	0·20	..	..	..	0·068	..	..	0·058
Recife City	15 (37%)	19 (35%)	0	..	2 (15%)	17 (52%)	2 (50%)	..	55 (37%)	16 (30%)	..
Metropolitan region	13 (32%)	10 (18%)	1 (50%)	..	8 (62%)	9 (27%)	1 (25%)	..	42 (28%)	24 (45%)	..
Other area of Pernambuco	13 (32%)	26 (47%)	1 (50%)	..	3 (23%)	7 (21%)	1 (25%)	..	51 (34%)	12 (23%)	..
Systemic features of infection											
Fever	21 (51%)	47 (85%)	1 (50%)	0·0003	11 (85%)	31 (94%)	4 (100%)	0·0063	114 (77%)	33 (62%)	0·037
Rash	37 (90%)	43 (78%)	0	0·12	10 (77%)	31 (94%)	2 (50%)	0·36	123 (83%)	43 (81%)	0·75
Pruritus	13 (32%)	18 (33%)	2 (100%)	0·92	5 (38%)	14 (42%)	3 (75%)	0·29	55 (37%)	15 (28%)	0·25
Non-purulent conjunctivitis	13 (32%)	15 (27%)	0	0·64	4 (31%)	8 (24%)	3 (75%)	0·70	43 (29%)	16 (30%)	0·88
Myalgia	20 (49%)	37 (67%)	2 (100%)	0·068	7 (54%)	25 (76%)	3 (75%)	0·24	94 (64%)	32 (60%)	0·69
Joint oedema	16 (39%)	36 (65%)	0	0·010	4 (31%)	20 (61%)	1 (25%)	0·82	77 (52%)	18 (34%)	0·024
Arthralgia	25 (61%)	47 (85%)	1 (50%)	0·0061	8 (62%)	28 (85%)	3 (75%)	0·67	112 (76%)	28 (53%)	0·0019
Vomiting	8 (20%)	16 (29%)	0	0·29	6 (46%)	13 (39%)	1 (25%)	0·048	44 (30%)	19 (36%)	0·41
Diarrhoea	3 (7%)	9 (16%)	0	0·19	4 (31%)	5 (15%)	0 (0%)	0·27	21 (14%)	10 (19%)	0·42
Sore throat	5 (12%)	4 (7%)	0	0·64	1 (8%)	3 (9%)	1 (25%)	>0·99	14 (9%)	4 (8%)	0·92
Difficulty breathing	7 (17%)	4 (7%)	0	0·24	5 (38%)	3 (9%)	0	0·33	19 (13%)	10 (19%)	0·29
Cough	9 (22%)	3 (5%)	0	0·016	2 (15%)	6 (18%)	2 (50%)	0·43	22 (15%)	4 (8%)	0·17

Data are median (IQR) for continuous data and n (%) evaluable for categorical data. Mono-infection was defined as laboratory test evidence of infection with a single virus; dual infection was defined as evidence of infection with more than one virus on the basis of a positive PCR or IgM test—infection might be concurrent or sequential; co-infection was defined as contemporaneous detection of RNA of more than one virus on PCR testing. Patients with dengue infection were excluded from the mono-infection comparison, because only two were assessed in the study, as well as from the dual-infection comparison, because only four patients were assessed in the study. Significant differences remained when we excluded the five patients positive for flavivirus classified as having Zika infection on epidemiological grounds. CHIKV=chikungunya virus. DENV=dengue virus. ZIKV=Zika virus. *p values comparing Zika mono-infection versus chikungunya mono-infection cases. †p values comparing Zika or chikungunya mono-infection versus Zika and chikungunya co-infection cases. ‡p values comparing cases with laboratory evidence of acute arbovirus infection with those without. §Overall, four (2%) of 201 patients included in the study reported previous yellow fever vaccination, 56 (28%) hypertension, and 21 (10%) diabetes—these baseline and comorbidity data did not differ between arbovirus diagnostic groups.

Table 1: **Demographics and clinical features of the 201 patients in the study population grouped by arboviral laboratory diagnosis**

symptoms and diagnosis) of patients across the different arboviral diagnostic groups. We used the χ^2 test for comparing categorical variables, or Fisher's exact test if the observations were fewer than five, in a two-tailed analysis. For continuous variables, which were not normally distributed, we compared medians by use of the K Sample equality-of-medians test.[32] Logistic regression models were used to provide estimates of the odds of having certain outcomes adjusted for demographic and baseline characteristics, including age, sex, history of hypertension, diabetes, and previous dengue infection. Missing data were coded as such and were included in analyses. We considered a two-sided p value lower than 0·05 to be significant (appendix 3, p 8). Analyses were done using Stata, version 14.1.

Role of the funding source

The funders of the study had no role in study design, data collection, data analyses, data interpretation, or writing of the Article. The corresponding authors had full access to all the data, and had final responsibility for the decision to submit for publication.

Results

Between Dec 4, 2014, and Dec 4, 2016, 1410 patients were admitted to the neurology service. 249 had suspected arbovirus-associated neurological disease, of whom 37 (15%) were too unwell to be recruited to the study and died shortly after hospital admission, and three (1%) declined participation. Of the remaining 209 (84%) patients, 201 (81%) had sufficient samples for diagnostic

testing and thus were included in the study (figure 1). Patients came from across the Pernambuco state (figure 2). The median age was 48 years (IQR 34–60) and 106 (53%) of 201 patients were women (table 1). Four (2%) of 201 patients included in the study reported previous yellow fever vaccination, 56 (28%) hypertension, and 21 (10%) diabetes. Neuroimaging was done in 150 (75%) patients (118 [59%] had MRI, while the other 32 [16%] patients had CT scans only), while 24 (12%) had electrophysiological peripheral nerve studies, and 11 (5%) had EEG.

We collected 363 serum samples and 171 CSF samples from the 201 patients: 151 (75%) patients had serum alone tested, 49 (24%) had a sufficient sample for both serum and CSF arbovirus testing after routine CSF analysis, and one (<1%) patient had CSF alone tested. 109 (54%) patients had serial serum samples and seven (3%) had more than one CSF sample. Overall, 148 (74%) patients tested positive for arbovirus infection: 98 (49%) with a single virus (mono-infection), and 50 (25%) with dual infection, including 13 (6%) with co-infection (table 2; appendix 3, p 9). Of the 148 arbovirus-positive patients, 41 (28%) had Zika mono-infection: 17 (11%) were PCR positive (with or without a positive IgM) and 24 (16%) were IgM positive alone (including 22 [15%] confirmed by PRNT). Two of these 24 patients were positive for both Zika and dengue IgM and could not be differentiated by PRNT, and thus they were classified as having Zika virus infection on epidemiological grounds. 55 (37%) patients had chikungunya mono-infection: five (3%) were PCR positive (with or without IgM) and 50 (34%) IgM positive alone. More patients with Zika than with chikungunya infection were included in our study in 2015, whereas more patients with chikungunya than with Zika infection were included in 2016 (figure 2). Only two (1%) patients had dengue mono-infection, both were serum IgM positive and confirmed by PRNT (table 2).

