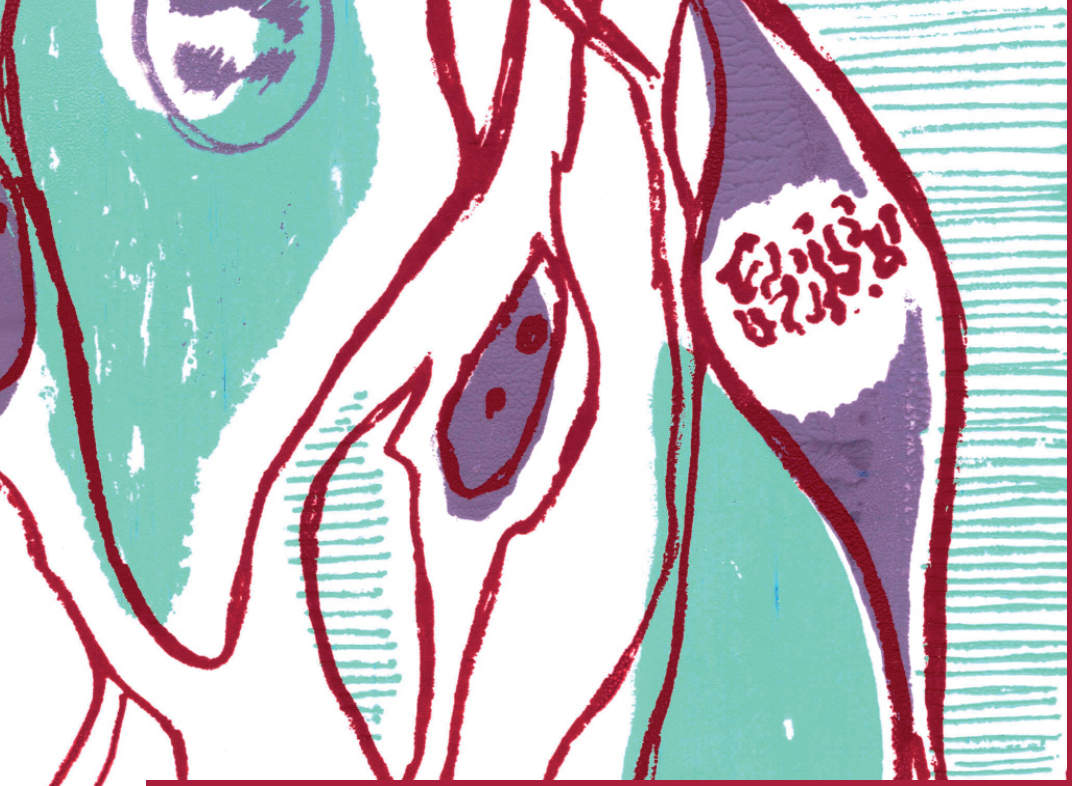
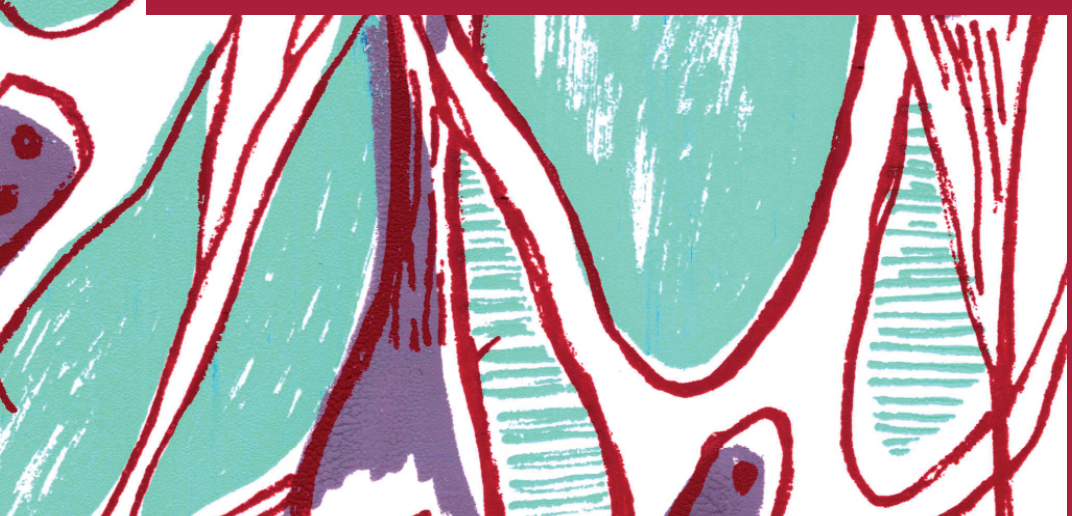


Infections and the Guillain-Barre Syndrome

SONJA E. LEONHARD

from endemic to pandemic



Infections and the Guillain-Barré Syndrome - from endemic to pandemic

Sonja Emily Leonhard

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van endemie tot pandemie

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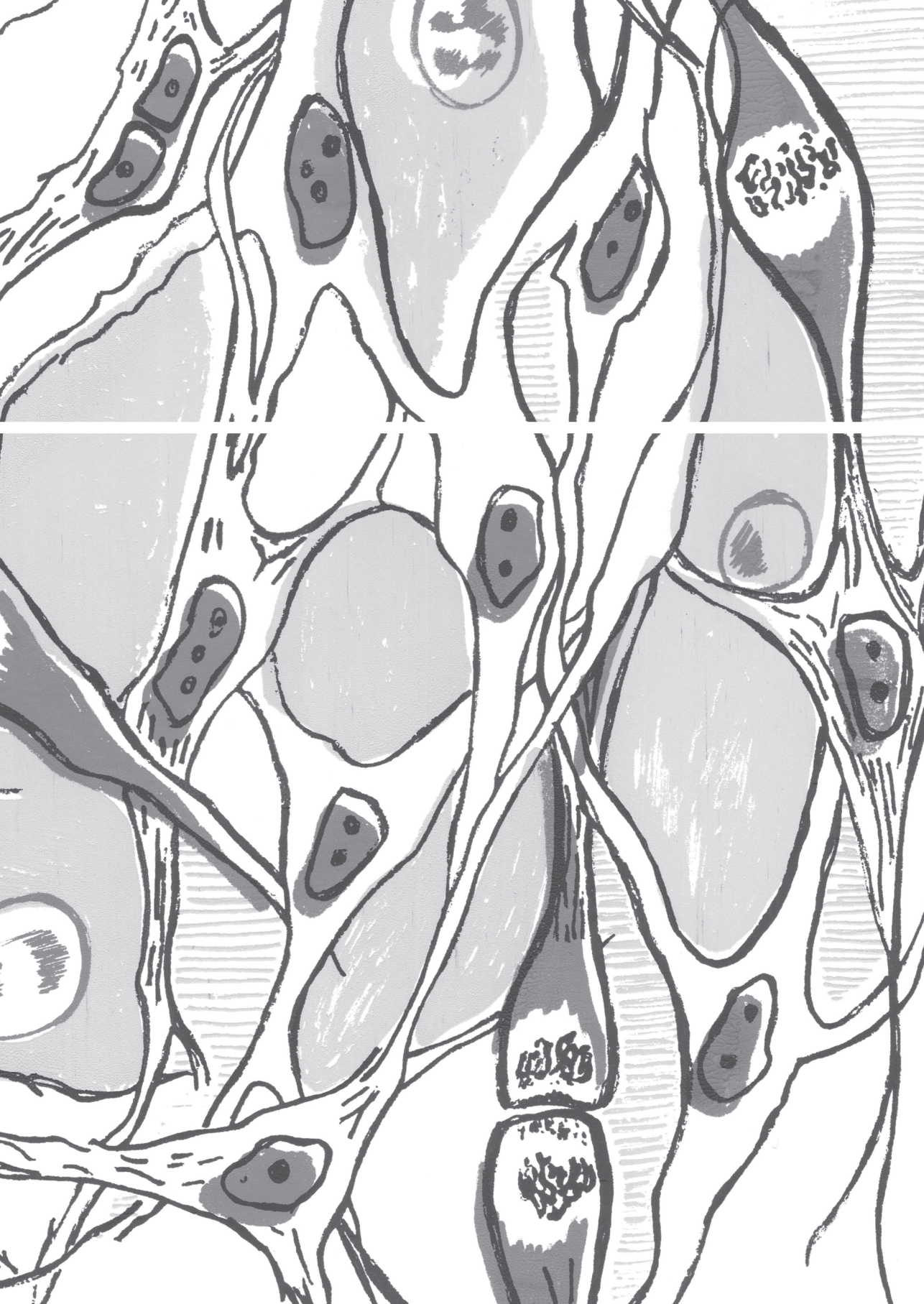


General introduction and scope of the thesis

In the past decades the world confronted several pandemics of emerging infectious diseases, including Zika virus and most recently Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Guillain-Barré syndrome (GBS), a rare and severe disease of the peripheral nerves, is one of the neurological complications reported in relation to these infections. In the past, GBS has also been associated with vaccines and other infections that commonly occur in the general population, such as *Campylobacter jejuni* and cytomegalovirus (CMV). And although GBS is a rare complication of these inflammatory agents, endemic infections occurring at a high rate, epidemics, or vaccine campaigns may induce peaks in the incidence of GBS. The growing threat of infectious disease pandemics, as well as the development of vaccines for Zika virus and SARS-CoV-2, warrant further study into the relation between these and other infectious agents and GBS.

GUILLAIN-BARRÉ SYNDROME

Guillain-Barré syndrome (GBS) is an acute inflammatory polyradiculoneuropathy. It is the most common cause of acute flaccid paralysis world-wide, and has an annual global incidence of approximately 1–2 per 100,000 person–years.[1] Jean-Baptiste Octave Landry was the first to describe the typical clinical features of GBS, but the syndrome was named after Georges Guillain and Jean Alexandre Barré, who, together with André Strohl, first described the typical combination of a normal cell count and increased protein level in the cerebrospinal fluid (CSF) in patients with clinical features as described by Landry.[2] This 'dissociation albumino-cytolique' was at the time essential in distinguishing GBS from infectious causes of acute flaccid paralysis, in particular poliomyelitis. GBS is characterized by rapidly progressive weakness and numbness that usually starts in the lower legs and within hours to weeks ascends to the trunk, arm, facial, extra-ocular, pharyngeal and tongue muscles.[3] Clinical examination reveals loss of tendon reflexes in most patients.[4, 5] Depending on which nerves are affected by the inflammation, the clinical symptoms can vary. This heterogeneity of clinical symptoms includes differences in the distribution of weakness, and the presence of sensory signs, autonomic- and cranial nerve involvement. Based on these differences, several distinct clinical variants of GBS have been described , including the sensorimotor variant, pure motor variant (only weakness without sensory signs), and paraparetic variant (only weakness of the legs), and the Miller Fisher syndrome (MFS, ophthalmoplegia, ataxia and areflexia).[6]

GBS is a monophasic disease and the progressive phase usually lasts less than 4 weeks and is followed by a plateau- and recovery phase. Depending on the severity

of the nerve damage, the disease course can vary, with some patients fully recovered within weeks, and others bedridden for months.[4, 8, 9] Nerve conduction studies can help support the diagnosis of GBS and can identify the nature of the damage to the peripheral nervous system, distinguishing between patients with predominantly damage to the axons and those with predominantly damage to the myelin sheaths or paranodal Schwann cell membranes of the peripheral nerves and nerve roots.[10, 11] Effective treatment strategies focus on reducing inflammation to prevent further neural damage and include intravenously distributed immunoglobulins and plasma exchange.[12] However, even with the best care available, still approximately 20% of patients are unable to walk 6 months after disease onset and ±2-7% of patients die as a consequence of the disease.[8, 13, 14]

PRECEDING INFECTIONS IN GBS

In the original publication, Guillain, Barré, and Strohl wrote: *"The pathogenesis of this radiculoneuropathic syndrome cannot be precisely defined. Although an infection or toxic insult should be considered, we have found no supporting evidence for this."*[2] We now know, a century later, that infectious agents indeed play an important role in the etiology and pathogenesis of GBS. In the early 1960s the first papers appeared that postulated an association between infections and GBS, by showing that two-thirds of patients had infectious symptoms in the weeks preceding the onset of neurological signs.[15-17] Subsequently these observations were confirmed in several larger cohort- and epidemiological studies and a number of specific infections were linked to GBS in case-control studies, including *C. jejuni*, CMV, Epstein-Barr virus (EBV), *Mycoplasma pneumoniae*, and hepatitis E virus (HEV).[18-24] It is hypothesized that the immune response to these infections triggers the onset of GBS.[18] In cases with a preceding *C. jejuni* infection, it has been shown that epitopes of *C. jejuni* resemble epitopes of nerve tissue, and antibodies directed against the pathogen also inadvertently attack the nerves.[25] This mechanism, known as molecular mimicry, has also been postulated in other preceding infections in GBS.[26, 27] Related to their role in triggering the onset of GBS, infections are also thought to play an important part in the clinical and electrophysiological phenotype and disease course of patients with GBS. For instance, *C. jejuni* has been associated with a severe form of GBS in which patients have a pure motor variant and predominantly axonal damage on nerve conduction studies.[20, 21] These previous studies on preceding infections in GBS were often limited in size and focused on just one infection, and results between studies differ regarding the frequencies of preceding infections and the demographic and clinical features. A large international study is necessary to better understand the distribu-

tion of these preceding infections in GBS patients globally, the role of co-infections, and the association with clinical and electrophysiological phenotypes of GBS.

OUTBREAKS OF GBS

Although GBS is usually a sporadic disease, outbreaks have been reported in association with epidemics of infectious or immunological agents.**(Figure 1)** The first outbreak of GBS linked to an immunological agent was the 1976 'swine' flu vaccine that was followed by a disturbing 7.3 fold increase in risk of GBS after widespread distribution in the United States of America (USA).[28] The disquiet that ensued over the association between this vaccine and GBS led to the introduction of surveillance systems to study the relation between GBS and influenza vaccination in the USA and other countries, and the development of case definitions for GBS.[29] And although other studies on seasonal influenza vaccines have either shown no relation, or an increase of only one GBS case per one million vaccinated persons, apprehension in the distribution of new influenza vaccines remains, as exemplified by the concern expressed in the Netherlands during the vaccine program following the H1N1 ('Mexican flu') pandemic in 2009 regarding the possible association with GBS.[30-33] The first infectious disease outbreak in relation to GBS was reported in 1995 in Northern China, when clusters of patients with a pure motor axonal variant of GBS

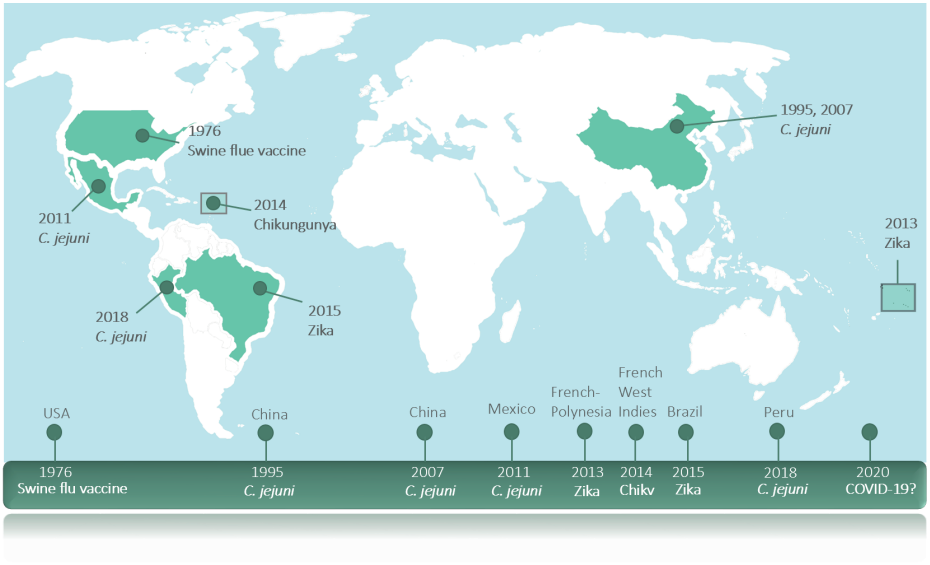


Figure 1 | Timeline of outbreaks of GBS. Graph showing the location and year of previous outbreaks of GBS and the infectious and inflammatory agents that were linked to these outbreaks.

and preceding diarrhea appeared. They were eventually linked to a local outbreak of *C. jejuni*.[34] Other outbreaks of GBS linked to *C. jejuni* occurred in China (2007), Mexico (2011), and Peru (2018).[35-37] But the most remarkable infectious disease epidemic linked to GBS in the past century has to be the Zika virus pandemic, occurring between 2015 and 2017 in Latin America.

ZIKA VIRUS AND GBS

Before 2015 not many people will have heard of the Zika virus, even though the virus was already discovered in 1947, when it was isolated from a nonhuman primate in the Zika forest in Uganda.[38] Zika virus is primarily transmitted via the bite of a mosquito, and is therefore classified as an arthropod-borne virus (arbovirus). Its most prolific vector is the *Aedes aegypti*, a mosquito that is widespread in tropical regions globally.[39]

In the first 60 years after its discovery, Zika virus spread silently across Africa and Asia, responsible for fewer than 20 described cases.[40] The first outbreak of Zika virus was reported on the Yap island in Micronesia in 2007, and was followed by a larger outbreak in French-Polynesia in 2013.[41, 42] People who became infected during these outbreaks generally were either asymptomatic or had mild and self-limiting symptoms of fever, rash, conjunctivitis, and arthritis, and there was therefore little public concern over this virus. This changed in late 2015 when, during a large outbreak of the Zika virus in Brazil, an increased number of infants were born with microcephaly: a severe birth defect caused by abnormal development of the brain.[43, 44] During the same period, increased incidence of GBS was reported in regions where the Zika virus transmission peaked.[45] When the virus began to spread rapidly across Latin America, the World Health Organization designated Zika virus a Public Health Emergency of International Concern in February 2016. Given the known link between GBS and other infectious diseases, the possible association between Zika virus and GBS was readily made and case-control and cohort studies were set up that provided evidence of an association between the two.[46-49]

Several important questions regarding Zika virus in relation to GBS remained unanswered in these publications. It was not clear whether Zika virus-related GBS was, like GBS related to other preceding infections, associated with specific clinical and electrophysiological features or disease severity. Controversy also remained whether Zika virus-related GBS is a typical post-infectious disease, or if Zika may cause GBS through a para-infectious mechanism or a direct infection of nerve roots

or nerves.[47, 50, 51] Furthermore, some studies indicated that Zika virus may be related to other neurological complications besides microcephaly and GBS, such as myelitis, but the potential spectrum of neurologic complications associated with Zika virus remained undefined.[52] Additionally, other arbovirus infections transmitted by the same mosquito, such as chikungunya virus and dengue virus, had been linked to GBS in case reports or series, but their potential to trigger GBS was still unclear.[53-55]

PREPARING FOR FUTURE OUTBREAKS

The Zika virus pandemic and the subsequent surge of patients with GBS also laid bare limitations in diagnosis and management of GBS that clinicians experience globally. No internationally applicable guidelines for the diagnosis and management of GBS were available, and therapeutic options for GBS are expensive and not accessible to all patients across the world.[56] Especially low- and middle income countries, that are most vulnerable to outbreaks of infectious diseases, are likely to be affected by these limitations.[57]

The intensified global transport of people and products shifts the risk of emerging infectious diseases from intermediate vectors (e.g. birds) to direct man-to-man transmission, and the SARS-CoV-2 pandemic exemplifies this ongoing threat.[57] As was seen in previous pandemics, disquiet has again ensued regarding a possible relation between SARS-CoV-2 and GBS and there is apprehension in vaccine uptake of (ex-) GBS patients.[58] This signifies the importance of further investigating the role of infectious agents in the development of GBS and to advance the response to future outbreaks.

OBJECTIVES AND OUTLINE

The first objective of this thesis is to investigate the role of infectious diseases in the development of GBS during endemics and epidemics. The second objective is to determine the limitations in diagnosis and management of GBS that clinicians experience globally, and to explore opportunities to improve this in preparation of future outbreaks.

The outline of this thesis is as follows. In **Part I**, **Chapter 1** the occurrence of preceding infections in GBS patients and the relation to the clinical and electrophysiological phenotype and outcome of GBS is investigated in a large international cohort of

patients with GBS that were tested for a recent infection with *C. jejuni*, *M. pneumoniae,* CMV, EBV, and HEV. In **Part II** the relation between GBS and Zika virus is studied. In **Chapter 2** in a systematic review and meta-analysis of all published studies on GBS related to Zika virus infection, in **Chapter 3** in an observational cohort study of GBS patients prospectively collected during the Zika virus and chikungunya virus epidemics in Northeast Brazil, and in **Chapter 4** in a multicenter prospective case-control study of GBS patients during the epidemic and endemic phase of Zika virus in Brazil, Argentina and Malaysia. In **Part III** the spectrum of neurological disease associated with Zika virus infection is explored, in a review article in **Chapter 5** and in two case reports in **Chapters 6** and **7**. In **Part IV** the occurrence of GBS in relation to other epidemics of infectious disease is investigated. In **Chapter 8** an outbreak of GBS in Peru is investigated in a case-control study, and in **Chapter 9** patients with GBS collected in a large international cohort study during the SARS-CoV-2 pandemic are studied. **Part V** is dedicated to improving global research, diagnosis and management of GB. In **Chapter 10** the management of GBS in Brazil, during and prior to the Zika virus epidemic, is studied in a national survey among Brazilian neurologists. In **Chapter 11** a literature review of the diagnosis and management of GBS in low- and middle-income countries is provided. In **Chapter 12** an internationally applicable consensus-based guideline for the diagnosis and management of GBS is presented, and I conclude with **Chapter 13** where research, diagnosis, and management of GBS in times of pandemics is discussed, and recommendations on how to best prepare for the next outbreak are given.

REFERENCES

1. Sejvar JJ, Baughman AL, Wise M, Morgan OW. Population incidence of Guillain-Barré syndrome: a systematic review and meta-analysis. Neuroepidemiology 2011;36:123-133.
2. Guillain G. Sur un syndrome de radiculo-nevrite avec hyperalbuminose du liquode cephalo-rachidien sans reaction cellulaire: remarques sur les caracteres cliniques et graphiques des reflexes tendineux [Radiculoneuritis syndrome with hyperalbuminosis of cerebrospinal fluid without cellular reaction. Notes on clinical features and graphs of tendon reflexes]. Bell Mem Soc Med Paris 1916;40:1462-1470.
3. Willison HJ, Jacobs BC, van Doorn PA. Guillain-Barré syndrome. Lancet 2016;388:717-727.
4. Fokke C, van den Berg B, Drenthen J, Walgaard C, van Doorn PA, Jacobs BC. Diagnosis of Guillain-Barré syndrome and validation of Brighton criteria. Brain 2014;137:33-43.
5. Yuki N, Kokubun N, Kuwabara S, et al. Guillain-Barré syndrome associated with normal or exaggerated tendon reflexes. J Neurol 2012;259:1181-1190.
6. Wakerley BR, Uncini A, Yuki N, et al. Guillain–Barré and Miller Fisher syndromes—new diagnostic classification. Nat Rev Neurol 2014;10:537.
7. Wakerley BR, Yuki N. Mimics and chameleons in Guillain-Barré and Miller Fisher syndromes. Pract Neurol 2015;15:90-99.
8. Doets AY, Verboon C, van den Berg B, et al. Regional variation of Guillain-Barré syndrome. Brain 2018;141:2866-2877.
9. Walgaard C, Lingsma HF, Ruts L, van Doorn PA, Steyerberg EW, Jacobs BC. Early recognition of poor prognosis in Guillain-Barré syndrome. Neurology 2011;76:968-975.
10. Hafer-Macko C, Hsieh ST, Li CY, et al. Acute motor axonal neuropathy: an antibody-mediated attack on axolemma. Ann Neurol 1996;40:635-644.
11. Hadden RD, Cornblath DR, Hughes RA, et al. Electrophysiological classification of Guillain-Barré syndrome: clinical associations and outcome. Plasma Exchange/Sandoglobulin Guillain-Barré Syndrome Trial Group. Ann Neurol 1998;44:780-788.
12. Hughes RA, Swan AV, Raphael JC, Annane D, van Koningsveld R, van Doorn PA. Immunotherapy for Guillain-Barré syndrome: a systematic review. Brain 2007;130:2245-2257.
13. Van den Berg B, Bunschoten C, van Doorn PA, Jacobs BC. Mortality in Guillain-Barré syndrome. Neurology 2013;80:1650-1654.
14. Dourado ME, Felix RH, da Silva WK, Queiroz JW, Jeronimo SM. Clinical characteristics of Guillain-Barré syndrome in a tropical country: a Brazilian experience. Acta Neurol Scand 2012;125:47-53.
15. Takahashi M, Koga M, Yokoyama K, Yuki N. Epidemiology of Campylobacter jejuni isolated from patients with Guillain-Barré and Fisher syndromes in Japan. J Clin Microbiol 2005;43:335-339.
16. Melnick SC, Flewett TH. Role of Infection in the Guillain-Barr'e Syndrome. J Neurol Neurosurg Psychiatry 1964;27:395-407.
17. Campbell AM. The aetiology of polyneuritis. Proc R Soc Med 1958;51:157-159.
18. Jacobs BC, Rothbarth PH, van der Meche FG, et al. The spectrum of antecedent infections in Guillain-Barré syndrome: a case-control study. Neurology 1998;51:1110-1115.
19. Caudie C, Quittard Pinon A, Taravel D, et al. Preceding infections and anti-ganglioside antibody profiles assessed by a dot immunoassay in 306 French Guillain-Barré syndrome patients. J Neurol 2011;258:1958-1964.

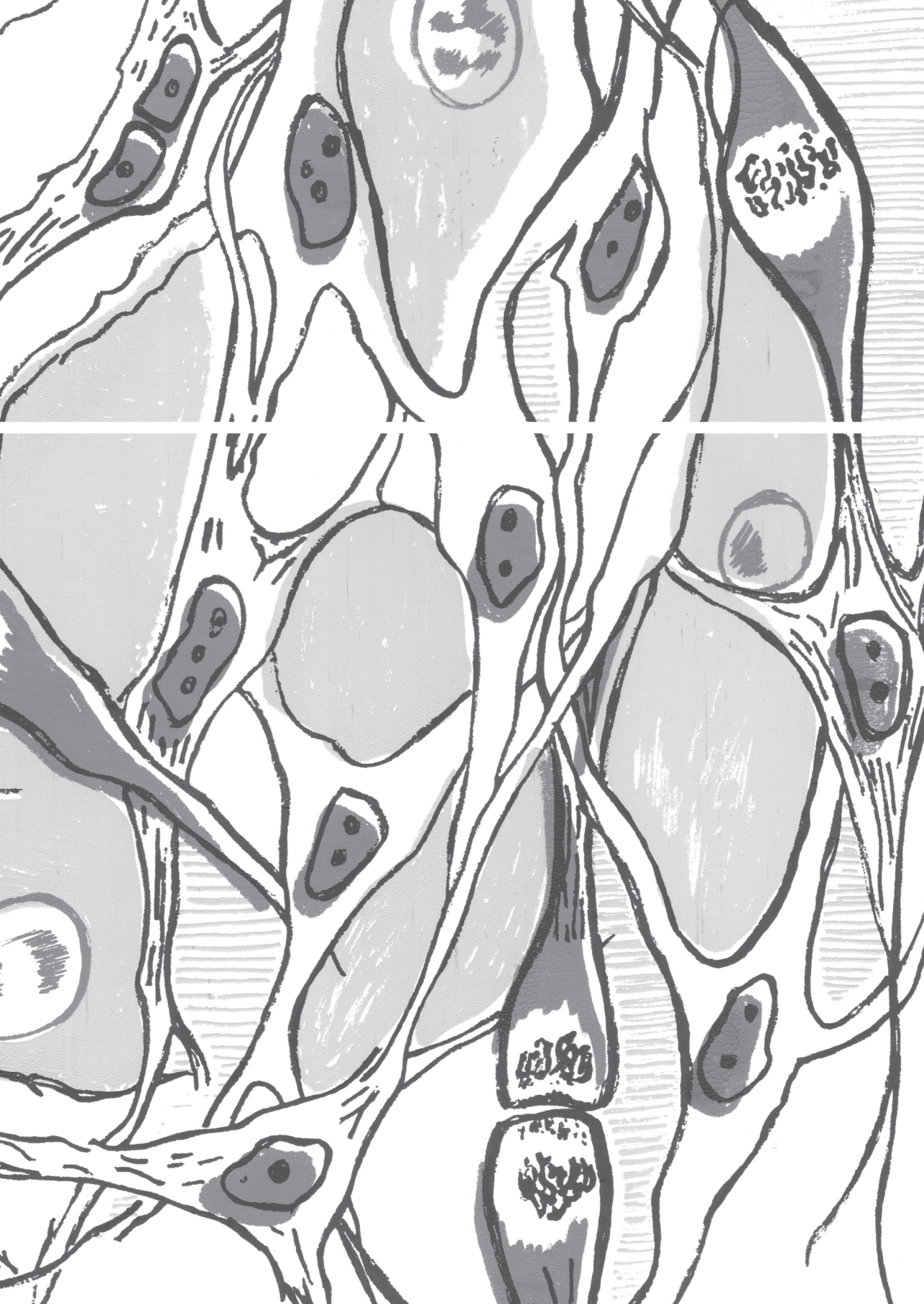
20. Rees JH, Soudain SE, Gregson NA, Hughes RA. Campylobacter jejuni infection and Guillain-Barré syndrome. N Engl J Med 1995;333:1374-1379.
21. Orlikowski D, Porcher R, Sivadon-Tardy V, et al. Guillain-Barré syndrome following primary cytomegalovirus infection: a prospective cohort study. Clin Infect Dis 2011;52:837-844.
22. Yuki N, Tagawa Y. Acute cytomegalovirus infection and IgM anti-GM2 antibody. J Neurol Sci 1998;154:14-17.
23. van den Berg B, van der Eijk AA, Pas SD, et al. Guillain-Barré syndrome associated with preceding hepatitis E virus infection. Neurology 2014;82:491-497.
24. Kaldor J, Speed BR. Guillain-Barré syndrome and Campylobacter jejuni: a serological study. Br Med J (Clin Res Ed) 1984;288:1867-1870.
25. Yuki N, Susuki K, Koga M, et al. Carbohydrate mimicry between human ganglioside GM1 and Campylobacter jejuni lipooligosaccharide causes Guillain-Barré syndrome. Proc Natl Acad Sci U S A 2004;101:11404-11409.
26. Kusunoki S, Chiba A, Hitoshi S, Takizawa H, Kanazawa I. Anti-Gal-C antibody in autoimmune neuropathies subsequent to mycoplasma infection. Muscle Nerve 1995;18:409-413.
27. Sumner AJ, Saida K, Saida T, Silberberg DH, Asbury AK. Acute conduction block associated with experimental antiserum-mediated demyelination of peripheral nerve. Ann Neurol 1982;11:469-477.
28. Schonberger LB, Bregman DJ, Sullivan-Bolyai JZ, et al. Guillain-Barré syndrome following vaccination in the National Influenza Immunization Program, United States, 1976-1977. Am J Epidemiol 1979;110:105-123.
29. Sejvar JJ, Kohl KS, Gidudu J, et al. Guillain-Barré syndrome and Fisher syndrome: case definitions and guidelines for collection, analysis, and presentation of immunization safety data. Vaccine 2011;29:599-612.
30. Jacobs BC, Wijnans L., Sturkenboom M., Van der Maas N. Guillain-Barré-syndroom en het nieuwe influenza A(H1N1)-virus. Ned Tijdschr Geneeskd 2009.
31. Principi N, Esposito S. Vaccine-preventable diseases, vaccines and Guillain-Barré syndrome. Vaccine 2018.
32. Ghaderi S, Gunnes N, Bakken IJ, Magnus P, Trogstad L, Håberg SE. Risk of Guillain-Barré syndrome after exposure to pandemic influenza A(H1N1)pdm09 vaccination or infection: a Norwegian population-based cohort study. European Journal of Epidemiology 2016;31:67-72.
33. Wise ME, Viray M, Sejvar JJ, et al. Guillain-Barré syndrome during the 2009-2010 H1N1 influenza vaccination campaign: population-based surveillance among 45 million Americans. American journal of epidemiology 2012;175:1110-1119.
34. Ho TW, Mishu B, Li CY, et al. Guillain-Barré syndrome in northern China. Relationship to Campylobacter jejuni infection and anti-glycolipid antibodies. Brain 1995;118 (Pt 3):597-605.
35. Jackson BR, Zegarra JA, López-Gatell H, et al. Binational outbreak of Guillain-Barré syndrome associated with Campylobacter jejuni infection, Mexico and USA, 2011. Epidemiol Infect 2014;142:1089-1099.
36. Zhang M, Li Q, He L, et al. Association study between an outbreak of Guillain-Barré syndrome in Jilin, China, and preceding Campylobacter jejuni infection. Foodborne Pathog Dis 2010;7:913-919.

37. Díaz-Soto S, Chavez K, Chaca A, Alanya J, Tirado-Hurtado I. Outbreak of Guillain-Barré syndrome in Peru. eNeurologicalSci 2019;14:89-90.
38. Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. Trans R Soc Trop Med Hyg 1952;46:509-520.
39. Musso D, Gubler DJ. Zika Virus. Clin Microbiol Rev 2016;29:487-524.
40. Faye O, Freire CCM, Iamarino A, et al. Molecular Evolution of Zika Virus during Its Emergence in the 20th Century. PLOS Neglected Tropical Diseases 2014;8:e2636.
41. Duffy MR, Chen TH, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med 2009;360:2536-2543.
42. Cao-Lormeau VM, Blake A, Mons S, et al. Guillain-Barré syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. Lancet 2016;387:1531-1539.
43. Panchaud A, Stojanov M, Ammerdorffer A, Vouga M, Baud D. Emerging Role of Zika Virus in Adverse Fetal and Neonatal Outcomes. Clinical Microbiology Reviews 2016;29:659-694.
44. Microcephaly Epidemic Research G. Microcephaly in Infants, Pernambuco State, Brazil, 2015. Emerg Infect Dis 2016;22:1090-1093.
45. World Health Organization. Zika situation report 10 March 2017. 2017.
46. Dirlikov E, Major CG, Medina NA, et al. Clinical Features of Guillain-Barré Syndrome With vs Without Zika Virus Infection, Puerto Rico, 2016. JAMA Neurol 2018;75:1089-1097.
47. Parra B, Lizarazo J, Jimenez-Arango JA, et al. Guillain-Barré syndrome associated with Zika virus infection in Colombia. N Engl J Med 2016;375:1513-1523.
48. Salinas JL, Walteros DM, Styczynski A, et al. Zika virus disease-associated Guillain-Barré syndrome-Barranquilla, Colombia 2015-2016. J Neurol Sci 2017;381:272-277.
49. Styczynski AR, Malta J, Krow-Lucal ER, et al. Increased rates of Guillain-Barré syndrome associated with Zika virus outbreak in the Salvador metropolitan area, Brazil. PLoS Negl Trop Dis 2017;11:e0005869.
50. Nascimento OJM, Frontera JA, Amitrano DA, Bispo de Filippis AM, Da Silva IRF, Group R-G-ZR. Zika virus infection-associated acute transient polyneuritis. Neurology 2017;88:2330-2332.
51. Leis AA, Stokic DS. Zika Virus and Guillain-Barré Syndrome: Is There Sufficient Evidence for Causality? Front Neurol 2016;7:170.
52. Mehta R, Soares CN, Medialdea-Carrera R, et al. The spectrum of neurological disease associated with Zika and chikungunya viruses in adults in Rio de Janeiro, Brazil: A case series. PLoS Negl Trop Dis 2018;12:e0006212.
53. Wielanek AC, Monredon JD, Amrani ME, Roger JC, Serveaux JP. Guillain-Barré syndrome complicating a Chikungunya virus infection. Neurology 2007;69:2105-2107.
54. Santos NQ, Azoubel AC, Lopes AA, Costa G, Bacellar A. Guillain-Barré syndrome in the course of dengue: case report. Arq Neuropsiquiatr 2004;62:144-146.
55. Soares CN, Cabral-Castro M, Oliveira C, et al. Oligosymptomatic dengue infection: a potential cause of Guillain Barré syndrome. Arq Neuropsiquiatr 2008;66:234-237.
56. Islam MB, Islam Z, Rahman S, et al. Small volume plasma exchange for Guillain-Barré syndrome in resource poor settings: a safety and feasibility study. Pilot Feasibility Stud 2017;3:40.

57. Jones KE, Patel NG, Levy MA, et al. Global trends in emerging infectious diseases. Nature 2008;451:990-993.
58. Gigli GL, Bax F, Marini A, et al. Guillain-Barré syndrome in the COVID-19 era: just an occasional cluster? J Neurol 2020.

Part I

Preceding infections in Guillain-Barré syndrome



Chapter 1

An international perspective on preceding infections in Guillain-Barré syndrome: the IGOS-1000 cohort

Sonja E. Leonhard, Annemiek A. van der Eijk, Henning Andersen, Giovanni Antonini, Samuel Arends, Shahram Attarian, Fabio A. Barroso, Kathleen Bateman, Manou R. Batstra, Luana Benedetti, Bianca van den Berg, Peter Van den Bergh, Jan Bürmann, Mark Busby, Carlos Casasnovas, David R. Cornblath, Amy Davidson, Alex Y. Doets, Pieter A. van Doorn, Charlotte Dornonville de la Cour, Thomas E. Feasby, Janev Fehmi, Tania Garcia-Sobrino, Jonathan M. Goldstein, Kenneth C. Gorson, Volkan Granit, Robert D.M. Hadden, Thomas Harbo, Hans-Peter Hartung, Imran Hasan, Jakob V. Holbech, James K.L. Holt, Israt Jahan, Zhahirul Islam, Summer M. Karafiath, Hans D. Katzberg, Ruud P. Kleyweg, Noah Kolb, Krista Kuitwaard, Motoi Kuwahara, Susumu Kusunoki, Linda W.G. Luijten, Satoshi Kuwabara, Edward Lee Pan, Helmar C. Lehmann, Marijke Maas, Lorena Martin Aguilar, James A.L. Miller, Quazi Deen Mohammad, Soledad Monges, Velina Nedkova-Hristova, Eduardo Nobile-Orazio, Julio Pardo, Yann Pereon, Luis Querol, Ricardo C. Reisin, Wouter van Rijs, Simon Rinaldi, Rhys C. Roberts, Joyce Roodbol, Nortina Shahrizaila, Soren H. Sindrup, Beth Stein, Cheng Y. Tan, Hatice Tankisi, Anne Tio, María J. Sedano Tous, Christine Verboon, Frederique H. Vermeij, Leo H. Visser, Ruth Huizinga, Hugh J. Willison, Bart C. Jacobs, the IGOS Consortium

Neurology –submitted November 2021

ABSTRACT

Background and objectives

Infections play a key role in the development of Guillain-Barré syndrome (GBS) and have been associated with specific clinical features and disease severity. The clinical variation of GBS across geographical regions has been suggested to be related to differences in the distribution of preceding infections, but this has not been studied on a large scale.

Methods

We analysed the first 1000 patients included in the International GBS Outcome Study with available biosamples (*n*=768) for the presence of a recent infection with: *Campylobacter jejuni*, hepatitis E virus, *Mycoplasma pneumoniae,* cytomegalovirus, and Epstein-Barr virus.

Results

Serological evidence of a recent infection with *C. jejuni* was found in 228 (30%), *M. pneumoniae* in 77 (10%), hepatitis E virus in 23 (3%), cytomegalovirus in 30 (4%) and Epstein-Barr virus in 7 (1%) patients. Evidence of more than one recent infection was found in 49 (6%) of these patients. Symptoms of antecedent infections were reported in 556 patients (72%), and this proportion did not significantly differ between those testing positive or negative for a recent infection. The proportions of infections were similar across continents. The sensorimotor variant and the demyelinating electrophysiological subtype were most frequent across all infection groups, although proportions were significantly higher in patients with a cytomegalovirus and significantly lower in those with a *C. jejuni* infection. *C. jejuni*–positive patients were more severely affected, indicated by a lower MRC sum score at nadir (*P*=0.004), and a longer time to regain the ability to walk independently (*P*=0.005). The pure motor variant and axonal electrophysiological subtype were more frequent in Asian compared to American or European *C. jejuni*-positive patients (*P*<0.001, resp. *P*= 0.001). Time to nadir was longer in the cytomegalovirus-positive patients (*P*=0.004).

Conclusion

Across geographical regions, the distribution of infections was similar but the association between infection and clinical phenotype differed. A mismatch between symptom reporting and serological results and the high frequency of co-infections, demonstrate the importance of broad serological testing in identifying the most likely infectious trigger. The association between infections and outcome indicates their value for future prognostic models.

INTRODUCTION

Guillain-Barré syndrome (GBS) is the most common cause of post-infectious flaccid paralysis world-wide. A preceding symptomatic infectious illness is reported in approximately two-thirds of cases. The infections that have most consistently been associated with GBS in case-control studies include: *Campylobacter jejuni*, hepatitis E virus (HEV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), *Mycoplasma pneumoniae*, and Zika virus.[1-4] Other infections and non-infectious preceding events such as vaccinations, surgery, and malignancy also have been linked to GBS in uncontrolled case series or surveillance studies.[5-11]

It is hypothesized that the immune response to these infections triggers the onset of GBS.[12] Cross-reactive antibodies induced by molecular mimicry have been demonstrated to play a key role in the pathophysiology of GBS after bacterial infections, but other mechanisms may also play a role.[13, 14] GBS is a heterogeneous disease, displaying a large variety of clinical and electrophysiological features, and outcome.[15, 16] These variations are thought to be attributable to differences in preceding infections.[17, 18] Geographical differences in phenotype and outcome of GBS found in previous studies are believed to be partly due to differences in the incidence and type of preceding infections between regions, but this has not yet been studied in a large international cohort.[3, 15, 19, 20]

We describe the distribution of a recent infection with *C. jejuni*, HEV, *M. pneumoniae,* CMV, and EBV, and the relation to clinical phenotypes and outcome in the largest international prospective observational cohort study on GBS.[21]

MATERIAL AND METHODS

Study design

Data were collected inthe International GBS Outcome Study (IGOS) study, a prospective, observational cohort study.[21] Patients were included from 154 hospitals (106 [69%] university or teaching hospitals, and 48 [31%] non-university hospitals) by the participating neurologist. All patients – independent of age, variant, disease severity, or treatment- were included within 2 weeks of onset of weakness. Patients were followed up at week 1, 2, 4, 8, 13, 26 and 52. The current study was based on the analysis of the first 1000 included patients with a sufficient amount of serum from study entry or week 1 available for laboratory testing. Patients were enrolled

between May 2012 and July 2015 from 120 active study sites in 18 countries across five continents.

Standard Protocol Approvals, Registrations, and Patient Consents

IGOS was approved by the review boards of Erasmus MC, University Medical Centre Rotterdam, The Netherlands (MEC-2011-477) and the local institutional review boards. Written informed consent was obtained from all patients or their legal representatives.

Data collection

All antecedent events recorded <8 weeks prior to onset of neurological symptoms were included in the analysis. The clinical variant of GBS (sensorimotor, pure motor, pharyngeal-cervical-brachial, Miller Fisher (overlap) syndrome (MFS), ataxic form, or other) was defined by the physician at week 4. If missing, the clinical variant recorded at week 2, week 1, or entry was used (in that order). 'Pure motor' variant of GBS was defined as the absence of sensory deficits in neurological examination in the first 4 weeks. Nadir was defined as the lowest MRC sum score during the first 4 weeks from study entry. If there was no weakness, the highest GBS disability score was used instead. Patients who reached nadir before study entry or who were lost to follow-up within 4 weeks were excluded from the nadir analysis. Albuminocytological dissociation in the CSF was defined as a cell count <50 cells/µl and a protein level >0.45 g/l.[21, 22] Raw data from the first nerve conduction study, local reference values and an algorithm were used to classify each study according to the criteria of Hadden et al. by an independent neurophysiologist (SA).[23] The ability to walk unaided (GBS disability score <3) at 6 and 12 months, and the time to reach this endpoint were used as outcome measures.

Laboratory testing

Sera from all patients were tested for a recent infection with *C. jejuni*, HEV, *M. pneumoniae*, CMV, and EBV according to standard diagnostic testing. Zika virus diagnostics were not performed as data were collected before the Zika virus pandemic. Evidence of a recent infection was based on immunoglobulin persistence, as: IgM positivity for *M. pneumoniae*, IgM and/or IgA positivity for *C. jejuni*, IgM and/or PCR positivity for HEV (**Supplementary Table 1**). For CMV and EBV, presence of IgM alone is insufficient evidence of a recent infection, as this may indicate a primary infection, secondary or re-infection, or serological cross-reactivity due to infection with different pathogens.[24] Therefore, we determined IgG values that are indicative of a recent infection in these patients. For CMV recent infection was defined as IgM positivity with negative IgG or IgG with low avidity, and for EBV viral capsid

antigen (VCA) IgM and IgG positivity with negative and EBV nuclear antigen (EBNA) IgG. As IVIg is is derived from IgG of blood donors, we were unable to determine if CMV or EBV IgG positivity was due to treatment or previous exposure to CMV or EBV in patients with samples collected post-IVIg treatment. For the purpose of this study, patients with samples collected post-IVIg or in whom timing of collection was unclear, who were CMV IgM positive with moderate/high IgG avidity or who were EBV VCA IgM and EBNA IgG positive, were considered negative for a recent CMV or EBV infection.

This cohort has also been tested for the presence of serum anti-ganglioside antibodies, and the results are currently being analysed. These results will be described in a separate paper. See the Supplementary Material for a description of laboratory methods.

Statistical analysis

We used SPSS Statistics 21.0 and R 3.6.1 for data analysis. Continuous data are presented as medians with interquartile ranges (IQR) and dichotomized or categorical data as numbers and proportions. Mann-Whitney U-test and Kruskal-Wallis test were used to compare continuous data, and Pearson Chi-Square Test or Fisher's exact test to compare proportions. A two-sided *P*-value of <0.05 was considered significant. Patients with a mono-infection with *C. jejuni*, *M. pneumoniae*, CMV, EBV, HEV, and patients who tested negative were compared. When groups were significantly different, each mono-infection group was pairwise compared to all other mono-infection groups and those testing negative. Significance was adjusted using the Bonferroni correction. Kaplan-Meier competing risk analysis was used to analyze the time to walk independently and the time to death during follow-up in patients unable to walk at nadir. We defined the time to walk as the median day between the the last visit that the patient was unable to walk independently and the first visit that they were able to walk independently again. Differences between groups were compared using the log-rank test. A Cox proportional hazards model was used to correct for other known risk factors, including age, residency in Bangladesh, and axonal subtype.[25] Residency in Bangladesh was included because this was a risk factor in a previous study.[15] R package 'dplyr 1.0.2' was used for preparing data and 'survival 3.1-8' for Kaplan-Meier and Cox proportional hazards analyses.

RESULTS

Of the 1000 patients in the cohort, we excluded 232 from the analysis because of alternative diagnoses (*n*=61), protocol violations (*n*=19), insufficient data (*n*=5) or insufficient serum sample (*n*=147).(**Figure 1**) The remaining cohort consisted of 768 patients. See **Table 1** for a description of the patients' characteristics.

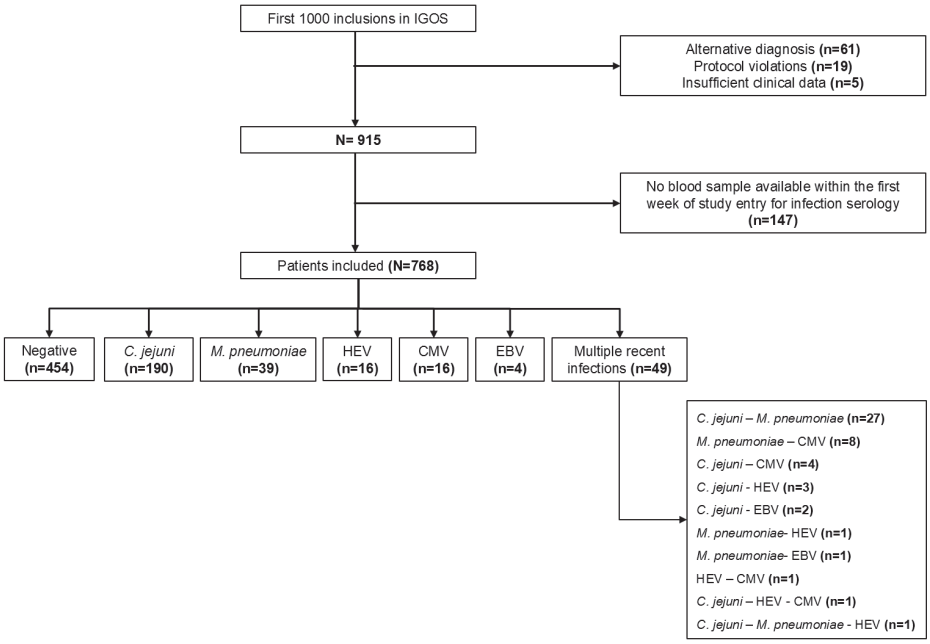


Figure 1. Flowchart
Flowchart of patient inclusions

Infection serology

Laboratory evidence of a recent infection with any of the tested pathogens was found in 314 patients (41%): *C. jejuni* in 228 (30%), *M. pneumoniae* in 77 (10%), HEV in 23 (3%), CMV in 30 (4%) and EBV in 7 (1%) patients. In total, 49 (6%) patients had evidence of two or more recent infections. When only considering mono-infections, *C. jejuni* was found in 190 (25%), *M. pneumoniae* in 39 (5%), HEV in 16 (2%), CMV in 16 (2%) and EBV in 4 (1%) patients.(**Figure 1, Figure 2**)

Two of 22 HEV IgM positive cases, and one of six HEV IgM borderline-positive case were confirmed by PCR, leading to a total of 23 patients with evidence of a recent HEV infection.

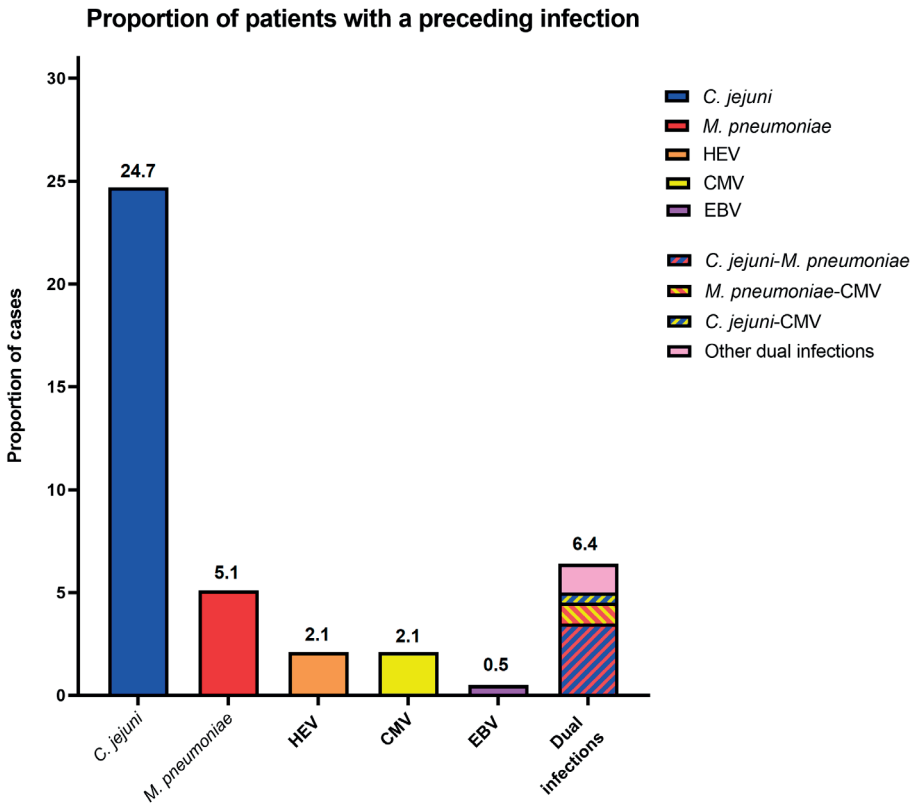


Figure 2. Proportions of preceding infections
HEV= Hepatitis E virus | CMV= Cytomegalovirus | EBV = Epstein Barr virus | Dual infections = Evidence of recent infection with more than one infectious agent. Fifty-nine percent of patients did not have evidence of a recent infection with any of these pathogens.

Blood samples were collected post-IVIg treatment in 230 (30%) patients and in a further 156 (20%) patients it was unknown whether samples were collected pre- or post-treatment. Seventy-three of these patients were IgM positive for CMV and/or EBV and were considered negative for a recent infection for the purpose of this study (see Methods). In a subanalysis where we included these patients as being positive for CMV or EBV, a total of 52 (7%) patients are CMV positive, and 64 (8%) EBV positive. Thirty-three of these patients (45%) had evidence of more than one infection. When excluding patients with evidence of more than one infection in this subanalysis, 24 (3%) are CMV positive and 30 (4%) EBV positive, and 10 patients (1%) positive for both EBV and CMV.

Table 1. Demographics, clinical characteristics and disease course

	All (n=768)	*C. jejuni* (n=190)	*M. pneumoniae* (n=39)	HEV (n=16)	CMV (n=16)	EBV (n=4)	Negative (n=454)	*P*-value
Sex (male)	459 (60)	126 (66)	19 (49)	14 (88)	10 (63)	1 (25)	258 (57)	0.012
Age (years)	51 (32-64)	49 (31-65)	*30 (17-50)**	35 (30-61)	42 (36-57)	24 (17-30)	*56 (37-66)**	**<0.001**
Children (<18 years old)	71 (9)	19 (10)	*12 (31)**	0 (0)	1 (6)	1 (25)	*24 (5)**	**<0.001**
Antecedent events (any)[a]	587 (77)	159 (84)	28 (72)	12 (75)	12 (75)	3 (75)	340 (75)	0.175
Respiratory tract/flu/fever	320 (42)	*42 (22)**	21 (54)	5 (31)	9 (56)	0 (0)	*234 (52)**	**<0.001**
Gastro-enteritis	211 (28)	*111 (58)**	*3 (8)**	4 (25)	2 (13)	1 (25)	*73 (16)**	**<0.001**
Vaccination[b]	23 (3)	4 (2)	2 (5)	2 (13)	0 (0)	1 (25)	13 (3)	0.053
Infectious symptoms - onset weakness (days)	10 (6-15)	9 (6-13)	14 (7-24)	20 (12-23)	13 (9-17)	11 (9-11)	11 (6-16)	0.016
Cranial nerve deficits[c]	381/766 (50)	78 (41)	25 (64)	9 (56)	6 (38)	2 (50)	236/452 (52)	0.040
Oculomotor	121/766 (16)	25 (13)	6 (15)	1 (6)	0 (0)	0 (0)	80/452 (18)	0.265
Facial	226/766 (30)	*39 (21)**	16 (41)	3 (19)	5 (31)	1 (25)	*151/452 (33)**	0.010
Bulbar	204/766 (27)	41 (22)	14 (36)	7 (44)	2 (13)	1 (25)	131/452 (29)	0.086
MRC sum score	46 (33-54)	*42 (24-54)**	45 (36-56)	37 (31-42)	49 (45-54)	51 (34-59)	48 (36-54)	0.025
Sensory symptoms	510 (67)	*90 (47)**	27 (69)	9 (56)	13 (81)	4 (100)	*335 (74)**	**<0.001**
Sensory deficits	465/744 (63)	*82/187 (44)**	23/36 (64)	11 (69)	11 (69)	4 (100)	*311/438 (71)**	**<0.001**
Ataxia	200/741 (27)	39/185 (21)	14/37 (38)	4 (25)	5 (31)	1 (25)	129/437 (30)	0.187
Onset weakness- nadir (days)	8 (4-12)	7 (4-11)	8 (4-13)	10 (7-12)	*12 (8-18)**	11 (5-15)	8 (4-12)	0.038
ICU admission	219 (29)	47 (25)	12 (31)	5 (31)	6 (38)	2 (50)	135 (30)	0.574
Mechanical ventilation	147 (19)	27 (14)	9 (23)	4 (25)	6 (38)	1 (25)	92 (20)	0.121
MRC sum score (nadir)[c]	42 (24-52)	38 (13-52)	44 (18-54)	36 (2-40)	44 (12-54)	50 (28-57)	44 (28-52)	0.079
MRC sum score (6 months)[d]	60 (56-60)	*58 (48-60)**	60 (56-60)	60 (56-60)	60 (60-60)	60 (60-60)	*60 (58-60)**	**<0.001**
Able to walk unaided (nadir)	131/685 (19)	34/168 (20)	4/35 (11)	2/13 (15)	7/15 (47)	2/4 (50)	74/397 (19)	0.055
Able to walk unaided (6 months)[e]	530/646 (82)	*121/163 (74)**	28/31 (90)	10/14 (71)	12/13 (92)	4 (100)	322/382 (84)	0.038

Patients with evidence of >1 recent infection are included in the full cohort and displayed separately in Table 2 and Supplementary Table 3. Data are presented as n/N reported (%) or median (IQR). *P*-value of comparison between *Campylobacter jejuni*, *Mycoplasma pneumoniae*, Hepatitis E virus (HEV), Cytomegalovirus (CMV), Epstein-Barr virus (EBV) and negative group. *=*P*-value is significant after Bonferroni correction for multiple testing (*P*<0.008). Clinical features are at entry. If'unable to examine' features were determined as 'missing', with the exception of ataxia as this is often unable to examine due to severe weakness.
[a]Other events, including urinary tract infection (n=9), surgery (n=8), rash/allergic reaction (n=4), combinations of different antecedent events (n=11), specific infection mentioned (n=21), including CMV or EBV (n=7), varicella zoster (n=3), measles (n=2). [b] influenza (n=12), tetanus (n=4), pertussis (n=3), hepatitis A/B (n=2), diphtheria (n=2), human papilloma virus (n=2), rubella (n=2), mumps (n=1), measles (n=1), yellow fever virus (n=1) and pneumococcus (n=1). [c]Nadir analysis (N=685): *C. jejuni* (n=171), *M. pneumoniae* (n=36), HEV (n=12), CMV (n=15), EBV (n=4), negative (n=402). [d]N=594, 174 patients missing, 33 patients died .[e]Patients who were able to walk at 13 weeks with missing data at week 26 were included in this category.

Demographics and clinical features

See **Table 1** for demographics, clinical features, management and outcome of the full cohort, stratified by mono-infection group. Patients with evidence of more than one recent infection are described separately in **Table 2** and **Supplementary Table 2**.

Patients were included from 19 countries, covering North and South America, Europe, Africa, and Asia. Most Asian patients were from Bangladesh. African patients were from South Africa (n=11) and Ghana (n=1). The proportions of patients testing positive for a recent infection were similar across continents.(**Figure 3**)

An antecedent event was reported in 587 (76%) patients, which included symptoms of an infection in 556 (72%) and other events in 31 (4%). The proportion of patients testing positive for a recent infection did not significantly differ between those who reported antecedent infectious symptoms (238/556 (43%)), and those who did not report such symptoms (67/181 (37%)). Of the patients who reported antecedent events other than symptoms of an infection, 23 reported a vaccination, of whom four received combinations of two or more vaccines.(**Table 1**) Median time between vaccination and onset of weakness was 16 days (IQR 12-26, range 7-36). Evidence of a recent infection with the tested pathogens was found in 10/23 (43%) patients reporting a vaccination. The proportion of patients receiving a vaccination did not significantly differ between those testing positive and negative for a recent infection.

Compared to the other patients, the group of patients who did not report an antecedent event to had a higher proportion of sensorimotor GBS (69% vs 59%, p=0.017). Other demographic or clinical features, including sex, age, proportion of children, continent of residence, electrophysiological subtype, and other GBS variants did not significantly differ.

Table 2. Demographic and clinical features of patients with laboratory evidence of >1 recent infection[a]

	C. jejuni + *M. pneumoniae* (n=27)	*M. pneumoniae* + CMV (n=8)	*C. jejuni* + CMV (n=4)
Sex (male)	16/27 (59)	5/8 (63)	3/4 (75)
Age (y)	26 (12-48)	23 (19-40)	34 (32-48)
Children (<18 years old)	10/27 (37)	2/8 (25)	0 (0)
Antecedent events (any)	17/27 (63)	7/8 (87)	4/4 100)
Respiratory tract infection	3/27 (11)	2/8 (25)	0 (0)
Gastro-enteritis	13/27 (48)	2/8 (25)	1/4 (25)
Flu/fever	0 (0)	1/8 (13)	1/4 (25)
Vaccination	0 (0)	0 (0)	0 (0)
'CMV infection'[b]	0 (0)	2/8 (25)	2/4 (50)
GBS variant			
Sensorimotor	10/27 (37)	7/7 (100)	3/4 (75)
Pure motor	14/27 (52)	0 (0)	0 (0)
MFS/MFS-overlap	3/27 (11)	0 (0)	1/4 (25)
Electrophysiological subtype			
Normal	1/19 (5)	0 (0)	0 (0)
Demyelinating	10/19 (53)	7/7 (100)	3/3 (100)
Axonal	3/19 (16)	0 (0)	0 (0)
Inexitable	0 (0)	0 (0)	0 (0)
Equivocal	5/19 (26)	0 (0)	0 (0)
ICU admission	6/27 (22)	2/8 (25)	1/4 (25)
Mechanical ventilation	5/27 (19)	0 (0)	1/4 (25)
Able to walk unaided (6m)[c]	17/21 (86)	6/6 (100)	3/4 (75)

Only subgroups of multiple recent infections with >3 patients have been included in this table. The other patients with multiple recent infections are displayed in Table 3. Data are presented as n/N reported (%) and median (IQR) [a]Combinations of recent infections with ≤3 cases have not been included in this table. [b]Antecedent event as reported by local investigator: 'fever by CMV infection' and 'CMV infection, IgM positive'. [c]Patients who were able to walk at 13 weeks and had missing data at week 26 have been included in this category

The most frequent clinical features at entry were weakness (683/765, 89%) and hypo- or areflexia (670/766, 88%). The most frequent clinical variant of GBS was the sensorimotor variant in 461 patients (61%), followed by the pure motor variant in 151 (20%). (**Figure 4**) The sensorimotor variant was more common in Europe (72%) and the Americas (68%), and less common in Bangladesh (26%), and other Asian countries (45%). The pure motor variant was more common in Bangladesh (71%), compared to other Asian countries (17%), Europe (11%) or the Americas (7%) ($P<0.001$).

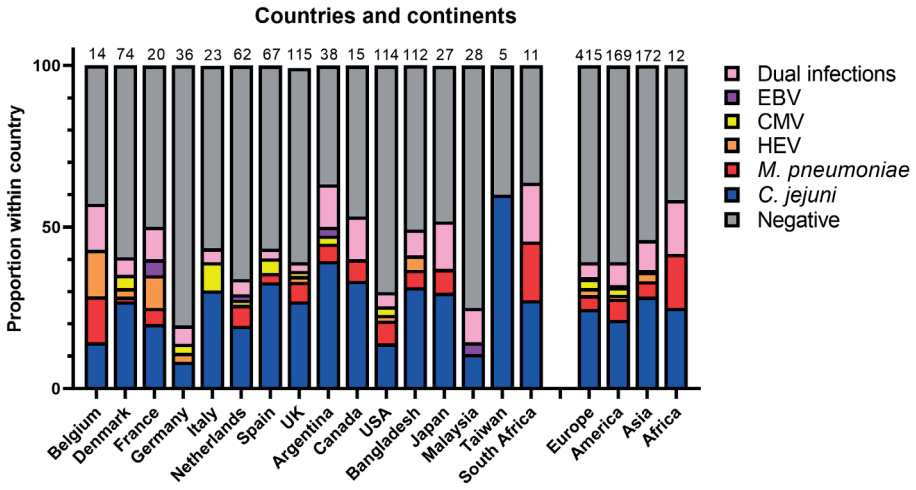


Figure 3. Percentage of tested preceding infections per continent and country of residence
Numbers of total included patient per country and continent displayed on top of each bar. | HEV= Hepatitis E virus | CMV= Cytomegalovirus | EBV = Epstein Barr virus | Dual infections = Evidence of recent infection with more than one infectious agent. Countries with <5 cases not shown: Greece (n=4), Puerto Rico (n=2), Ghana (n=1).

Ancillary investigations, treatment and outcome

In 682 (89%) patients CSF examination was performed. In all patients protein level, and in all but four leukocyte count was reported. Albumino-cytological dissociation was present in 458/678 (68%) patients and the median protein level was 0.66 g/L. Nerve conduction studies were performed in 641 (83%) patients. The most common classification across groups was the demyelinating subtype.(**Figure 4**) The axonal variant was more common in Bangladesh (33%) compared to other Asian countries (4%), Europe (7%) and the Americas (6%) (P<0.001). Of the 118 patients with a pure motor variant of GBS, 49 (42%) had a demyelinating, and 38 (32%) an axonal electrophysiological subtype.

Immunomodulatory treatment was given to 615 (80%) patients, of whom 561 (91%) received IVIg and 49 (8%) plasma exchange. The proportion of patients receiving treatment was lower in Bangladesh (12%) vs other Asian countries (85%), Europe (93%) or the Americas (92%) (P<0.001). At 6 months 530 of 646 patients with available data (82%) were able to walk independently. This proportion was lower in Bangladesh (67%) compared to other Asian countries (94%), Europe (83%) or the Americas (87%) (P=0.001).

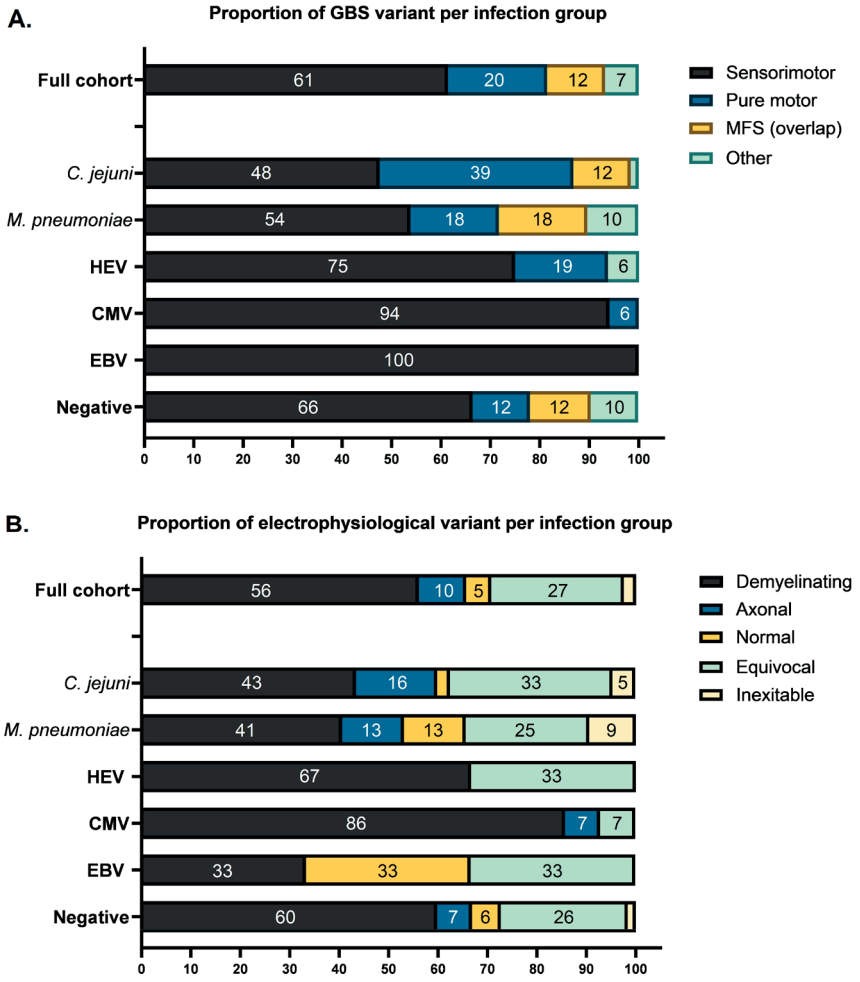


Figure 4. Clinical variant and electrophysiological subtype stratified by infection serology
Clinical variant determined in the first 4 weeks after onset of symptoms stratified by infection serology. N=751 patients with data on clinical variant (=number of cases in full cohort), N=48 patients with dual infections not displayed. (**A**). Electrophysiological subtype according to Hadden criteria stratified by infection serology (**B**). HEV= Hepatitis E virus | CMV= Cytomegalovirus | EBV = Epstein Barr virus | Dual infections = Evidence of recent infection with more than one infectious agent. N=628 patients with data on electrophysiological subtype (=number of cases in full cohort), N=48 patients with evidence of more than one recent infection not displayed.

Comparing infection groups

We compared patients with a mono-infection with *C. jejuni*, *M. pneumoniae*, CMV, EBV, HEV, and patients testing negative.(**Table 1**)

Demography

C. jejuni was the most frequent preceding infection across continents. Infection with CMV was not found in Asian or African patients.(**Figure 3**) The proportions of preceding infections did not significantly differ across continents, or when comparing Bangladesh to other regions. Age was lower ($P<0.0001$) and the proportion of patients <18 was higher ($P<0.0001$) in the *M. pneumoniae* infection group. Gastro-enteritis was more frequent in patients with a *C. jejuni* infection ($P<0.0001$). Respiratory tract infection was most frequently reported in patients with *M. pneumoniae* and CMV infection, although not significantly different from other groups.

Clinical features

Cranial nerve involvement was most common in patients with a preceding *M. pneumoniae*. Frequency of cranial nerve involvement was significantly lower in the *C. jejuni* group. Sensory symptoms or -deficits were most frequent in patients with a CMV and EBV infection, and significantly less common in the *C. jejuni* group. The sensorimotor variant was most commonacross infection groups and higher in the CMV group compared to the other groups ($P=0.007$). The pure motor variant and the axonal subtype were more frequent in the *C. jejuni* group ($P<0.0001$, resp. $P=0.001$). The frequency of the pure motor variant and the axonal subtype in *C. jejuni*-positive patients differed among continents. In 7/36 (19%) American patients with a recent *C. jejuni* infection a pure motor variant was found, versus 27/101 (27%) European, and 39/49 (80%) Asian patients. This variant was more common in *C. jejuni*-positive Bangladeshi patients (32/35, 91%), compared to other Asian countries (7/14 (50%) and the other continents ($P<0.001$). The axonal subtype was found in 13/26 (50%) Bangladeshi patients with a recent *C. jejuni* infection , versus 1/14 (7%) other Asian countries, 1/19 (5%) American, and 10/91 (11%) European patients. The difference between Bangladesh and the other regions was significant ($P<0.001$). The demyelinating subtype was the most common subtype across groups.

Disease severity and outcome

The time between onset of weakness and nadir was longer in patients with a CMV infection ($P=0.004$). MRC sum score at entry was lower in the *C. jejuni* group ($P=0.008$). The proportion of patients able to walk independently at 6 months was lowest in the HEV followed by the *C. jejuni* group. This proportion was lower ($P=0.003$) and the time to reach the ability to walk independently in the first year after onset of weakness longer (Kaplan-Meier log rank test, $P=0.005$) in patients with *C. jejuni* infection.(**Figure 5**) When controlling for age and residency in Bangladesh, recent *C. jejuni* infection remained a significant independent risk factor for a longer time to to walk independently ($P=0.005$).(**Supplementary Table 3**) When controlling for age

and an axonal electrophysiological subtype, *C. jejuni* was no longer an independent risk factor (*P*=0.07).(**Supplementary Table 4**) Almost all patients with a preceding CMV or EBV infection had normal strength and were able to walk independently at 6 months. The median protein level, treatment and ICU admission, mechanical ventilation or mortality did not differ significantly across infection groups.

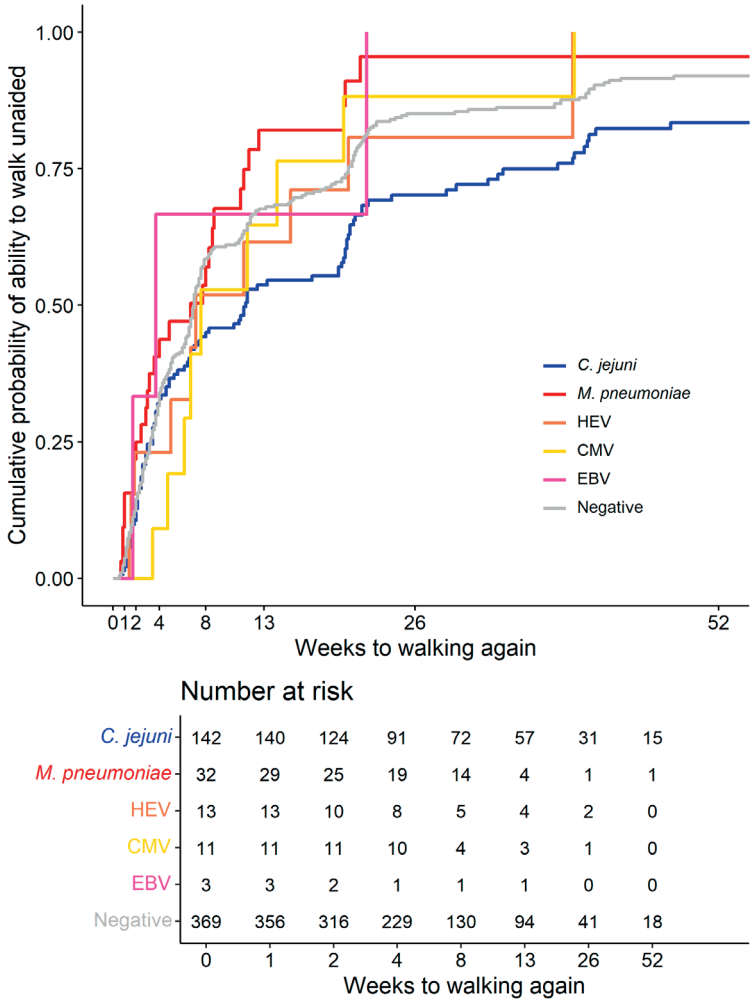


Figure 5. Time to walk independently stratified infection serology

Kaplan-Meier analysis of time to walk independently comparing all infection groups. Total number of patients included in the analysis: *n*=570, patients able to walk at nadir and those with more than one recent infection were excluded. Competing risk analysis was used to correct for time to death. See Supplementary Table 5 for the number of cases at risk in each group at each time point. See Supplementary Figure 1 for the Kaplan-Meier graph of all *C. jejuni* positive cases compared to those with other types of infection or no recent infection.

Multiple preceding infections

The 49 patients with evidence of more than one recent infection are described separately here. The majority had a dual *C. jejuni-M. pneumoniae* infection (*n*=27), followed by a *M. pneumoniae*-CMV infection (*n*=8), and *C. jejuni*-CMV infection (*n*=4) (**Table 2**), the other ten patients with more than one recent infection had <3 patients per subgroup (**Supplementary Table** 3). In some patients with evidence of more than one recent infection, preceding symptoms or clinical features of GBS were more typical for one of the diagnosed infections. Thirteen of 27 (48%) patients with a *C. jejuni-M. pneumoniae* infection had a preceding gastroenteritis, and 2/8 (25%) patients with a CMV-*M. pneumoniae* and 2/4 (50%) with a CMV-*C. jejuni* infection reported mononucleosis-like symptoms or diagnostic evidence of a CMV infection. Patients with a *C. jejuni-M. pneumoniae* infection were younger compared to patients with a *C. jejuni* mono-infection (*P*<0.0001), and patients with a CMV-*M. pneumoniae* infection were younger compared to those with a CMV mono-infection (*P*=0.023). A higher proportion of the pure motor variant was found in patients with a *C. jejuni-M. pneumoniae* compared to a *M. pneumoniae* mono-infection (*P*<0.001). In patients with a *M. pneumoniae*-CMV infection, the sensorimotor variant and demyelinating subtype was more frequent found compared to the *M. pneumoniae* mono-infection group (*P*=0.031, resp. *P*=0.008). ICU admittance, mechanical ventilation or ability to walk independently at 6 months did not differ between patients with multiple preceding infections to those with either one of the two.

DISCUSSION

In this large prospective international cohort study on GBS, *C. jejuni* was the most frequent preceding infection across the studied regions, and the different types of preceding infections were evenly distributed. This suggests that factors beyond the distribution of infections play a role in the regional variation in phenotype and outcome of GBS that has been found in this and previous studies.[15, 19] Laboratory evidence of more than one recent infection was found in 6% of patients. This demonstrates that testing for multiple infections can be useful when looking for the triggering agent in GBS and provides new clues for understanding the pathophysiology of the disease. Fifty-nine percent of cases had no laboratory evidence of a recent infection with the tested agents, which may be in part due to the strict criteria we used for defining a recent infection, or may indicate that infections we did not test for or other causes of immune activation triggered GBS. This may include pathogens that have been associated with GBS in previous studies, including *Haemophilus influenzae*, HIV, varicella zoster, herpes simplex and influenza virus.[1, 2, 26-30] Patients were

included before the Zika virus pandemic, but dengue virus and chikungunya virus may have been missed.[31, 32]

The frequency of a recent *C. jejuni* infection (30%) was comparable to most other cohorts of GBS patients.[2, 33, 34] The frequency of *M. pneumoniae* (10%) was higher compared to previous studies (2-5%), which may be explained by the relatively high proportion of children (9%) in our cohort.[1-3, 34] The frequency of CMV (4%) and EBV infection (1%) was lower compared to most other cohorts which reported CMV in 3-22% and EBV in 1-10%.[1-3, 34] This may be because some of these studies defined recent infection solely on presence of IgM.[1, 2, 34, 35] We based our definition on presence of both IgM and IgG, as IgM presence alone is insufficient evidence of a recent infection, and cross reactivity has been described between CMV and EBV and several other infections, including HEV.[24] We may also have underestimated the amount of CMV and EBV cases because we excluded CMV/EBV IgM positive patients with IgG results indicative of a past infection, in whom post-IVIg samples were used. Nevertheless, in a subanalysis where we included this group, almost half of these patients had evidence of more than one recent infection, likely reflecting the aspecificity of IgM positivity alone as evidence for a recent infection. The frequency of HEV infection was also slightly lower in our cohort (3%) compared to most other cohorts (5-6%), which may because we were unable to test the full cohort for HEV PCR, or because in other studies cross-reactivity was not taken into account.[36-38]

The demographic, clinical and electrophysiological profiles varied according to the preceding infection, suggesting that the type and parts of the nerves affected or the severity of inflammatory nerve damage is influenced by the infectious trigger.[2, 3, 37] In comparing groups, statistically significant differences were mostly found in the *C. jejuni*-positive group or those testing negative; there were no significant differences comparing the patients with a recent HEV, CMV, or EBV infection, but sample sizes were small. As expected, patients with a *C. jejuni* infection were more frequently male with a pure motor axonal variant and severe weakness at nadir.[19, 33] *C. jejuni*-positive patients were also significantly less likely to be able to walk independently at 6 months, and the time to walk independently was significantly longer compared to patients with other infections or those testing negative. When controlling for axonal electrophysiological subtype, the time to walk independently was no longer significantly different between these groups, indicating that the worse prognosis in *C. jejuni* patients is largely explained by a higher proportion of the axonal subtype in this group. Although patients from Bangladesh have limited access to treatment and a worse prognosis, correcting for this factor did not alter the results. *M. pneumoniae* infection was associated with a younger age, confirming results of a previous study.[2]

Patients with a HEV infection were almost all middle aged males, with a sensorimotor demyelinating GBS with severe weakness and bad long-term outcome. Whether HEV is associated with a severe form of GBS should be confirmed in future studies with higher case numbers.[37, 39] Patients with CMV or EBV generally had a sensorimotor demyelinating variant, normal strength and were able to walk independently at 6 months. The time between onset of weakness and nadir was significantly longer in the CMV group, suggesting a slower disease progession in this group, confirming results of a previous study.[20] However, our data also show that clinical profiles are not specific for each infection: the pure motor variant or axonal subtype were not solely reported in patients with a *C. jejuni* infection, and sensorimotor demyelinating GBS was the most frequent phenotype in all identified preceding infections. This contrasts with some previous studies, which indicated that *C. jejuni* is uniquely associated with a pure motor axonal subtype of GBS.[17, 18] These differences may be due to the severity of the infection or immune reponse (a more severe reponse presumably causing more axonal damage), variations in ganglioside distribution in nerve membranes or antibody binding ability, differences in *C. jejuni* strains, or the limited ability of nerve conduction studies to define pathology.[18] Another interesting finding was the relatively high frequency of the demyelinating electrophysiological subtype in patients with a pure motor variant of GBS, although this variant has in previous studies been closely associated with the axonal subtype.[40, 41] This may be due to differences in definition of the variants or electrophysiological subtyping between our and previous studies, or the limitations of the study protocol in capturing sensory signs at all timepoints in the disease course. Nevertheless, this indicates that patients with a primarily demyelinating GBS can present with predominantly motor signs.