Zika and chikungunya dual infection was found in 46 (31%) of 148 patients: 13 (9%) had co-infection, with both viruses detected by PCR (six [4%] in serum and five [3%] in CSF; two [1%] patients were Zika positive in CSF and chikungunya positive in serum); 26 (18%) were Zika positive with PCR and chikungunya IgM positive (eight [5%] were Zika PCR positive in CSF, and 18 [12%] in serum); one (<1%) was chikungunya PCR positive (in CSF) and Zika IgM positive (CSF and serum); six (4%) were positive for Zika and chikungunya IgM antibodies in serum alone. Three (2%) of these patients with dual infection had evidence of both Zika and dengue IgM and a comparable neutralising antibody titre on PRNT, but were defined as Zika because of epidemiological linkage. Four (3%) patients had chikungunya and dengue dual infection (on the basis of IgM in serum). Overall, 179 (89%) of 201 patients had anti-dengue IgG antibodies, indicating previous exposure; this did not predispose to a specific neurological syndrome, nor did it affect clinical outcome.

For the 148 patients positive on arbovirus testing with either mono-infection or dual infection, the most frequent systemic arbovirus symptoms overall were rash (in 123 [83%] patients), fever (114 [77%]), arthralgia (112 [76%]), and myalgia (94 [64%]; table 1). Fever, arthralgia, and joint oedema were more common in patients with a positive arbovirus test than in those with a negative test (table 1). Patients with chikungunya mono-infection were

	Mono-infection (n=98)			Dual infection (n=50)		
	ZIKV (n=41)	CHIKV (n=55)	DENV (n=2)	ZIKV + CHIKV co-infection (n=13)	ZIKV + CHIKV (n=33)	CHIKV + DENV (n=4)
ZIKV						
CSF						
PCR alone	2 (5%)	..	..	4 (31%)	6 (18%)	..
IgM alone	2 (5%)	..	..	0	0	..
PCR and IgM	1 (2%)	..	..	0	0	..
Serum						
PCR alone	4 (10%)	..	..	3 (23%)	15 (45%)	..
IgM alone	19 (46%)	..	..	0	6 (18%)	..
PCR and IgM	7 (17%)	..	..	3 (23%)	1 (3%)	..
CSF and serum						
PCR CSF, PCR serum	0	..	..	1 (8%)	0	..
PCR CSF, IgM serum	0	..	..	0	0	..
IgM CSF, IgM serum	3 (7%)	..	..	0	1 (3%)	..
IgM CSF, PCR serum	0	..	..	0	1 (3%)	..
PCR CSF, PCR serum, IgM CSF	1 (2%)	..	..	0	1 (3%)	..
PCR CSF, IgM serum, IgM CSF	1 (2%)	..	..	1 (8%)	0	..
PCR CSF, PCR serum, IgM serum	0	..	..	1 (8%)	1 (3%)	..
IgM CSF, PCR serum, IgM serum	1 (2%)	..	..	0	1 (3%)	..
PCR CSF, PCR serum, IgM CSF, IgM serum	0	..	..	0	0	..
CHIKV						
CSF						
PCR alone	..	0	..	3 (23%)	0	0
IgM alone	..	0	..	0	0	0
PCR and IgM	..	0	..	0	0	0
Serum						
PCR alone	..	2 (4%)	..	3 (23%)	0	0
IgM alone	..	50 (91%)	..	0	31 (94%)	4 (100%)
PCR and IgM	..	3 (5%)	..	5 (38%)	0	0
CSF and serum						
PCR CSF, PCR serum	..	0	..	0	0	0
PCR CSF, IgM serum	..	0	..	1 (8%)	1 (3%)	0
IgM CSF, IgM serum	..	0	..	0	1 (3%)	0
IgM CSF, PCR serum	..	0	..	0	0	0
PCR CSF, PCR serum, IgM CSF	..	0	..	0	0	0
PCR CSF, IgM serum, IgM CSF	..	0	..	1 (8%)	0	0
PCR CSF, PCR serum, IgM serum	..	0	..	0	0	0
IgM CSF, PCR serum, IgM serum	..	0	..	0	0	0
PCR CSF, PCR serum, IgM CSF, IgM serum	..	0	..	0	0	0

(Table 2 continues on next page)

	Mono-infection (n=98)			Dual infection (n=50)		
	ZIKV (n=41)	CHIKV (n=55)	DENV (n=2)	ZIKV + CHIKV co-infection (n=13)	ZIKV + CHIKV (n=33)	CHIKV + DENV (n=4)
(Continued from previous page)						
DENV						
CSF						
PCR alone	..		0			0
IgM alone	..	..	0	..	..	0
PCR and IgM	..	..	0	..	..	0
Serum						
PCR alone	..	..	0	..	..	0
IgM alone	..	..	2 (100%)	..	..	4 (100%)
PCR and IgM	..	..	0	..	..	0

Mono-infection was defined as laboratory test evidence of infection with a single virus; dual infection was defined as evidence of infection with more than one virus on the basis of a positive PCR or IgM test—infection might be concurrent or sequential; co-infection was defined as contemporaneous detection of RNA of more than one virus on PCR testing. CHIKV=chikungunya virus. DENV=dengue virus. ZIKV=Zika virus.

Table 2: **Summary of diagnostic test results for 148 patients who were positive for an arbovirus**

significantly more likely than those with Zika mono-infection to have fever (47 [85%] of 55 patients with chikungunya infection *vs* 21 [51%] of 41 with Zika infection; p=0·0003), joint oedema (36 [65%] *vs* 16 [39%]; p=0·010), or arthralgia (47 [85%] *vs* 25 [61%]; p=0·0061) and were less likely to report cough (three [5%] *vs* nine [22%]; p=0·016). However, rash and conjunctivitis, often suggested as symptoms that can help distinguish Zika from chikungunya infection, did not differ significantly between these groups. Compared with patients with mono-infection, those with dual infection more frequently reported fever (68 [71%] of 96 patients with mono-infection *vs* 42 [91%] of 46 with dual infection; p=0·0063) and vomiting (24 [25%] *vs* 19 [41%]; p=0·048). These results remained significant when we excluded the five patients with positive flavivirus tests who were classified as having Zika infection on epidemiological grounds.