The proportions of infections were evenly distributed across regions, comparing Europe to the Americas, Africa (mostly South-African patients), Bangladesh, and other Asian countries. This is a surprising finding, as the higher rate of pure motor and axonal GBS in patients from Bangladesh was previously attributed to a higher frequency of *C. jejuni*.[15, 40] This variation may instead be explained by differences in the association between the preceding infection and GBS phenotype across continents, indicated by the higher rate of the pure motor variant in Asian patients and of the axonal electrophysiological subtype in specifically Bangladeshi patients with a *C. jejuni* infection compared to those from Europe or the Americas. This may be due to regional differences in bacterial or viral strains, host susceptibility, or patient selection, such as the selection of more severe cases in Bangladesh.

Antecedent symptoms of an infection did not always predict the identified preceding infection in our cohort. Of the 181 patients who did not report preceding symptoms of an infection, 67 (37%) had evidence of a recent infection with the tested pathogens. And in 10/23 (43%) patients who reported a vaccination, serological evidence of an infection was found. This indicates that the reported preceding events are not predictive of the demonstrated infection, and broad serological testing is important in defining the most likely infectious trigger in GBS patients. Specifically in light of the vaccine campaign for SARS-CoV-2, this highlights the importance for clinicians and public health officials of thoroughly investigating other infectious causes in patients developing GBS in the weeks after receiving a vaccine, because co-incidences may happen.[42]

The 49 patients with evidence of more than one recent infection may represent coincidental infections, or may indicate that presence of several recent infections causes a more robust immune response, increasing the risk of developing GBS. This 'dual hit hypothesis' may also in part explain why some people develop GBS after certain common infections, while others do not.[43] Another explanation is polyclonal B-cell activation as a response to one infection, which may lead to false positive serologic tests results for others. Polyclonal B-cell activation may be caused by cytokines, superantigens, direct infection of B cells or Toll-like receptor mediated B-cell activation.[44, 45] The same may apply to patients with evidence of a recent infection who reported a recent vaccination, although coincidence is more likely in these cases as insufficient evidence exists of an association between GBS and most vaccines.[46] Some patients with evidence of more than one recent infection had symptoms more typical for one of the infections. Whether this suggests that one infection is the 'true trigger' of GBS and the other is a false-positive, or that one of the infections produces predominant symptoms, is not clear.[47, 48] Correlating our findings with serum anti-glycolipid antibody profiles in future studies may be informative in discriminating which infection triggered GBS.

Our study has several limitations. First, we are unable to determine if the studied infections occur more frequently in GBS patients compared to the general population this due to the absence of a control group, although this is plausible, based on evidence from previous case-control studies and the association with clinical phenotypes in our cohort..[2, 3, 33, 37] We could only find two population-wide IgM seroprevalence studies, in which the HEV IgM seroprevalence was magnitudes lower than that found in our study (0.1-0.5% vs 4%).[49, 50] Second, the cohort may not be representative of GBS in the general population, as participating IGOS centers are often academic or teaching hospitals, and several regions, especially the South American, African

and parts of the Asian continent, are underrepresented. Third, we used strict criteria for determining a recent infection and therefore may have incorrectly labeled some patients as negative. Conversely, as we were not able to perform PCR or culture, we may have missed some cases that had not (yet) mounted a serological response.

In conclusion, *C. jejuni* was the most common preceding infection in this large international study on GBS, and infections were evenly distributed across the studied regions. Although testing for infections in GBS patients often has been limited to research activities, our findings suggest that serological studies may have a role in the evaluation of GBS patients in clinical practice by providing information about the clinical course and long-term prognosis. Broad serological testing is advised when looking for the triggering agent in GBS patients as dual infections occur and antecedent infectious symptoms are non-specific in predicting the underlying infection.

REFERENCES

1. Boucquey D, Sindic CJ, Lamy M, Delmee M, Tomasi JP, Laterre EC. Clinical and serological studies in a series of 45 patients with Guillain-Barre syndrome. *J Neurol Sci* 1991;104:56-63.
2. Jacobs BC, Rothbarth PH, van der Meche FG, et al. The spectrum of antecedent infections in Guillain-Barré syndrome: a case-control study. *Neurology* 1998;51:1110-1115.
3. Caudie C, Quittard Pinon A, Taravel D, et al. Preceding infections and anti-ganglioside antibody profiles assessed by a dot immunoassay in 306 French Guillain-Barre syndrome patients. *J Neurol* 2011;258:1958-1964.
4. Styczynski AR, Malta J, Krow-Lucal ER, et al. Increased rates of Guillain-Barré syndrome associated with Zika virus outbreak in the Salvador metropolitan area, Brazil. *PLoS Negl Trop Dis* 2017;11:e0005869.
5. Mehta R, Soares CN, Medialdea-Carrera R, et al. The spectrum of neurological disease associated with Zika and chikungunya viruses in adults in Rio de Janeiro, Brazil: A case series. *PLoS Negl Trop Dis* 2018;12:e0006212.
6. Umapathi T, Lim CS, Ooi EE, et al. Asymptomatic dengue infection may trigger Guillain-Barre syndrome. *J Peripher Nerv Syst* 2016;21:375-377.
7. Brannagan TH, 3rd, Zhou Y. HIV-associated Guillain-Barré syndrome. *J Neurol Sci* 2003;208:39-42.
8. Hiew FL, Ramlan R, Viswanathan S, Puvanarajah S. Guillain-Barré Syndrome, variants & forms fruste: Reclassification with new criteria. *Clin Neurol Neurosurg* 2017;158:114-118.
9. Principi N, Esposito S. Vaccine-preventable diseases, vaccines and Guillain-Barré syndrome. *Vaccine* 2019;37:5544-5550.
10. Hocker S, Nagarajan E, Rubin M, Wijdicks EFM. Clinical factors associated with Guillain-Barré syndrome following surgery. *Neurol Clin Pract* 2018;8:201-206.
11. De Sanctis P, Doneddu PE, Vigano L, Selmi C, Nobile-Orazio E. Guillain-Barre syndrome associated with SARS-CoV-2 infection. A systematic review. *Eur J Neurol* 2020;27:2361-2370.
12. Willison HJ, Jacobs BC, van Doorn PA. Guillain-Barré syndrome. *Lancet* 2016;388:717-727.
13. Yuki N, Susuki K, Koga M, et al. Carbohydrate mimicry between human ganglioside GM1 and Campylobacter jejuni lipooligosaccharide causes Guillain-Barre syndrome. *Proc Natl Acad Sci U S A* 2004;101:11404-11409.
14. Kuwahara M, Samukawa M, Ikeda T, et al. Characterization of the neurological diseases associated with Mycoplasma pneumoniae infection and anti-glycolipid antibodies. *J Neurol* 2017;264:467-475.
15. Doets AY, Verboon C, van den Berg B, et al. Regional variation of Guillain-Barre syndrome. *Brain* 2018;141:2866-2877.
16. Leonhard SE, Mandarakas MR, Gondim FAA, et al. Diagnosis and management of Guillain-Barre syndrome in ten steps. *Nat Rev Neurol* 2019;15:671-683.
17. Drenthen J, Yuki N, Meulstee J, et al. Guillain-Barré syndrome subtypes related to Campylobacter infection. *J Neurol Neurosurg Psychiatry* 2011;82:300-305.
18. Kuwabara S, Ogawara K, Misawa S, et al. Does Campylobacter jejuni infection elicit "demyelinating" Guillain-Barre syndrome? *Neurology* 2004;63:529-533.
19. Islam Z, Jacobs BC, van Belkum A, et al. Axonal variant of Guillain-Barré syndrome associated with Campylobacter infection in Bangladesh. *Neurology* 2010;74:581-587.

20. Visser LH, van der Meche FG, Meulstee J, et al. Cytomegalovirus infection and Guillain-Barré syndrome: the clinical, electrophysiologic, and prognostic features. Dutch Guillain-Barre Study Group. *Neurology* 1996;47:668-673.
21. Jacobs BC, van den Berg B, Verboon C, et al. International Guillain-Barré Syndrome Outcome Study: protocol of a prospective observational cohort study on clinical and biological predictors of disease course and outcome in Guillain-Barré syndrome. *J Peripher Nerv Syst* 2017;22:68-76.
22. Hadden RD, Karch H, Hartung HP, et al. Preceding infections, immune factors, and outcome in Guillain-Barré syndrome. *Neurology* 2001;56:758-765.
23. Hadden RD, Cornblath DR, Hughes RA, et al. Electrophysiological classification of Guillain-Barre syndrome: clinical associations and outcome. Plasma Exchange/Sandoglobulin Guillain-Barre Syndrome Trial Group. *Ann Neurol* 1998;44:780-788.
24. Hyams C, Mabayoje DA, Copping R, et al. Serological cross reactivity to CMV and EBV causes problems in the diagnosis of acute hepatitis E virus infection. *J Med Virol* 2014;86:478-483.
25. Bewick V, Cheek L, Ball J. Statistics review 12: survival analysis. *Crit Care* 2004;8:389-394.
26. Ju YY, Womersley H, Pritchard J, Gray I, Hughes RA, Gregson NA. Haemophilus influenzae as a possible cause of Guillain-Barré syndrome. *J Neuroimmunol* 2004;149:160-166.
27. Thornton CA, Latif AS, Emmanuel JC. Guillain-Barre syndrome associated with human immunodeficiency virus infection in Zimbabwe. *Neurology* 1991;41:812-815.
28. Stowe J, Andrews N, Wise L, Miller E. Investigation of the temporal association of Guillain-Barré syndrome with influenza vaccine and influenzalike illness using the United Kingdom general practice research database. *American Journal of Epidemiology* 2009;169:382-388.
29. Tam CC, O'Brien SJ, Petersen I, Islam A, Hayward A, Rodrigues LC. Guillain-Barré Syndrome and Preceding Infection with Campylobacter, Influenza and Epstein-Barr Virus in the General Practice Research Database. *PLOS ONE* 2007;2:e344.
30. Islam B, Islam Z, GeurtsvanKessel CH, et al. Guillain-Barre syndrome following varicella-zoster virus infection. *Eur J Clin Microbiol Infect Dis* 2018;37:511-518.
31. Grijalva I, Grajales-Muñiz C, González-Bonilla C, et al. Zika and dengue but not chikungunya are associated with Guillain-Barré syndrome in Mexico: A case-control study. *PLoS Negl Trop Dis* 2020;14:e0008032.
32. Stegmann-Planchard S, Gallian P, Tressières B, et al. Chikungunya, a Risk Factor for Guillain-Barré Syndrome. *Clinical Infectious Diseases* 2019.
33. Rees JH, Soudain SE, Gregson NA, Hughes RA. Campylobacter jejuni infection and Guillain-Barré syndrome. *N Engl J Med* 1995;333:1374-1379.
34. Hao Y, Wang W, Jacobs BC, et al. Antecedent infections in Guillain-Barre syndrome: a single-center, prospective study. *Ann Clin Transl Neurol* 2019;6:2510-2517.
35. Jacobs BC, van Doorn PA, Groeneveld JH, Tio-Gillen AP, van der Meche FG. Cytomegalovirus infections and anti-GM2 antibodies in Guillain-Barré syndrome. *J Neurol Neurosurg Psychiatry* 1997;62:641-643.
36. Stevens O, Claeys KG, Poesen K, Saegeman V, Van Damme P. Diagnostic Challenges and Clinical Characteristics of Hepatitis E Virus-Associated Guillain-Barre Syndrome. *JAMA Neurol* 2017;74:26-33.
37. Van den Berg B, van der Eijk AA, Pas SD, et al. Guillain-Barré syndrome associated with preceding hepatitis E virus infection. *Neurology* 2014;82:491-497.

38. Fukae J, Tsugawa J, Ouma S, Umezu T, Kusunoki S, Tsuboi Y. Guillain-Barre and Miller Fisher syndromes in patients with anti-hepatitis E virus antibody: a hospital-based survey in Japan. *Neurol Sci* 2016;37:1849-1851.
39. Geurtsvankessel CH, Islam Z, Mohammad QD, Jacobs BC, Endtz HP, Osterhaus AD. Hepatitis E and Guillain-Barré syndrome. *Clin Infect Dis* 2013;57:1369-1370.
40. Kuwabara S, Yuki N. Axonal Guillain-Barré syndrome: concepts and controversies. *Lancet Neurol* 2013;12:1180-1188.
41. Visser LH, Van der Meche FG, Van Doorn PA, et al. Guillain-Barre syndrome without sensory loss (acute motor neuropathy). A subgroup with specific clinical, electrodiagnostic and laboratory features. Dutch Guillain-Barre Study Group. *Brain* 1995;118 (Pt 4):841-847.
42. Keddie S, Pakpoor J, Mousele C, et al. Epidemiological and cohort study finds no association between COVID-19 and Guillain-Barré syndrome. *Brain* 2020.
43. Huizinga R, van den Berg B, van Rijs W, et al. Innate Immunity to Campylobacter jejuni in Guillain-Barré Syndrome. *Ann Neurol* 2015;78:343-354.
44. Kuijf ML, Samsom JN, van Rijs W, et al. TLR4-Mediated Sensing of Campylobacter jejuni by Dendritic Cells Is Determined by Sialylation. *The Journal of Immunology* 2010;185:748-755.
45. Nothelfer K, Sansonetti PJ, Phalipon A. Pathogen manipulation of B cells: the best defence is a good offence. *Nat Rev Microbiol* 2015;13:173-184.
46. Principi N, Esposito S. Vaccine-preventable diseases, vaccines and Guillain-Barré syndrome. *Vaccine* 2018.
47. Zanghellini F, Boppana SB, Emery VC, Griffiths PD, Pass RF. Asymptomatic primary cytomegalovirus infection: virologic and immunologic features. *J Infect Dis* 1999;180:702-707.
48. Abbott RJ, Pachnio A, Pedroza-Pacheco I, et al. Asymptomatic Primary Infection with Epstein-Barr Virus: Observations on Young Adult Cases. *J Virol* 2017;91:e00382-00317.
49. Ding X, Li TC, Hayashi S, et al. Present state of hepatitis E virus epidemiology in Tokyo, Japan. *Hepatol Res* 2003;27:169-173.
50. Fukuda S, Ishikawa M, Ochiai N, et al. Unchanged high prevalence of antibodies to hepatitis E virus (HEV) and HEV RNA among blood donors with an elevated alanine aminotransferase level in Japan during 1991–2006. *Archives of Virology* 2007;152:1623-1635.
51. Ang CW, Krogfelt K, Herbrink P, et al. Validation of an ELISA for the diagnosis of recent Campylobacter infections in Guillain-Barré and reactive arthritis patients. *Clin Microbiol Infect* 2007;13:915-922.
52. Koning L, Pas SD, de Man RA, et al. Clinical implications of chronic hepatitis E virus infection in heart transplant recipients. *J Heart Lung Transplant* 2013;32:78-85.
53. Pas SD, de Man RA, Mulders C, et al. Hepatitis E virus infection among solid organ transplant recipients, the Netherlands. *Emerg Infect Dis* 2012;18:869-872.
54. Gyarmati P, Mohammed N, Norder H, Blomberg J, Belák S, Widén F. Universal detection of hepatitis E virus by two real-time PCR assays: TaqMan and Primer-Probe Energy Transfer. *J Virol Methods* 2007;146:226-235.
55. Tribble DR, Baqar S, Scott DA, et al. Assessment of the Duration of Protection in Campylobacter jejuni; Experimental Infection in Humans. *Infection and Immunity* 2010;78:1750.
56. El-Kamary SS, Strickland GT. Hepatitis, Viral. In: Quah SR, ed. International Encyclopedia of Public Health (Second Edition). Oxford: Academic Press, 2017: 611-620.

57. Baylis SA, Blümel J, Mizusawa S, et al. World Health Organization International Standard to harmonize assays for detection of hepatitis E virus RNA. *Emerging infectious diseases* 2013;19:729-735.
58. Moule JH, Caul EO, Wreghitt TG. The specific IgM response to Mycoplasma pneumoniae infection: interpretation and application to early diagnosis. *Epidemiol Infect* 1987;99:685-692.
59. Prince HE, Lapé-Nixon M. Role of cytomegalovirus (CMV) IgG avidity testing in diagnosing primary CMV infection during pregnancy. *Clin Vaccine Immunol* 2014;21:1377-1384.
60. Revello MG, Gerna G. Diagnosis and Management of Human Cytomegalovirus Infection in the Mother, Fetus, and Newborn Infant. *Clinical Microbiology Reviews* 2002;15:680.
61. Delforge ML, Desomberg L, Montesinos I. Evaluation of the new LIAISON(®) CMV IgG, IgM and IgG Avidity II assays. *J Clin Virol* 2015;72:42-45.
62. De Paschale M, Clerici P. Serological diagnosis of Epstein-Barr virus infection: Problems and solutions. *World J Virol* 2012;1:31-43.
63. François C, Segard C, Bouvier M, et al. Comparison of Abbott Architect(®), Siemens Immulite(®), and Diasorin Liaison(®) for determination of Epstein-Barr virus serological diagnosis. *Diagn Microbiol Infect Dis* 2018;90:96-101.
64. Lupo J, Germi R, Semenova T, Buisson M, Seigneurin JM, Morand P. Performance of two commercially available automated immunoassays for the determination of Epstein-Barr virus serological status. *Clin Vaccine Immunol* 2012;19:929-934.

SUPPLEMENTARY MATERIAL

Methods

Antibodies against *C. jejuni* were determined for all patients using an indirect enzyme-linked immunosorbent assay (ELISA) for IgG and antibody class capture ELISAs for IgM and IgA antibodies, as previously described.[51] The presence of anti-*C. jejuni* antibodies was expressed as a ratio of optical density between a test sample and a reference serum sample.(**Supplementary Table 1**) IgM and IgG antibodies against HEV were determined for all patients using commercially available ELISAs (Wantai, Beijing, PR China), according to the manufacturer's instructions. In all positive and borderline positive samples, the ELISA was repeated and these samples were screened for HEV RNA by an internally controlled quantitative real-time reverse transcription PCR (RT-PCR), as described previously.[52, 53] Because of insufficient sample volume, samples from Bangladesh were tested locally for HEV RT-PCR according to previously described methods.[54] Presence of anti-*M. pneumoniae* IgM and IgG antibodies was tested using a commercially available ELISA (Serion ELISA classic *M. pneumoniae*, Serion GmbH, Würzburg, Germany). In IgM positive or borderline-positive samples, ELISA was repeated. Discrepancies between the repeated ELISA results were arbitrated by one of the authors (AAvdE). The presence of IgM and IgG antibodies and IgG avidity against CMV and of viral capsid antigen (VCA) IgM and VCA IgG and EBV nuclear antigen (EBNA), was determined by LIAISON®XL (DiaSorin, Italy); a semi-automated system, which uses chemiluminescent immunoassay (CLIA) technology for detection of antibodies in human serum samples.

Supplementary Table 1. Diagnostic tests and definition or recent infection

Pathogen	Diagnostic tests	Location	Definition recent infection	Immunoglobulin persistance	Sensitivity / Specificity
C. jejuni	ELISA IgM, IgA, IgG	SSDZ, Delft, The Netherlands	Optical density ratio between a test sample and a reference serum sample for IgM and/or IgA antibodies higher than 1.0	IgM and IgA appear shortly after acute infection and persist for weeks up to 2 months[55]	IgM and/or IgA: 96% / 95% IgG: 96% / 95%[51]
HEV	ELISA IgM, IgG, PCR	Dept of Viroscience, Erasmus MC, Rotterdam, The Netherlands	>1.1 signal-to-noise ratio for IgM upon repeated testing and/or PCR positivity. IgM values between 0.9-1.1 in one or repeated samples were considered positive if IgG levels were elevated (>1.1).	IgM appears shortly after acute infection, falls to undetectable levels within weeks, but can last up to 6 months in some patients[56]	IgM: 97% / 98% IgG: 99% / 99,9% (Wantai) RNA: lower limit of detection (95% hit rate) of 143 IU/ml. World Health Organization standard.[57]
M. pneumoniae	ELISA IgM, IgG	Dept of Viroscience, Erasmus MC, Rotterdam, The Netherlands	IgM >17 AU/ml upon repeated testing. IgM 13-17 AU/ml values in one or repeated samples were considered positive if IgG levels were elevated (>30 AU/ml for adults and >15AU/mL for children)	IgM appears at ±7 days after acute infection, peaks at 10-30 days, and falls to undetectable levels within 3-6 months[58]	IgM: 96 % / 93% IgG: >99% / 89% (Serion)
CMV	IgM, IgG, avidity	Dept of Viroscience, Erasmus MC, Rotterdam, The Netherlands	pre-IVIg : -positive IgM (>22,0 AU/mL) and negative IgG (<14 AU/mL) or positive IgG (>14 AU/mL) with low (<0.15 index value) avidity -negative IgM (<22 AU/mL) and positive IgG with low avidity post-IVIg: -positive IgM and negative IgG or positive IgG with low avidity -positive IgM and positive IgG with high avidity are considered negative in this study	IgM appears <10 days after infection, usually drops after 2-3 months, but can persist up to a year. Can be positive upon reactivation. IgG avidity only low after primo infection, remains low <2 months after onset in most[59, 60]	IgM: 90.1% / 99.1%*[61] IgG: 99.5% / 99.7%*

Pathogen	Diagnostic tests	Location	Definition recent infection	Immunoglobulin persistance	Sensitivity / Specificity
EBV	IgG EBNA, IgM VCA, IgG VCA	Dept of Viroscience, Erasmus MC, Rotterdam, The Netherlands	pre-IVIg: - negative EBNA (<5 U/mL) and positiveVCA IgM (>40U/mL) and IgG (>40U/mL) -negative EBNA and VCA IgG and positive VCA IgM post-IVIg: identical to pre-IVIg, with the exception of samples with positive EBNA and positive VCA IgM and IgG – these samples are considered negative in this study	VCA IgM: appears during acute infection and usually disappears after a few weeks, but may persist for several months. IgG appears during acute infection and stays for life[62]	IgM: 97.8% / 99.2%*[63, 64] EBNA: 98.8% / 97.6%*

*According to DiaSorin Assay prescription, based on the referenced studies

Supplementary Table 2. Demographic and clinical features of patients with evidence of >1 recent infection, with ≤3 cases per subgroup

	Sex and age	Antecedent event	GBS clinical variant	Electrophysiological subtype	ICU & Ventilation	Outcome 6m
C. jejuni + HEV (n=3)	2/3 Male 50-60 y/o	1/3 RTI 2/3 None	2/3 Sensorimotor 1/3 Pure motor	3/3 Demyelinating	2/3 ICU 1/3 Ventilated	1/2 GBSDS 2 1/2 GBSDS 6
C. jejuni + EBV (n=2)	2 y/o Male & 48 y/o Male	1/2 GE 1/2 None	1/2 Pure motor 1/2 Ataxic form	1/1 Equivocal	None	NR
M. pneumoniae + HEV (n=1)	28 y/o Male	RTI	MFS	No EMG	Not admitted to ICU or ventilated	Healthy state
M. pneumoniae + EBV (n=1)	18 y/o Female	"Symptoms of EBV"	Sensorimotor	Equivocal	Not admitted to ICU or ventilated	Able to walk unaided
HEV + CMV (n=1)	20 y/o Male	Vaccination (undefined)	Sensorimotor	Demyelinating	ICU & Ventilated	NR
C. jejuni + HEV- CMV (n=1)	56 y/o Male	None	Sensorimotor	Demyelinating	Not admitted to ICU or ventilated	Able to walk unaided
C. jejuni +*M. pneumoniae* + HEV (n=1)	51 y/o Female	None	Sensorimotor	Demyelinating	Not admitted to ICU or ventilated	NR

RTI= Respiratory tract infection | GE = gastro-enteritis | NR= not reported | MFS= Miller Fisher syndrome | GBSDS= GBS disability score

Supplementary Table 3. Cox Regression analysis for the time to walk unaided, controlling for age and residency in Bangladesh

	Coef	*P*	95% CI – coef
C. jejuni	-0.299	0.007	-0.52 - -0.08
Age	-0.010	3.25e-05	-0.015 - -0.005
Bangladesh	-0.415	0.003	-0.690 - -0.139

Comparing all patients with a *C. jejuni*-infection to all patients with evidence of another infection or those tested negative. Patients able to walk at nadir, or with insufficient follow-up data to determine time to walk, were excluded from this analysis (total included patients *n*=570).

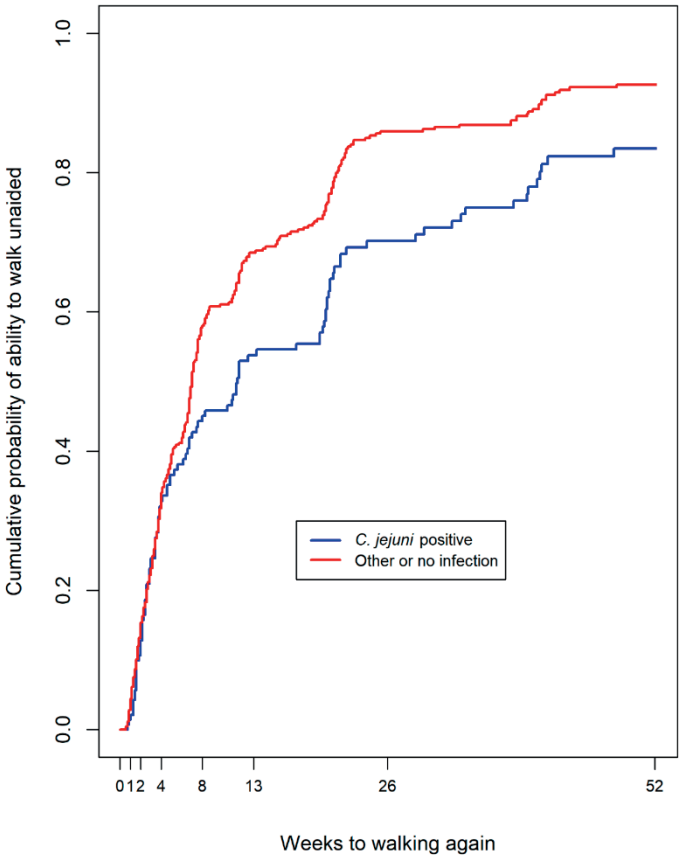
Supplementary Table 4. Cox Regression analysis for the time to walk unaided, controlling for age and axonal electrophysiological subtype

	Coef	*P*	95% CI – coef
C. jejuni	-0.22	0.070	-0.46 – 0.02
Age	-0.01	0.0001	-0.02 – -0.005
Axonal	-0.55	0.004	-0.92 – -0.17

Comparing all patients with a *C. jejuni*-infection to all patients with evidence of another infection or those tested negative, excluding the patients with evidence of more than one recent infection. Patients able to walk at nadir, with insufficient follow-up data to determine time to walk, and those without nerve conduction studies were excluded from this analysis (total included patients *n*=495).

Supplementary Table 5. Number of cases 'at risk' to be able to walk again at each time point

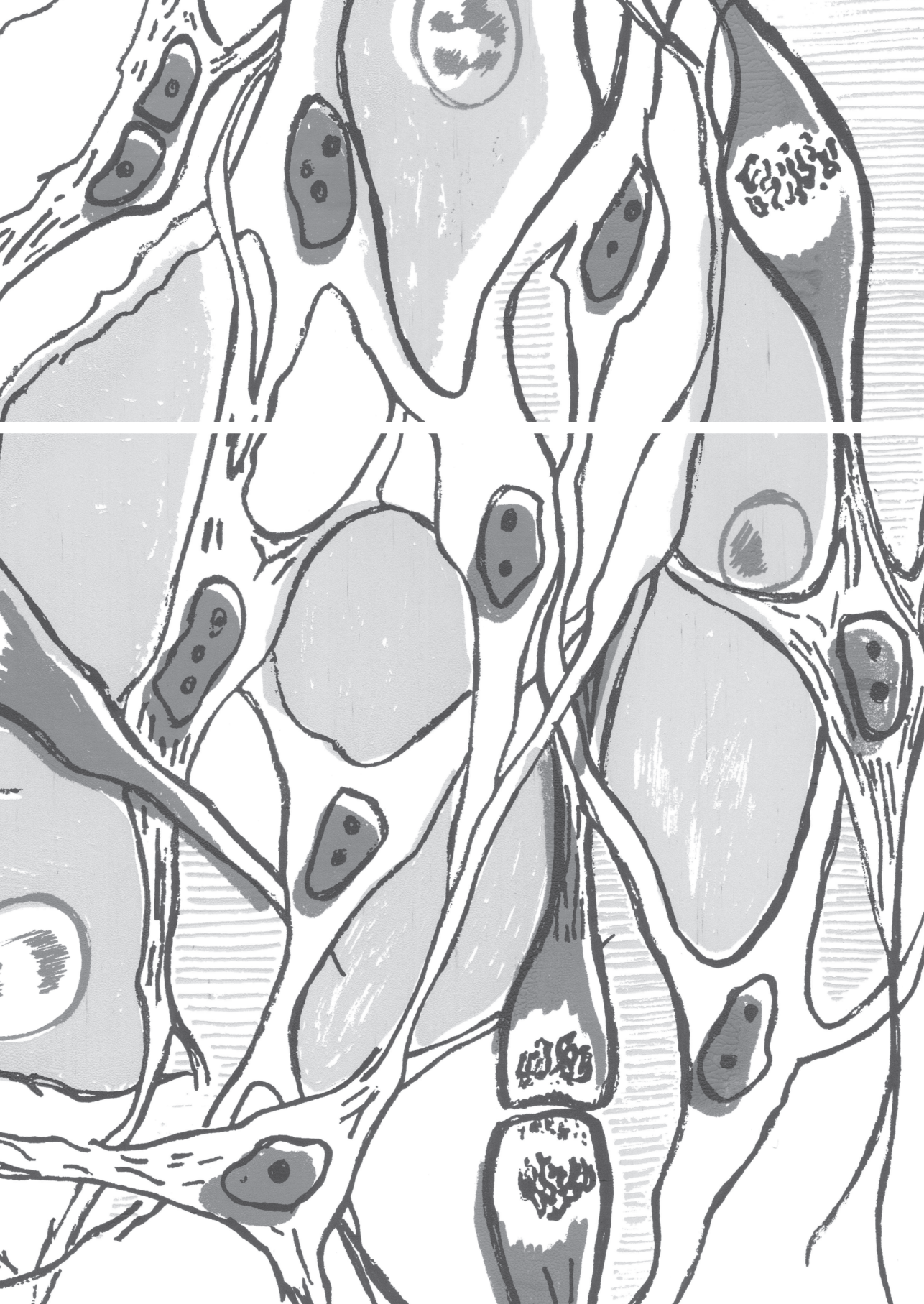
	0	week 1	week 2	week 4	week 8	week 13	week 26	week 52
C. jejuni	142 (0)	140 (0)	124 (0)	91 (2)	72 (4)	57 (6)	31 (11)	15 (14)
M. pneumoniae	32 (0)	29 (0)	25 (0)	19 (0)	14 (0)	4 (1)	1 (2)	1 (2)
HEV	13 (0)	13 (0)	10 (0)	8 (0)	5 (1)	4 (1)	2 (1)	0 (1)
CMV	11 (0)	11 (0)	11 (0)	10 (0)	4 (2)	3 (2)	1 (2)	0 (2)
EBV	3 (0)	3 (0)	2 (0)	1 (0)	1 (0)	1 (0)	0 (0)	0 (0)
Negative	369 (0)	356 (3)	316 (4)	229 (18)	130 (25)	94 (29)	41 (32)	18 (34)
Total	570 (0)	552 (3)	488 (4)	358 (20)	226 (32)	163 (39)	76 (48)	34 (53)



Supplementary Figure 1. Time to walk independently comparing *C. jejuni* positive cases to all other cases Total number of patients included in the analysis: *n*=570, patients able to walk at nadir and those with more than one recent infection were excluded. Competing risk analysis was used to correct for time to death.

Part II

Guillain-Barré syndrome during the Zika virus epidemic



Chapter 2

Guillain-Barré syndrome related to Zika virus infection: a systematic review and meta-analysis of the clinical and electrophysiological phenotype

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ABSTRACT

Background

The Zika virus (ZIKV) has been associated with Guillain-Barré syndrome (GBS) in epidemiological studies. Whether ZIKV-associated GBS is related to a specific clinical or electrophysiological phenotype has not been established. To this end, we performed a systematic review and meta-analysis of all published studies on ZIKV-related GBS.

Methods

We searched Pubmed, EMBASE and LILACS, and included all papers, reports or bulletins with full text in English, Spanish or Portuguese, reporting original data of patients with GBS and a suspected, probable or confirmed recent ZIKV infection. Data were extracted according to a predefined protocol, and pooled proportions were calculated.

Results

Thirty-five studies were included (13 single case reports and 22 case series, case-control or cohort studies), reporting on a total of 601 GBS patients with a suspected, probable or confirmed ZIKV infection. Data from 21 studies and 587 cases were available to be summarized. ZIKV infection was confirmed in 21%, probable in 22% and suspected in 57% of cases. ZIKV PCR was positive in 30% (95% CI 15-47) of tested patients. The most common clinical features were: limb weakness 97% (95% CI 93-99), diminished/absent reflexes 96% (95% CI 88-100), sensory symptoms 82% (95% CI 76-88), and facial palsy 51% (95% CI 44-58). Median time between infectious and neurological symptoms was 5-12 days. Most cases had a demyelinating electrophysiological subtype and half of cases were admitted to the Intensive Care Unit (ICU). Heterogeneity between studies was moderate to substantial for most variables.

Conclusions

The clinical phenotype of GBS associated with ZIKV infection reported in literature is generally a sensorimotor demyelinating GBS with facial palsy and a severe disease course often necessitating ICU admittance. Time between infectious and neurological symptoms and negative PCR in most cases suggests a post-infectious disease mechanism. Heterogeneity between studies was considerable and results may be subject to reporting bias. This study was registered on the international Prospective Register of Systematic Reviews (CRD42018081959).

INTRODUCTION

Guillain-Barré syndrome (GBS) is the most common cause of acute flaccid paralysis worldwide, with an incidence rate of approximately 1 per 100,000 person-years.[1] GBS is an acute immune-mediated polyradiculoneuropathy, and is presumed to be triggered by preceding infections with specific pathogens, such as *Campylobacter jejuni*, cytomegalovirus (CMV), and Epstein-Barr virus (EBV).[2] Recently, the incidence of GBS increased during Zika virus (ZIKV) epidemics in French Polynesia (2013) and Latin America (2015-2016) and an association between GBS and ZIKV was established through epidemiological studies.[3, 4]

The classic form of GBS is characterized by a rapidly progressive and symmetrical weakness of the limbs, with sensory symptoms and reduced or absent tendon reflexes.[4] Cranial nerve involvement is frequent, with facial and bulbar muscles most often affected.[5] Electrophysiological studies help to confirm the diagnosis of GBS, and can indicate different subtypes, including acute inflammatory demyelinating polyradiculoneuropathy (AIDP), acute motor axonal neuropathy (AMAN), and acute motor and sensory axonal neuropathy (AMSAN).[4] The majority of patients will lose the ability to walk during the acute phase of the disease and about 25% of patients need to be mechanically ventilated at the Intensive Care Unit (ICU).[6] Clinical presentation and severity of GBS can vary extensively between patients. This variability is thought to be, in part, caused by differences in the type of preceding infections. For instance, *C. jejuni* has been associated with a pure motor axonal form of GBS with a severe disease course, while CMV has been linked to a sensorimotor GBS with pronounced respiratory insufficiency.[6-8]

Since the ZIKV epidemics, numerous studies have been published on ZIKV-related GBS, but it has not been established if there is a specific clinical and electrophysiological phenotype of GBS after ZIKV, and whether this differs from GBS triggered by other pathogens.[3, 4] Therefore, we have performed a systematic review and meta-analysis of all published studies on ZIKV-related GBS, and give a comprehensive overview of demographic characteristics, clinical features, diagnostic investigations, and outcome of ZIKV-related GBS patients.

METHODS

This systematic literature review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and was registered on the

international Prospective Register of Systematic Reviews (PROSPERO) with number CRD42018081959.[9]

Information sources and search strategy

First, by selecting key words from relevant articles, search strategies were constructed for the Pubmed, EMBASE and LILACS databases (**Figure 1**), which were searched on 24 November 2017 and on 24 January 2019. Second, the titles and abstracts were screened by two researchers (JDLB and SC) to identify the key words ('Guillain-Barre Syndrome', 'viruses', 'virus', 'Zika virus' and 'Zika'), and to exclude *in vitro* or *in animal* studies, and reports from meetings and congresses. The selected papers were read in full by two independent reviewers (CCBS, MFPMA) and a third reviewer (SEL) was consulted in case of disagreement.

We included all papers, reports or bulletins with available full text in English, Spanish or Portuguese, without restriction in year of publication, reporting original data of patients with GBS and a suspected, probable or confirmed recent ZIKV infection, of any age, gender and in any setting. Predefined exclusion criteria were: GBS within 3 months after a vaccination or other proven triggering infection (e.g. *C. jejuni*), and studies with no information on age, residence, and at least one clinical variable of interest. When the study population of reported cases overlapped with cases published in other papers, the paper reporting the highest amount of cases was included. When only part of the cases in a study fulfilled our inclusion criteria, only these cases were included, but if separate data of these cases were not available after contacting the corresponding author, the article was excluded.

Data extraction and management

Data were extracted independently by one of three reviewers (CCBS, MFPMA, SEL) according to a predefined protocol. The data extraction was then checked by one of the other two reviewers, and discrepancies were solved by discussion among all of them. Variables of interest comprised demographics, clinical characteristics (symptoms and signs of arbovirus infection and GBS), ancillary diagnostic investigations (electrophysiology and CSF), treatment, clinical course, and outcome of GBS. The corresponding authors were requested to share data on variables of interest that were not reported.

Cases were classified according to diagnostic certainty levels for GBS and ZIKV infection. To classify the diagnosis GBS we employed the Brighton Collaboration Criteria (2011).[10] If the Brighton Criteria were not reported, these were defined based on available reported data, and if the clinical description did not correspond to the

reported Brighton level, cases were reclassified after clarification was sought with the corresponding author. The diagnostic certainty of ZIKV infection was classified as confirmed, probable or suspected, according to the Centers for Disease Control and Prevention (CDC) criteria[11] (**Table 1**), based on the results of laboratory tests: case-by-case in case reports and series, and all cases combined in larger studies.

Table 1. Zika virus disease case definition

Suspected	Acute onset of fever (measured or reported), OR maculopapular rash, OR arthralgia, OR conjunctivitis; OR Guillain-Barré syndrome (not explained by another etiology)*
Probable	Suspected ZIKV disease AND Epidemiologic linkage AND Laboratory evidence of recent ZIKV or flavivirus infection by: • Positive ZIKV IgM (serum/CSF) with: ○ Positive neutralizing antibody titers against ZIKV and DENV (or other flaviviruses endemic to region of exposure) OR ○ Negative DENV IgM and no neutralizing antibody testing performed.
Confirmed	Suspected ZIKV disease AND Laboratory evidence of recent ZIKV infection by: • Positive ZIKV culture, viral antigen or RNA (serum, CSF, tissue, or other specimen) OR • Positive ZIKV IgM (serum/CSF) with positive ZIKV and negative DENV (or other flaviviruses endemic to region of exposure) neutralizing antibody titers

Zika virus case definition according to the Centers for Disease Control (CDC).[11] ZIKV= Zika virus | DENV= Dengue virus | CSF= cerebrospinal fluid | RNA=Ribonucleic acid
*During a ZIKV epidemic

Clinical characteristics were retrieved as the number of patients in whom the variable was present in the numerator, and the total number of reported cases in the denominator: n/N (%). For arbovirus symptoms, we assumed symptoms were absent rather than missing if they were not cited in the manuscript, to account for the reporting bias, and therefore described as zero (n) out of the total number of reported cases (N). For the neurologic findings, variables not cited were considered missing data, because a risk of measurement bias was deemed higher than a risk of a reporting bias for these variables. If clinical characteristics were reported at multiple time points, data representing the full disease course were presented. Continuous variables (age, time between infectious and neurologic symptoms, duration of progression and plateau phase of GBS, duration hospital admission) were extracted as medians and or means, depending on how they were presented in the original article.

Statistical analysis

First, we calculated the proportions per study of each variable of interest, and then the pooled proportions with data from all included studies reporting more than one GBS case. We were unable to summarize continuous variables, as in most studies

these were reported as medians without availability of individual data or means. To address the possibility of an ascertainment bias of ZIKV infection among study populations, we then performed a subgroup sensitivity analysis, repeating the pooled analysis with grouped data of only probable or confirmed ZIKV cases (overall study populations comprising only probable/confirmed ZIKV cases, and subsamples of probable/confirmed cases from studies that also included suspected ZIKV cases, when available). We also performed sensitivity analyses by excluding papers that recruited only ICU patients, to account for selection bias in the pooled proportion of mechanical ventilation and ICU assistance.

The pooled proportions and the 95% confidence intervals were estimated using the random effects model and the Freedman Tukey double arcsine transformation, to account for proportions near 0 and 1. Heterogeneity between studies was calculated using the Chi-square test and I^2 statistics, which was interpreted as follows: not important (I^2= 0-40%); moderate (I^2= 30-60%); substantial (I^2= 50-90%); considerable (I^2= 75-100%).[12] The meta-analysis was done using the *metaprop* command in STATA 15.1.[13]

RESULTS

Study selection

We identified 1716 articles in the databases researched, of which 35 studies were included in our systematic review. The 35 selected studies reported on a total of 601 GBS cases with a suspected, probable or confirmed ZIKV infection with reported data of at least one variable of interest, and consisted of 13 single case reports and one cohort in which only one case fulfilled our inclusion criteria (n=14, **Table 2**), and 14 case series and seven case-control studies (n=587, **Table 3**). For the pooled analysis of the studies, we were only able to use the studies that reported on more than one case (**Table 3**). For the subgroup meta-analysis or probable/confirmed ZIKV cases, data of 165 GBS cases with probable or confirmed ZIKV infection, from 14 studies, could be pooled (**Figure 1**).

Study characteristics: case selection, case ascertainment and risk of bias

In **Table 2**, the single case reports are presented alphabetically with a brief clinical description per case. Eleven cases were from ZIKV epidemic or endemic regions and three were travelers returning from epidemic regions. Eight cases were positive for ZIKV PCR, four for IgM and plaque-reduction neutralization test (PRNT), and two

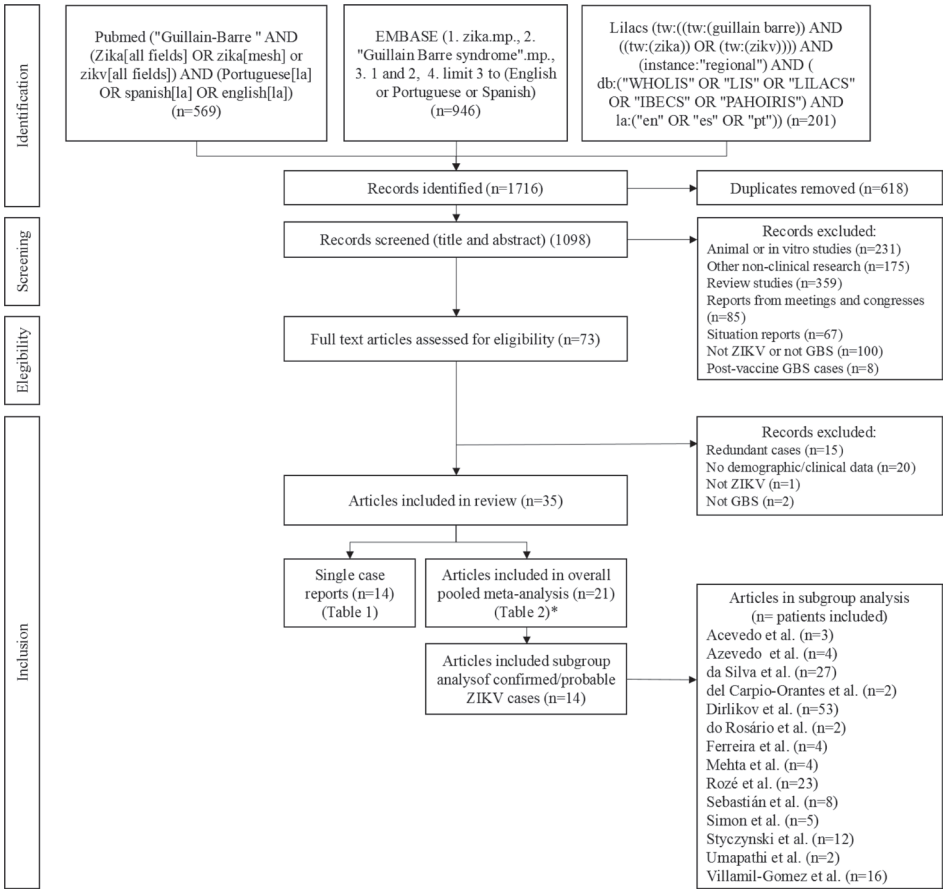


Figure 1. PRISMA Flowchart of search and selection of studies on GBS associated with recent ZIKV infection.*

*excluding Geurtsvankessel et al. (only one GBS case associated with a recent ZIKV infection)

were reported to be ZIKV positive with no further information provided. Six of eight cases of whom the Brighton classification was reported, fulfilled level 1. The most frequent clinical phenotype was a demyelinating sensorimotor GBS with facial and/or bulbar palsy.

In **Table 3**, the 21 studies reporting more than one patient are displayed according to the location and time-period of cases, in line with the global spread of the ZIKV epidemics on the Pacific islands (Oct 2013-Dec 2014) and Latin America (Dec 2014-2017). The first study was from French Polynesia in 2013-2014,[4] and the last was from Mexico in 2016-2017.[46] One study reported cases during and outside of a ZIKV outbreak period in Singapore[47]

Table 2. Single case reports of GBS associated with recent ZIKV infection.

First author	Journal, year	City, country	Period	Clinical description	ZIKV diagnosis
Beattie[14]	Infect Dis Clin Pract 2018	Dominican Republic (DR)	2016	**64 y/o woman** returning from DR to USA. Paresthesias, sensory signs, tetraparesis, areflexia, difficulty walking, facial palsy. Preceding (10d) fever, rash, malaise, arthralgia, conjunctivitis, headache, cough, rhinorrhea. CSF: ACD. EMG: AIDP with axonal damage. Brighton level 1.Treatment: IVIg. ICU and MV. Discharge at 35d (tetraparesis).	ZIKV PCR+ (S,U) IgM: ZIKV- (S,CSF), DENV/CHIKV- (S) VNT ZIKV< DENV (S)
Brasil[15]	Lancet 2016	Rio de Janeiro, Brazil	Jun 2014	**24 y/o woman**. Paresthesias, tetraparesis, areflexia, difficulty walking. Concurrent fever, rash, headache, ocular pain, conjunctivitis, edema. Normal CSF and EMG. Brighton level 3. No treatment. Discharge at 13d (recovered).	PCR: ZIKV+ (S,CSF,U,Sa), DENV/CHIKV-(S, CSF)
Fabrizius[16]	Am J Trop Med Hyg 2016	Guyana	Mar 2016	**44 y/o man**. Paresthesias, sensory signs, ataxia, LL paresis, areflexia. Preceding (8d) fever, headache, rash, arthralgia, arthritis, conjunctivitis. CSF: ACD. EMG: sensorimotor peripheral neuropathy. Brighton 1. Treatment: IVIg. Discharge at 15d (walking with aid).	PCR: ZIKV/DENV/CHIKV- (S), ZIKV+ (U) IgM: ZIKV+ (S,CSF); DENV/CHIKV- (S) VNT ZIKV=DENV
Fontes[17]	Neuroradiol 2016	Rio de Janeiro, Brazil	2016	**51 y/o woman**. LL paresis, difficulty walking, facial palsy. Preceding (?d) rash, myalgia, arthralgia, conjunctivitis. CSF: ACD. EMG: AIDP. Treatment: IVIg. Clinical improvement. Discharge NR	ZIKV+ (S,U – type tests NR)
Gonzalez-Escobar[18]	Rev Panam Salud Publica 2016	Tunapuna, Trinidad Tobago	Aug 2016	**29 y/o man**. Paresthesias, ataxia, LL paresis and progressing to tetraparesis. Preceding (7d) fever, rash, headache, malaise. Treatment: IVIg. Mild weakness at 10m.	PCR: ZIKV+(S), DENV/CHIKV-(S) IgM: ZIKV/DENV/CHIKV- (S)
Geurtsvankessel[a19]	Ann Clin Transl Neur, 2018	Dhaka, Bangladesh	Nov 2013-Dec 2015	**58 y/o woman.** Distal hypesthesia, tetraparesis, facial and bulbar palsy, autonomic symptoms (constipation). EMG: not done. Treatment: IVIg. Independent walking at 3m.	IgM, IgG, VNT: ZIKV+ (S) PCR: ZIKV-(S)

First author	Journal, year	City, country	Period	Clinical description	ZIKV diagnosis
Hamer[20b]	Ann Intern Med 2017	Suriname	May 2015-Feb 2016	**60 y/o woman** Returning from Surinam to the Netherlands. Tetraparesis, bulbar and bilateral facial palsy, areflexia, sensory signs. Preceding (?d) fever, myalgia, diarrhea, vomiting. CSF: ACD. EMG: AIDP. Brighton 1. Hospitalized 15d.	PCR: ZIKV+(U, S) IgM: ZIKV+(CSF).
Kassavetis[21]	Neurology 2016	Haiti	Jan 2016	**35 y/o man**. Paresthesias, sensory signs, bulbar and bilateral facial palsy, ophtalmoplegia, ataxia, areflexia. Preceding (1d) fever, headache, ocular pain, nasal congestion. CSF: ACD. Brighton 2 (MFS overlap). Treatment: IVIg. Discharge at 5d, walking with aid at 3w.	IgM&VNT: ZIKV+(S,CSF)
Miller[22]	J Neurol Sci 2017	Dominican Republic	May 2016	**55 y/o woman**. Paresthesias, LL paresis progressing to tetraparesis, bulbar and sensory signs, ataxia, areflexia. Concurrent asthenia, malaise, myalgia. CSF: ACD. EMG: AIDP. Brighton 1. Treatment: IVIg. Discharge at 22d (walking with aid).	PCR: ZIKV-(S,CSF,U), IgM: ZIKV+ (S,CSF), DENV+(S), CHIKV-(S), VNT ZIKV< DENV (S)
Rabelo[23]	Front Microbiol 2018	Rio de Janeiro, Brazil	Jun 2016	**28 y/o pregnant woman** (stillbirth). LL paresis progressing to tetraparesis, unable to walk, areflexia, paresthesias, sensory, autonomic, and respiratory signs. Preceding (20d) rash, vomiting. CSF: normal. EMG: AMSAN. Treatment: IVIg. Discharge at 28d, walking with aid at 40d.	ZIKV confirmed in placental and fetal tissues IgM: ZIKV/DENV/CHIKV- (S)
Raboni[24]	Transpl Infect Dis 2017	Maranhão, Brazil	Jun 2015	**9 y/o girl**. LL paresthesia, paresis, unable to walk, progressing to respiratory dysfunction. Preceding (90d) hematopoietic stem cell transplant. CSF: raised cell count and protein level. EMG: AIDP. Treatment: IVIg and PE. ICU, MV. Hospitalized (?d). Recovered at 4m.	PCR: ZIKV/DENV-(S), DENV NS1- IgM: ZIKV/DENV+(S), VNT ZIKV< DENV
Reyna-Villasmil[25]	Med Clin 2016	Zulia, Venezuela	2016	**28 y/o pregnant woman** (normal birth). Tetraparesis, bulbar palsy, areflexia, progressing to respiratory dysfunction. Preceding (10d) fever, rash, myalgia, conjunctivitis. CSF: ACD. EMG: AIDP. Brighton 1.Treatment: IVIg. ICU and MV. Discharge at 21d (recovered).	Serology for ZIKV+ (type tests NR)
Siu[26]	Neurology 2016	Tonga, Polynesia	2016	**47 y/o man**. Returning from Tonga to New Zealand. Paresthesias, progressive tetraparesis, areflexia, sensory and respiratory signs. Preceding (6d) edematous leg with pustular lesions. CSF: ACD. EMG: AIDP. Brighton 1. Treatment: IVIg and PE. ICU and MV. Discharge at 33d (bedbound).	PCR: ZIKV/DENV/CHIKV-(CSF), ZIKV+/DENV/CHIKV-(S), DENV NS1- IgM: ZIKV/DENV+(S)

First author	Journal, year	City, country	Period	Clinical description	ZIKV diagnosis
Zambrano[27]	Am J Trop Med Hyg 2016	Guayaquil, Ecuador	Mar 2016	**57 y/o woman**. Paresthesia, facial palsy, tetraparesis, areflexia. Preceding (5d) headache, fever, lumbar back pain. CSF: ACD. EMG: results NR. Treatment: PE. ICU. Discharge at 10d.	PCR: ZIKV/CHIKV+/DENV-(S,CSF,U)

NR= Not Reported | y/o= year-old | USA= United States of America | LL= lower limbs | UL= upper limbs | ICU= Intensive Care Unit | MV= mechanical ventilation | CSF= cerebrospinal fluid | ACD= albuminocytological dissociation | EMG= electromyography/nerve conduction studies | IVIg= intravenous immunoglobulin | PE= plasma exchange | Brighton= Brighton Collaboration Criteria level | MFS= Miller Fisher Syndrome | ZIKV= Zika virus | CHIKV= Chikungunya virus | DENV= Dengue virus | PCR= polymerase chain reaction | VNT= virus neutralization test | DENV NS1= NS1 antigen of DENV | S= serum | Sa= saliva | CSF= cerebrospinal fluid | U= urine.

[a]Not published as a case report but only one case fulfilling our criteria for suspected/probable/confirmed ZIKV in larger cohort of 418 cases.

[b]Returning travelers with suspected, probable or confirmed ZIKV infection reported to the GeoSentinel Surveillence Network. 93 cases reported, 2 GBS cases, one is already described in the Kassavetis' paper, the other is described here.

Inclusion criteria, case selection and setting differed between studies. A diagnosis of GBS was the inclusion criterion in 14 studies, and seven studies also included other acute neurologic illnesses besides GBS.[29, 32, 34-37, 41] Six studies included all GBS patients in their reference population,[4, 28, 38, 44, 45, 47] one study included all GBS patients >12 years old,[31] and one study included all arbovirus-related neurologic manifestations.[35] All other studies included a convenience sample of patients seen at one or more health-care centres. Three studies only included patients admitted to the ICU,[32, 37, 40] and nine studies only included GBS patients with a clinical suspicion or laboratory evidence of a ZIKV infection.[30, 32, 33, 36, 37, 40-42, 46] Seven studies were set in a specialized hospital (academic or reference centre),[4, 28, 29, 34, 39, 42, 45] and two multi-centre studies were set in both specialized and non-specialized hospitals.[36, 40] These differences are potential sources of selection bias within studies and heterogeneity across studies.

Sixteen studies reported the criteria that were applied for diagnostic certainty of GBS, and 13 used the Brighton Criteria. In four studies the Brighton Criteria were prospectively applied by a physician; in seven, retrospectively through records review; two studies gave no information on how the Brighton level was assessed; and three employed other criteria. The risk of ascertainment bias of GBS is likely to be low or very low, as the vast majority of all cases in this review fulfilled Brighton levels 1-3 (396/407, 97%).

Regarding the ascertainment of ZIKV infection, 13 studies tested their cases for both PCR and IgM,[29, 32-34, 36, 40, 42-45, 47, 50, 51] five only for PCR,[30, 33, 35, 37, 39, 41] and three only for IgM.[28, 31] Based on the CDC ZIKV case definition, more than a half of all GBS cases in our review had a suspected ZIKV infection (324/570, 57%), which gives a high risk for ascertainment bias within studies and heterogeneity across studies.

Patient characteristics

Demographics

The median age of the study populations varied between 34 and 61 years, and only 11 pediatric patients were included in four studies.[31, 33, 35, 38] The majority of patients was male (62%) and the male:female ratio of all studies combined was 1.63. In multicenter studies or those including all GBS cases in the reference population, the male:female ratio was 1:1, with the exception of studies from French Polynesia[4] and Martinique[45], which had ratios of 3:1 and 2:1, respectively (**Table 3**).

Table 3. Demographic characteristics, case selection and ascertainment in studies reporting more than one Guill

First author	Journal, year	Provenance (city, country)	ZIKV outbreak	Study design	Incidence period
Cao-Lormeau[b4]	Lancet 2016	Papeete, Tahiti, French-Polynesia	Oct 2013-Apr 2014	Prospective case-control	Oct 2013 - Mar 2014
Simon[28]	J Neurovirol 2018	Noumea, New Caledonia Melanesia	Jan-Dec 2014	Prospective case-control	Jan - Dec 2014
Ferreira[29]	Am J Trop Med Hyg 2016	Recife, Brazil	Nov 2014-2015	Case series	15 Dec 2014 - 30 Jun 2015
Nóbrega[30]	Epidemiol Serv Saude 2018	Recife, Brazil	Nov 2014-2015	Case series	23 Dec 2014 - 19 Jun 2015
Styczynski[31]	PLoS Negl Trop Dis 2017	Salvador, Brazil	Jan 2015-May 2016	Retrospective case-control	1 Jan 2015 - 31 Aug 2015
do Rosário[32]	Am J Trop Med Hyg 2016	Salvador, Brazil	Jan 2015-May 2016	Case series	15 May - 30 Jul 2015
Keesen[33]	Lancet 2017	João Pessoa, Brazil	2016	Case series	2016
da Silva[34]	JAMA Neurol 2017	Rio de Janeiro, Niteroi and SãoGonçalo, Brazil	May 2015-Nov 2016	Cohort	5 Dec 2015-10 May 2016

ain-Barré syndrome case with a recent Zika virus infection

	Study population	Ascertainment GBS[a]	Ascertainment ZIKV	N cases in analysis	Median age
	All GBS inpatients in French Polynesia during ZIKV outbreak	Brighton 1-3 by neurologist or intensivist	PCR: ZIKV(S) VNT, IgM&IgG: ZIKV, DENV(S)	42 (11:31)	42 (36-56)
	All GBS adult patients in New Caledonia during ZIKV outbreak	Brighton 1-2	PCR, IgM&IgG: ZIKV, DENV(S) VNT: ZIKV(S)	5[c] (3:2)	52 (mean) [29-75]
	First six adults with acute neurological illness and ZIKV PCR+, in reference neurology hospital	Criteria NR, data compatible with Brighton 1 and 4 (2:2)	PCR: ZIKV,DENV(S) IgM&IgG: ZIKV,DENV(S)	4 (1:3)	33.5 [25-48]
	All GBS inpatients in metropolitan region identified in the Hospital Information System, with arboviral symptoms (<60d) and/or laboratory positivity	Brighton 1-4 by medical records review	ZIKV PCR tested in 1 case (S) DENV IgM tested in 1 case (S)	18 (9:9)	44 [14-62]
	All GBS cases (≥ 12y/o) reported to the Bahia Epidemiologic Surveillance Center	Brighton 1-3 by medical records review	IgM: ZIKV,DENV(S) VNT: ZIKV,DENV(S)	50[d] (19:22)	44 [32-54]
	Adult patients admitted to ICU with ascending paresis, preceding exanthema, ZIKV IgM+	Wakerley Criteria, 2014	PCR: ZIKV, DENV, CHIKV(S) IgM&IgG:ZIKV,DENV, CHIKV Panel of 18 arboviruses in S VNT:ZIKV,DENV, CHIKV (S)	2 (1:1)	46,5 [22 and 49]
	GBS cases in Paraiba province admitted to neurology reference hospital during the ZIKVepidemic in 2016, ZIKV PCR verified	NR	PCR: ZIKV IgM&IgG: DENV,CHIKV (type biosample NR)	12 (8:4)	35,5 [7-73]
	All adults with <60d of onset of transverse myelitis, meningo-encephalitis or GBS admitted to neuromuscular expertise center	Brighton	ZIKV PCR if -: ZIKV IgM(S,CSF) ZIKV IgM if+: DENV IgM	28[e] (9:19)	42 (22-67)

First author	Journal, year	Provenance (city, country)	ZIKV outbreak	Study design	Incidence period
Azevedo[35]	Rev Soc Bras Med Trop 2018	Rio de Janeiro, Brazil	May 2015-Nov 2016	Case series	Jun 2015 - Dec 2016
Mehta[36]	PLoS Negl Trop Dis 2018	Rio de Janeiro, Brazil	May 2015-Nov 2016	Case series	1 Nov 2015- 1 Jun 2016
Sebastián[37]	J Crit Care 2017	7 Latin American countries[g]	2015-2016	Case series	1 Dec 2015-2 Apr 2016
Salinas[38]	J Neurol Sci 2017	Barranquilla, Colombia	Oct 2015-Apr 2016	Retrospective case-control	1 Oct 2015-2 Apr 2016
Parra[39]	NEJM 2016	Cucuta, Medellin, Cali, Barranquilla, Neiva, Colombia	Oct 2015-Apr 2016	Prospective case-control	Jan - Mar 2016
Villamil-Gomez[40]	Travel Med Infect Dis 2017	Sucre, Colombia	Oct 2015-Apr 2016	Case series	2016
Acevedo[41]	Front Microbiol 2017	Guayaquil, Ecuador	Jan-Oct 2016	Case series	1 Feb - 31 Aug 2016
Langerak[42]	Front Neurol 2016	Paramaribo, Suriname	Oct 2015-2016	Cases series	Jan - Mar 2016
Dirlikov-a[43]	MMWR 2016	Puerto Rico	Dec 2015-Dec 2016	Case series	1 Jan - 31 Jul 2016

Study population	Ascertainment GBS[a]	Ascertainment ZIKV	N cases in analysis	Median age
All non-congenital neurologic disorders reported to Information System for Notifiable Diseases and Arboviral Neurologic Manifestation Report	PAHO criteria	PCR: ZIKV, CHIKV(S) IgM: DENV, CHIKV(S) IgG: CHIKV(S)	72 (NR)	45
Patients ≥12 y/o admitted to one of 11 participating hospitals, with an acute neurologic disease, suspected and tested for ZIKV	Brighton by medical records review	PCR:ZIKV,DENV,CHIKV (S,CSF,U) IgM&IgG: ZIKV(S), DENV,CHIKV (S,CSF)	7[f] (4:3)	41 [19-67]
Adults with a confirmed ZIKV infection in one of 24 ICUs of the Latin America Surveillance Network	Brighton by intensivist or neurologist (results NR)	PCR: ZIKV(S)	8 (2:6)	38 [18-67]
All GBS cases in Barranquilla reported to the national and the local surveillance system[h]	Brighton 1-3 by medical records review	IgM&VNT: ZIKV,DENV(S)	47 (25:22)	49 [10-83]
All patients with GBS at six university-based hospitals	Brighton by neurologist or internist	PCR: ZIKV (S,CSF,U), DENV(S,CSF) IgM&IgG: DENV(S,CSF)	68[i] (30:38)	47 (35-57)
Adults with confirmed ZIKV infection and GBS, admitted to ICU of two major clinical reference centers in Sincelejo-Sucre	NR	PCR: ZIKV; DENV NS1 IgM&IgG: DENV,CHIKV (samples NR)**	16 (4:12)	53 (47-68)
16 adult patients with neurological symptoms and PCR+ ZIKV, DENV or CHIKV in CSF, admitted to emergency room or ICU of largest hospital of Guayaquil	Criteria NR, data compatible with Brighton 1, 2, 4	PCR: ZIKV, DENV, CHIKV(CSF)	3 (1:2)	54 [18-62]
Consecutive adult patients diagnosed with GBS and preceding ZIKV infection	Criteria NR, data compatible with Brighton 1	PCR: ZIKV(S,CSF,U); IgM&IgG: ZIKV,DENV(S) VNT: ZIKV(S), DENV NS1(S)	3 (0:3)	50 [40-60]
GBS cases admitted at 13 hospitals, identified by the GBS Passive Surveillance System-Puerto Rico Department of Health	Brighton by medical records review	PCR: ZIKV,DENV,CHIKV(S,CSF) IgM: ZIKV,DENV,CHIKV(S,CSF)	34 (20:14)	55 [21-88]

First author	Journal, year	Provenance (city, country)	ZIKV outbreak	Study design	Incidence period
Dirlikov-b[44]	JAMA Neurology 2018	Puerto Rico	Dec 2015-Dec 2016	Case series	Jan - Dec 2016
Rozé[45]	Clin Infect Dis 2017	Martinique, French Caribbean	Jan-Oct 2016	Case series	Jan - Oct 2016
del Carpio-Orantes[46]	Neurología 2018	Veracruz, Mexico	2016	Case series	2016 - 2017
Umapathi[47]	J Peripher Nerv Syst 2018	Singapore, Singapore	Aug-Nov 2016	Prospective case-control	May - Dec 2016
Total					

Age as median and (interquartile range) or [range] unless indicated otherwise. NA= not applicable | Brighton= Brighton Collaboration Criteria levels[10] | EMG= electromyography/nerve conduction studies | y/o = years old | ICU= Intensive Care Unit | ZIKV= Zika virus | CHIKV= Chikungunya virus | DENV= Dengue virus | PCR= polymerase chain reaction | VNT= virus neutralization test | DENV NS1= DENV NS1 antigen | NR= Not Reported | S= serum | CSF= cerebrospinal fluid | U= urine | Sa= saliva | fem=female, mal=male | ICD10= 10th revision of the International Statistical Classification of Diseases and Related Health Problems).

[a]Patients not fulfilling the Brighton Criteria were included: da Silva (n=3), Mehta (n=1), Parra (n=6). [b]Additional data retrieved from previous publication by Watrin et al,[48] 2016. [c]Clinical data available for 5 cases with laboratory evidence of ZIKV infection (IgM & IgG positive). [d]Age, infectious symptoms and laboratory data available for 41 cases included in case-control study, neurologic signs and symptoms available for all 50 reported cases. [e]One post-vaccine case was excluded from data extraction, data on CSF examination were available for all 29 cases, age and clinical data were available for 27 ZIKV positive cases. [f]A total of 13 GBS cases with suspected/probable/confirmed ZIKV were reported but data were available for only 7 cases with positive arbovirus tests. [g]Colombia, Venezuela, Salvador, Guatemala, Puerto Rico, Ecuador, Perú and Chile. [h] Colombia National Surveillance System (Sivigila) and *Secretaria de Salud de Barranquilla*. [i]Five cases from Barranquilla may overlap with cases reported by Salinas *et al*. [jf]A total of 123 GBS cases with suspected/probable/confirmed ZIKV were reported but clinical and laboratory data were available for 107 cases tested for ZIKV. [k]Laboratory data available for all cases and clinical data for 23 cases with laboratory evidence of ZIKV. [l]Clinical data of 8 cases additionally retrieved from previous publication by del Carpio-Orantes *et al*, 2017.[49] [m]A total of 14 cases were reported, data were extracted from 11 cases collected during the ZIKV outbreak plus one case with laboratory evidence of recent ZIKV infection before the outbreak.

Study population	Ascertainment GBS[a]	Ascertainment ZIKV	N cases in analysis	Median age
All GBS cases admitted at all the 57 general hospitals of Puerto Rico and identified by the GBS Passive Surveillance System.	Brighton1-3 by medical records review	PCR: ZIKV,DENV,CHIKV (S,CSF,U,Sa) IgM: ZIKV, DENV, CHIKV(S,CSF)	107[j] (47:60)	54 [4-88]
All GBS inpatients at only specialized center in the country	Brighton 1-2 by neurologist	PCR:ZIKV,DENV, CHIKV (S,CSF,U) IgM&IgG: ZIKV, DENV,CHIKV(S) VNT ZIKV (if ZIKV PCR-&IgM- or ZIKV&DENV IgM+)	30[k] (8:15)	61 (56-71)
All GBS cases documented by *Instituto Mexicano del Seguro Social* with GBS and tested for arboviruses	Brighton 1-3 by medical records review	PCR: ZIKV,DENV, CHIKV(S) IgM&IgG: ZIKV(S) IgM: DENV/ CHIKV(S)	18	47 [19-70]
All GBS cases from all public and private hospitals in Singapore before and during ZIKV outbreak	ICD10 G61.0 records in electronic databases	PCR: ZIKV, DENV(S,U) VNT, IgM & IgG: ZIKV,DENV(S)	12[m] (7:5)	55,5 [25-81]
			587	

Certainty levels of GBS diagnosis and ZIKV infection

Separate proportions of each Brighton level (1-4) were available in ten studies[29, 31, 32, 34, 39, 41-43, 46] (295 cases): 110 cases fulfilling level 1; 146 level 2; 26 level 3 and 13 level 4. Miller Fisher Syndrome (MFS) was reported in only four studies, one study from Singapore (five cases),[47] and three studies from Latin America (six cases).[34, 39, 46] ZIKV infection was confirmed in 118 (21%), probable in 128 (22%) and suspected in 324 (57%) of all cases with reported separate proportions of each ZIKV certainty level. In the overall pooled estimates of study populations with available proportions of at least the Brighton level 1 and a suspected ZIKV infection, 57% of cases had Brighton level 1 and 44% had a suspected ZIKV infection (**Figure 2, Supplementary Figure 2**). We re-calculated these pooled frequencies after excluding two studies that only included cases with Brighton levels 1-2,[28, 45] finding 51% (95% CI: 28-74; I^2 89.2%) with Brighton 1 (105/290), and re-calculated pooled frequencies after excluding eight studies that only included cases with probable/confirmed ZIKV,[28, 29, 32, 36, 37, 40-42] finding 65% (95% CI: 47-80; I^2 93.2%) with a suspected ZIKV infection (319/522).

Table 4. Demographics and clinical characteristics of GBS cases associated with ZIKV reported in 21 case series.

	All cases (N 587)		Probable/confirmed ZIKV infection (N 165)	
Demographics				
Adults % (n/N)	98% (550/563)		100% (165/165)	
Female % (n/N)	38% (216/570)		41% (67/165)	
Infectious symptoms	**n/N**	**Pooled proportion (95%CI; I^2)**	**n/N**	**Pooled Proportion (95%CI; I^2)**
Arboviral symptoms				
Rash	253/544	56% (43-69; 83%)	86/149	61% (37-82; 78%)
Fever	228/539	45% (33-57; 77%)	66/149	42% (21-64; 75%)
Arthralgia	150/539	35% (21-49; 86%)	50/149	31% (15-50; 64%)
Myalgia	126/550	25% (12-41; 89%)	40/149	29% (7-55; 83%)
Headache	106/550	22% (8-38; 91%)	32/149	25% (5-50; 83%)
Conjunctivitis	98/539	17% (8-28; 80%)	30/149	15% (7-24; 14%)
Ocular pain	24/550	1% (0-6; 74%)	3/149	0% (0-3; 41%)
Gastrointestinal[a]	59/550	8% (3-14; 66%)	15/149	6% (0-21; 67%)
Rhinorrhea	12/550	0% (0-1; 0%)	1/149	0% (0-0; 0%)
Cough or chest pain	28/550	2% (0-7; 71%)	9/149	2% (0-13; 61%)
Neurologic symptoms				
Sensory symptoms	333/421	82% (76-88; 30%)	97/119	86% (73-96; 34%)
Dysphagia	133/351	30% (17-45; 90%)	49/112	34% (7-67; 85%)
Dysarthria	64/281	11% (1-25; 78%)	3/13	17% (0-60; 48%)
Diplopia	11/234	0% (0-4; 33%)	1/13	2% (0-25; 0%)
Neurologic signs				
Facial palsy	246/486	51% (44-58; 36%)	75/139	56% (42-71; 38%)
Bulbar palsy	60/182	25% (10-42; 70%)	4/11	32% (0-76; 33%)
Ocular palsy	22/232	5% (0-12; 46%)	0/11	0% (0-19; 0%)
Any limb paresis	544/582	97% (93-99; 49%)	153/165	98% (93-100; 17%)
Tetraparesis	153/251	64% (51-77; 53%)	79/110	74% (61-87; 25%)
Paraparesis	69/251	24% (18-31; 0%)	21/110	15% (7-24; 0%)
Sensory deficits	155/317	49% (29-68; 86%)	59/104	59% (39-78; 48%)
Areflexia or hyporeflexia	400/435	96% (88-100; 79%)	131/142	97% (86-100, 56%)
Ataxia	76/317	17% (4-35; 87%)	34/91	29% (4-61; 74%)
Respiratory dysfunction[b]	124/369	23% (13-35; 77%)	37/104	24% (10-41; 38%)
Dysautonomia	73/359	13% (5-24; 71%)	21/102	16% (8-26; 0%)
GBS classification				
Brighton criteria				
Level 1-3	396/407	100% (97-100; 56%)	128/135	99% (93-100; 49%)
Level 4	13/407	0% (3-100; 62%)	7/135	1% (0-11; 54%)
Miller Fisher Syndrome	11/419	0% (0-2; 53%)	1/137	0% (0-0; 0%)

	All cases (N 587)		Probable/confirmed ZIKV infection (N 165)	
Other variants	3/419	0% (0-0; 0%)	0/137	0% (0-0; 0%)

Brighton level= Brighton Collaboration Criteria[10] levels. [a]Nausea, vomiting or diarrhea. [b]Reported as 'trouble breathing', 'difficulty breathing' or 'respiratory dysfunction'

Clinical characteristics

All but one study reported the presence of clinical symptoms of infection. [35] Two or more symptoms were present in 91% of cases (378/444; 95% CI 84-96, I^2 61.2%). The most common symptoms were rash, fever and arthralgia, with similar pooled frequencies between overall estimates and the probable/confirmed subgroup (**Table 4**). The median time between the start of infectious symptoms and neurologic symptoms ranged from -1 to 12 days in the 16 studies reporting on this (**Figure 3**). For arbovirus symptoms the heterogeneity ranged from considerable (I^2= 75-100%), in the overall analysis, to substantial (I^2= 50-90%), in the probable/confirmed subgroup.

Among neurologic findings, paresis was reported in all studies, and almost all studies reported on sensory symptoms, tendon reflexes, and facial palsy, while other symptoms were reported less frequently. The most frequent neurological findings were limb paresis, sensory symptoms, and hypo/areflexia. Other frequent symptoms were facial palsy in about half, and bulbar palsy and respiratory dysfunction in about a quarter of cases. Frequencies of tetraparesis, sensory deficits, bulbar palsy and ataxia were higher in the probable/confirmed cases compared to all reported cases (**Table 4**). Separate data on tetraparesis vs paraparesis were reported in ten studies.[4, 28, 29, 31, 32, 34, 36, 41, 42, 44] Paraparesis was present in 69 of 251 reported cases (24% 95% CI 18-31). This included reports of cases with only lower limb weakness at nadir (30/251), cases with only lower limb weakness at an unclear time point in the disease (33/251), and cases that were reported as having a paraparetic variant of GBS (6/251). Heterogeneity in the analysis of all cases combined was substantial (I^2= 50-90%) for dysarthria, dysphagia, bulbar palsy, sensory deficits, areflexia/hyporeflexia, ataxia, respiratory dysfunction and dysautonomia. In the probable/confirmed subgroup analysis this was substantial only for dysphagia and ataxia.