The patients who tested positive for Zika, chikungunya, or dengue virus—either with mono-infection or dual infection—presented with various neurological manifestations (table 3). The disease syndromes were grouped into five disease categories: CNS, PNS, mixed CNS and PNS, cranial neuropathy, and other neurological disease (table 4). Of the 41 patients with Zika mono-infection, six (15%) had CNS disease, 26 (63%) had PNS disease (of whom 25 [61%] had Guillain-Barré syndrome), two (5%) had mixed CNS and PNS disease, three (7%) had cranial neuropathies, and four (10%) had other neurological disease. Of 55 patients with chikungunya mono-infection, 26 (47%) had CNS disease, nine (16%) had PNS disease (of whom seven [13%] had Guillain-Barré syndrome), three (5%) had mixed CNS and PNS disease, nine (16%) had cranial neuropathies (including two [4%] with abducens nerve paresis), and eight (15%) had other neurological disease. One (50%) patient with dengue had myelitis and the other (50%) had Guillain-Barré syndrome. In 46 patients with dual infection with Zika and chikungunya, 13 (28%) had CNS disease, 15 (33%) had PNS disease, two (4%) had mixed CNS and PNS disease, six (13%) had cranial neuropathies, and ten (22%) had other neurological disease.

We observed an overall difference in neurological manifestations depending on the infecting arbovirus. When comparing the clinical features of patients with Zika mono-infection with those of patients with chikungunya mono-infection, patients diagnosed with Zika infection presented more often with facial weakness, quadriparesis, or hyporeflexia on examination (table 3) and were more often diagnosed with PNS disease (26 [63%] of 41 patients with Zika infection *vs* nine [16%] of 55 with chikungunya infection, p<0·0001; odds ratio [OR] 0·11, 95% CI 0·04–0·29), mainly Guillain-Barré syndrome (table 4). Patients with chikungunya mono-infection presented more frequently with confusion, hyperreflexia, or sensory level on examination (table 3) and were more commonly diagnosed with CNS disease (26 [47%] with chikungunya infection *vs* six [15%] with Zika infection, p=0·0008; OR 5·23, 95% CI 1·90–14·43; table 4). These results were not affected by adjustment for demographic factors or comorbidities, and almost all clinical differences persisted when we excluded patients who presented with neurological disease more than 3 months after arbovirus infection (appendix 3, pp 10, 12). Patients with dual Zika and chikungunya infection presented more often with a stroke or transient ischaemic attack than those with either infection alone (eight [17%] of 46 with dual infection *vs* five [6%] of 96 with mono-infection, p=0·047; OR 3·83, 95% CI 1·18–12·47). The significance of this result was lost when adjusting for age and comorbidities (appendix 3, p 11). We did not find a difference in the frequency of neurological syndromes between patients with a positive arbovirus test and those with a negative test (table 4).

We assessed in more detail the neurological syndromes seen most frequently in the patients with a positive arbovirus test in our study, namely encephalitis (16 [11%] patients); myelitis (22 [15%]); ADEM (eight [5%]); and Guillain-Barré syndrome (47 [32%]). All 16 patients with arboviral encephalitis presented with fever, rash, or both, followed by neurological disease; the median interval was 5 days (IQR 2–5) for patients with Zika mono-infection compared with 9 days (6–47) for those with chikungunya infection (p=0·18). Most patients (14 [88%] of 16) had altered behaviour or reduced consciousness—only patients with cerebellitis or rhombencephalitis did not—and seven (44%) had seizures; whether patients had seizures or not did not differ with viral diagnosis. Of the 16 patients with arboviral encephalitis, one (6%) with Zika infection also had meningism; of those with chikungunya infection, three (19%) had limb weakness, two (13%) had cerebellar signs, and one (6%) had ophthalmoplegia plus bulbar weakness; two (13%) patients with Zika and chikungunya dual infection had weakness in all four limbs

	Mono-infection (n=98)				Dual infection (n=50)				All cases (n=201)		
	ZIKV (n=41)	CHIKV (n=55)	DENV (n=2)	p value*	ZIKV + CHIKV co-infection (n=13)	ZIKV + CHIKV (n=33)	CHIKV + DENV (n=4)	p value†	Laboratory evidence of acute arbovirus infection (n=148)	No laboratory evidence of acute arbovirus infection (n=53)	p value‡
Time from systemic arbovirus infection to neurological symptom onset (days)	7 (5–25)	15 (7–46)	16 (14–17)	0·062	9 (7–126)	12 (4–36)	9 (7–48)	0·84	10 (5–33)	10 (5–30)	0·48
Neurological signs and symptoms											
Headache	25 (61%)	35 (64%)	1 (50%)	0·79	6 (46%)	23 (70%)	3 (75%)	0·95	93 (63%)	37 (70%)	0·36
Photophobia	4 (10%)	5 (9%)	0	0·91	1 (8%)	2 (6%)	0	0·83	12 (8%)	3 (6%)	0·56
Altered consciousness	7 (17%)	16 (29%)	0	0·17	3 (23%)	12 (36%)	2 (50%)	0·28	40 (27%)	13 (25%)	0·72
Confusion	3 (7%)	16 (29%)	0	0·0081	3 (23%)	9 (27%)	1 (25%)	0·40	32 (22%)	10 (19%)	0·67
Abnormal behaviour	2 (5%)	8 (15%)	0	0·13	3 (23%)	4 (12%)	0	0·41	17 (11%)	6 (11%)	0·97
Seizures	3 (7%)	6 (11%)	0	0·55	1 (8%)	2 (6%)	2 (50%)	0·83	15 (10%)	5 (9%)	0·88
Visual changes	7 (17%)	11 (20%)	0	0·72	2 (15%)	7 (21%)	1 (25%)	0·91	28 (19%)	15 (28%)	0·15
Cranial neuropathy of cranial nerve III, IV, or VI	3 (7%)	7 (13%)	0	0·61	2 (15%)	1 (3%)	0	0·68	13 (9%)	5 (9%)	0·89
Facial weakness	16 (39%)	9 (16%)	1 (50%)	0·012	4 (31%)	7 (21%)	0	0·79	37 (25%)	13 (25%)	0·95
Bulbar symptoms	9 (22%)	7 (13%)	0	0·23	2 (15%)	10 (30%)	0	0·19	28 (19%)	16 (30%)	0·090
Autonomic dysfunction§	7 (17%)	5 (9%)	0	0·24	1 (8%)	1 (3%)	0	0·21	14 (9%)	7 (13%)	0·44
Limb weakness	31 (76%)	38 (69%)	2 (100%)	0·48	9 (69%)	22 (67%)	4 (100%)	0·58	106 (72%)	35 (60%)	0·45
Hemiparesis	1 (2%)	6 (11%)	0	0·11	1 (8%)	5 (15%)	0	0·42	13 (9%)	5 (9%)	0·89
Paraparesis	6 (15%)	15 (27%)	1 (50%)	0·14	2 (15%)	4 (12%)	2 (50%)	0·21	30 (20%)	16 (30%)	0·14
Quadriparesis	24 (61%)	17 (31%)	1 (50%)	0·007	6 (46%)	13 (39%)	2 (50%)	0·87	63 (43%)	14 (26%)	0·038
Abnormal reflexes											
Hyporeflexia or absent reflexes	25 (61%)	18 (33%)	0	0·0059	7 (54%)	11 (33%)	0	0·52	61 (41%)	17 (32%)	0·24
Hyperreflexia	3 (7%)	13 (24%)	1 (50%)	0·034	2 (15%)	7 (21%)	1 (25%)	0·67	27 (18%)	14 (26%)	0·21
Impaired coordination	8 (20%)	5 (9%)	0	0·14	1 (8%)	2 (6%)	0	0·22	16 (11%)	3 (6%)	0·27
Sensory deficit on examination	30 (73%)	33 (60%)	2 (100%)	0·18	8 (62%)	21 (64%)	2 (50%)	0·76	96 (65%)	35 (66%)	0·88
Sensory level	3 (7%)	13 (24%)	1 (50%)	0·034	2 (15%)	9 (27%)	2 (50%)	0·31	29 (20%)	5 (9%)	0·090
Sphincter dysfunction	8 (20%)	18 (33%)	1 (100%)	0·15	3 (23%)	10 (28%)	2 (50%)	0·88	42 (28%)	8 (15%)	0·055
Unable to walk	14 (34%)	16 (29%)	1 (100%)	0·60	5 (38%)	10 (30%)	2 (50%)	0·87	48 (32%)	14 (26%)	0·42
Investigations											
White blood cell count (10^9 cells per L)	9 (7–11)	9 (7–11)	5 (NA)	0·80	11 (8–13)	11 (7–12)	11 (6–17)	0·047	10 (7–12)	8 (5–10)	0·17
Platelets (10^9 cells per L)	271 (220–321)	250 (199–316)	236 (NA)	0·67	259 (203–355)	251 (187–330)	237 (117–237)	>0·99	251 (216–311)	265 (200–329)	0·95
CSF cell count (10^6 cells per L)	1·17 (0·33–4·58)	3·50 (0·33–14·33)	98·16 (4·00–192·00)	0·36	2·33 (0·33–8·00)	1·33 (0·33–17·00)	2·83 (0·17–8·50)	0·94	2·17 (0·33–13·50)	1·66 (0·50–9·00)	0·33
CSF protein (g/L)	91 (48–156)	59 (41–84)	136 (80–191)	0·57	59 (37–67)	51 (33–111)	40 (27–45)	0·10	64 (40–101)	45 (33–67)	0·010
Treatment											
Steroids	12 (29%)	31 (61%; n=51)	1 (50%)	0·0026	6 (46%)	12 (41%; n=29)	2 (66%; n=3)	0·68	64 (46%; n=139)	27 (53%; n=51)	0·40
Immunoglobulin	24 (58%)	12 (24%; n=51)	0	0·00063	6 (46%)	9 (31%; n=29)	0 (0%; n=3)	0·71	51 (37%; n=139)	18 (35%; n=51)	0·86
Antivirals	4 (10%)	5 (9%; n=51)	0	>0·99	0	2 (7%; n=29)	0 (n=3)	0·54	11 (8%; n=139)	1 (2%; n=51)	0·14
Anticonvulsants	1 (2%)	1 (2%; n=51)	0	>0·99	1 (8%)	2 (7%; n=29)	1 (33%; n=3)	0·36	5 (4%; n=139)	2 (4%; n=51)	0·92

(Table 3 continues on next page)

	Mono-infection (n=98)				Dual infection (n=50)				All cases (n=201)		
	ZIKV (n=41)	CHIKV (n=55)	DENV (n=2)	p value*	ZIKV + CHIKV co-infection (n=13)	ZIKV + CHIKV (n=33)	CHIKV + DENV (n=4)	p value†	Laboratory evidence of acute arbovirus infection (n=148)	No laboratory evidence of acute arbovirus infection (n=53)	p value‡
(Continued from previous page)											
Inpatient progress and outcome											
Intubated	1 (3%; n=31)	1 (2%)	0	>0·99	2 (17%; n=12)	3 (9%; n=32)	0	0·087	7 (5%; n=136)	4 (9%; n=47)	0·40
Admitted to intensive treatment unit	3 (9%; n=33)	1 (2%)	0	0·29	2 (17%; n=12)	3 (9%; n=32)	0	0·27	9 (7%; n=138)	6 (13%; n=46)	0·28
Number of days in hospital	17 (9–23)	17 (9–22)	20 (14–25)	0·94	19 (13–23)	22 (8–29)	12 (7–17)	0·34	17 (9–24)	18 (9–27)	0·73
Disability at discharge¶	37 (93%; n=40)	47 (92%; n=51)	2 (100%)	>0·99	11 (85%)	26 (81%; n=32)	3 (100%; n=3)	0·15	126 (89%; n=141)	43 (91%; n=47)	0·92
Died	0	1 (2%)	0	>0·99	0	0	0	>0·99	1 (1%)	1 (2%)	0·45

Data are median (IQR) for continuous data and n (%) or n (%; N) evaluable for categorical data. Mono-infection was defined as laboratory test evidence of infection with a single virus; dual infection was defined as evidence of infection with more than one virus on the basis of a positive PCR or IgM test—infection might be concurrent or sequential; co-infection was defined as contemporaneous detection of RNA of more than one virus on PCR testing. Neurological symptom onset was estimated from the patient history (if onset preceded presentation with neurological disease) or was based on the presence of symptoms on examination in hospital (if onset of neurological features and infection were concurrent). Patients with dengue infection were excluded from the mono-infection comparison, because only two were assessed in the study, as well as from the dual-infection comparison, because only four patients were assessed in the study. Where data were not available for all cases, the denominator is indicated. Significant differences remained when we excluded the five patients positive for flavivirus who were classified as having Zika infection on epidemiological grounds. CHIKV=chikungunya virus. DENV=dengue virus. NA=not applicable. ZIKV=Zika virus. *p values comparing Zika mono-infection versus chikungunya mono-infection cases. †p values comparing Zika or chikungunya mono-infection versus Zika and chikungunya dual infection cases. ‡p values comparing cases with laboratory evidence of acute arbovirus infection with those without. §Symptoms of autonomic dysfunction include fluctuations in blood pressure, arrhythmias, vasomotor dysfunction, and abnormalities in gastrointestinal motility. ¶Data on neurological disability at discharge was available for 188 (94%) of 199 discharged patients.