Diagnostic investigations

PCR, principally in serum, was the most frequently performed test for ZIKV diagnosis, although anti-ZIKV IgM was positive twice more often (**Table 5**). In the CSF, ZIKV PCR was positive in only 10 of 244 tested cases. Presence of neutralizing antibodies against ZIKV in the serum was tested in eight studies.[4, 28, 31, 32, 38, 42, 45, 47] To differentiate ZIKV from DENV, IgM antibodies against DENV were tested in 18 studies (426

Table 5. Ancillary investigations, treatment and disease progression of GBS cases associated with ZIKV reported in 21 case series

Ancillary investigations	All cases (N 587)		Cases with probable/confirmed ZIKV infection (N 165)	
	n/N	Pooled proportion (CI; I^2)	n/N	Pooled proportion (CI; I^2)
Zika virus certainty level				
Confirmed	118/570	24% (11-40; 92%)	88/165	63% (32-90; 90%)
Probable	128/570	14% (3-30; 93%)	75/165	36% (9-67; 90%)
Suspected	324/570	44% (28-62; 92%)	----	----
Arboviral tests				
ZIKV infection[a]				
PCR (any sample)	118/470	30% (15-47; 90%)	88/153	71% (40-95; 88%)
PCR Serum	43/409	10% (1-24; 87%)	42/134	32% (5-66; 89%)
PCR CSF	10/244	3% (0-16; 74%)	6/78	11% (0-38; 80%)
PCR Urine	48/253	28% (7-54; 90%)	31/69	63% (21-97; 81%)
IgM (any sample)	254/375	68% (49-85; 90%)	126/137	97% (87-100; 52%)
IgM Serum	228/374	67% (45-85; 91%)	124/137	94% (81-100; 66%)
IgM CSF	36/111	60% (7-100; 95%)	33/50	77% (23-100; 91%)
PRNT ZIKV	121/154	86% (62-100; 86%)	23/23	100% (94-100; 0%)
PRNT ZIKV>DENV	20/105	16% (7-26; 14%)	11/18	67% (20-100; 52%)
DENV infection (PCR)	3/235	0% (0-1; 0%)	2/75	0% (0-10; 35%)
CHIKV infection (PCR or IgM)	16/187	1% (0-8; 56%)	4/88	0% (0-10; 29%)
DENV and CHIKV co-infection	6/165	1% (0-14; 71%)	2/84	0% (0-8; 42%)
CSF analysis	**425/537**	92% (79-100; 92%)	**122/139**	99% (87-100; 65%)
Increased protein level[b]	253/289	94% (89-98; 19%)	64/70	97% (89-100; 0%)
ACD	276/335	89% (80-96; 64%)	91/99	98% (92-100; 0%)
Electrophysiological exam	**245/477**	68% (49-85; 93%)	**86/145**	77% (46-98; 88%)
AIDP	143/244	62% (38-83; 89%)	62/86	68% (44-88; 59%)
AMAN	58/244	16% (0-41; 92%)	11/85	13% (1-33; 56%)
AMSAN	13/244	1% (0-6; 51%)	9/85	3% (0-11; 8%)
Equivocal	9/240	0% (0-2; 0%)	0/86	0% (0-0; 0%)
Unexcitable	4/240	0% (0-0; 0%)	1/86	0% (0-1; 0%)
Normal	11/245	0% (0-4; 26%)	2/86	0% (0-1; 0%)
Immunomodulatory treatment	**458/555**	92% (81-99; 88%)	**153/160**	100% (97-100; 8%)
IVIg	441/555	89% (77-97; 90%)	152/160	99% (94-100; 27%)
Plasma exchange	6/555	0% (0-0; 0%)	1/160	0% (0-0; 0%)
IVIg and plasma exchange	11/555	0% (0-1; 25%)	0/160	0% (0-0; 0%)
Disease progression				
Admission to ICU	287/544	49% (35-62; 86%)	82/146	57% (29-84; 86%)
Mechanical ventilation	118/567	21% (15-28; 44%)	35/140	19% (7-34; 57%)
Died	23/485	1% (0-3; 0%)	4/133	0% (0-2; 0%)

Abbreviations: ZIKV= Zika virus | CHIKV= Chikungunya virus | DENV= Dengue virus | PCR= polymerase chain reaction | CSF= cerebrospinal fluid | ACD=Albuminocytological dissociation | AIDP= acute inflammatory demyelinating polyradiculoneuropathy | AMAN= acute motor axonal neuropathy | AMSAN= acute motor sensory axonal neuropathy | IVIg = intravenous immunoglobulin | ICU= Intensive Care Unit
[a]Proportions calculated per case, not per biological sample. [b]Definitions of increased protein level in CSF differed per study (>45 mg/dL, >51mg/dL or no cut-off reported).

cases),[4, 28-34, 36, 38-40, 42-47] and were positive in 70 patients. Of these patients, 54 were also positive for ZIKV PCR, IgM and/or ZIKV neutralizing antibodies, and in 16 cases no separate information on ZIKV test results was available. Infection with CHIKV was investigated in nine studies and 187 cases,[32, 33, 35, 36, 40, 41, 44, 46] of which 16 were PCR or IgM positive.

Only five studies tested all (ZIKV suspected) cases for other infections that have been associated with GBS (*C. jejuni*, CMV, EBV, Hepatitis E virus, *Mycoplasma pneumoniae*).[4, 28, 32, 42, 45] And all tested cases (80/587 (14%)) were negative for recent infection. None of the studies tested for all of these pathogens. Heterogeneity was considerable for all ZIKV laboratory tests (I^2= 75-100%).

CSF was examined in most studies, and information on protein level and cell count was provided by about half of these. Increased protein level and albuminocytological dissociation were present in the vast majority of cases and results were similar between all studies combined and the probable/confirmed subgroup. Eleven studies reported the CSF cell count, which did not exceed 55 cells/mm^3, and medians were below 5 cells/mm^3 (**Figure 4**).[4, 29-32, 34, 36, 39, 41, 42, 45] Heterogeneity was limited for increased protein level and albuminocytological dissociation in all studies combined and the probable/confirmed subgroup.

Electrophysiological studies were done in about half of reported cases. In five studies no information on electrophysiological examination was reported.[29, 30, 33, 35, 40] Criteria used to classify cases into the different electrophysiological subtypes were reported in only five studies,[28, 34, 39, 42, 45] and included criteria by Hadden et al, Ho et al, and Rajabally et al.[52-54]. The most frequent electrophysiological subtype was AIDP in 62% (95% CI 38-83), followed by AMAN in 16% (95% CI 0-41), with both similar pooled proportions in the probable/confirmed ZIKV subgroup. In most studies, the majority of cases had an AIDP subtype, except for the study from French-Polynesia[4] where all cases were classified as AMAN, three studies with similar percentages of AMAN and AIDP,[31, 37, 41] a study from Singapore[47] with similar frequencies of AIDP and a normal EMG (in patients with MFS), and a Brazilian case series[36] reporting only a normal EMG and AMAN or AMSAN subtypes.

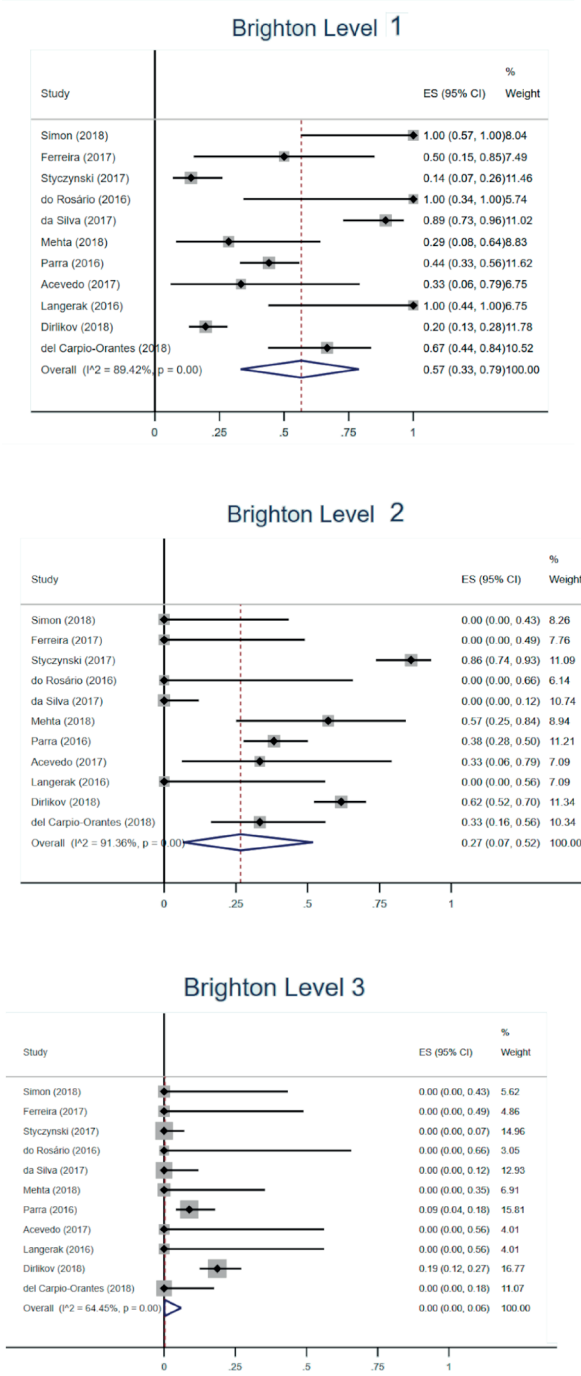
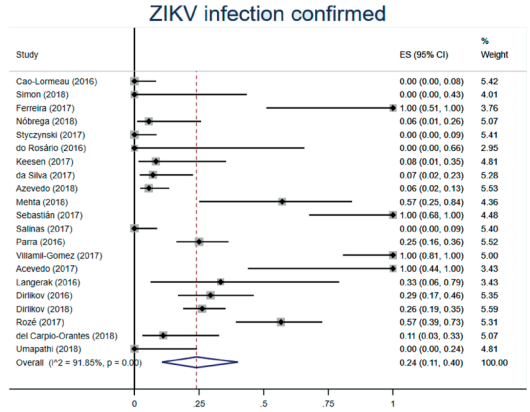
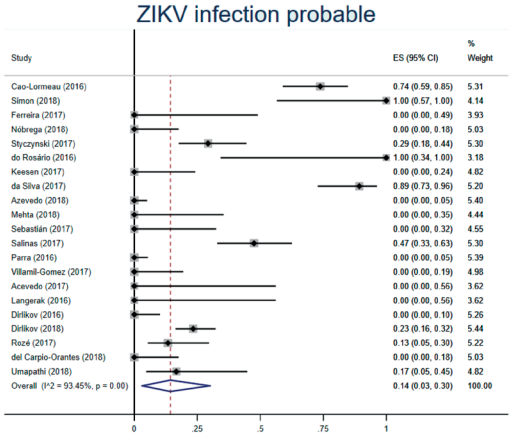
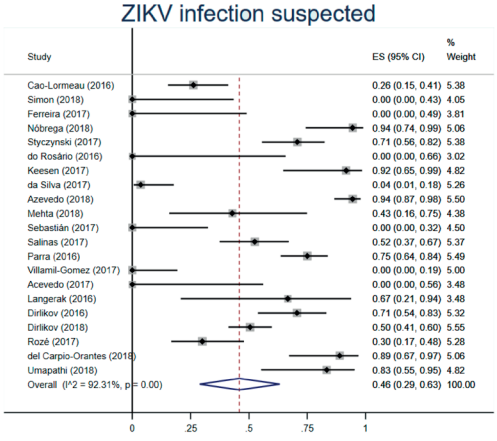


Figure 2. Pooled proportions (forest plots) of Brighton classification and ZIKV infection certainty levels of GBS cases during ZIKV epidemics







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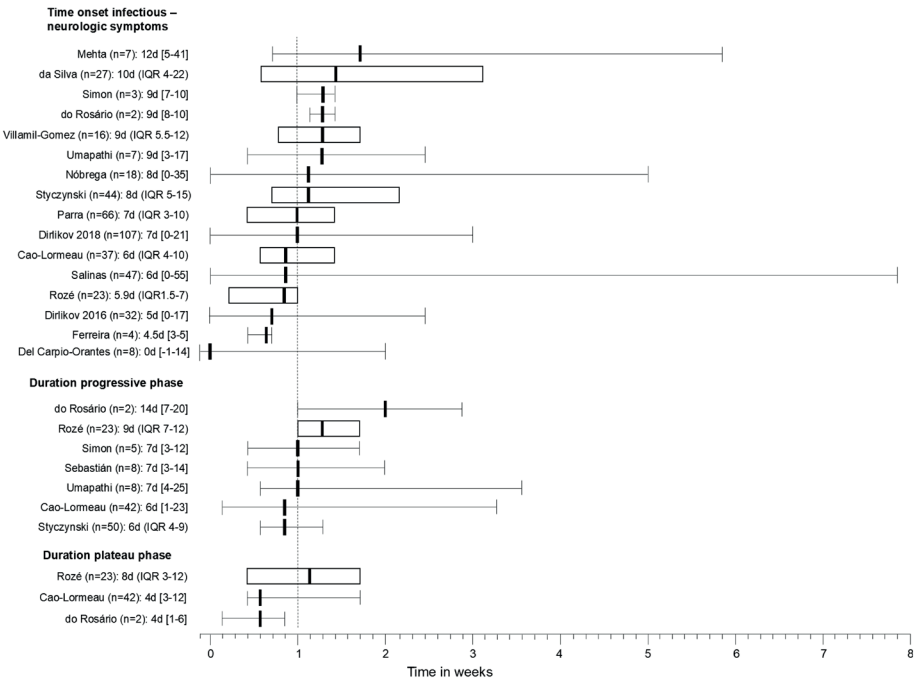

Figure 3. Per study medians and ranges of days of time between onset of infectious and neurologic symptoms, and the progressive and plateau phase of GBS cases.

Treatment and disease progression

All but three studies[33, 41, 47] provided information on treatment, and in most studies, almost all cases were treated with IVIg, except for three large studies, from Colombia[38, 39] and Brazil[35], where only 55-70% of patients were treated with immunomodulating therapy. Three studies provided no information on ICU admission,[28, 30, 33] which was necessary in about 50% of all reported cases, and even more frequent in the probable/confirmed subgroup (57%, 95% CI 29-84). Mechanical ventilation (MV) was necessary in about 20% of both all cases and the probable/confirmed subgroup. Death was infrequent in all cases combined and the probable/confirmed subgroup. Heterogeneity was substantial for immunomodulatory treatment and ICU admission, and moderate for MV (**Table 5**). We recalculated the pooled proportions of ICU, MV and death after excluding three studies that only selected cases admitted to the ICU,[32, 37, 40] and found that ICU admissions (261/518) were lower although still frequent (40%, 95% CI: 28-52), frequency of MV (111/441) was unchanged (22%, 95% CI: 16-28), and frequency of death (22/475) was similar (2%, 95%CI 0-4%), with comparable frequencies in the probable/confirmed subgroup.

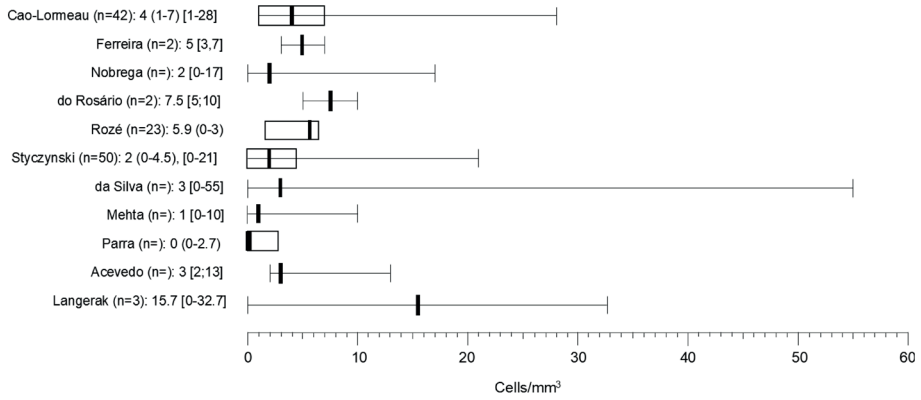

Figure 4. Overview of cell count in the CSF in reported studies.
Cell count in medians, () = inter quartile range, [] = range.

Eight studies informed about the time between onset and nadir of neurologic deficits (progressive phase), and only three studies reported the duration of the plateau phase (**Figure 3**). Only one large study from French-Polynesia[4] informed about the functional evaluation of mobility of patients at nadir, showing incapacity to walk in 27/42 and difficulty to walk in 3/42. The mobility of patients at 6 months after onset of disease was described in a study from Brazil[31] (33/50 walking without aid, 17/50 incapacity to walk) and a study from Puerto Rico[44] (48/79 able to walk 10 meters without aid, 39/79 any difficulty walking, and 12/79 incapacity to walk).

DISCUSSION

Our systematic review and meta-analysis shows that published studies on ZIKV-related GBS typically report a classic sensorimotor type of GBS with frequently a facial palsy and a demyelinating electrophysiological subtype. The disease course is often severe with high rates of respiratory dysfunction and ICU admission. The time between onset of infectious and neurologic symptoms and negative PCR in most patients suggests a post-infectious rather than a direct infectious disease mechanism. These results should however be interpreted with caution as the studies included in this systematic review are variable in study design and setting, selection criteria, diagnostic ascertainment, and reporting of variables, which are potential sources of bias.

The combination of sensorimotor signs with facial palsy and respiratory insufficiency and a demyelinating electrophysiological subtype has previously been

described in GBS patients with other preceding virus infections, such as CMV, indicating that such a clinical and electrophysiological profile may be related to preceding virus infections in general, in contrast to a bacterial infection with *C. jejuni*, that is associated with a pure motor axonal type of GBS.[7, 8, 55, 56] Additionally, although GBS is generally more common in men than in women, we found equal distributions of male and female frequencies in larger studies, similar to previous reports on GBS after other virus infections, suggesting that females may be more prone to virus-related GBS.[7, 55] This finding could however also be due to a higher incidence of ZIKV disease in females compared to males as has been shown in some studies.[57, 58] Another interesting finding was the high frequency of paraparesis (24%) compared to previous literature on GBS (1-11%), indicating that this may be a GBS variant related to ZIKV, although a lower percentage of paraparesis in the subgroup of patients with probable/confirmed ZIKV makes this feature less specific.[5, 59, 60] Furthermore, in some studies it is not clear if the paraparesis evolved to tetraparesis at a later time point, and whether myelitis, which has been linked to ZIKV in other studies, was excluded.[5, 59-61]

Some included studies diverged from the generally reported phenotype. Most importantly, the study from French Polynesia[4], in which all 42 patients had an AMAN electrophysiological subtype, 17 (40%) had a paraparesis and only 26 (62%) had hypo- or areflexia; and the study from Singapore[47], in which 4 out of 12 patients (33%) had MFS and one (8%) had MF overlap syndrome. The high percentage of MFS in Singapore is in line with other publications that show high prevalence of MFS in Asian countries, but whether an AMAN subtype is typical for the Pacific region has not been studied.[5] As most of the other studies described cases from Latin America and the Caribbean, these discrepancies may be due to regional differences in host and/or environmental factors, including differences in the ZIKV strains.[5, 62] However, some dissimilarities could also be due to differences in diagnostic and electrophysiological accuracy between studies. For instance, the interpretation of electrophysiological data in the study from French Polynesia[4] has previously been questioned, as the prolonged distal motor latencies, found at first examination and persisting after 4 months, would be more consistent with the AIDP subtype.[63]

The median time between the onset of infectious symptoms and the start of neurologic symptoms varied between 5 and 12 days, which is similar to other infections preceding GBS.[7, 64, 65] Considering that the incubation period of ZIKV infection is estimated at 1-2 weeks, the latency between ZIKV infection and GBS was more than a week for most cases, suggesting a post-infectious immunopathogenesis, rather than a direct neuronal damage or a para-infectious mechanism, as has been sug-

gested in previous publications.[66, 67] A low frequency of ZIKV PCR positivity in blood and CSF, and a low cell count in the CSF in the majority of cases, further argues against a direct infection. These findings are in line with an *in vivo* study that showed resistance of peripheral nerve cells to infection by ZIKV.[68]

Remarkably, half of all cases combined and more than a half of probable/confirmed cases was admitted to the ICU. This proportion is higher than expected based on other literature[69, 70] where percentages vary between 15 and 30, and remained higher (40%) after we excluded papers that only included patients admitted to the ICU. These data may indicate that GBS following ZIKV infection is often severe enough to necessitate ICU admission. However, the percentage (20%) of mechanically ventilated patients is similar to most other publications.[5, 60, 71, 72] It is not clear what causes this discrepancy. A possible explanation is that presence of autonomic symptoms, rapid progression, severe weakness, or respiratory problems that did not evolve into respiratory insufficiency, were reasons to admit to the ICU, especially during the ZIKV epidemic when an increased vigilance for GBS may have lowered the threshold for intensive care monitoring. Furthermore, many studies were done in specialized centres that may receive more severely affected patients referred from other centres, or may more easily admit patients to the ICU for monitoring compared to non-specialized centres.

The large variability of study designs and settings, selection criteria, diagnostic ascertainment and reporting of variables were important sources of bias within studies and heterogeneity across studies, which is a critical limitation of our meta-analysis. Most importantly, diagnostic ascertainment of GBS and ZIKV differed, and electrophysiological criteria were not reported in most studies. Diagnostic certainty of ZIKV infection was limited in most studies, and other preceding infections in GBS were often not excluded. Furthermore, the type of hospital may have biased the inclusion of severe cases, causing heterogeneity in both clinical signs and disease progression. We calculated the I^2 to quantify this heterogeneity between studies, and have performed a sensitivity analysis to estimate the pooled frequencies among a subgroup of cases with only probable/confirmed ZIKV to analyse the clinical picture of GBS among cases with a high ascertainment of ZIKV infection.

The I^2 was considerable for most infectious symptoms, which is likely due to recall and reporting bias. As we assumed infectious symptoms were absent, rather than missing, if not reported, we may have increased this heterogeneity. Heterogeneity in neurologic symptoms and signs was considerable for some variables, and may be due to differences in study design and methodology and geographical location.

Heterogeneity of arboviral test results was also considerable, which may be due to differences between timing of sample collection and variation in incubation and viremia periods. In general, the variables with considerable heterogeneity are difficult to interpret and preclude any firm conclusions to be drawn from these data. However, the I^2 in the probable/confirmed ZIKV subgroup was generally lower than in all cases combined, indicating that the heterogeneity was partly caused by differences in the diagnostic certainty of ZIKV infection, providing more evidence for a specific clinical and electrophysiological phenotype of ZIKV-related GBS.

CONCLUSION

Published studies on ZIKV-related GBS generally report a sensorimotor demyelinating GBS with a facial palsy and a severe disease course that often necessitates ICU admittance. The paraparetic variant of GBS is also common, which should caution clinicians to exclude myelitis in ZIKV-related cases. The time between onset of infectious and neurologic symptoms and absence of viral genome detected by PCR in most cases, suggest a post-infectious, rather than a direct infection or para-infectious mechanism.

SUPPLEMENTARY MATERIAL

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REFERENCES

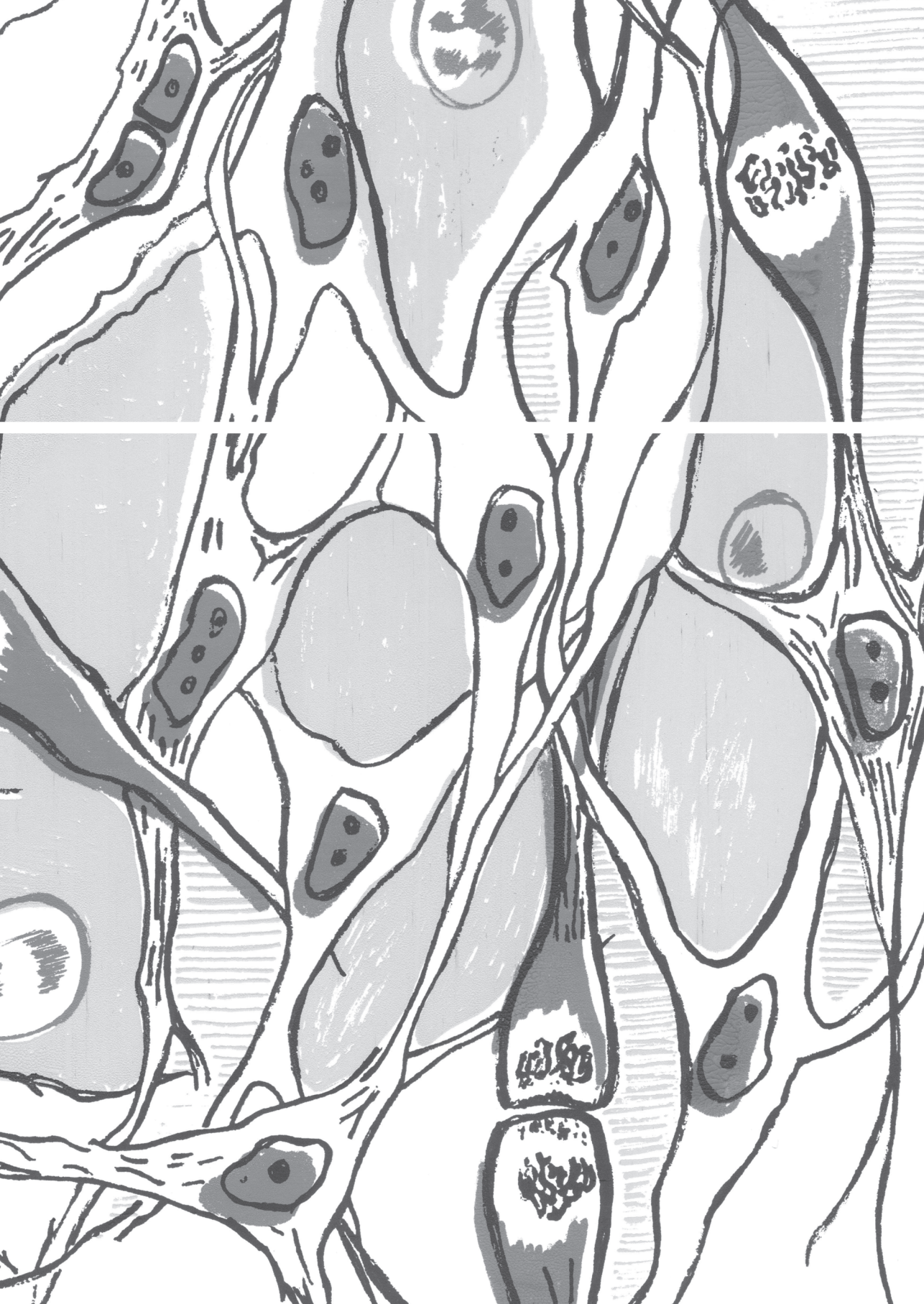
1. Sejvar JJ, Baughman AL, Wise M, Morgan OW. Population incidence of Guillain-Barré syndrome: a systematic review and meta-analysis. *Neuroepidemiology* 2011;36:123-133.
2. Jacobs BC, Rothbarth PH, van der Meche FG, et al. The spectrum of antecedent infections in Guillain-Barré syndrome: a case-control study. *Neurology* 1998;51:1110-1115.
3. World Health Organization. Zika situation report 5 February 2016. *World Health Organization* 2016:Available from: https://www.who.int/emergencies/zika-virus/situation-report/5-february-2016/en/.
4. Cao-Lormeau VM, Blake A, Mons S, et al. Guillain-Barre Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *Lancet* 2016;387:1531-1539.
5. Doets AY, Verboon C, van den Berg B, et al. Regional variation of Guillain-Barré syndrome. *Brain* 2018;141:2866-2877.
6. van den Berg B, Walgaard C, Drenthen J, Fokke C, Jacobs BC, van Doorn PA. Guillain-Barré syndrome: pathogenesis, diagnosis, treatment and prognosis. *Nat Rev Neurol* 2014;10:469-482.
7. Orlikowski D, Porcher R, Sivadon-Tardy V, et al. Guillain-Barre syndrome following primary cytomegalovirus infection: a prospective cohort study. *Clin Infect Dis* 2011;52:837-844.
8. Jacobs BC, van Doorn PA, Groeneveld JH, Tio-Gillen AP, van der Meche FG. Cytomegalovirus infections and anti-GM2 antibodies in Guillain-Barre syndrome. *J Neurol Neurosurg Psychiatry* 1997;62:641-643.
9. Moher D, Shamseer L, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* 2015;4:1.
10. Sejvar JJ, Kohl KS, Gidudu J, et al. Guillain-Barré syndrome and Fisher syndrome: case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* 2011;29:599-612.
11. Prevention CfDCa. Zika Virus Disease and Zika Virus Infection 2016 Case Definition. 2016.
12. Higgins JPT GS. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. *The Cochrane Collaboration* 2011:12.
13. Nyaga VN, Arbyn M, Aerts M. Metaprop: a Stata command to perform meta-analysis of binomial data. *Arch Public Health* 2014;72:39.
14. Beattie J, Parajuli S, Sanger M, et al. Zika Virus-Associated Guillain-Barre Syndrome in a Returning US Traveler. *Infect Dis Clin Pract (Baltim Md)* 2018;26:e80-e84.
15. Brasil P, Sequeira PC, Freitas AD, et al. Guillain-Barre syndrome associated with Zika virus infection. *Lancet* 2016;387:1482.
16. Fabrizius RG, Anderson K, Hendel-Paterson B, Kaiser RM, Maalim S, Walker PF. Guillain-Barre Syndrome Associated with Zika Virus Infection in a Traveler Returning from Guyana. *Am J Trop Med Hyg* 2016;95:1161-1165.
17. Fontes CA, Dos Santos AA, Marchiori E. Magnetic resonance imaging findings in Guillain-Barre syndrome caused by Zika virus infection. *Neuroradiology* 2016;58:837-838.
18. Gonzalez-Escobar G, Valadere AM, Adams R, et al. Prolonged Zika virus viremia in a patient with Guillain-Barre syndrome in Trinidad and Tobago. *Rev Panam Salud Publica* 2018;41:e136.

19. GeurtsvanKessel CH, Islam Z, Islam MB, et al. Zika virus and Guillain-Barre syndrome in Bangladesh. *Ann Clin Transl Neurol* 2018;5:606-615.
20. Hamer DH, Barbre KA, Chen LH, et al. Travel-Associated Zika Virus Disease Acquired in the Americas Through February 2016: A GeoSentinel Analysis. *Ann Intern Med* 2017;166:99-108.
21. Kassavetis P, Joseph JM, Francois R, Perloff MD, Berkowitz AL. Zika virus-associated Guillain-Barre syndrome variant in Haiti. *Neurology* 2016;87:336-337.
22. Miller E, Becker Z, Shalev D, Lee CT, Cioroiu C, Thakur K. Probable Zika virus-associated Guillain-Barre syndrome: Challenges with clinico-laboratory diagnosis. *J Neurol Sci* 2017;375:367-370.
23. Rabelo K, Souza LJ, Salomao NG, et al. Placental Inflammation and Fetal Injury in a Rare Zika Case Associated With Guillain-Barre Syndrome and Abortion. *Front Microbiol* 2018;9:1018.
24. Raboni SM, Bonfim C, Almeida BM, et al. Flavivirus cross-reactivity in serological tests and Guillain-Barré syndrome in a hematopoietic stem cell transplant patient: A case report. *Transpl Infect Dis* 2017;19.
25. Reyna-Villasmil E, Lopez-Sanchez G, Santos-Bolivar J. Guillain-Barré syndrome due to Zika virus during pregnancy. *Med Clin (Barc)* 2016;146:331-332.
26. Siu R, Bukhari W, Todd A, Gunn W, Huang QS, Timmings P. Acute Zika infection with concurrent onset of Guillain-Barré Syndrome. *Neurology* 2016;87:1623-1624.
27. Zambrano H, Waggoner JJ, Almeida C, Rivera L, Benjamin JQ, Pinsky BA. Zika Virus and Chikungunya Virus CoInfections: A Series of Three Cases from a Single Center in Ecuador. *Am J Trop Med Hyg* 2016;95:894-896.
28. Simon O, Acket B, Forfait C, et al. Zika virus outbreak in New Caledonia and Guillain-Barré syndrome: a case-control study. *J Neurovirol* 2018;24:362-368.
29. Brito Ferreira ML, Antunes de Brito CA, Moreira AJP, et al. Guillain-Barre Syndrome, Acute Disseminated Encephalomyelitis and Encephalitis Associated with Zika Virus Infection in Brazil: Detection of Viral RNA and Isolation of Virus during Late Infection. *Am J Trop Med Hyg* 2017;97:1405-1409.
30. Nobrega M, Araujo ELL, Wada MY, Leite PLE, Dimech GS, Percio J. Outbreak of Guillain-Barre syndrome possibly related to prior Zika virus infection, Metropolitan Region of Recife, Pernambuco, Brazil, 2015. *Epidemiol Serv Saude* 2018;27:e2017039.
31. Styczynski AR, Malta J, Krow-Lucal ER, et al. Increased rates of Guillain-Barré syndrome associated with Zika virus outbreak in the Salvador metropolitan area, Brazil. *PLoS Negl Trop Dis* 2017;11:e0005869.
32. do Rosario MS, de Jesus PA, Vasilakis N, et al. Guillain-Barre Syndrome After Zika Virus Infection in Brazil. *Am J Trop Med Hyg* 2016;95:1157-1160.
33. Keesen TSL, de Almeida RP, Gois BM, et al. Guillain-Barre syndrome and arboviral infection in Brazil. *Lancet Infect Dis* 2017;17:693-694.
34. da Silva IRF, Frontera JA, Bispo de Filippis AM, Nascimento O, Group R-G-ZR. Neurologic Complications Associated With the Zika Virus in Brazilian Adults. *JAMA Neurol* 2017;74:1190-1198.
35. Azevedo MB, Coutinho MSC, Silva MAD, et al. Neurologic manifestations in emerging arboviral diseases in Rio de Janeiro City, Brazil, 2015-2016. *Rev Soc Bras Med Trop* 2018;51:347-351.

36. Mehta R, Soares CN, Medialdea-Carrera R, et al. The spectrum of neurological disease associated with Zika and chikungunya viruses in adults in Rio de Janeiro, Brazil: A case series. *PLoS Negl Trop Dis* 2018;12:e0006212.
37. Sebastian UU, Ricardo AVA, Alvarez BC, et al. Zika virus-induced neurological critical illness in Latin America: Severe Guillain-Barre Syndrome and encephalitis. *J Crit Care* 2017;42:275-281.
38. Salinas JL, Walteros DM, Styczynski A, et al. Zika virus disease-associated Guillain-Barré syndrome-Barranquilla, Colombia 2015-2016. *J Neurol Sci* 2017;381:272-277.
39. Parra B, Lizarazo J, Jimenez-Arango JA, et al. Guillain-Barré syndrome associated with Zika virus infection in Colombia. *N Engl J Med* 2016;375:1513-1523.
40. Villamil-Gomez WE, Sanchez-Herrera AR, Hernandez H, et al. Guillain-Barré syndrome during the Zika virus outbreak in Sucre, Colombia, 2016. *Travel Med Infect Dis* 2017;16:62-63.
41. Acevedo N, Waggoner J, Rodriguez M, et al. Zika Virus, Chikungunya Virus, and Dengue Virus in Cerebrospinal Fluid from Adults with Neurological Manifestations, Guayaquil, Ecuador. *Front Microbiol* 2017;8:42.
42. Langerak T, Yang H, Baptista M, et al. Zika Virus Infection and Guillain-Barre Syndrome in Three Patients from Suriname. *Front Neurol* 2016;7:233.
43. Dirlikov E, Major CG, Mayshack M, et al. Guillain-Barre Syndrome During Ongoing Zika Virus Transmission - Puerto Rico, January 1-July 31, 2016. *MMWR Morb Mortal Wkly Rep* 2016;65:910-914.
44. Dirlikov E, Major CG, Medina NA, et al. Clinical Features of Guillain-Barre Syndrome With vs Without Zika Virus Infection, Puerto Rico, 2016. *JAMA Neurol* 2018;75:1089-1097.
45. Roze B, Najioullah F, Ferge JL, et al. Guillain-Barré Syndrome Associated With Zika Virus Infection in Martinique in 2016: A Prospective Study. *Clin Infect Dis* 2017;65:1462-1468.
46. Del Carpio-Orantes L, Peniche Moguel KG, Sanchez Diaz JS, et al. Guillain-Barre syndrome associated with Zika virus infection: Analysis of a cohort from the region of northern Veracruz in 2016-2017. *Neurologia* 2018.
47. Umapathi T, Kam YW, Ohnmar O, et al. The 2016 Singapore Zika virus outbreak did not cause a surge in Guillain-Barre syndrome. *J Peripher Nerv Syst* 2018;23:197-201.
48. Watrin L, Ghawche F, Larre P, Neau JP, Mathis S, Fournier E. Guillain-Barre Syndrome (42 Cases) Occurring During a Zika Virus Outbreak in French Polynesia. *Medicine (Baltimore)* 2016;95:e3257.
49. Del Carpio Orantes L, Juarez Rangel FJ, Garcia-Mendez S. Incidence of Guillain-Barre syndrome at a secondary centre during the 2016 zika outbreak. *Neurologia* 2017.
50. Cao-Lormeau VM, Blake A, Mons S, et al. Guillain-Barré syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *Lancet* 2016;387:1531-1539.
51. Del Carpio-Orantes L, Peniche Moguel KG, Sanchez Diaz JS, et al. Guillain-Barre syndrome associated with Zika virus infection: Analysis of a cohort from the region of northern Veracruz in 2016-2017 *Neurologia (Engl Ed)* 2020;35:429-431.
52. Hadden RD, Cornblath DR, Hughes RA, et al. Electrophysiological classification of Guillain-Barre syndrome: clinical associations and outcome. Plasma Exchange/Sandoglobulin Guillain-Barre Syndrome Trial Group. *Ann Neurol* 1998;44:780-788.

53. Ho TW, Mishu B, Li CY, et al. Guillain-Barre syndrome in northern China. Relationship to Campylobacter jejuni infection and anti-glycolipid antibodies. *Brain* 1995;118 (Pt 3):597-605.
54. Rajabally YA, Durand MC, Mitchell J, Orlikowski D, Nicolas G. Electrophysiological diagnosis of Guillain-Barré syndrome subtype: could a single study suffice? *J Neurol Neurosurg Psychiatry* 2015;86:115-119.
55. Caudie C, Quittard Pinon A, Taravel D, et al. Preceding infections and anti-ganglioside antibody profiles assessed by a dot immunoassay in 306 French Guillain-Barre syndrome patients. *J Neurol* 2011;258:1958-1964.
56. Rees JH, Hughes RA. Campylobacter jejuni and Guillain-Barré syndrome. *Ann Neurol* 1994;35:248-249.
57. Coelho FC, Durovni B, Saraceni V, et al. Higher incidence of Zika in adult women than adult men in Rio de Janeiro suggests a significant contribution of sexual transmission from men to women. *Int J Infect Dis* 2016;51:128-132.
58. Lozier M, Adams L, Febo MF, et al. Incidence of Zika Virus Disease by Age and Sex - Puerto Rico, November 1, 2015-October 20, 2016. *MMWR Morb Mortal Wkly Rep* 2016;65:1219-1223.
59. Wakerley BR, Kokubun N, Funakoshi K, Nagashima T, Hirata K, Yuki N. Clinical classification of 103 Japanese patients with Guillain-Barre syndrome. *J Neurol Sci* 2016;369:43-47.
60. Fokke C, van den Berg B, Drenthen J, Walgaard C, van Doorn PA, Jacobs BC. Diagnosis of Guillain-Barré syndrome and validation of Brighton criteria. *Brain* 2014;137:33-43.
61. Hiew FL, Ramlan R, Viswanathan S, Puvanarajah S. Guillain-Barré Syndrome, variants & forms fruste: Reclassification with new criteria. *Clin Neurol Neurosurg* 2017;158:114-118.
62. Beaver JT, Lelutiu N, Habib R, Skountzou I. Evolution of Two Major Zika Virus Lineages: Implications for Pathology, Immune Response, and Vaccine Development. *Front Immunol* 2018;9:1640-1640.
63. Uncini A, Shahrizaila N, Kuwabara S. Zika virus infection and Guillain-Barré syndrome: a review focused on clinical and electrophysiological subtypes. *J Neurol Neurosurg Psychiatry* 2017;88:266-271.
64. Rees JH, Soudain SE, Gregson NA, Hughes RA. Campylobacter jejuni infection and Guillain-Barré syndrome. *N Engl J Med* 1995;333:1374-1379.
65. Takahashi M, Koga M, Yokoyama K, Yuki N. Epidemiology of Campylobacter jejuni isolated from patients with Guillain-Barré and Fisher syndromes in Japan. *J Clin Microbiol* 2005;43:335-339.
66. Muñoz LS, Parra B, Pardo CA, Neuroviruses Emerging in the Americas S. Neurological Implications of Zika Virus Infection in Adults. *The Journal of infectious diseases* 2017;216:S897-S905.
67. Fourié T, Grard G, Leparc-Goffart I, Briolant S, Fontaine A. Variability of Zika Virus Incubation Period in Humans. *Open Forum Infect Dis* 2018;5:ofy261-ofy261.
68. Cumberworth SL, Barrie JA, Cunningham ME, et al. Zika virus tropism and interactions in myelinating neural cell cultures: CNS cells and myelin are preferentially affected. *Acta Neuropathol Commun* 2017;5:50.
69. Gracey DR, McMichan JC, Divertie MB, Howard FM, Jr. Respiratory failure in Guillain-Barré syndrome: a 6-year experience. *Mayo Clin Proc* 1982;57:742-746.