Table 3: **Neurological symptoms, treatment, and outcome of study population grouped by arbovirus diagnosis**

and one (6%) had dysarthria and dysphonia. Two (13%) patients with Zika infection and three (19%) with chikungunya infection had CSF pleiocytosis. MRI was done in 13 (81%) patients and showed mostly cortical lesions. Six (38%) patients were treated with aciclovir for possible herpes simplex virus encephalitis, and ten (63%) were given steroids (table 3). The median time of hospitalisation for patients with arboviral encephalitis was 17 days (range 10–24) and ten (63%) of 16 patients had motor or cognitive deficits at discharge, with one (6%) having ongoing seizures; these results did not differ according to viral diagnosis.

The 22 patients with arbovirus-positive myelitis had a preceding fever, rash, or both and a median of 12 days (IQR 7–28) before the onset of neurological symptoms—this did not differ according to viral diagnosis. These patients typically had paraparesis (14 [64%] patients) or quadriparesis (seven [32%]), which was initially flaccid in four (18%) patients (all with chikungunya infection), often with sensory (21 [95%]) or sphincter involvement (18 [82%]); this pattern did not differ according to viral infection. Eight (36%) patients with myelitis, all with chikungunya mono-infection or dual infection, also had features of encephalopathy. 13 (59%) patients with myelitis had CSF pleiocytosis, and six (27%) had elevated protein only. MRI was done in 16 (73%) of these patients and confirmed the diagnosis of arboviral myelitis in 14 (64%) patients, showing thoracic cord inflammation—sometimes with cervical or brainstem lesions—but not the more diffuse changes seen typically in ADEM. Two (9%) patients with myelitis with a normal MRI and six (27%) patients without MRI had clinical features of myelopathy, of whom four (18%) also had CSF pleiocytosis, indicating inflammation. One (5%) 24-year-old patient with myelitis, unilateral visual loss, and Zika and chikungunya dual infection had an extensive longitudinal cord lesion plus a contrast-enhancing right optic nerve lesion; negative on anti-aquaporin-4 antibody testing, he was diagnosed with neuromyelitis optica spectrum disorder. 16 (73%) patients had corticosteroids and three (14%), with more extensive disease, received immunoglobulin. At discharge, after a median 22 days (IQR 17–30) of hospitalisation, all 22 patients had ongoing disability.

The eight patients with arbovirus-positive ADEM presented with various neurological features a median of 9 days (IQR 5–96) after systemic symptoms. Of these, five (63%) patients had reduced consciousness and confusion, three (38%) had behavioural changes, and one (13%) had a focal seizure. Seven (88%) patients with ADEM had motor deficits, five (63%) also with sensory loss, and two (25%) with cranial nerve involvement (one had ophthalmoplegia, the other facial and bulbar weakness). Five (63%) patients with ADEM had CSF pleiocytosis, and in all seven (88%) patients for whom MRI was done, it revealed inflammatory white matter changes in the brain, cervical, and thoracic spinal cord (figure 3A–C). One (13%) patient with encephalopathy and asymmetrical quadriparesis did not have MRI and was diagnosed clinically as suspected ADEM. All patients with ADEM received steroids and three (38%) also received intravenous immunoglobulin. Patients were discharged after a median 23 days (IQR 11–29), all with ongoing disability.

	Mono-infection (n=98)				Dual infection (n=50)				All cases (n=201)		
	ZIKV (n=41)	CHIKV (n=55)	DENV (n=2)	p value*	ZIKV + CHIKV co-infection (n=13)	ZIKV + CHIKV (n=33)	CHIKV + DENV (n=4)	p value†	Laboratory evidence of acute arbovirus infection (n=148)	No laboratory evidence of acute arbovirus infection (n=53)	p value‡
CNS											
Total	6 (15%)	26 (47%)	1 (50%)	0·0008	2 (15%)	11 (33%)	3 (75%)	0·54	49 (33%)	15 (28%)	0·52
(Meningo) encephalitis	3 (7%)	9 (16%)	0	0·19	1 (8%)	3 (9%)	0	0·50	16 (11%)	5 (9%)	0·78
Myelitis	1 (2%)	12 (22%)	1 (50%)	0·0060	1 (8%)	5 (15%)	2 (50%)	0·94	22 (15%)	4 (8%)	0·17
ADEM	1 (2%)	5 (9%)	0	0·37	0	2 (6%)	0	0·98	8 (5%)	6 (11%)	0·26
Isolated seizure	1 (2%)	0	0	0·85	0	1 (3%)	1 (25%)	>0·99	3 (2%)	0	0·79
PNS											
Total	26 (63%)	9 (16%)	1 (50%)	<0·0001	6 (46%)	9 (27%)	0	0·65	51 (34%)	19 (36%)	0·86
GBS	25 (61%)	7 (13%)	1 (50%)	<0·0001	6 (46%)	8 (24%)	0	0·73	47 (32%)	18 (34%)	0·77
CIDP	0	1 (2%)	0	>0·99	0	0	0	>0·99	1 (1%)	0	>0·99
Radiculitis	1 (2%)	1 (2%)	0	>0·99	0	0	0	0·91	2 (1%)	0	>0·99
Sensory polyneuropathy	0	0	0	..	0	1 (3%)	0	0·65	1 (1%)	1 (2%)	0·92
Mixed CNS and PNS disease											
Total	2 (5%)	3 (5%)	0	>0·99	1 (8%)	1 (3%)	0	>0·99	7 (5%)	3 (6%)	>0·99
Meningoradiculopathy	1 (2%)	0	0	0·85	0	0	0	>0·99	1 (1%)	0	>0·99
Myeloradiculopathy	1 (2%)	1 (2%)	0	>0·99	1 (8%)	1 (3%)	0	0·78	4 (3%)	3 (6%)	0·54
Myelopolyneuropathy	0	1 (2%)	0	>0·99	0	0	0	>0·99	1 (1%)	0	>0·99
Polyneuropathy with meningism and encephalopathy	0	1 (2%)	0	>0·99	0	0	0	>0·99	1 (1%)	0	>0·99
Cranial nerves disease											
Total	3 (7%)	9 (16%)	0	0·19	1 (8%)	5 (15%)	0	0·93	18 (12%)	10 (19%)	0·23
Optic neuritis	3 (7%)	6 (11%)	0	0·82	1 (8%)	4 (12%)	0	0·99	14 (9%)	9 (17%)	0·14
Abducens nerve paresis§	0	2 (4%)	0	0·65	0	0	0	0·91	2 (1%)	1 (2%)	>0·99
Facial nerve paresis	0	1 (2%)	0	>0·99	0	1 (3%)	0	>0·99	2 (1%)	0	>0·99
Other neurological disease¶											
Total	4 (10%)	8 (15%)	0	0·48	3 (23%)	7 (21%)	1 (25%)	0·16	23 (16%)	6 (11%)	0·45
Stroke or transient ischaemic attack	2 (5%)	3 (5%)	0	>0·99	2 (15%)	6 (18%)	0	0·047	13 (9%)	4 (8%)	>0·99
Ocular myositis	0	1 (2%)	0	>0·99	1 (8%)	0	0	>0·99	2 (1%)	0	>0·99
Myositis	1 (2%)	2 (4%)	0	>0·99	0	1 (3%)	1 (25%)	>0·99	5 (3%)	2 (4%)	>0·99
Multiple sclerosis relapse	1 (2%)	1 (2%)	0	>0·99	0	0	0	0·91	2 (1%)	0	>0·99
Amyotrophic lateral sclerosis-like syndrome‖	0	1 (2%)	0	>0·99	0	..	0	>0·99	1 (1%)	0	>0·99

Data are median (IQR) for continuous data and n (%) evaluable for categorical data. Mono-infection was defined as laboratory test evidence of infection with a single virus; dual infection was defined as evidence of infection with more than one virus on the basis of a positive PCR or IgM test—infection might be concurrent or sequential; co-infection was defined as contemporaneous detection of RNA of more than one virus on PCR testing. Patients with dengue infection were excluded from the mono-infection comparison, because only two were assessed in the study, as well as from the dual-infection comparison, because only four patients were assessed in the study. Significant differences remained when we excluded the five patients positive for flavivirus who were classified as having Zika infection on epidemiological grounds. ADEM=acute disseminated encephalomyelitis. CHIKV=chikungunya virus. CIDP=chronic inflammatory demyelinating polyneuropathy. DENV=dengue virus. GBS=Guillain Barré syndrome. PNS=peripheral nervous system. ZIKV=Zika virus. *p values comparing Zika mono-infection versus chikungunya mono-infection cases. †p values comparing Zika or chikungunya mono-infection versus Zika and chikungunya dual infection cases. ‡p values comparing cases with laboratory evidence of acute arbovirus infection with those without. §Two (4%) of 55 patients positive for chikungunya virus presented with ophthalmoplegia, diagnosed as abducens nerve paresis. ¶Syndromes not widely associated with arbovirus infection were defined as other neurological disease. ‖One (2%) of 55 patients positive for chikungunya virus presented 7 months after an episode of viral illness with progressive lower limb weakness followed by upper limb weakness, difficulty walking, and dysphagia and was classified as having an amyotrophic lateral sclerosis-like syndrome.

Table 4: **Neurological disease diagnoses of study population grouped by arbovirus diagnosis**

47 patients with Guillain-Barré syndrome were positive for arbovirus on laboratory testing. Almost all (46 [98%]) had a prodrome of fever, rash, or both (one had myalgia and pruritus only). These symptoms started a median of 7 days (IQR 3–17) before neurological symptom onset for Zika-associated Guillain-Barré syndrome, which was shorter than the median 26 days (15–61) for chikungunya-associated Guillain-Barré syndrome (p=0·0028), but similar to the median 9 days (5–20) for patients with Zika and chikungunya dual infection. 46 (98%) patients with Guillain-Barré syndrome presented with progressive paralysis. Facial paralysis was seen in 26 (55%) patients, more often bilateral (18 [69%]) than unilateral (8 [31%]). 16 (34%) of these patients developed bulbar symptoms. CSF cytoalbuminological dissociation was seen in 42 (93%) patients. Neurophysiological examination was