70. van Leeuwen N, Lingsma HF, Vanrolleghem AM, et al. Hospital Admissions, Transfers and Costs of Guillain-Barré Syndrome. *PLOS ONE* 2016;11:e0143837.
71. Durand MC, Porcher R, Orlikowski D, et al. Clinical and electrophysiological predictors of respiratory failure in Guillain-Barre syndrome: a prospective study. *Lancet Neurol* 2006;5:1021-1028.
72. Ruts L, Drenthen J, Jongen JL, et al. Pain in Guillain-Barré syndrome: a long-term follow-up study. *Neurology* 2010;75:1439-1447.



Chapter 3

Guillain-Barré syndrome during the Zika virus outbreak in Northeast Brazil: an observational cohort study

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ABSTRACT

Objective

To determine the clinical phenotype of Guillain-Barré syndrome (GBS) after Zika virus (ZIKV) infection, the anti-glycolipid antibody signature, and the role of other circulating arthropod-borne viruses, we describe a cohort of GBS patients identified during ZIKV and chikungunya virus (CHIKV) outbreaks in Northeast Brazil.

Methods

We prospectively recruited GBS patients from a regional neurology center in Northeast Brazil between December 2014 and February 2017. Serum and CSF were tested for ZIKV, CHIKV, and dengue virus (DENV), by RT-PCR and antibodies, and serum was tested for GBS-associated antibodies to glycolipids.

Results

Seventy-one patients were identified. Forty-eight (68%) had laboratory evidence of a recent arbovirus infection; 25 (52%) ZIKV, 8 (17%) CHIKV, 1 (2%) DENV, and 14 (29%) ZIKV and CHIKV. Most patients with a recent arbovirus infection had motor and sensory symptoms (72%), a demyelinating electrophysiological subtype (67%) and a facial palsy (58%). Patients with a recent infection with ZIKV and CHIKV had a longer hospital admission and more frequent mechanical ventilation compared to the other patients. No specific anti-glycolipid antibody signature was identified in association with arbovirus infection, although significant antibody titres to GM1, GalC, LM1, and GalNAc-GD1a were found infrequently.

Conclusion

A large proportion of cases had laboratory evidence of a recent infection with ZIKV or CHIKV, and recent infection with both viruses was found in almost one third of patients. Most patients with a recent arbovirus infection had a sensorimotor, demyelinating GBS. We did not find a specific anti-glycolipid antibody signature in association with arbovirus-related GBS.

INTRODUCTION

Zika virus (ZIKV), a positive sense single stranded RNA flavivirus transmitted by the *Aedes aegypti* mosquito, has caused major outbreaks in the Americas between 2015 and 2017. Brazil was severely affected by the epidemic and the incidence was especially high in the Northeast region of the country.[1] Over the last decades, Brazil also faced outbreaks of dengue virus (DENV) and chikungunya virus (CHIKV), that are transmitted by the same mosquito and, like ZIKV, can cause febrile illness with myalgia, arthralgia, and rash.[2-4] And although most infections with ZIKV are asymptomatic, or cause mild disease, in some patients severe neurological complications occur, and the most frequently reported neurological complication in adults is the Guillain-Barré syndrome (GBS).[5-9] In patients with DENV and CHIKV infection neurological complications, including GBS, have also been reported in smaller studies.[10-13]

GBS is an immune-mediated polyradiculoneuropathy that is triggered by preceding infections. Some types of infections have been shown to be associated with a specific clinical phenotype of GBS and presence of specific anti-glycolipid antibodies directed against gangliosides (a type of sialylated glycolipid) on the nerve axon.[14, 15]

However, a uniform description of the clinical phenotype or the anti-ganglioside antibody signature of ZIKV-related GBS has not emerged in previous studies.[5, 8, 16-20] Furthermore, little is known about the role of other circulating arboviruses, such as DENV and CHIKV, as potential triggers for GBS.[10]

To study the relation between GBS and circulating arbovirus infections, we describe a large, well-defined, and unselected cohort of GBS patients with evidence of a preceding arbovirus infection from a single center in Northeast Brazil that was tested for arboviruses and a broad spectrum of anti-ganglioside antibodies. The area of the study hospital is endemic for DENV and cases were collected during a ZIKV and a CHIKV outbreak.

METHODS

Study setting, population, design and ethics

All patients with a suspected preceding arbovirus infection and an acute neurological disease identified between December 2014 and December 2016 at Hospital da Restauração, a public hospital with a tertiary neurology service in Northeast Brazil, were consecutively recruited. In total, 201 neurological disease cases were identi-

fied, as we have previously described.[21] The most frequent neurological diagnoses were GBS, myelitis, and (meningo)encephalitis. For the current study, the 65 patients diagnosed with GBS from this cohort were selected and analyzed. Additionally, all GBS patients with a history of arbovirus symptoms identified between December 2016 and February 2017 were included in this study (n=6). (**Supplementary Figure 1**) A suspected arbovirus infection was defined as fever, arthralgia or rash within 12 months before the onset of neurological symptoms. We chose a 12 month window because we did not want to make presumptions about the latency between infection and neurological disease onset. We did a separate analysis of the cases presenting within 3 months after onset of infectious symptoms, recognizing that most GBS cases occur within this time window. Diagnosis of GBS was classified according to the Brighton Collaboration criteria, and GBS variants other than Miller Fisher syndrome were defined according to other published criteria.[22, 23] To enhance diagnostic accuracy, the clinical history of all patients was reviewed by MLBF, SEL and SBL, and in case of disagreement arbitrated by BCJ. All patients signed informed consent forms. The study protocol was reviewed and approved by the Oswaldo Cruz Foundation - FIOCRUZ, Instituto Aggeu Magalhães Ethics Committee (CAAE #511.06115.8 000 5190).

Clinical Data Procedures

Clinical information was recorded on standardized case report forms and included demographics, history of suspected arbovirus infection and neurological examination, ancillary investigations and disease progression that were collected until 12 months after onset of neurological symptoms.(See **Supplementary Material**) The online registry for mortality of the Brazilian Ministry of Health was consulted to document mortality following hospital discharge within the study period. For **Figure 1**, the number of GBS cases was based on hospital records reviewed by MLBF, and the outbreak periods of ZIKV, DENV and CHIKV were based on reported epidemiological data from the Instituto Aggeu Magalhães, Fiocruz Pernambuco (2000-2006), and the Brazilian Ministry of Health (Ministério de Saúde, Secretaria de Vigilância em Saúde, 2006-2018).[24, 25] As these numbers were defined around routine surveillance they should be interpreted with caution.

Diagnostic virology

Serum and cerebrospinal fluid (CSF) samples were collected and sent to the Flavivirus Reference Laboratory, Oswaldo Cruz Foundation, Recife, Brazil for arbovirus diagnostic testing. Viral RNA was extracted from serum samples using the QIAamp Viral RNA kit (Qiagen, Hilden - Germany). ZIKV, CHIKV and DENV real time RT-PCR (rRT-PCR) reactions were performed from purified RNA serum samples.[26-28] Anti-

DENV and anti-CHIKV IgM and IgG antibodies were detected using commercially available capture enzyme-linked immunosorbent assay (ELISA) kits (dengue- Panbio, Alere - USA; chikungunya - EuroImmun AG, Luebeck - Germany). ZIKV specific IgM antibodies were detected by IgM-Capture ELISA (MAC-ELISA), which uses ZIKV and DENV antigens in parallel.[29] Serotype-specific anti-dengue antibodies and anti-Zika antibodies were assessed by 50% plaque reduction neutralization tests (PRNT), following a previously described protocol. The cut-off for positivity was defined based on a 50% reduction in plaque count ($PRNT_{50}$).[30]

We considered there to be evidence of recent ZIKV, CHIKV or DENV infection if there was viral RNA or specific IgM antibodies in patient serum or CSF, as defined previously.[4, 27-29] Presence of ZIKV neutralizing antibodies on PRNT and negative IgM was considered as insufficient evidence of a recent ZIKV infection. In samples IgM-positive for both ZIKV and DENV, the PRNT assay was used to quantify neutralizing antibody titers to ZIKV and DENV serotypes 1-4 and determine viral diagnosis. If patients had neutralizing antibodies against both viruses without a PCR positive test confirming infection with one or the other, we deemed this an indeterminate flavivirus infection and, given the epidemiological linkage, presumed it to be Zika as others have previously.[7, 30]

Anti-glycolipid serology

Glycolipid microarray analysis of serum samples was performed at the University of Glasgow, United Kingdom, to detect IgM and IgG antibodies against 16 commonly studied glycolipids in GBS: GM1, GM2, phosphatidylserine, GM4, GA1, GD1a, GD1b, GT1a, GT1b, GQ1b, GD3, SGPG, LM1, GalNAc-GD1a, GalC and sulfatide, plus their possible heterodimeric complexes as previously described.[31] Matrixes were scanned using Genepix 4300A (Molecular Devices, California, USA) and heat maps were created using MeV software. Due to the heterogeneous pattern of anti-glycolipid antibodies found in GBS, the small sample size, the known presence of naturally occurring anti-carbohydrate antibodies in the normal population and the lack of baseline control sera, statistical comparison of the array results was limited. Therefore, for the purpose of assay standardization, the anti-glycolipid antibody profile in patients with GBS were compared to the profile obtained from the sera of patients with other neurological diseases seen during the same study period at the same hospital, either with or without evidence of a recent arbovirus infection.

Statistical analysis

We used IBM SPSS Statistics 25® for data analysis, comparing clinical features between the different arbovirus diagnostic groups with the Mann-Whitney U test or

the Kruskal-Wallis test for continuous data, and the Chi square or Fisher's exact test for proportions.

Proportions were described as number of patients with the variable present divided by the number of patients with the variable reported, excluding those with missing values. A two-sided P-value < 0.05 was considered significant.

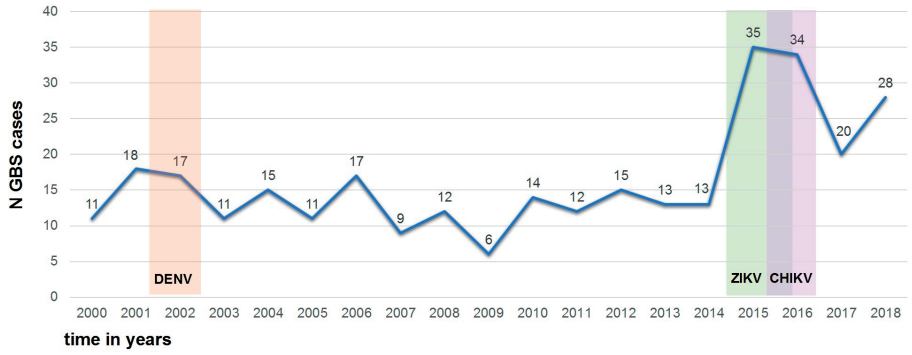


Figure 1. Number of GBS cases in study hospital in relation to outbreak periods of Dengue, Zika and Chikungunya virus

RESULTS

A total of 71 patients with GBS were identified for the study between December 2014 and February 2017 (**Supplementary Figure 1**). During the recruitment period, at the time of the ZIKV and CHIKV outbreak, a peak in GBS admissions was seen in the study hospital compared with the previous years (**Figure 1**).[30, 32]

GBS cases in the study hospital in Recife, Pernambuco, Brazil between 2000 and 2018 in relation to periods of outbreaks of dengue virus (DENV, orange), Zika virus (ZIKV, green) and chikungunya virus (CHIKV, purple). The numbers in the line graph indicate the number of new GBS patients identified at the hospital per year. Outbreak periods were defined based on epidemiological data of the Pernambuco state from the Brazilian Ministry of Health. The number of notified DENV cases in 2002(±116,000) and the number of notified CHIKV cases in 2016 (±50,000) were 5-10 times higher compared to previous and following years. The ZIKV outbreak in 2014-2016 was based on the high number of suspected DENV cases (±110,000 in 2015) that were in later studies determined as probable ZIKV cases.[33]

Table 1: Demographic, infectious and neurological symptoms

	All cases (n=71)	No lab evidence of recent arbovirus (n=23)	ZIKV (n=25)	CHIKV (n=8)	ZIKV + CHIKV (n=14)	*p value*
Age	46 (32-56)	45 (34-57)	39 (30-50)	51 (37-58)	50 (32-57)	*p=0.59*
Male: Female (ratio)	35:36 (0.97)	9:14 (0.64)	14:11 (1.27)	3:5 (0.6)	8:6 (1.33)	
Infectious symptoms						
Rash	65 (92)	18 (78)	25 (100)	8 (100)	13 (93)	***p=0.01***
Arthralgia	40/70 (57)	13/22 (59)	13 (52)	6 (75)	8 (57)	*p=0.77*
Myalgia	39/70 (56)	16/22 (73)	9 (36)	6 (75)	7 (50)	*p=0.05*
Fever	38/70(54)	11 (48)	10 (40)	5 (63)	12 (86)	***p=0.04***
Headache	38/70 (54)	12/22 (55)	11 (44)	4 (50)	10 (71)	*p=0.44*
Infectious- neurological symptoms (days)*	8 (4-24)	6 (4-15)	7 (3-12)	29 (18-111)	9 (6-31)	***p=0.007***
Neurological symptoms						
Facial weakness	36 (51)	11 (48)	14 (56)	5 (63)	5 (36)	*p=0.58*
Bulbar symptoms	25 (35)	10 (44)	8 (32)	3 (38)	4 (29)	*p=0.80*
Limb weakness	69 (97)	22 (96)	24 (96)	8 (100)	14 (100)	*p=1.0*
Sensory symptoms	61 (86)	17 (74)	23 (92)	8 (100)	12 (86)	*p=0.25*
Neurological examination						
Cranial neuropathy	39/70 (56)	12/23 (52)	16 (67)	5 (63)	5 (36)	*p=0.31*
Oculomotor weakness	2 (3)	1 (4)	1 (4)	0 (0)	0 (0)	*p=1.00*
Facial palsy	38/70 (54)	10/22 (46)	16 (64)	5 (63)	6 (43)	*p=0.48*
Bulbar palsy	17 (24)	7 (30)	5 (20)	3 (38)	2 (14)	*p=0.52*
Limb weakness	67 (94)	22 (96)	23 (92)	8 (100)	13 (93)	*p=1.00*
Tetraparesis	60 (85)	17 (74)	21 (74)	8 (100)	13 (93)	*p=0.34*
Paraparesis	7 (10)	5 (22)	2 (8)	0 (0)	0 (0)	*p=0.17*
Reflexes absent or low	61/70 (86)	19 (83)	22 (92)	6 (75)	14 (100)	*p=0.18*
Sensory deficits	28 (39)	10 (44)	16 (64)	6 (75)	7 (50)	*p=0.67*
Ataxia	8/68 (12)	1/22 (5)	5 (22)	1 (13)	1 (7)	*p=0.34*
Unable to walk	36 (52)	14 (61)	9 (39)	4 (50)	9 (64)	*p=0.39*
Dysautonomia†	18/68 (27)	7/21 (33)	7 (28)	2 (25)	2 (15)	*p=0.66*

Data are presented as n/N(%) or median (IQR). Statistical analysis of categorical variables with Chi square/ Fisher's exact, of continuous variables with Mann-Whitney U test or the Kruskal-Wallis. The p-value is the comparison between ZIKV, CHIKV, ZIKV-CHIKV and arbovirus-negative groups. *When excluding the 7 patients with time onset infectious – neurologic symptoms of >3months , differences between the ZIKV, CHIKV, ZIKV-CHIKV and no recent infection groups were still significant (p=0.02) . †hypo- or hypertension (n=10), excessive transpiration (n=6), tachycardia (n=4)

Demographic, clinical and diagnostic features

Demographic and clinical features are shown in **Table 1**. The median age was 46 (interquartile range (IQR) 32-56) years. Thirty-six patients (51%) were female. One child, aged 9, was included in the study.

Rash (92%), arthralgia (57%), and myalgia (56%) were the most frequently reported symptoms of a preceding infection. The median time between infectious and neurological symptoms was 8 days (IQR) 4-24), two patients developed infectious and neurological symptoms on the same day, and 35 (49%) developed neurological symptoms within 1 week. (**Supplementary Figure 2**)

The median time between onset of neurological symptoms and hospital admission was 5 days (IQR 2-11). Limb weakness and absent or diminished reflexes were found in the vast majority of patients. Sixty-one (86%) patients had either sensory symptoms or sensory loss identified in neurological examination. Cranial neuropathy was found in 39 (56%) patients, and facial and bulbar palsy were most frequently reported. Twelve patients (17%) had a clinical variant form of GBS: paraparetic (n=7), pure sensory (n=1), Miller Fisher syndrome (MFS) (n=1), MF-GBS-overlap syndrome (n=1), and bilateral facial paralysis with sensory signs (n=2).

CSF was examined for cell count and protein level in all patients. A combination of a normal cell count and increased (>45 mg/dL) protein level (albumino-cytological dissociation) was found in 89%. Sixty-four (90%) patients had a cell count of ≤5 cells/uL and none had a cell count of >20. Electrophysiological studies were performed in 21 (30%) patients, ten (62%) had features of a demyelinating, and six (28%) of an axonal motor or axonal motor and sensory neuropathy (**Table 2**). The date of electrophysiological studies was available in 15 (71%) cases, and studies were performed at a median of 24 days (IQR 13-47) after onset of neurological symptoms. Cranial or spinal computed tomography or magnetic resonance imaging was done to exclude alternative diagnoses in 35 (47%) patients.

Thirteen (18%) patients fulfilled Brighton criteria level 1, 45 (63%) level 2, and 13 (18%) level 4.[22] Of the patients with Brighton Level 4, three had a variant form of GBS, eight had normal or increased tendon reflexes, in one data on reflexes was missing, and one reached their nadir after 28 days. Twelve (92%) of these patients had either albumino-cytological dissociation in the CSF or electrophysiological studies compatible with GBS.

Table 2: Ancillary investigations, treatment and outcome

	All cases (N=71)	No lab evidence of recent arbovirus (N=23)	ZIKV (n=25)	CHIKV (n=8)	ZIKV + CHIKV (n=14)	*p value*
Ancillary investigations						
Cell count (cells/uL)	1 (0.33-2.7)	1 (0.33-2)	1 (0.33-3.33)	0.33 0.33-1.83)	0.67 (0.33-2.33)	*p=0.80*
<50 cells/uL	71 (100)	23 (100)				
Protein level (mg/dL)	95 (60-172)	72 (58-140)	102 (90-172)	124 (49-197)	66 (51-172)	*p=0.13*
>45 mg/dL	63 (89)	20 (87)	24 (96)	7 (88)	11 (79)	*p=0.35*
Nerve conduction studies	21 (30)	6 (26)	6 (24)	4 (50)	5 (36)	
AIDP	13/21 (62)	3/6 (50)	5/6 (83)	2/4 (50)	3/5 (60)	*p=0.64*
AMAN	3/21 (14)	2/6 (33)	0/6 (0)	1/4 (25)	0/5 (0)	
AMSAN	3/21 (14)	1/6 (17)	0/6 (0)	1/4 (25)	1/5 (20)	
Equivocal/other	2/21 (10)	0/6 (0)	1/6 (17)	0/4 (0)	1/5 (20)	
Treatment						
Immunomodulating therapy	70 (99)	23 (100)	25 (100)	8 (100)	13 (93)	*p=0.31*
IVIg	63 (89)	21 (91)	24 (96)	7 (88)	11 (79)	*p=0.30*
Steroids	7 (10)	2 (9)	1 (4)	1 (13)	2 (14)	*p=0.57*
Disease progression						
Duration of hospital admission	19 (13-24)	19 (9-25)	16 (11-20)	17 (15-20)	24 (20-29)	***p=0.02***
Respiratory insufficiency	12 (17)	2 (9)	3 (12)	2 (25)	5 (36)	*p=0.15*
Intensive Care Unit	14/69 (20)	7/22 (32)	1 (4)	1 (13)	5 (36)	***p=0.031***
Duration Intensive Care Unit	16 (8-52)	17 (6-90)	73	9	14 (14-19)	*p=0.55*
Intubated	9/66 (14)	3/20 (15)	1 (4)	0 (0)	5 (36)	***p=0.049***
Outcome						
Died	0 (0)	0 (0)				
Sequela at discharge	64/68 (94)	21 (91)	22 (92)	7 (88)	13 (93)	*p=0.38*
Recovered last follow-up	11/27 (41)	1/10 (10)	3/7 (43)	4/5 (80)	3/4 (75)	***p=0.02***

Data are presented as n/N(%) or median [range], (IQR). IVIg= intravenous immunoglobulin, onset= onset of neurological symptoms . Time in days. Statistical analysis of categorical variables with Chi square/Fisher's exact, of continuous variables with Mann-Whitney U test or the Kruskal-Wallis. The p-value represents the comparison between ZIKV, CHIKV, ZIKV-CHIKV and arbovirus negative groups. When patient groups had zero patients to compare, no p-value was calculated.

Table 3. Arbovirus test results

			ZIKV	CHIKV	ZIKV-CHIKV	DENV	all cases
virus	**sample**	**test**	**n=25**	**n=8**	**n=14**	**n=1**	**n=72**
ZIKV	serum	PCR only	5/25	-	5/12	-	10/66
		IgM only	13/23	-	4/14	-	17/68
		PCR & IgM	1/23	-	0/12	-	1/66
	CSF	PCR only	0/11	-	2/6	-	2/19
		IgM only	1/8	-	0/6	-	1/16
		PCR & IgM	0/8	-	1/6	-	1/15
	CSF & serum	PCR CSF, PCR serum	0/11	-	1/6	-	1/19
		IgM CSF, IgM serum	3/7	-	1/6	-	3/15
		IgM CSF, PCR & IgM serum	1/7	-	0/6	-	1/15
		PCR & IgM CSF, PCR serum	1/8	-	0/6	-	1/15
CHIKV	serum	PCR only	-	0/8	2/13	-	2/64
		IgM only	-	8/8	7/14	-	15/71
		PCR & IgM	-	0/8	1/13	-	1/64
	CSF	PCR only	-	-	1/6	-	1/12
	CSF & serum	PCR CSF, IgM serum	-	-	3/6	-	3/12
DENV	serum	IgM only	2/25	0/7	4/14	1/1	7/71
	CSF	IgM only	0/8	-	1/6	-	1/57

Arbovirus test results stratified according to infection with Zika virus (ZIKV) chikungunya (CHIKV), dengue virus (DENV), and Zika and chikungunya virus (ZIKV-CHIKV). Number of positive tested patients is displayed in relation to total number of patients tested for each test or combination of tests (n/N) for each diagnostic category (ZIKV, CHIKV, ZIKV-CHIKV, DENV). PCR= polymerase-chain-reaction, IgM= immunoglobulin M, CSF=cerebrospinal fluid

Arbovirus diagnostics

In total, 112 serum samples and 19 CSF samples were available for arbovirus testing and in 28 patients serial serum samples were available. Forty-eight (68%) had evidence of a recent arbovirus infection of which 25 (52%) had a recent ZIKV, 8 (17%) CHIKV, one (2%) DENV, and 14 (29%) had evidence of both a recent ZIKV and CHIKV infection. (**Table 3, Figure 2**) Serum or CSF was IgM positive for both ZIKV and DENV in eight patients, six of these were ZIKV PCR positive, in one the neutralizing titer for ZIKV was higher than DENV, and in one no PRNT was done and this case was classified as a recent ZIKV infection on epidemiological grounds.[7, 34](**Supplementary Figure 2 and 3**)

Of the patients with samples collected within the first 2 months after onset of neurological symptoms, 77% had evidence of a recent arbovirus infection, whereas after 2

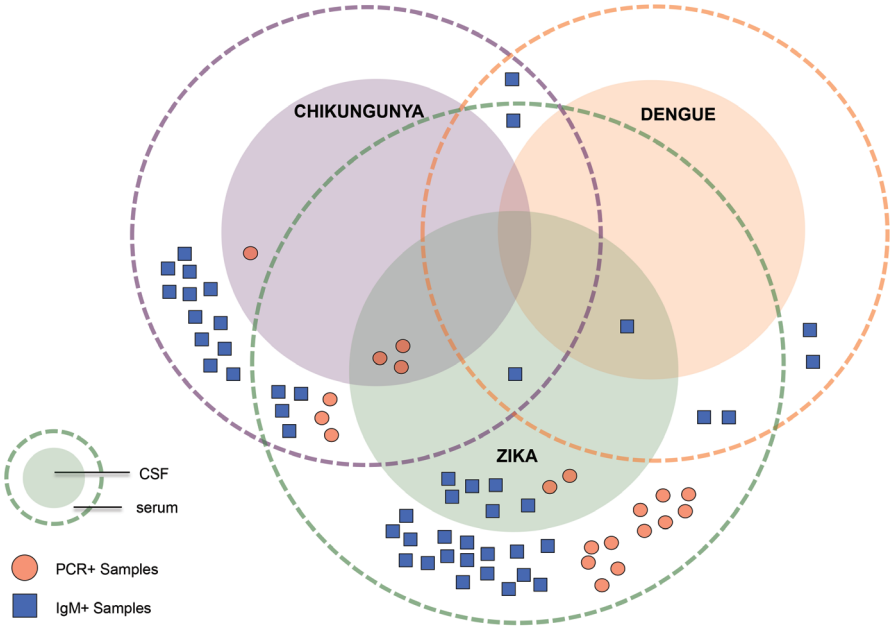


Figure 2. Venn diagram of arbovirus diagnostic groups
Overview of positive PCR and IgM samples for Zika virus (ZIKV), chikungunya virus (CHIKV) and dengue virus (DENV) in serum and cerebrospinal fluid (CSF). Of the patients with samples collected within the first 2 months after onset of neurological symptoms, 77% had evidence of a recent arbovirus infection, whereas after 2 months 52% did. In the 29 cases with late samples available, 14 (48%) neutralization assays were done, of which 12 (86%) were positive.

months 52% did. In the 29 cases with late samples available, 14 (48%) neutralization assays were done, of which 12 (86%) were positive.

Demographic or clinical features did not differ significantly between arbovirus diagnostic groups, with some exceptions. The median time between infectious and neurological symptoms was significantly longer in patients with CHIKV, and paraparesis was found more frequently in laboratory negative- compared to the other patients. No differences were found in frequency of electrophysiological subtypes between groups.

In the post-hoc analysis, the median time between onset of infection to onset of neurologic symptoms was 7 days (IQR 4-15). The findings in this analysis did not differ from the overall analysis, with the exception that the percentage of cases with rash and fever was not significantly different across groups.

Glycolipid antibody testing

Anti-glycolipid IgG and IgM antibody testing was performed on a subset of 52 GBS cases and a group of 40 controls with other neurological diseases. Of the 52 GBS sera examined, 41 (79%) tested positive for a recent arbovirus infection and of the 40 control sera, 27 (68%) had evidence of a recent arbovirus infection. We did not detect a glycolipid antigen-specific marker for arbovirus-associated GBS. The typical antibody signature (anti-GM1, anti-GM1b, anti-GD1a, anti-GalNAc-GD1a) most frequently associated with the axonal form of GBS was not seen in this cohort. In serum samples where anti-glycolipid antibodies were detected, most antibody reactivities were of very low intensity and not significantly different between GBS cases and other neurological controls, either with or without evidence of a recent arbovirus infection (Supplementary figure 4). Regardless of the group analysis, rare samples contained significant antibody titres to individual or groups of nerve-enriched glycolipids including GM1 (patient #169), GalC (patient #92), LM1 (patients #92 and 97) and GalNAc-GD1a (patient #39). Whilst these never reached significance in a group analysis, they were absent from the control group at these titres, but their relevance in individual cases is unclear and notably pathophysiologically unproven. The case with MFS did not have significant antibody titres to GQ1b, which is detected in ~90% MFS patients.[35] Of the patients with significant glycolipid antibody titers, only patient #169 had nerve conduction studies done, which showed an acute motor-sensory axonal neuropathy.

Treatment and disease progression

The median duration of hospital admission was 19 days (IQR 13-24). The majority of patients were treated with intravenous immunoglobulin (IVIg), and seven (10%) received steroids (as monotherapy) in another hospital, prior to admission to the study hospital. Fourteen of 69 reported patients (20%) were admitted to the Intensive Care Unit (ICU) and 9 of 66 (14%) were intubated. Patients with laboratory evidence of both a recent ZIKV and CHIKV infection had a longer duration of hospitalization, were admitted to the ICU, and intubated significantly more frequently than the other patients (**Table 2**). PCR-positive patients more often were intubated (5/17 vs 1/29, p=0.02), had respiratory insufficiency (8/19 vs 2/29, p=0.008) and had a longer duration of hospitalization (p=0.027) compared to those with only serological evidence of a recent arbovirus infection. In patients with evidence of both ZIKV and CHIKV infection, a larger proportion of those who were PCR-positive compared to those who were negative had respiratory insufficiency (0/4 vs 5/10), were admitted to the ICU (0/4 vs 5/10), or intubated (0/4 vs 5/10), although findings were not significant in this small subgroup.

None of the patients died during hospitalization. At discharge, 94% of patients had functional disability. Of the 27 patients followed up for 6 months or longer, 11 (41%) had recovered completely at last follow-up, six (22%) still had weakness in arms or legs, and seven (26%) had persisting facial weakness, which was still present more than 3 years after onset in five patients. Although numbers between groups were small, patients with laboratory evidence of a recent arbovirus infection were more likely than those without laboratory evidence to have recovered at last follow-up and presence of facial weakness was less common in this group (**Table 2**).

DISCUSSION

A large proportion of GBS patients in this Brazilian cohort had laboratory evidence of a recent infection with ZIKV or CHIKV, and recent infection with both of these viruses was found in almost one third of patients. This indicates that both of these viruses may be associated with GBS, building upon evidence from previous studies.[4, 10, 12] A recent DENV infection was found in just one patient in this cohort. This may be because there was no outbreak of DENV during the study period, also, there have been conflicting reports in literature about the presumed association between DENV and GBS.[34, 36] A larger proportion of cases with a recent infection with both ZIKV and CHIKV was admitted to the ICU and mechanically ventilated compared to the other patients, and the duration of hospital admission was longer in this group. This is important information for clinicians, as the geographic distributions of these arboviruses largely overlap and populations are therefore potentially at risk of contracting both infections. Furthermore, although the *A. aegypti* mosquito is the most prolific vector for both viruses, CHIKV is also effectively transmitted by *A. albopictus*, which populates more temperate regions, including southern Europe.[37] Therefore, clinicians working in these areas should be aware of this virus as a possible trigger for GBS.

The finding that a recent infection with both ZIKV and CHIKV could lead to a more severe severe form of GBS may be due to a larger underlying pathological immune response or a higher viral load. A more severe disease progression in PCR-positive versus -negative patients further suggests that viral load may be a factor in disease severity, as has been shown previously.[38] Most patients with a recent infection with both ZIKV and CHIKV developed neurological symptoms more than 1 week after infectious disease onset, and as the acute phase of ZIKV and CHIKV infections usually lasts a week, it seems unlikely that acute infectious symptoms alone caused

the severe disease progression in these patients. However, in patients with CHIKV infection, polyarthralgia lasting weeks to months has been described.[3]

Our cohort was younger and more often female than expected based on other studies on GBS.[39] A similar demographic profile has previously been described in GBS following other viral infections, including cytomegalovirus.[40, 41] This indicates that females and a younger age group may be more prone to develop GBS after a viral infection. However, young women have also been shown to be at highest risk for ZIKV infection, and the Latin American population is younger compared to Europe and North America, where most previous GBS studies have been conducted.[42-44] The general clinical profile of GBS following a recent arbovirus infection with ZIKV and/or CHIKV in our study was a sensorimotor GBS with facial palsy. Electrophysiological studies showed demyelination in most, although not all, cases. This is again similar to what has been described in GBS after other virus infections and is in contrast to the clinical profile of GBS after a *C. jejuni* infection, that has been associated with higher frequencies of a pure motor GBS variant and an axonal electrophysiological subtype.[40, 41]

It has been suggested that ZIKV-related GBS is caused by direct infection or para-infectious nerve damage, due to the short time between onset of infectious and neurological symptoms.[7] However, although some patients developed neurological symptoms on the same day as the onset of infectious symptoms, the median time between infectious and neurologic symptoms in our cohort was 8 days, which is similar to GBS followed by other infections and is in accordance with a post-infectious pathogenesis of GBS.[45] The incubation time of ZIKV is estimated at 7-14 and of CHIKV and DENV at 2-10 days, which may in part explain the differences we found in time between infectious- and neurological symptoms.[46, 47]

We did not find a specific anti-ganglioside antibody signature associated with arbovirus-related GBS. There was clear variation in basal levels of antibodies to the different glycolipid targets assessed across the tested population, irrespective of arbovirus or neurological status, as can be demonstrated upon visual inspection of the heat map (**Supplementary Figure 4**). Due to the absence of healthy control samples, we were unable to validate whether there was an increased frequency compared with baseline levels in the local population of anti-GA1 antibodies, which we previously observed in the smaller French Polynesian ZIKV-GBS cohort.[5] The low intensity antibodies that were observed may represent low affinity naturally occurring anti-carbohydrate antibodies in this population, or an epiphenomenon of neurological disease pathology. Our results contradict a Brazilian cohort study of

patients with acute ZIKV infection without neurological disease that had elevated levels of anti-GD3 antibodies.[48] It was hypothesized that during a subsequent infection these antibodies would breach a critical threshold, resulting in neurological pathology. However, a subsequent study by the same group did not identify GD3 as a sole antibody target in patients with ZIKV-GBS, instead, they reported a universal increase in anti-glycolipid antibodies.[49] This is likely due to differences in assay methodology including the setting of background assay noise and the restricted use of control samples, thereby under-estimating the extensive variation of non-specific binding amongst individuals observed in our assay platform.

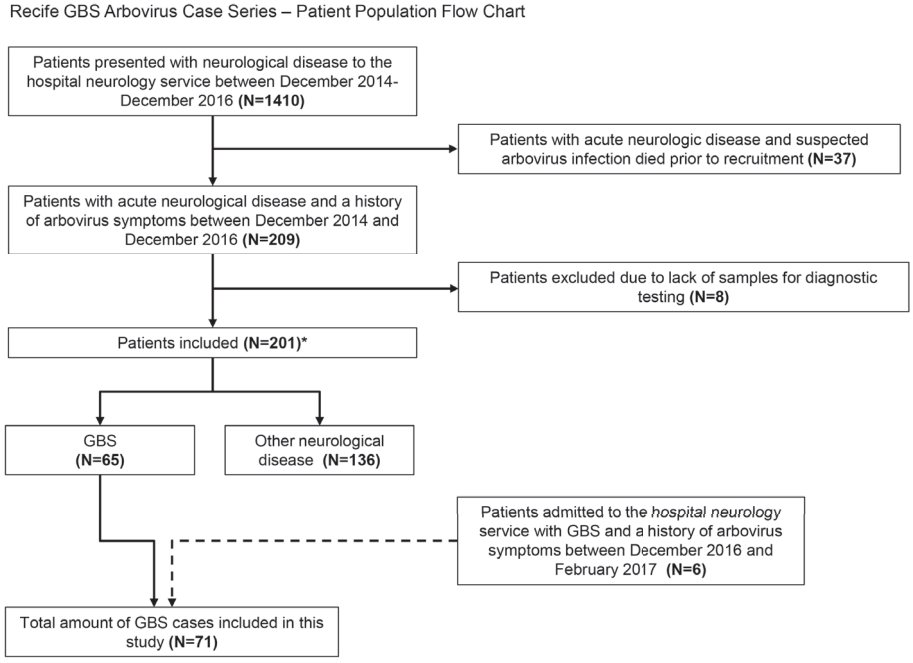
The peak in GBS cases that was observed in Recife before epidemiological surveillance for ZIKV was set up in the area, indicates the potential of GBS to act as a sentinel for the occurrence of outbreaks of arbovirus infection in areas where monitoring of such outbreaks is difficult. However, careful exclusion of other potential causes is crucial, as was seen in a recent outbreak of GBS in Peru, that was thought to be linked to ZIKV but later associated with *C. jejuni* and the typical anti-ganglioside antibody profile associated with this bacterium.[50]

Our study has several limitations. Clinical data and biological material could not always be collected in the acute phase of the disease, and we were unable to collect healthy controls for a case-control analysis. This study was therefore not designed to determine causality and evidence of a recent infection does not necessarily mean that this was indeed the infection triggering the onset of GBS, especially as we were unable to test for other infections associated with GBS. The late collection of samples may have led to falsely classifying patients as negative that may no longer have had virus RNA or IgM antibodies detectable, suggested by the lower frequency of positive results by PCR and IgM in patients with samples collected >2 months after start of neurological symptoms, but the high percentage (86%) of positive neutralization tests in these later samples. Furthermore, EMG examination was performed infrequently owing to a paucity of equipment and expertise in this study setting and was not classified on a uniform basis. The Brighton criteria were helpful in showing the diagnostic certainty based on the information available for all reported patients. These limitations are naturally inherent to studies conducted in an outbreak setting, in a low income region of Brazil.

In conclusion, our study indicates that besides ZIKV, CHIKV may be associated with GBS. No specific anti-glycolipid antibody signature was identified in our cohort in connection to arbovirus-related GBS. The severity of disease in patients with GBS and evidence of both a recent ZIKV and CHIKV infection emphasizes the impact of

arbovirus infections on patients and healthcare services. As threats of emerging infectious diseases persist it is important to advance our response to future outbreaks of GBS.[51]

SUPPLEMENTARY MATERIAL



Supplementary Figure 1. Population Flow Chart
Patient population flowchart.
*Published as a general overview paper of all patients with acute neurological disease and symptoms of a recent arbovirus infection.[21]

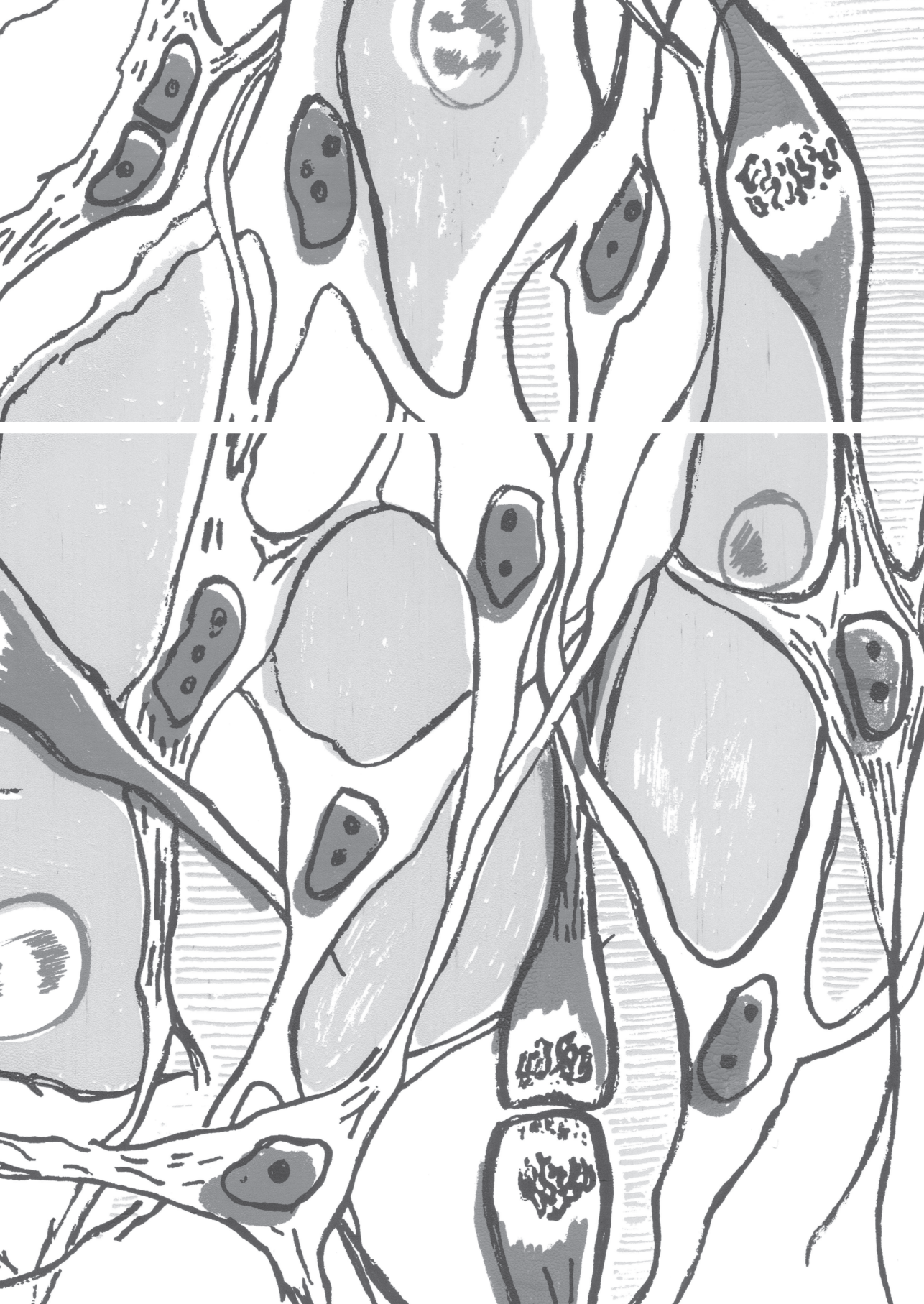
Supplementary Figure 2, 3 and 4 are available online at: https://doi.org/10.1016/j.jns.2020.117272

REFERENCES

1. Pan American Health Organization. Epidemiological Report Brazil. September 2017.
2. Petersen LR, Jamieson DJ, Powers AM, Honein MA. Zika Virus. New England Journal of Medicine 2016;374:1552-1563.
3. Weaver SC, Lecuit M. Chikungunya Virus and the Global Spread of a Mosquito-Borne Disease. New England Journal of Medicine 2015;372:1231-1239.
4. Mehta R, Soares CN, Medialdea-Carrera R, et al. The spectrum of neurological disease associated with Zika and chikungunya viruses in adults in Rio de Janeiro, Brazil: A case series. PLoS Negl Trop Dis 2018;12:e0006212.
5. Cao-Lormeau VM, Blake A, Mons S, et al. Guillain-Barre Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. Lancet 2016;387:1531-1539.
6. Brito Ferreira ML, Antunes de Brito CA, Moreira AJP, et al. Guillain-Barre Syndrome, Acute Disseminated Encephalomyelitis and Encephalitis Associated with Zika Virus Infection in Brazil: Detection of Viral RNA and Isolation of Virus during Late Infection. Am J Trop Med Hyg 2017;97:1405-1409.
7. Parra B, Lizarazo J, Jimenez-Arango JA, et al. Guillain-Barré syndrome associated with Zika virus infection in Colombia. N Engl J Med 2016;375:1513-1523.
8. Leonhard SE, Bresani-Salvi CC, Lyra Batista JD, et al. Guillain-Barré syndrome related to Zika virus infection: A systematic review and meta-analysis of the clinical and electrophysiological phenotype. PLoS Negl Trop Dis 2020;14:e0008264.
9. Dirlikov E, Medina NA, Major CG, et al. Acute Zika Virus Infection as a Risk Factor for Guillain-Barre Syndrome in Puerto Rico. JAMA 2017;318:1498-1500.
10. Brito CAA, Azevedo F, Cordeiro MT, Marques ETA, Jr., Franca RFO. Central and peripheral nervous system involvement caused by Zika and chikungunya coinfection. PLoS neglected tropical diseases 2017;11:e0005583-e0005583.
11. Carod-Artal FJ, Wichmann O, Farrar J, Gascon J. Neurological complications of dengue virus infection. Lancet Neurol 2013;12:906-919.
12. Stegmann-Planchard S, Gallian P, Tressières B, et al. Chikungunya, a Risk Factor for Guillain-Barré Syndrome. Clinical Infectious Diseases 2019.
13. Umapathi T, Lim CS, Ooi EE, et al. Asymptomatic dengue infection may trigger Guillain-Barre syndrome. J Peripher Nerv Syst 2016;21:375-377.
14. Drenthen J, Yuki N, Meulstee J, et al. Guillain-Barré syndrome subtypes related to Campylobacter infection. J Neurol Neurosurg Psychiatry 2011;82:300-305.
15. Yuki N, Susuki K, Koga M, et al. Carbohydrate mimicry between human ganglioside GM1 and Campylobacter jejuni lipooligosaccharide causes Guillain-Barre syndrome. Proc Natl Acad Sci U S A 2004;101:11404-11409.
16. Gongora-Rivera F, Grijalva I, Infante-Valenzuela A, et al. Zika Virus infection and Guillain-Barré syndrome in Northeastern Mexico: A case-control study. PLOS ONE 2020;15:e0230132.
17. Uncini A, Gonzalez-Bravo DC, Acosta-Ampudia YY, et al. Clinical and nerve conduction features in Guillain-Barre syndrome associated with Zika virus infection in Cucuta, Colombia. Eur J Neurol 2018;25:644-650.

18. Dirlikov E, Major CG, Medina NA, et al. Clinical Features of Guillain-Barre Syndrome With vs Without Zika Virus Infection, Puerto Rico, 2016. JAMA Neurol 2018;75:1089-1097.
19. Chang AY, Lynch R, Martins K, et al. Long-term clinical outcomes of Zika-associated Guillain-Barre syndrome. Emerg Microbes Infect 2018;7:148.
20. Roze B, Najioullah F, Ferge JL, et al. Guillain-Barré Syndrome Associated With Zika Virus Infection in Martinique in 2016: A Prospective Study. Clin Infect Dis 2017;65:1462-1468.
21. Brito Ferreira ML, Militão de Albuquerque MdFP, de Brito CAA, et al. Neurological disease in adults with Zika and chikungunya virus infection in Northeast Brazil: a prospective observational study. The Lancet Neurology 2020;19:826-839.
22. Sejvar JJ, Kohl KS, Gidudu J, et al. Guillain-Barré syndrome and Fisher syndrome: case definitions and guidelines for collection, analysis, and presentation of immunization safety data. Vaccine 2011;29:599-612.
23. Wakerley BR, Uncini A, Yuki N, et al. Guillain–Barré and Miller Fisher syndromes—new diagnostic classification. Nat Rev Neurol 2014;10:537.
24. Cordeiro MT, Schatzmayr HG, Nogueira RMR, Oliveira VFd, Melo WTd, Carvalho EFd. Dengue and dengue hemorrhagic fever in the State of Pernambuco, 1995-2006. Revista da Sociedade Brasileira de Medicina Tropical 2007;40:605-611.
25. Ministry of Health Brazil. National System in Health Surveillance: situation report: Pernambuco. 2011.
26. Lanciotti RS, Kosoy OL, Laven JJ, et al. Chikungunya virus in US travelers returning from India, 2006. Emerg Infect Dis 2007;13:764-767.
27. Lanciotti RS, Kosoy OL, Laven JJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis 2008;14:1232-1239.
28. Santiago GA, Vergne E, Quiles Y, et al. Analytical and clinical performance of the CDC real time RT-PCR assay for detection and typing of dengue virus. PLoS Negl Trop Dis 2013;7:e2311.
29. Centers for Disease Control. Zika MAC-ELISA instructions for use. 2016.
30. Magalhaes T, Braga C, Cordeiro MT, et al. Zika virus displacement by a chikungunya outbreak in Recife, Brazil. PLoS Negl Trop Dis 2017;11:e0006055.
31. Halstead SK, Kalna G, Islam MB, et al. Microarray screening of Guillain-Barre syndrome sera for antibodies to glycolipid complexes. Neurol Neuroimmunol Neuroinflamm 2016;3:e284.
32. de Oliveira WK, Carmo EH, Henriques CM, et al. Zika Virus Infection and Associated Neurologic Disorders in Brazil. N Engl J Med 2017;376:1591-1593.
33. Brito CA, Brito CC, Oliveira AC, et al. Zika in Pernambuco: rewriting the first outbreak. Rev Soc Bras Med Trop 2016;49:553-558.
34. Simon O, Billot S, Guyon D, et al. Early Guillain-Barre Syndrome associated with acute dengue fever. J Clin Virol 2016;77:29-31.
35. Uchibori A, Gyohda A, Chiba A. Ca(2+)-dependent anti-GQ1b antibody in GQ1b-seronegative Fisher syndrome and related disorders. J Neuroimmunol 2016;298:172-177.
36. Fragoso YD, Gomes S, Brooks JB, et al. Guillain-Barre syndrome and dengue fever: report on ten new cases in Brazil. Arq Neuropsiquiatr 2016;74:1039-1040.
37. Angelini R, Finarelli AC, Angelini P, et al. Chikungunya in north-eastern Italy: a summing up of the outbreak. Euro Surveill 2007;12:E071122 071122.

38. Lannuzel A, Fergé JL, Lobjois Q, et al. Long-term outcome in neuroZika: When biological diagnosis matters. Neurology 2019;92:e2406-e2420.
39. Doets AY, Verboon C, van den Berg B, et al. Regional variation of Guillain-Barré syndrome. Brain 2018;141:2866-2877.
40. Caudie C, Quittard Pinon A, Taravel D, et al. Preceding infections and anti-ganglioside antibody profiles assessed by a dot immunoassay in 306 French Guillain-Barre syndrome patients. J Neurol 2011;258:1958-1964.
41. Orlikowski D, Porcher R, Sivadon-Tardy V, et al. Guillain-Barre syndrome following primary cytomegalovirus infection: a prospective cohort study. Clin Infect Dis 2011;52:837-844.
42. Coelho FC, Durovni B, Saraceni V, et al. Higher incidence of Zika in adult women than adult men in Rio de Janeiro suggests a significant contribution of sexual transmission from men to women. Int J Infect Dis 2016;51:128-132.
43. Lozier M, Adams L, Febo MF, et al. Incidence of Zika Virus Disease by Age and Sex - Puerto Rico, November 1, 2015-October 20, 2016. MMWR Morb Mortal Wkly Rep 2016;65:1219-1223.
44. United Nations DoEaSA, Population Division,. World Population Prospects 2019. Online Edition Rev 1 2019.
45. Takahashi M, Koga M, Yokoyama K, Yuki N. Epidemiology of Campylobacter jejuni isolated from patients with Guillain-Barré and Fisher syndromes in Japan. J Clin Microbiol 2005;43:335-339.
46. Fourié T, Grard G, Leparc-Goffart I, Briolant S, Fontaine A. Variability of Zika Virus Incubation Period in Humans. Open Forum Infect Dis 2018;5:ofy261-ofy261.
47. Rudolph KE, Lessler J, Moloney RM, Kmush B, Cummings DAT. Incubation periods of mosquito-borne viral infections: a systematic review. Am J Trop Med Hyg 2014;90:882-891.
48. Nico D, Conde L, Rivera-Correa JL, et al. Prevalence of IgG Autoantibodies against GD3 Ganglioside in Acute Zika Virus Infection. Frontiers in Medicine 2018;5.
49. Rivera-Correa J, de Siqueira IC, Mota S, et al. Anti-ganglioside antibodies in patients with Zika virus infection-associated Guillain-Barré Syndrome in Brazil. PLOS Neglected Tropical Diseases 2019;13:e0007695.
50. Ramos AP LS, Halstead SK, Cuba MS, Castañeda CC, Dioses JA, Tipismana MS, Abanto JT, Llanos A, Gourlay D, Grogl M, Ramos M, Rojas JD, Meza R, Puiu D, Sherman RM, Salzberg SL, Simner PJ, Willison HJ, Jacobs BC, Cornblath DR, Umeres HF, Pardo CA. Guillain-Barré syndrome outbreak in Peru 2019 associated with Campylobacter jejuni infection. Neurology: Neuroimmunology & Neuroinflammation 2020, accepted for publication.
51. Leonhard SE, Cornblath DR, Endtz HP, Sejvar JJ, Jacobs BC. Guillain-Barre syndrome in times of pandemics. J Neurol Neurosurg Psychiatry 2020;91:1027-1029.



Chapter 4

Antecedent infections in Guillain-Barré syndrome in endemic areas of arbovirus transmission: A multinational case-control study

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ABSTRACT

Background and Aims

Half of the world's population is at risk of arthropod-borne virus (arbovirus) infections. Several arbovirus infections have been associated with Guillain-Barré syndrome (GBS). We investigated whether arboviruses are driving GBS beyond epidemic phases of transmission and studied the antibody response to glycolipids.

Methods

The protocol of the International Guillain-Barré syndrome Outcome Study (IGOS), an observational prospective cohort study, was adapted to a case-control design. Serum samples were tested for a recent infection with Zika virus (ZIKV), dengue virus (DENV), chikungunya (CHIKV) virus, hepatitis E virus, Epstein-Barr virus (EBV), cytomegalovirus (CMV), *Campylobacter jejuni, Mycoplasma pneumoniae,* and for antibodies to glycolipids.

Results

Forty-nine patients were included from Brazil (63%), Argentina (14%) and Malaysia (22%). Evidence of a recent infection was found in 27/49 (55%) patients: *C. jejuni* (n=15, 31%), *M. pneumoniae* (n=5, 10%), CHIKV (n=2, 4%), EBV (n=1, 2%), *C. jejuni* and *M. pneumoniae* (n=2, 4%), CMV and DENV (n=1, 2%), and *C. jejuni* and DENV (n=1, 2%). In 22 patients 35 paired controls were collected. Odds ratio for recent infections did not significantly differ between cases and controls. No typical anti-ganglioside antibody binding was associated with recent arbovirus infection.

Interpretation

Arbovirus infections occur in GBS patients outside of epidemic viral transmission, although not significantly more than in controls. Broad infection and anti-ganglioside antibody serology are important to establish the most likely pathogenic trigger in GBS patients. Larger studies are necessary to determine the association between arboviruses and GBS.

INTRODUCTION

Guillain-Barré syndrome (GBS) is an immune-mediated polyradiculoneuropathy and the most common cause of acute flaccid paralysis worldwide.[1] GBS is usually preceded by an infection and several pathogens have been associated with GBS in case-control studies, including *Campylobacter jejuni*, hepatitis E virus (HEV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), and *Mycoplasma pneumoniae*.[2-5] During the Zika virus (ZIKV) epidemic in 2015-2016 in Latin America, an increased incidence of GBS patients was observed and an association between ZIKV and GBS has later been confirmed.[6-8]

ZIKV is a flavivirus that is transmitted by the *Aedes aegypti* mosquito. Other arthropod-borne viruses (arboviruses) transmitted by the same mosquito, including dengue virus (DENV) and chikungunya virus (CHIKV), have also been associated with GBS, although evidence of an association is limited in comparison to ZIKV.[6, 9-18] Most studies on DENV and GBS are limited to case series,[10, 18-22] although two surveillances studies[17, 18] showed a temporal association between the incidence of GBS and DENV, and one case-control study provided evidence of an association between GBS and DENV.[23] Several studies have linked clusters of GBS cases with outbreaks of CHIKV,[15, 24-26] and a case-control study[9] demonstrated that CHIKV is a risk factor for GBS. Arboviruses have been increasingly recognized as a global health threat, as their geographic distribution has spread dramatically over the past decades.[12, 27, 28] Roughly half of the world's population is currently living in areas at risk for transmission of these viruses, and especially countries in Latin America and Southeast Asia are at risk.[29]

Previous studies that demonstrated a link between GBS and ZIKV or other arboviruses were done during epidemic phases of viral transmission, and it is unknown whether these viruses also play a role in the occurrence of GBS in endemic phases. Another aspect of arbovirus-related GBS that has not been illuminated, is the possible role of co-infections with other known triggers of GBS, as most previous studies only tested for arbovirus infections. Furthermore, the underlying pathophysiology and the role of antibodies to specific gangliosides and other glycolipids on the nerve axon, has not been uniformly demonstrated for GBS related to arboviruses.[26, 30-33]

The International Guillain-Barré syndrome Outcome Study (IGOS) is an international observational prospective cohort study on the disease course and outcome of GBS patients.[34] The protocol and infrastructure of this study were used and adapted to develop a case-control study ('IGOS-Zika study') to investigate the association between GBS and arboviruses, and specifically whether these infections drive

the occurrence of GBS beyond the peaks of epidemics. Samples were tested for a broad range of infections that are known to trigger GBS and for antibodies against glycolipids to investigate the role of co-infections and anti-glycolipid antibodies in arbovirus-related GBS.

METHODS

Study design

The study protocol of IGOS has been published elsewhere.[34] This protocol was adapted to investigate the association between arbovirus infections and GBS. Additional questions regarding immunization history and preceding symptoms and signs of arbovirus infections were collected. Where possible, two hospital-based controls were collected for every case. Controls were sex- and age matched (age difference <10 years), were treated in the same hospital and collected within 10 days of the included case. Controls were excluded if they had been diagnosed with GBS 1 year prior or if they were admitted for a (post-)infectious disorder. The same questions on arbovirus history and a serum sample were collected from the controls. Otherwise the protocol was identical to the original IGOS protocol. Patients were enrolled in two study sites in Brazil, four sites in Argentina, and one site in Malaysia. The IGOS study (MEC-2011-477) and the amendment of the study protocol (NL38706.078.11) were approved by the review boards of Erasmus MC University Medical Center, Rotterdam, The Netherlands. The study protocol was also approved by the local institutional review boards of all participating hospitals or universities. Written informed consent was obtained from all patients or their legal representatives.

Data collection

Data were collected on demography, antecedent events, and neurological symptoms and signs of GBS at study entry and at 1, 4, and 26 weeks.[34] Additional collection of data at week 2, 8, 13 and 52 was optional. Muscle strength was recorded by the Medical Research Council (MRC) score and disability by the GBS disability score.[35, 36] Disease nadir was defined as the first visit that the lowest MRC sum score was found during the first 4 weeks from study entry. When there was no muscle weakness, GBS disability score was used instead. Results of routine cerebrospinal fluid (CSF) examination and nerve conduction studies were collected. To determine the electrophysiological subtype, raw data of the first nerve conduction study, local reference values and an algorithm were used to classify each nerve conduction study according to the criteria of Hadden et al. by two independent clinical neurophysiologists (SA, JD).[37] Patients were categorized based on the Brighton Collaboration criteria based on

the available data.[38] Insufficient data were available to categorize the Miller Fisher syndrome (MFS) patients according to the published criteria, and all patients with clinical variants of GBS without limb weakness were categorized as Level 4. The ability to walk at 6 months was used to determine outcome. For patients with missing data at the 6 month visit, who were able to walk independently at the previous visit (week 13 or week 8), this visit was used to determine outcome.

Diagnostic virology and bacteriology

All patients and controls with available serum samples were tested for a recent infection with *C. jejuni*, HEV, *M. pneumoniae*, CMV, EBV, DENV, ZIKV and CHIKV. Serum samples collected at entry or week 1 were used where possible, otherwise samples collected at week 2 or 4 were used. Antibodies against *C. jejuni* were determined using an indirect enzyme-linked immunosorbent assay (ELISA) for IgG and antibody class capture ELISAs for IgM and IgA antibodies, as previously described.[39] IgM and IgG antibodies against HEV and *M. pneumoniae* were determined using commercially available ELISAs (Wantai, Beijing, PR China, respectively Serion ELISA classic *M. pneumoniae*, Serion GmbH, Würzburg, Germany). The presence of IgM and IgG antibodies and IgG avidity against CMV and of VCA IgM and viral capsid antigen (VCA) IgG and EBV nuclear antigen (EBNA), was determined by LIAISON®XL (DiaSorin, Italy); a semi-automated system, which uses chemiluminescent immunoassay (CLIA) technology for detection of antibodies. The presence of IgM and IgG antibodies against ZIKV and DENV were determined using commercially available ELISA (Euro-Immun, Lübeck, Germany). The presence of IgM and IgG antibodies against CHIKV was determined using a commercially available ELISA (Novatec) and immunofluorescence was done to verify presence of IgM. Immunofluorescence was leading in the interpretation of the results. In all patients that were IgM or IgG positive against ZIKV, a virus neutralization test (VNT) was done to differentiate between a recent DENV and ZIKV infection.[40] In general, IgM positivity is a good marker for a recent arbovirus infection, as studies have shown that ZIKV, CHIKV and DENV IgM become positive starting the first week after onset of symptoms and usually persist for up to 2-3 months.[41-43] Evidence of a recent infection was defined as: IgM positivity for *M. pneumoniae* and HEV, and IgM and/or IgA positivity for *C. jejuni*. For CMV, IgM positivity with negative IgG or IgG with low avidity, and for EBV, VCA IgM and VCA IgG positivity with negative EBNA IgG was considered indicative of a recent infection. For ZIKV, IgM positivity confirmed by VNT, and for CHIKV, IgM positivity in immunofluorescence was considered indicative of a recent infection. For DENV, NS1 positivity was considered indicative of a recent (re)infection as well as the combination of IgM and IgG positivity. Low positive or borderline IgM with positive IgG

was considered indicative of a previous infection (with possible reinfection with a different DENV strain). (**Supplementary table 1**)

Anti-glycolipid serology

Sera were tested with ELISA for IgG and IgM antibodies against GM1, GM2, GA1, GD1a, GD1b, GT1a, GQ1b, and GD3, and using combinatorial glycoarray for IgM and IgG anti-glycolipid antibodies against GM1, GM2, phosphatidylserine, GA1, GD1a, GD1b, GT1a, GQ1b, GD3, GalC, lactosylceramide and sulfatide, plus their possible heterodimeric complexes.[44, 45] Combinatorial glycoarray was done using a thin-layer chromatography autosampler which spotted glycolipids and glycolipid-combinations onto in-house made glass slides containing a polyvinylidene difluoride (PVDF) membrane.[46] Antibodies were detected using AF647-conjugated goat anti-human IgM and Cy3-conjugated goat anti-human IgG (Jackson ImmunoResearch). Fluorescent intensity was measured using the appurtenant LuxScan™ software. The mean and standard deviation was calculated for each glycolipid (-complex) using the fluorescent intensities of the control patients. Fluorescent intensities were considered positive if more than the mean plus three times the standard deviation.

Statistical analysis

We used SPSS Statistics 21.0 for data analysis. Continuous data are presented as medians with interquartile ranges (IQR) and dichotomized or categorical data as numbers and proportions. We used the Mann-Whitney U-test and Kruskal-Wallis test to compare continuous data, and the χ^2-test or Fisher's exact test to compare proportions. A two-sided *P-value* of <0.05 was considered significant. For the case-control analysis, crude odds ratios were calculated (not matching for pairs) using contingency tables and 95% confidence interval are calculated according to Altman, 1991.[47, 48] The Cox proportional hazards model was used for the individually paired case-control analysis (SPSS COXREG function), adjusting for age and sex.[49, 50] We used *R* version 3.6.1., packages dplyr 1.0.5 and ggplot2 3.3.2 for the development of the heatmaps. Raw data were clustered based on a distance matrix using Pearsons correlation and hierarchical cluster algorithm (Ward.2D) and clipped at a 10,000 upper limit.[51]

RESULTS

In total, 54 patients were included between July 2017 and December 2019. Five patients were excluded, four because of insufficient clinical data and one because of an alternative diagnosis (chronic inflammatory demyelinating polyradiculoneuropa-

thy). For 22 of the remaining 49 patients paired controls were collected, and they were included in the case-control analysis part of the study.(**Figure 1**) Demographic and clinical features, ancillary investigations and outcome of the full cohort are described in **Table 1**.

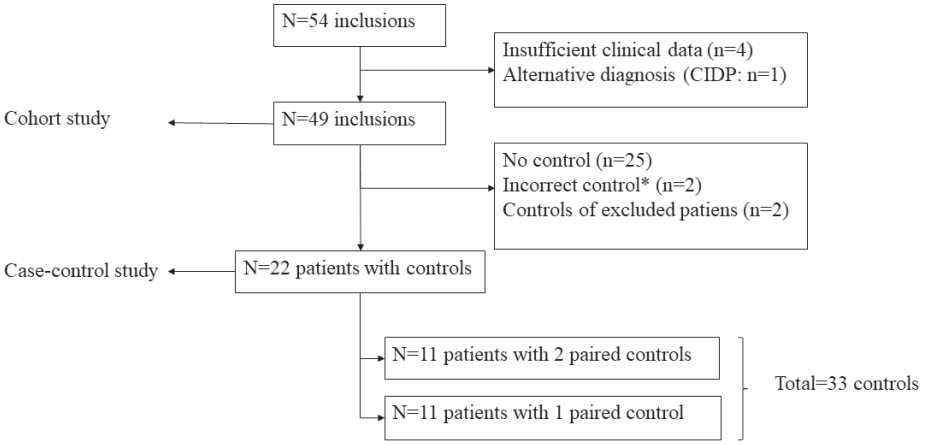

Figure 1. Flowchart Flowchart of inclusions in cohort and case-control part of the analysis.
*Family control (brother) instead of hospital control (n=1), hospital control admitted with Alzheimer's and chikungunya fever (n=1)

Viral and bacterial serology

Evidence of a recent infection was found in 27/49 (55%) of patients, and included arbovirus infections in four patients (8%), including CHIKV in two (4%), DENV and CMV in one (2%), and DENV and *C. jejuni* in one patient (2%). In addition, in one patient, a low-positive IgM, positive IgG and negative NS1 indicated a possible reinfection with a different DENV strain, and in one patient a borderline-positive IgM, and positive IgG and VNT for ZIKV indicated a possible recent ZIKV infection. For the purpose of this study, these patients were not considered positive for a recent infection with these viruses. Details of serological test results for arbovirus infection-positive cases are shown in **Supplementary table 2**.The patients with a recent CHIKV infection and the patient with a recent DENV and *C. jejuni* infection were included in Northeast Brazil between May and July 2019. The patient with a DENV and CMV infection was included in Malaysia in August 2019 (**Table 2**). *C. jejuni* was the most common preceding infection in 15 patients (31%), followed by *M. pneumoniae* in five (10%), and one additional patient had evidence of a recent infection with both these pathogens. Evidence of a recent EBV infection was found in one patient (2%), and none of the patients had evidence of a recent HEV infection. Samples were collected at a median of 11 days (IQR 7-19) after onset of weakness.

Table 1. Demography, clinical features at entry and outcome of the full cohort of patients with GBS

	All cases (n=49)
Sex (male)	32 (65)
Age (years)	42 (23-57)
<18 years old	7 (14)
Country of inclusion	
Brazil	31 (63)
Argentina	7 (14)
Malaysia	11 (22)
Antecedent event - onset weakness (days)	7 (4-15)
Antecedent symptom (any)	36 (74)
Fever	20/36 (56)
Respiratory tract infection[a]	15/36 (42)
Gastro-intestinal infection[b]	18/36 (50)
Rash	4/36 (11)
Cranial nerve deficits	29/48 (60)
Oculomotor	10/48 (21)
Facial	18/48 (38)
Bulbar	10/48 (21)
Limb weakness	37/48 (77)
MRC sum score	45 (32-58)
Hypo-/areflexia	42/48 (88)
Sensory deficits[c]	23/47 (49)
Sensory symptoms	27/41 (66)
Ataxia[c]	13/41 (32)
Onset weakness- nadir (days)	10 (5-15)
GBS clinical variant	
Sensorimotor	19/48 (40)
Pure motor	14/48 (29)
MFS (overlap)	10/48 (20)
Other	5/48 (10)
Nerve conduction studies[d]	48/49 (98)
Demyelinating	28/48 (58)
Axonal	6/48 (13)
Equivocal	13/48 (27)
Immunomodulatory treatment	44/49 (90)
IVIg	43/49 (88)
Plasmapheresis	1/49 (2)
ICU admission	20 (41)
Mechanical ventilation	12 (25)
Able to walk unaided at 6 months[e]	28/33 (85)

Data are presented as n/N reported (%) or median (IQR). Clinical features presented are at study entry. [a]Sore throat, nasal cold and/or cough, [b]Diarrhea or Nausea/vomiting, [c]If 'unable to examine'coded as missing, [d]One patient tested negative had an inexcitable EMG, [e]Patients able to walk at 8 or 13 weeks and missing data at week 26 were included in this category

Table 2. Demographic and clinical features of GBS patients with evidence of a recent arbovirus infection

	Sex, age, country	Antecedent event	Clinical features (entry)	GBS clinical variant	EMG subtype	Treatment, ICU & Ventilation	Disease nadir	Outcome last follow-up
CHIKV[a]	male, 72 y/o, Brazil	Nasal cold (20d prior)	Brighton Level 1. Bulbar & oculomotor palsy, limb weakness, sensory deficits, blood pressure dysfunction	MFS-GBS overlap	Demyelinating	IVIg (5d), admitted to ICU (7d) and MV (3d)	MRC-SS =32, GBS-DS=5. Onset-nadir 9d	MRC-SS=60, GBS-DS w8=0 (w8 last follow-up)
CHIKV[b]	female, 37 y/o, Brazil	Fever, joint pain, rash (4d prior)	Brighton Level 1. Bulbar & facial palsy, limb weakness, sensory deficits, blood pressure dysfunction	Sensorimotor	Demyelinating	IVIg (5d), admitted to ICU (21d) and MV (17d)	MRC-SS=28, GBS-DS=5 Onset-nadir 11d	MRC-SS=58, GBS-DS=4 (w8 last follow-up)
DENV & CMV	male, 30 y/o, Malaysia	Fever, myalgia, arthralgia, headache, retro-ocular pain (13d prior)	Brighton Level 4. Facial palsy, sensory deficits, ataxia	Ataxic form	Demyelinating	IVIg (5d), no ICU or MV	MRC-SS=60, GBS-DS=3 Onset-nadir 7d	MRC-SS=60, GBS-DS=0 (w26 last follow-up)
DENV & *C. jejuni*	male, 19 y/o, Brazil	Fever, diarrhea (5d prior)	Brighton Level 2. Limb weakness	Pure motor	Axonal	IVIg (5d), no ICU or MV	MRC-SS=40, GBS-DS=3 Onset-nadir 5d	MRC-SS=54, GBS-DS=2 (w26 last follow-up)

CHIKV= chikungunya virus | DENV= dengue virus | CMV= cytomegalovirus *C. jejuni*= *Campylobacter jejuni* | y/o = years old | MFS= Miller Fisher syndrome | IVIg= intravenous immunoglobulins | ICU= Intensive Care Unit | MV= mechanical ventilation | MRC-SS= MRC sum score | GBS-DS= GBS disability score. [a]P40 in Figure 2, [b]P39 in Figure 2.

Clinical features, ancillary investigations and outcome of the full cohort

The median time between onset of neurological symptoms and hospital admission was 6 days (IQR 3-10). Lumbar puncture was done in 46/48 reported patients (96%). In 73% an increased protein level (>0.45 g/L) and a cell count below 50 cells/μL was found (albuminocytological dissociation).[34, 52] The median cell count was 1.0 (1.0-3.5), and none of the patients had a cell count above 50 cells/μL. Nerve conduction studies were done in 48 (98%) patients. To exclude differential diagnoses, MRI of the spinal cord was performed in eight patients, and was normal in six and showed enhancement of the cauda equina in two. According to the Brighton Collaboration Criteria, 25 (51%) had Level 1, 7 (14%) Level 2, and 17 (35%) Level 4. Patients were categorized as Brighton Level 4 because of: time to nadir >28 days (n=2), normal (n=4) or increased tendon reflexes (n=1), clinical variant of GBS without limb weakness (n=9) and missing data on time to nadir (n=1). Four out of five patients with normal or increased tendon reflexes had evidence of a recent *C. jejuni* infection. Nerve conduction studies showed signs typical of a poly(radiculo)neuropathy in 16/17 patients (96%), and 9/17 (53%) had an albuminocytological dissociation in the CSF.

At disease nadir 79% of patients were unable to walk unaided (GBS disability score ≥3), and the median MRC sum score was 43 (IQR 31-46). When including patients with missing data at 6 months, but who were able to walk at week 8 or week 13 after study inclusion, 28/33 (85%) were able to walk unaided at 6 months. Eighteen of 19 patients who were followed-up to 1 year or more (95%) were able to walk unaided at 1 year. One patient died due to complications of pulmonary tuberculosis 5 months after onset of GBS.

Comparison of infection groups

Preceding symptoms of an infection were reported in 36 (74%) of the patients, and included fever, gastro-intestinal and respiratory tract infection. Of the patients with preceding symptoms of an infection, 16 (44%) had no serological evidence of a recent infection. Vice-versa, of the 27 patients with serological evidence of a recent infection, 7 (26%) did not have preceding infectious symptoms. Antecedent events other than infectious symptoms included vaccination (n=4) and surgery (n=1). The types of vaccination were influenza, polio, and tetanus. All patients that reported a recent vaccination had serological evidence of a recent infection; with *C. jejuni* (n=1), *M. pneumoniae* (n=1), EBV (n=1) and CHIKV (n=1). The patient with surgery also had preceding infectious symptoms, including fever, gastro-intestinal complaints and joint pain. She was negative for the tested infections.

The clinical features of the patients with evidence of a recent arbovirus infection are shown in **Table 2**. The two patients with a recent CHIKV infection had different clinical variants (MFS-overlap and sensorimotor), the same electrophysiological subtype (demyelinating) and a similar clinical progression; both were admitted to ICU and ventilated, had a low MRC sum score at nadir, but near complete recovery of strength at 8 weeks follow-up. One of these patients had typical antecedent symptoms of CHIKV infection, including fever, joint pain and rash; the other reported a nasal cold 20 days prior. The patient with a recent DENV and CMV infection reported preceding symptoms of fever, myalgia, arthralgia, headache and retro-ocular pain and had an ataxic variant and demyelinating subtype of GBS. The patient with a recent DENV and *C. jejuni* infection had preceding symptoms of a gastro-enteritis and a pure motor variant and axonal subtype of GBS.

In patients with a recent *C. jejuni* infection, gastro-enteritis was the most common reported antecedent event (78%). The pure motor variant of GBS was most frequently reported (12/15, 80%), cranial nerve involvement was infrequent (5/15, 33%), and the MRC sum score at entry was relatively low (41 (IQR 30-46)). Nine out of 12 reported patients (75%) were able to walk unaided at 6 months. The five patients with a *M. pneumoniae* infection were frequently <18 years old (2/5, 40%), had a relatively long time between antecedent event and onset of weakness (18 days (IQR 11-21)), a high MRC sum score at entry (59 (IQR 56-60)), and 2/2 reported patients had fully recovered at 8 weeks. The patient with a recent EBV infection was 9 years old, had preceding symptoms of headache and nausea, a sensorimotor demyelinating variant, and full recovery of disability at 13 weeks follow-up. Details on the clinical features per infection group are displayed in **Supplementary Table 3**.

Anti-ganglioside antibodies

The presence of serum anti-ganglioside antibodies (IgM and IgG) against 12 commonly studied glycolipids in GBS was tested in ELISA and combinatorial glycoarray.

In ELISA, 21 patients (43%) were positive for one or more of these antibodies (IgM or IgG), versus none of the 32 tested controls.(**Table 3**) In patients with a CHIKV or EBV infection, no antiganglioside antibodies were found in ELISA. In patients with a *C. jejuni* infection antibodies against GM1, GM2, and GD1a were most frequently reported. In the patient with a *C. jejuni* and DENV infection, IgM antibodies against GM1, GM2 and IgM and IgG against GD1a were found, and in the patient with a CMV and DENV infection IgM antibodies against GM2 were found. The presence of anti-ganglioside antibodies (IgM or IgG) was found in patients with an axonal (4/6, 67%) as well as in patients with a demyelinating electrophysiological subtype of GBS (14/28, 50%).

Table 3. Anti-ganglioside antibodies in serum (ELISA)

	Controls (n=32)*		All cases (n=49)		*C. jejuni* (n=15)		*M. pneumoniae* (n=5)	
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
Any	0 (0)	0 (0)	11 (22)	15 (31)	6 (40)	9 (60)	1 (20)	1 (20)
GM1	0 (0)	0 (0)	6 (12)	5 (10)	4 (27)	4 (27)	1 (20)	0 (0)
GM2	0 (0)	0 (0)	6 (12)	1 (2)	4 (27)	1 (7)	0 (0)	0 (0)
GD1a	0 (0)	0 (0)	4 (8)	5 (10)	4 (27)	4 (27)	0 (0)	0 (0)
GD1b	0 (0)	0 (0)	1 (2)	6 (12)	0 (0)	3 (20)	0 (0)	1 (20)
GD3	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
GQ1b	0 (0)	0 (0)	0 (0)	3 (6)	0 (0)	0 (0)	0 (0)	0 (0)

*In 32/35 controls sufficient serum sample was available for anti-ganglioside antibody testing

In glycoarray, 19 patients (39%) were positive for IgM and 25 (51%) for IgG antibodies against single glycolipids, and 26 patients (53%) were positive for IgM and 36 (74%) for IgG antibodies against glycolipids in complexes. In contrast, of the 32 controls, 2 (6%) were positive IgM and 6 (19%) for IgG antibodies against single glycolipids, and 11 (34%) for IgM and 10 (31%) for IgG antibodies against glycolipids in complexes. In **Figure 2** glycoarray findings are visualized in a heatmap. Binding of IgG antibodies to glycolipids is clearly lower in cases versus controls although some reactivity against GalC, lactosylceramide and sulfatide is seen in both cases and controls. Similar to the ELISA results, no or only low reactivity was found in patients with (arbo)virus infections. The patient with a recent CHIKV infection that had a MFS-GBS overlap variant (P40) was positive for IgG and IgM antibody binding to GD3 in complex with several other glycolipids, including GQ1b, but binding was low and not visible in the heatmap (**Figure 2**). In the other patients with a recent CHIKV infection (P39) no antibody binding to glycolipids was found. In one patient with *M. pneumoniae* infection and a sensorimotor variant of GBS (P49) reactivity was found against complexes with GD1a, GD1b, GD3 and GQ1b. In patients with a *C. jejuni* infection, a large variety of reactivity was found, but clusters were mostly seen in complexes with GM1, GD1a and GD1b. The patient with a *C. jejuni* and DENV infection (P41) showed complex reactivity similar to that of other patients with a *C. jejuni* infection.