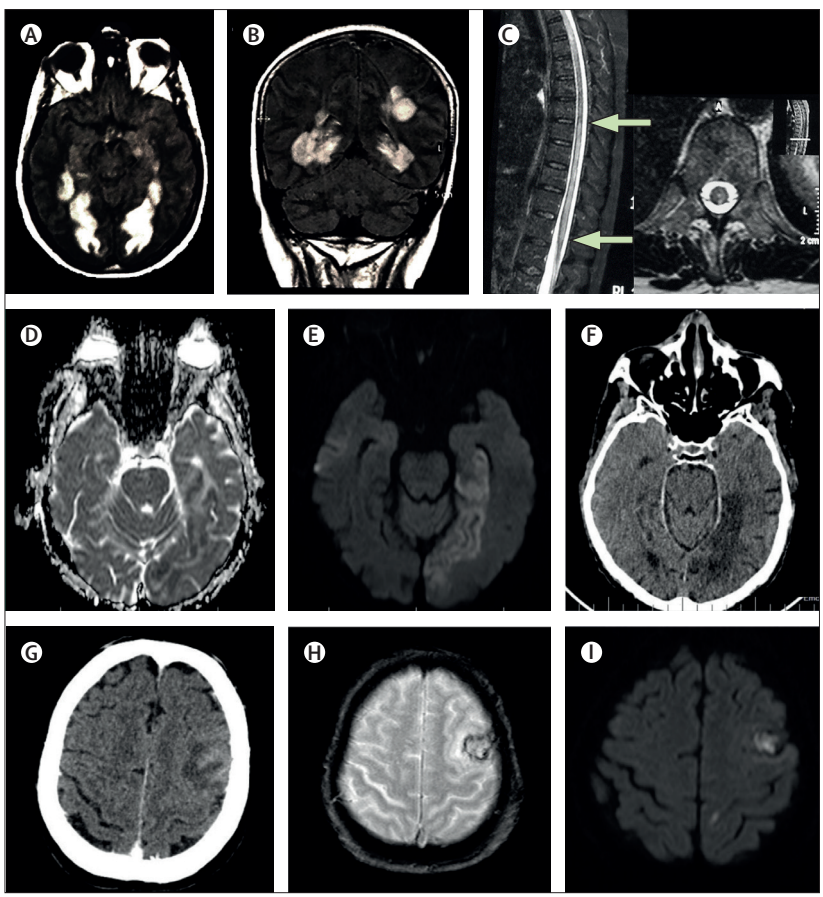

Figure 3: **Neuroimaging findings**
(A) FLAIR MRI of the brain showing bilateral asymmetric hyperintense signal changes in the cortex, most likely representing acute demyelination, in a 57-year-old woman with acute disseminated encephalomyelitis. The patient presented 14 days after having systemic symptoms of arboviral infection with a reduced level of consciousness, bilaterally reduced visual acuity, bilateral lower limb weakness with brisk reflexes, and a T6 sensory level; MRI imaging was done on the day of admission. CSF samples were positive for Zika and chikungunya viruses on PCR and IgM testing, and serum samples were positive for Zika virus on PCR testing. (B) Coronal FLAIR MRI of the brain of the same patient. (C) T2-weighted MRI of the spinal cord of the same patient showing a longitudinally extensive intramedullary signal change within the lower thoracic cord extending to the conus (indicated by arrows). T2-weighted MRI (D) and DWI MRI (E) of a 78-year-old man positive for chikungunya virus with a stroke. The patient, with a history of hypertension, developed left-sided hemiparesis and dysarthria 7 days after onset of systemic symptoms of rash, myalgia, arthralgia, and joint oedema. Serum was positive for chikungunya virus IgM antibodies. MRI shows features of acute ischaemia, including an area of restricted diffusion involving the left PCA territory (medial temporal lobe and the left occipital lobe acute medially, including the lingual gyrus). (F) Follow-up CT at 6 weeks in the same patient, showing an established left PCA territory infarct with involution. CT of the head (G), T2-weighted MRI (H), and DWI MRI of the brain (I) of a patient positive for Zika and chikungunya virus with a stroke. The patient, a 64-year-old woman with hypertension, developed a left-sided hemiparesis 12 weeks after onset of systemic viral symptoms. Serum was positive for Zika virus on PCR and IgM testing, and chikungunya virus on IgM testing. She presented at hospital again 3 months after neurological symptom onset and imaging was done. (G) CT showing an area of gyral hyperdensity with surrounding low density change in the left frontal lobe and a mass effect. (H, I) MRI imaging showing an area of signal abnormality in the left frontal lobe (middle frontal gyrus) with restricted diffusion and areas of susceptibility. These features are suggestive of ischaemia with areas of haemorrhage within it, indicative of a subsequent, more recent event after the initial presentation 3 months before. DWI=diffusion-weighted imaging. FLAIR=transverse fluid attenuated inversion recovery. PCA=posterior cerebral artery.

done for 14 (30%) patients, of whom six (43%) had Zika, three (21%) chikungunya, and five (36%) Zika and chikungunya dual infection. Of these 14 patients, ten (21%) had an acute inflammatory demyelinating polyneuropathy, two (4%) had acute motor-sensory axonal polyneuropathy, and two (4%) had equivocal findings. 16 (34%) patients with Guillain-Barré syndrome had spinal cord MRI, with none showing inflammation. 41 (87%) patients with Guillain-Barré syndrome received immunoglobulin, and the remainder received steroids (13%); nine (19%) patients were admitted to intensive care, with six (13%) requiring invasive ventilation. More patients with Zika and chikungunya dual infection than those with mono-infection were admitted to intensive care (five [36%] of 14 *vs* two [6%] of 32, p=0·041) and were mechanically ventilated (five [36%] of 14 *vs* one [3%] of 32, p=0·014); their hospital stay was also longer (median 24 days [IQR 20–30] for patients with dual infection *vs* 17 days [10–20] for those with mono-infection; p=0·0028). These differences remained when we excluded the three patients with more than 3 months from systemic infection to neurological symptom onset (appendix 3, p 13). At discharge, 46 (98%) patients with Guillain-Barré syndrome had disability, but this did not differ according to arboviral diagnosis.

We identified 13 patients admitted with a stroke (n=11) or transient ischaemic attack (n=2) who had a preceding fever or rash syndrome and were positive for arbovirus on laboratory testing: two (15%) patients with Zika infection, three (23%) with chikungunya infection, and eight (62%) infected with both viruses, including two with co-infection. The interval between systemic arbovirus symptoms and neurological symptom onset was a median of 119 days (IQR 103–134) for patients with Zika infection, 51 days (7–124) for those with chikungunya infection, and 88 days (14–102) for those with dual infection. 12 (92%) of these patients presented with hemiparesis, of whom three (23%) had dysarthria, and one (8%) presented with a seizure and reduced consciousness only. All patients with stroke or transient ischaemic attack underwent imaging: four (31%) had MRI, eight (62%) had CT, and one (7%) had both. Seven (54%) of these patients had evidence of ischaemic changes only and three (23%) had evidence of ischaemia with haemorrhagic transformation (figure 3D–I; appendix 3, p 14). No distinguishing clinical or radiological features were found between patients with mono-infection or dual infection who had a stroke or transient ischaemic attack. The increased risk of cerebrovascular disease after dual infection versus mono-infection remained significant, even after excluding patients who presented more than 3 months after their febrile syndrome (six [15%] of 40 patients *vs* two [2·5%] of 81, p=0·031). We also adjusted for age and comorbidities, which are known risk factors for stroke. Although age did not differ significantly between stroke patients with mono-infection compared to dual infection, when adjusting for this and other variables, dual infection was no longer a significant risk factor for acute cerebrovascular disease (appendix 3, p 11). Median age of patients with arbovirus infection and who presented acutely with cerebrovascular disease was 65 years (IQR 61–78), which was greater than the median age of patients with an acute neurological disease other than stroke or transient ischaemic attack who tested positive for an arbovirus. Additionally, almost all these patients

(12 [92%] of 13) had hypertension, compared with 33 (24%) of 135 patients with a positive arbovirus laboratory test who did not have cerebrovascular disease (p<0·0001); five (38%) had diabetes, and one (8%) had a previous stroke.

Discussion

To our knowledge, our study is the largest of its kind to compare Zika-associated and chikungunya-associated neurological disease. We have shown that, although the viruses cause a similar broad range of CNS and PNS manifestations, Guillain-Barré syndrome is predominant in patients with Zika infections, whereas CNS disease is more common in patients with chikungunya infections.

The past two decades have seen a remarkable spread in the distribution of these emerging arboviruses. Chikungunya virus, first identified in Tanzania in 1953, caused outbreaks in Africa and southeast Asia in the 1960s and 1970s. From 2004 onwards, chikungunya virus caused large epidemics, spreading to the Pacific Islands in 2011, and the Americas in 2013.[6] Zika virus has spread even more rapidly. Isolated initially in Uganda in 1947, the virus was considered a benign cause of occasional febrile illness.[33] However, Zika virus caused large outbreaks in Micronesia in 2007, French Polynesia in 2014 (where the Guillain-Barré syndrome link was first recognised), and Brazil in 2015, where its role in causing microcephaly was established.[8,11,34] As arboviruses spread, the need to understand their severe manifestations and the effect of dual infection becomes even more crucial, particularly because the areas affected by different viruses overlap increasingly.[4,13,22]

We found that, while both Zika and chikungunya viruses can cause Guillain-Barré syndrome, patients with Zika infection presented more rapidly after their prodromal illness than those with chikungunya infection (median 7 *vs* 26 days). The rapid onset of Guillain-Barré syndrome after Zika infection has been suggested as a useful distinguishing feature,[9,10] reflecting para-infectious rather than post-infectious mechanisms,[35] but whether this time interval is significantly shorter than that for other pathogens is unclear.[36] High incidence of facial involvement has also been suggested as a marker of Zika-associated Guillain-Barré syndrome,[5] but we found that facial involvement was equally common (just higher than 50%) in patients with chikungunya-associated Guillain-Barré syndrome.

Approximately half of our patients with chikungunya infection with CNS presentations had myelitis. Although myelitis has been described with chikungunya infection before,[6] its importance compared with encephalitis has not been emphasised previously. In our 2018 systematic review, 322 (37%) of 856 patients with chikungunya-associated neurological disease had encephalopathy, but just 19 (2%) had myelopathy.[6] We also saw myelitis in patients with Zika mono-infection and dual infection in our study. Considerable attention has been given to acute flaccid myelitis caused by enteroviruses, particularly enterovirus D68;[37] our study is a pertinent reminder that arboviruses should also be considered in such cases in endemic areas or in returning travellers. ADEM was another important presentation seen in our study, especially after chikungunya infection; this finding suggests that infection can result in post-infectious immune-mediated CNS disease.

We were interested particularly in whether dual arbovirus neurological infections were more severe than mono-infections, as suggested by preliminary reports[13,22] and seen in other brain infections.[20,21] We found that dual infections were common, accounting for approximately a third of patients with an arbovirus-positive laboratory test, and probably reflecting an overlap of two epidemics.[4] This prevalence of dual infections is higher than that in a Brazilian study of non-neurological arboviral disease (with 9% of patients having dual arbovirus infection),[38] but lower than that of a small neurological disease study from Ecuador (where 75% of patients had arbovirus co-infection).[22] We found that patients with Guillain-Barré syndrome and dual infection more often required intensive care support and mechanical ventilation, and spent on average nearly 7 days longer in hospital than those with Zika or chikungunya mono-infections, which suggests that patients with dual infection had more severe disease. We found no evidence that patients with previous dengue infection had more severe disease than those without, although we observed a high rate of previous exposure to dengue across all disease groups.

Intriguingly, presentations with cerebrovascular disease were nearly 3 times more common in patients with dual infection than in those with mono-infection (17% *vs* 6%; OR 3·83, 95% CI 1·18–12·47), although significance was lost when accounting for other much stronger risk factors (age, hypertension, and diabetes; appendix 3, p 11). Case reports exist of stroke after chikungunya or Zika virus infection.[14,39] The risk of stroke after infection is known to be increased, especially for varicella zoster virus, which causes vasculitis, and HIV, which is thought to disrupt the vascular endothelium.[24,40] Stroke is also being reported increasingly in association with COVID-19, caused by severe acute respiratory syndrome coronavirus 2.[41] A retrospective, population-based cohort study of 13 787 patients in Taiwan suggested that stroke risk might also be increased after dengue infection.[42] Our findings strengthen the evidence for a link between cerebrovascular disease and arboviral infection, although our patients had other risk factors, suggesting that this disease is multifactorial. Clearly, a case-control approach is needed to better understand this question.

Our study was limited by the challenges of doing research during outbreaks; for example, sample availability was limited, and we could not study some patients in extremis (eg, those who died before inclusion in the study). We only recruited patients admitted under the neurology service with preceding symptoms of systemic arbovirus

infection, meaning that we would not have captured patients with disease manifestations that were neurological alone. Although distinguishing Zika from dengue virus can be challenging, we adopted the most robust approaches available, including the use of confirmatory neutralisation antibody testing in cases when doubt remained. Nevertheless, for five patients, we were unable to distinguish serologically between dengue and Zika viruses and classified them as Zika on the basis of the epidemiology, an approach used previously.[9] Excluding these five patients did not alter our findings overall.

In summary, we have shown that Zika virus infection was associated with various CNS and PNS manifestations beyond Guillain-Barré syndrome, whereas for chikungunya virus infection, encephalitis, and myelitis were the most important presentations. The overall effect of arboviral outbreaks on neurology services is large: 148 (10%) of our 1410 neurology department admissions over 2 years were patients with laboratory-confirmed arboviral infection. We see about 70% of all neurological patients in the public health system of Pernambuco (population 9·5 million), so we believe that our findings are fairly representative. Clinicians and public health officials need to be aware of the wide spectrum of neurological diseases linked to arboviruses and appreciate that dual infection is common and might affect disease pattern and severity.

Contributors

MLBF, MFPMA, CAAB, RFOF, AJPM, CMTM, RAAX, LJP, and TS devised the idea for the study. MLBF, MFPMA, CAAB, RFOF, CMTM, and RAAX wrote the protocol and submitted the ethics and research and development applications. MLBF, MFPMA, CAAB, RFOF, AJPM, MIMM, RPM, RM-C, SDM, MLS, RM, RRS, SEL, LJP, MTC, and SL collected data. MLBF, MFPMA, CAAB, RFOF, AJPM, MIMM, RPM, RM-C, SDM, MLS, RM, RRS, SEL, ME, AR-H, GB, LT, MJG, BCJ, HJW, LJP, CAP, RAAX, CMTM, DWGB, MTC, SL, and TS analysed and interpreted data. RFOF, RM-C, LJP, and MTC completed and interpreted laboratory diagnostics. MLBF and MB reviewed neuroimaging. MLBF, MFPMA, and TS provided overall supervision of the study and writing. MLBF, MFPMA, CAAB, RFOF, RM-C, RM, CMTM, RAAX, SL, and TS wrote the initial draft of the paper. All authors contributed to, reviewed, and approved the final draft of the paper.

Declaration of interests

BCJ received grants from Grifols, CSL-Behring, Annexon, Prinses Beatrix Spierfonds, Hansa Biomedical, and GBS-CIDP Foundation International and is on the Global Medical Advisory Board of the GBS CIDP Foundation International. TS is an adviser to the GlaxoSmithKline Ebola Vaccine programme and chairs a Siemens Diagnostics clinical advisory board. TS has a test for bacterial meningitis based on a blood test, filed for patent (GB 1606537.7, April 14, 2016), approval pending. All other authors declare no competing interests.

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