Case-control study

In total, 35 paired controls were collected of 23 cases. One of these cases was excluded because of an alternative diagnosis, leaving 22 patients with 33 paired controls for the paired case-control analysis.(**Supplementary table 4**) None of the cases or controls included in this analysis had evidence of a recent infection with ZIKV, CHIKV or EBV. Calculated crude odds ratio and adjusted odds ratio of recent infections were not significant.

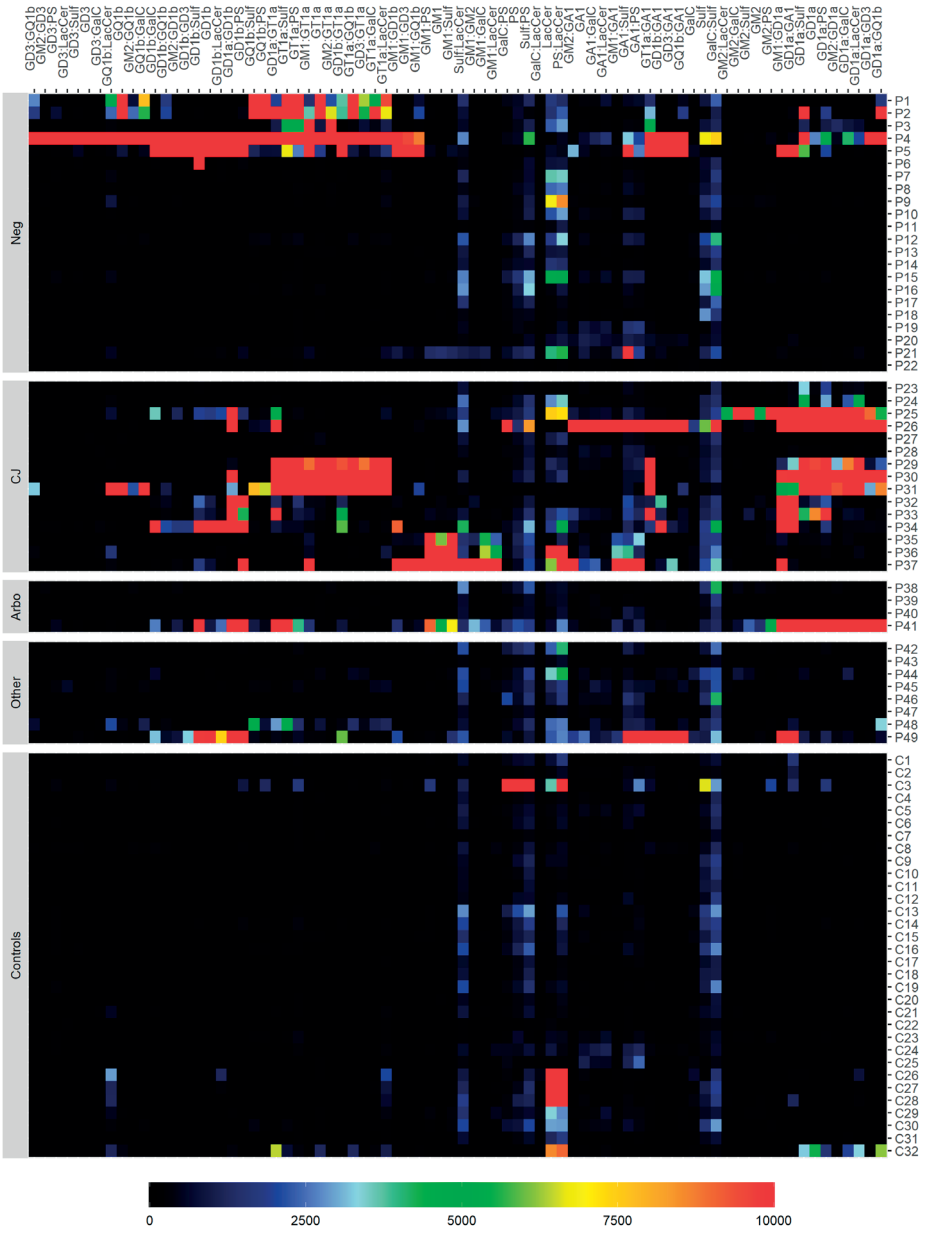

Figure 2. Heatmap of IgG antibody binding to glycolipids as assessed by glycoarray.
Each row presents one patient (P1-P49) or control (C1-C23), each column presents one of the tested glycolipid antibodies (single or in complex). Raw data was clustered based on a distance matrix using Pearsons correlation and hierarchical cluster algorithm, and clipped at a 10,000 upper limit.

We also performed an unpaired case-control analysis, comparing all 49 cases to all 35 controls (**Table 4**). Although all infections occurred more frequently in cases versus controls, calculated crude odds ratio were not significant. Evidence of a recent infection with DENV, CHIKV, CMV or EBV was only found in cases. Furthermore, two cases had a possible recent arbovirus infection (one ZIKV infection and one DENV reinfection), and in none of the controls were such borderline results found.

Table 4. Unpaired case-control analysis

Evidence of recent infection[a]	Controls (n=35)	Cases (n=49)	Crude odds ratio (CI)[b]	P-value
Dengue virus	0/35 (0%)	2 (4%)	3.737 (0.174 - 80.290)	0.3996
Chikungunya virus	0/31 (0%)	2 (4%)	3.316 (0.154 - 71.403)	0.4440
C. jejuni	6/30 (20%)	18 (37%)	2.323 (0.799 - 6.748)	0.1215
M. pneumoniae	4/31 (13%)	7 (14%)	1.125 (0.301 - 4.212)	0.8612
Cytomegalovirus	0/27 (0%)	1/46 (2%)	1.813 (0.0713 - 46.089)	0.7185
Epstein-Barr virus	0/27 (0%)	1/46 (2%)	1.813 (0.0713 - 46.089)	0.7185

Proportions are shown as number positive/number tested.[a] Zika virus and Hepatitis E virus are not displayed in this table as none of the cases and none of the controls had evidence of a recent infection with these viruses. Not all cases and controls were tested for all infections. [b] Odds ratio was calculated using the Haldane-Anscombe correction if one of the two groups had zero subjects.

DISCUSSION

Previous studies conducted during epidemic phases of arboviral transmission have demonstrated evidence of an association between GBS and ZIKV, CHIKV and DENV. However, literature on the occurrence of arbovirus infections in GBS patients during endemic phases of transmission is limited.[53] In this observational multinational cohort and case-control study on GBS in relation to arbovirus infections, we found that these infections do occur at low rates in GBS patients during endemic phases of viral transmission. Of the 49 patients included in the study, a recent arbovirus infection was found in four cases (8%) that were collected in Northeast Brazil and Malaysia during times when no epidemics of arbovirus infections were reported, and included CHIKV (n=2) and DENV (n=2). Two additional patients had evidence of a possible recent infection with ZIKV and DENV. In contrast, we did not find evidence of a (possible) recent arbovirus infection in any of the 35 controls. Odds ratio did not significantly differ between cases and controls, most likely because our study was underpowered, indicated by the broad confidence intervals. The absence of Zika-related GBS in the current study, conducted in a period of low viral transmission, is in accordance with the results of a meta-analysis that estimated the overall risk of reported GBS at 2.0 (95% CI 0.5-4.5) per 10,000 ZIKV cases.[54] Nevertheless, this estimated risk is magnitudes higher than the annual global incidence of GBS

(±1–2 cases per 100,000 person–years), indicating the potential of ZIKV to cause large outbreaks of GBS during epidemics. The risk of GBS after CHIKV or DENV has not been defined in detail, but based on our results it is likely that these infections may also be an infrequent trigger of GBS during endemic phases of transmission. No data on IgM seroprevalence is available during the time period (2017-2019) and in the specific regions of our study. A seroprevalence study done in a different area in Brazil in 2018 showed an IgM seroprevalance of 5% for CHIKV and 2% for DENV and ZIKV.[55] One study from Malaysia performed between 2012 and 2017 showed 0.6-2.2% seropositivity for ZIKV neutralizing antibodies,[56] and another study performed in 2015 in a rural area showed ±11% IgM seroprevalence of DENV.[57] We were not able to find reliable data on CHIKV IgM seroprevalence in Malaysia or of any of the three arboviruses for Argentina. Although the proportion of positive cases found in this study is higher than most of these seroprevalence studies, we are unable to draw any conclusions due to the differences in study population.

We also tested our cohort for other infections that have previously been associated with GBS, and found evidence of a recent *C. jejuni* infection in 18 (37%), *M. pneumonia* in 7 (14%), CMV in one (2%) and EBV in one (2%). Infections with *C. jejuni* were specifically frequent in Brazilian patients in our study. Studies on GBS in Brazil outside of the ZIKV pandemic are scarce, and other infections have rarely been tested.[58, 59] These results indicate that, as in other countries, *C. jejuni* is the most common trigger in Brazil. The two patients with a recent DENV infection also had evidence of another infection; one with *C. jejuni* and one with CMV. It is not clear what the significance of these co-infections is. Presence of several recent infections may cause a more severe immune response that increases the risk of development of GBS,[60] or polyclonal B-cell activation as a response to one infection may lead to false positive serologic test results for other pathogens.[61, 62] Nevertheless, this finding indicates that previous studies only testing for arboviruses may have missed patients who also had evidence of another infection associated with GBS.

In our study preceding symptoms of an infection were only partly correlated with the serological evidence of a recent infection. In almost half of the patients who had preceding symptoms of an infection, no serological evidence of a recent infection was found. It may be that part of these cases were false negative for the tested infections, as we were not able to perform PCR or culture and therefore may have missed some cases that did not (yet) mount a detectable serological response. Alternatively or in addition, some of these patients may have had infections that were not tested for in this cohort, which may include *H. influenzae* or varicella zoster virus, as these have been linked to GBS in some previous studies.[63-65] However, it is important to

note that in 26% of the patients *with* serological evidence of an infection no preceding infectious symptoms were reported, which may indicate minor symptoms or asymptomatic infection. Furthermore, in all patients who reported a vaccination, serological evidence of an infection was found. This is expected as for most vaccines no evidence exists of an association with GBS, and it highlights the importance of also investigating other infectious causes in patients developing GBS in the weeks after receiving a vaccine.[66, 67]

The clinical and electrophysiological profile of GBS in relation to the preceding infections confirmed findings from previous studies. The patients with a preceding *C. jejuni* infection frequently had a pure motor variant and axonal electrophysiological subtype and more severe muscle weakness and slower recovery.[5, 68] A minority of these patients had normal or increased tendon reflexes, as has been reported in other patients with *C. jejuni*-associated GBS.[69] Patients with a recent *M. pneumonia* infection were younger, and patients with preceding virus infections, including those with recent CHIKV infection, generally had a demyelinating electrophysiological subtype of GBS and a relatively fast recovery.[2, 5, 26, 70] Both patients with CHIKV infection were admitted to the ICU and ventilated. In previous studies on arbovirus-related GBS, higher proportions of ICU admission and mechanical ventilation were found compared to other GBS cohorts.[23, 26, 71] This may indicate that arbovirus-related GBS is associated with a more severe initial disease course and/or respiratory insufficiency, but patient numbers are too small to draw conclusions.

Serology of anti-ganglioside antibodies clearly showed higher reactivities in patients compared to controls, both in ELISA and glycoarray, confirming the role of these antibodies in the pathophysiology of GBS.[2, 30] The patients with a recent *C. jejuni* infection mainly displayed binding of GM1, GM2, GD1a, and GT1a, as has been reported previously.[72-74] In one of the patients with a recent CHIKV infection, low binding of GD3 antibodies in complex was found, and in the other CHIKV-positive case no binding was found. The patient with a recent DENV and *C. jejuni* infection had an anti-glycolipid complex reactivity similar to that of the patients with a *C. jejuni* mono-infection, and in the patient with a recent DENV and CMV infection, IgM antibodies against GM2 were found, similar to previously published cases of CMV-related GBS.[75, 76] This is in line with a previous study from Northeast Brazil where we did not find a specific anti-ganglioside antibody profile related to arbovirus infections.[26] Although a study on ZIKV-related GBS in French-Polynesia demonstrated antibody activity against GD1a, this has not been replicated in any study on GBS conducted during the Latin American ZIKV epidemic.[31] In general anti-ganglioside antibodies have rarely been demonstrated in GBS patients with preceding virus

infections, indicating that the underlying pathophysiology may be different from bacterium-related GBS.

The fact that the anti-ganglioside antibody profile of the patients with a DENV was more typical for their co-infection suggests that the CMV and *C. jejuni* infection were the actual trigger of GBS in these cases, and the DENV infection was a coincidental finding. The patient with a *C. jejuni* and DENV infection also had a clinical profile most compatible with a *C. jejuni* infection, with preceding diarrhea and a pure motor axonal variant of GBS. This is similar to findings of a study from Bangladesh conducted during an endemic phase of ZIKV transmission, where 9/18 ZIKV-positive GBS cases also had evidence of a recent *C. jejuni* infection, and a clinical phenotype typical for that infection.[77]

Our study has several limitations. Most importantly the case-control study was underpowered. It was not always possible in clinical practice to collect two paired controls for every case, as per the original protocol; an unfortunate but unavoidable feat in a multinational study. Second, participating centers were mostly academic or teaching hospitals, and inclusion of patients may have been biased towards more complicated or severe cases. Third, although we used sophisticated serological testing to identify presence of arbovirus and other preceding infections, we were not able to perform PCR (for the viruses) or culture (for the bacteria) due to sample and cost limitations, and may have missed patients that did not (yet) mount a serological response.

In conclusion, we found that preceding infections with CHIKV and DENV occur in GBS patients outside of epidemics, although not significantly more often than in controls. Broad serological testing, anti-ganglioside antibody diagnostics, as well as clinical and electrophysiological findings may be helpful in determining the actual trigger in GBS patients with co-infections. Larger studies on arbovirus-related GBS are necessary to further study the association with GBS in endemic phases of transmission.

SUPPLEMENTARY MATERIAL

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REFERENCES

1. Sejvar JJ, Baughman AL, Wise M, Morgan OW. Population incidence of Guillain-Barré syndrome: a systematic review and meta-analysis. Neuroepidemiology 2011;36:123-133.
2. Caudie C, Quittard Pinon A, Taravel D, et al. Preceding infections and anti-ganglioside antibody profiles assessed by a dot immunoassay in 306 French Guillain-Barre syndrome patients. J Neurol 2011;258:1958-1964.
3. Geurtsvankessel CH, Islam Z, Mohammad QD, Jacobs BC, Endtz HP, Osterhaus AD. Hepatitis E and Guillain-Barré syndrome. Clin Infect Dis 2013;57:1369-1370.
4. Rees JH, Soudain SE, Gregson NA, Hughes RA. Campylobacter jejuni infection and Guillain-Barre syndrome. N Engl J Med 1995;333:1374-1379.
5. Jacobs BC, Rothbarth PH, van der Meche FG, et al. The spectrum of antecedent infections in Guillain-Barre syndrome: a case-control study. Neurology 1998;51:1110-1115.
6. Styczynski AR, Malta J, Krow-Lucal ER, et al. Increased rates of Guillain-Barré syndrome associated with Zika virus outbreak in the Salvador metropolitan area, Brazil. PLoS Negl Trop Dis 2017;11:e0005869.
7. World Health Organization. Zika situation report 5 February 2016. World Health Organization 2016:Available from: https://www.who.int/emergencies/zika-virus/situation-report/5-february-2016/en/.
8. Parra B, Lizarazo J, Jimenez-Arango JA, et al. Guillain-Barré syndrome associated with Zika virus infection in Colombia. N Engl J Med 2016;375:1513-1523.
9. Stegmann-Planchard S, Gallian P, Tressières B, et al. Chikungunya, a Risk Factor for Guillain-Barré Syndrome. Clinical Infectious Diseases 2019.
10. Simon O, Billot S, Guyon D, et al. Early Guillain-Barre Syndrome associated with acute dengue fever. J Clin Virol 2016;77:29-31.
11. Grijalva I, Grajales-Muniz C, Gonzalez-Bonilla C, et al. Zika and dengue but not chikungunya are associated with Guillain-Barre syndrome in Mexico: A case-control study. PLoS Negl Trop Dis 2020;14:e0008032.
12. Hills SL, Fischer M, Petersen LR. Epidemiology of Zika Virus Infection. J Infect Dis 2017;216:S868-S874.
13. Keesen TSL, de Almeida RP, Gois BM, et al. Guillain-Barré syndrome and arboviral infection in Brazil. The Lancet Infectious Diseases 2017;17:693-694.
14. Mehta R, Gerardin P, de Brito CAA, Soares CN, Ferreira MLB, Solomon T. The neurological complications of chikungunya virus: A systematic review. Rev Med Virol 2018;28:e1978.
15. Matos AMB, Maia Carvalho FM, Malta DL, et al. High proportion of Guillain-Barré syndrome associated with chikungunya in Northeast Brazil. Neurol Neuroimmunol Neuroinflamm 2020;7.
16. Mello CdS, Cabral-Castro MJ, Silva de Faria LC, Peralta JM, Puccioni-Sohler M. Dengue and chikungunya infection in neurologic disorders from endemic areas in Brazil. Neurol Clin Pract 2020;10:497-502.
17. Pastula DM, Khan AS, Sharp TM, et al. Investigation of a Guillain-Barré syndrome cluster in the Republic of Fiji. J Neurol Sci 2017;372:350-355.
18. Suryapranata FST, Ang CW, Chong LL, Murk J-L, Falconi J, Huits RMHG. Epidemiology of Guillain-Barré Syndrome in Aruba. The American journal of tropical medicine and hygiene 2016;94:1380-1384.

19. Santos NQ, Azoubel AC, Lopes AA, Costa G, Bacellar A. Guillain-Barre syndrome in the course of dengue: case report. Arq Neuropsiquiatr 2004;62:144-146.
20. Fragoso YD, Gomes S, Brooks JB, et al. Guillain-Barre syndrome and dengue fever: report on ten new cases in Brazil. Arq Neuropsiquiatr 2016;74:1039-1040.
21. Umapathi T, Lim CS, Ooi EE, et al. Asymptomatic dengue infection may trigger Guillain-Barre syndrome. J Peripher Nerv Syst 2016;21:375-377.
22. Soares CN, Cabral-Castro M, Oliveira C, et al. Oligosymptomatic dengue infection: a potential cause of Guillain Barré syndrome. Arq Neuropsiquiatr 2008;66:234-237.
23. Tan CY, Razali SNO, Goh KJ, Sam IC, Shahrizaila N. Association of dengue infection and Guillain-Barre syndrome in Malaysia. J Neurol Neurosurg Psychiatry 2019;90:1298-1300.
24. Oehler E, Fournier E, Leparc-Goffart I, et al. Increase in cases of Guillain-Barre syndrome during a Chikungunya outbreak, French Polynesia, 2014 to 2015. Euro Surveill 2015;20:30079.
25. Wielanek AC, Monredon JD, Amrani ME, Roger JC, Serveaux JP. Guillain-Barre syndrome complicating a Chikungunya virus infection. Neurology 2007;69:2105-2107.
26. Leonhard SE, Halstead S, Lant SB, et al. Guillain-Barre syndrome during the Zika virus outbreak in Northeast Brazil: An observational cohort study. J Neurol Sci 2020;420:117272.
27. Girard M, Nelson CB, Picot V, Gubler DJ. Arboviruses: A global public health threat. Vaccine 2020;38:3989-3994.
28. Vairo F, Haider N, Kock R, Ntoumi F, Ippolito G, Zumla A. Chikungunya: Epidemiology, Pathogenesis, Clinical Features, Management, and Prevention. Infectious Disease Clinics of North America 2019;33:1003-1025.
29. Messina JP, Brady OJ, Golding N, et al. The current and future global distribution and population at risk of dengue. Nature Microbiology 2019;4:1508-1515.
30. Yuki N, Susuki K, Koga M, et al. Carbohydrate mimicry between human ganglioside GM1 and Campylobacter jejuni lipooligosaccharide causes Guillain-Barre syndrome. Proc Natl Acad Sci U S A 2004;101:11404-11409.
31. Cao-Lormeau VM, Blake A, Mons S, et al. Guillain-Barre Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. Lancet 2016;387:1531-1539.
32. Nico D, Conde L, Rivera-Correa JL, et al. Prevalence of IgG Autoantibodies against GD3 Ganglioside in Acute Zika Virus Infection. Frontiers in Medicine 2018;5.
33. Rivera-Correa J, de Siqueira IC, Mota S, et al. Anti-ganglioside antibodies in patients with Zika virus infection-associated Guillain-Barré Syndrome in Brazil. PLOS Neglected Tropical Diseases 2019;13:e0007695.
34. Jacobs BC, van den Berg B, Verboon C, et al. International Guillain-Barre Syndrome Outcome Study: protocol of a prospective observational cohort study on clinical and biological predictors of disease course and outcome in Guillain-Barre syndrome. J Peripher Nerv Syst 2017;22:68-76.
35. Kleyweg RP, van der Meche FG, Schmitz PI. Interobserver agreement in the assessment of muscle strength and functional abilities in Guillain-Barre syndrome. Muscle Nerve 1991;14:1103-1109.
36. Hughes RA, Newsom-Davis JM, Perkin GD, Pierce JM. Controlled trial prednisolone in acute polyneuropathy. Lancet 1978;2:750-753.

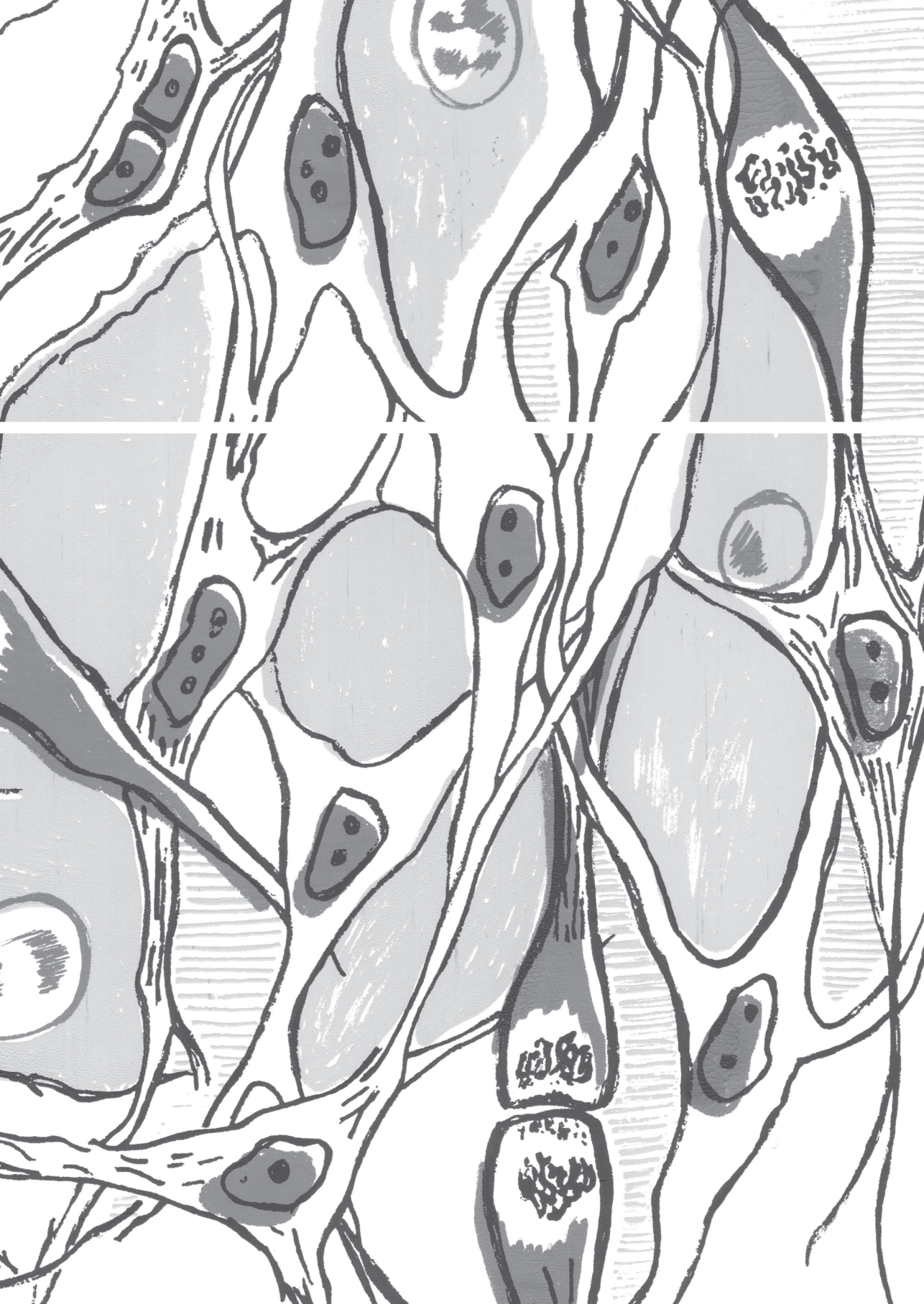
37. Hadden RD, Cornblath DR, Hughes RA, et al. Electrophysiological classification of Guillain-Barre syndrome: clinical associations and outcome. Plasma Exchange/Sandoglobulin Guillain-Barre Syndrome Trial Group. Ann Neurol 1998;44:780-788.
38. Sejvar JJ, Kohl KS, Gidudu J, et al. Guillain-Barré syndrome and Fisher syndrome: case definitions and guidelines for collection, analysis, and presentation of immunization safety data. Vaccine 2011;29:599-612.
39. Ang CW, Krogfelt K, Herbrink P, et al. Validation of an ELISA for the diagnosis of recent Campylobacter infections in Guillain-Barré and reactive arthritis patients. Clin Microbiol Infect 2007;13:915-922.
40. van Meer MPA, Mögling R, Klaasse J, et al. Re-evaluation of routine dengue virus serology in travelers in the era of Zika virus emergence. J Clin Virol 2017;92:25-31.
41. Centers for Disease Control and Prevention. Testing for Zika virus.
42. World Health Organization. Chikunungya virus fact sheet.
43. Centers for Disease Control and Prevention. Serologic tests for Dengue virus.
44. Kuijf ML, van Doorn PA, Tio-Gillen AP, et al. Diagnostic value of anti-GM1 ganglioside serology and validation of the INCAT-ELISA. J Neurol Sci 2005;239:37-44.
45. Taams NE, Notermans NC, Fokkink WR, et al. Clinical relevance of serum antibodies to GD1b in immune-mediated neuropathies. J Peripher Nerv Syst 2018;23:227-234.
46. Rinaldi S, Brennan KM, Kalna G, et al. Antibodies to heteromeric glycolipid complexes in Guillain-Barre syndrome. PLoS One 2013;8:e82337.
47. Altman DG. Practical Statistics for Medical Research: Chapman and Hall, 2020.
48. MedCalc. Odds ratio calculator. https://wwwmedcalcorg/calc/odds_ratiophp.
49. Hosmer DWLS. Applied Logistic Regression. 2000.
50. Pearce N. Analysis of matched case-control studies. BMJ 2016;352:i969.
51. Halstead SK, Kalna G, Islam MB, et al. Microarray screening of Guillain-Barre syndrome sera for antibodies to glycolipid complexes. Neurol Neuroimmunol Neuroinflamm 2016;3:e284.
52. Hadden RD, Karch H, Hartung HP, et al. Preceding infections, immune factors, and outcome in Guillain-Barré syndrome. Neurology 2001;56:758-765.
53. Akrami KM, de Nogueira BMF, do Rosário MS, et al. The re-emergence of Zika in Brazil in 2020: a case of Guillain Barré Syndrome during the low season for arboviral infections. J Travel Med 2020;27.
54. Mier YT-RL, Delorey MJ, Sejvar JJ, Johansson MA. Guillain-Barré syndrome risk among individuals infected with Zika virus: a multi-country assessment. BMC Med 2018;16:67.
55. Barreto FKA, Alencar CH, Araújo FMC, et al. Seroprevalence, spatial dispersion and factors associated with flavivirus and chikungunha infection in a risk area: a population-based seroprevalence study in Brazil. BMC Infect Dis 2020;20:881.
56. Sam IC, Montoya M, Chua CL, Chan YF, Pastor A, Harris E. Low seroprevalence rates of Zika virus in Kuala Lumpur, Malaysia. Trans R Soc Trop Med Hyg 2019;113:678-684.
57. Dhanoa A, Hassan SS, Jahan NK, et al. Seroprevalence of dengue among healthy adults in a rural community in Southern Malaysia: a pilot study. Infect Dis Poverty 2018;7:1.
58. Dourado ME, Duarte RC, Ferreira LC, et al. Anti-ganglioside antibodies and clinical outcome of patients with Guillain-Barré Syndrome in northeast Brazil. Acta Neurol Scand 2003;108:102-108.

59. Souza CO, Vieira M, Batista FMA, et al. Serological Markers of Recent Campylobacter jejuni Infection in Patients with Guillain-Barré Syndrome in the State of Piauí, Brazil, 2014-2016. Am J Trop Med Hyg 2018;98:586-588.
60. Huizinga R, van den Berg B, van Rijs W, et al. Innate Immunity to Campylobacter jejuni in Guillain-Barré Syndrome. Ann Neurol 2015;78:343-354.
61. Hyams C, Mabayoje DA, Copping R, et al. Serological cross reactivity to CMV and EBV causes problems in the diagnosis of acute hepatitis E virus infection. J Med Virol 2014;86:478-483.
62. Kuijf ML, Samsom JN, van Rijs W, et al. TLR4-Mediated Sensing of Campylobacter jejuni by Dendritic Cells Is Determined by Sialylation. The Journal of Immunology 2010;185:748-755.
63. Ju YY, Womersley H, Pritchard J, Gray I, Hughes RA, Gregson NA. Haemophilus influenzae as a possible cause of Guillain-Barré syndrome. J Neuroimmunol 2004;149:160-166.
64. Hao Y, Wang W, Jacobs BC, et al. Antecedent infections in Guillain-Barré syndrome: a single-center, prospective study. Ann Clin Transl Neurol 2019;6:2510-2517.
65. Islam B, Islam Z, GeurtsvanKessel CH, et al. Guillain-Barre syndrome following varicella-zoster virus infection. Eur J Clin Microbiol Infect Dis 2018;37:511-518.
66. Principi N, Esposito S. Vaccine-preventable diseases, vaccines and Guillain-Barré syndrome. Vaccine 2018.
67. Lunn MP, Cornblath DR, Jacobs BC, et al. COVID-19 vaccine and Guillain-Barre syndrome: let's not leap to associations. Brain 2021;144:357-360.
68. Kuwabara S, Ogawara K, Misawa S, et al. Does Campylobacter jejuni infection elicit "demyelinating" Guillain-Barre syndrome? Neurology 2004;63:529-533.
69. Yuki N, Kokubun N, Kuwabara S, et al. Guillain-Barré syndrome associated with normal or exaggerated tendon reflexes. J Neurol 2012;259:1181-1190.
70. Orlikowski D, Porcher R, Sivadon-Tardy V, et al. Guillain-Barre syndrome following primary cytomegalovirus infection: a prospective cohort study. Clin Infect Dis 2011;52:837-844.
71. Leonhard SE, Bresani-Salvi CC, Lyra Batista JD, et al. Guillain-Barre syndrome related to Zika virus infection: A systematic review and meta-analysis of the clinical and electrophysiological phenotype. PLoS Negl Trop Dis 2020;14:e0008264.
72. Sekiguchi Y, Uncini A, Yuki N, et al. Antiganglioside antibodies are associated with axonal Guillain-Barre syndrome: a Japanese-Italian collaborative study. J Neurol Neurosurg Psychiatry 2012;83:23-28.
73. Ogawara K, Kuwabara S, Mori M, Hattori T, Koga M, Yuki N. Axonal Guillain-Barre syndrome: relation to anti-ganglioside antibodies and Campylobacter jejuni infection in Japan. Ann Neurol 2000;48:624-631.
74. Zhang M, Gilbert M, Yuki N, et al. Association of Anti-GT1a Antibodies with an Outbreak of Guillain-Barre Syndrome and Analysis of Ganglioside Mimicry in an Associated Campylobacter jejuni Strain. PLoS One 2015;10:e0131730.
75. Jacobs BC, van Doorn PA, Groeneveld JH, Tio-Gillen AP, van der Meche FG. Cytomegalovirus infections and anti-GM2 antibodies in Guillain-Barre syndrome. J Neurol Neurosurg Psychiatry 1997;62:641-643.
76. Irie S, Saito T, Nakamura K, et al. Association of anti-GM2 antibodies in Guillain-Barré syndrome with acute cytomegalovirus infection. J Neuroimmunol 1996;68:19-26.

77. GeurtsvanKessel CH, Islam Z, Islam MB, et al. Zika virus and Guillain-Barre syndrome in Bangladesh. Ann Clin Transl Neurol 2018;5:606-615.

Part III

The spectrum of neurological disease associated with Zika virus infection



Chapter 5

Zika virus infection in the returning traveler: what every neurologist should know

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ABSTRACT

Zika virus has been associated with a wide range of neurological complications. Neurologists in areas without current active transmission of the virus may be confronted with Zika-associated neurological disease, as a large number of returning travelers with Zika virus infection have been reported and the virus continues to spread to previously unaffected regions. This review provides an overview of Zika virus associated neurological disease to date and aims to support neurologists who may encounter patients returning from ZIKV endemic areas.

INTRODUCTION

The exponential increase in traveling has major consequences for the epidemiology of infectious and post-infectious disease, constituting a global public health challenge.[1] The most recent example of this is the Zika virus (ZIKV) epidemic in Latin America that has now spread to 84 countries globally and has been associated with severe neurological sequelae, including microcephaly, Guillain-Barrė syndrome (GBS), and central nervous system (CNS) disorders.[2] Regions without active transmission, including Europe, East Asia and North America, should still be on high alert for people with ZIKV infections, as large numbers of returning travelers with ZIKV infection have been reported and parts of these regions are predicted to be at risk for active viral transmission in summer months.[3-5] This review aims to support neurologists who may see travellers with neurological complications returning from ZIKV endemic areas.

Transmission

ZIKV is an enveloped positive-strand RNA member of the *Flavivirus* genus in the Flaviviridae family. Other flaviviruses include dengue (DENV), yellow fever (YFV), West Nile virus (WNV) and Japanese encephalitis virus (JEV), many of which are associated with neurological disease.[6] Like these viruses, ZIKV is principally transmitted by a mosquito bite and is thus described as an arthropod-borne virus or 'arbovirus'. Its most prolific vector is the *Aedes aegypti* mosquito, which transmits the virus between humans and is widespread in tropical regions globally. Other *Aedes* mosquitos that populate more temperate regions, such as *Aedes albopictus*, are also able to facilitate viral spread but appear to do so less effectively.[7]

Direct transmission between humans has been demonstrated occurring vertically from mother to foetus, via blood transfusion and as a result of sexual contact, the latter route constituting an estimated 1% of ZIKV cases in Europe.[8, 9] Thus, whilst human-to-human transmission is far less common than conventional arboviral transmission, it remains important to elicit a history of potential exposure to contacts that might have led to subsequent infection.

Geographical Spread

ZIKV derives its name from the Zika forest in Uganda where it was identified in 1947.[10] The first outbreak of ZIKV was reported in Micronesia in 2007, 60 years after its discovery.[11] The link between ZIKV and neurological complications was first identified when an epidemic in French Polynesia in 2013 was followed by a twenty-fold increase in incidence of GBS cases.[12] It is unclear if a change in viral strain led

to altered pathogenicity, or whether the high incidence of infection resulted in a noticeably large number of otherwise rare neurological manifestations. Recent data suggest a mutation in the virus' non-structural 1 protein, which occurred around 2013, might promote the acquisition of the virus by its mosquito vector, thus enhancing its transmission in recent epidemics.[13]

Cases were next reported in Brazil in early 2015, where ZIKV went on to affect an estimated 0.5 to 1.5 million people. During these months, alarming increases of microcephaly and GBS cases were reported, prompting the World Health Organization (WHO) to declare ZIKV a Public Health Emergency of International Concern on 1 February 2016. The virus has since spread to a further 84 countries in the Americas, Africa and Asia, including 225 cases through presumed local mosquito-borne transmission in Southern states of the USA.[2]

Despite the decreasing trend of ZIKV infection in the Americas in 2017, the global health community advocates vigilance as it is unclear where and when the next outbreak will take place. Models predict that South- and Southeast Asia and Oceania are at high risk for future outbreaks, with seasonal transmission a possible threat in southern parts of North America, China and Europe.[3, 4]

Whilst no local spread has been reported in Europe and transmission has ceased in North America, there have been approximately 2130 travel-imported cases in Europe and around 5,500 travel-imported cases in the USA since the start of the Brazilian epidemic.[14, 15] In clinical practice, suspicion of ZIKV or related arboviral infections including DENV and chikungunya virus (CHIKV), should be high in anyone returning from - or in close contact with individuals returning from - endemic areas. We recommend checking the websites of Public Health England, WHO, Centre of Disease Control (CDC) or European Centre for Disease Prevention and Control (ECDC) for the latest updates regarding ZIKV spread and travel advice (**Box 1**).

Box 1: Useful Links

- **Public Health England**
  https://www.gov.uk/government/collections/zika-virus-zikv-clinical-and-travel-guidance
- **WHO**
  http://www.who.int/emergencies/zika-virus/en/
- **Centre of Disease Control (CDC)**
  https://www.cdc.gov/zika/
- **European Centre for Disease Prevention and Control (ECDC)**
  ecdc.europa.eu/en/zika-virus-infection

Systemic Symptoms

ZIKV has an estimated incubation period of 3-10 days and can remain asymptomatic in approximately 80% of cases.[11, 16] Therefore, absence of a history of a febrile illness should not exclude the diagnosis. Symptomatic infection is characterized by fever, rash, non-purulent conjunctivitis and arthralgia lasting up to a week, but may often also only present as just rash without fever or other accompanying signs and symptoms. [11] These symptoms resemble those of other vector-borne viruses such as DENV and CHIKV, although a mild disease course and the presence of conjunctivitis is more specific for ZIKV (**Table 1**). As such, it is the neurological features, rather than the systemic ones, which are the main cause of disability and death.

Table 1 Clinical features of arboviruses

	Zika virus	Dengue virus	Chikungunya virus
Fever	++	+++	+++
Rash (maculopapular)	+++	+	++
Conjunctivitis	++	–	–
Arthralgia	++	+	+++
Myalgia	+	++	+
Headache	+	++	++
Shock	–	+	+/–
Haemorrhage	–	+	–

Reproduced from the Centers for Disease Control and Prevention.

Neurological complications of Zika virus in the adult

ZIKV is known to be neurotropic and in neural progenitor cells infection has been shown to halt proliferation and induce cell death, which is the likely disease mechanism of ZIKV-related microcephaly cases.[17, 18] Beyond congenital Zika, direct viral invasion as well as a para-infective or postinfective autoimmune response may contribute to pathogenesis. **Box 2** lists the wide range of neurological problems associated with acute ZIKV infection.[19]

As such, any traveler returning from an area with ZIKV transmission or with a sexual partner who has returned from such an area, who develops an acute neurological illness should be screened for ZIKV infection. Other co-circulating arboviruses should also be considered - such as CHIKV and DENV, which have been linked to neurological complications.

Box 2: Neurological conditions associated with Zika

- **Congenital Zika Syndrome**
- **Peripheral Nervous System**
  - **Guillain-Barré Syndrome**
  - **Chronic inflammatory demyelinating polyneuropathy**
  - **Acute transient polyneuritis**
  - **Myasthenia gravis**
- **Central Nervous System**
  - **Myelitis**
  - **(Meningo)Encephalitis**
  - **Acute disseminated encephalomyelitis (ADEM)**
  - **Encephalopathy**

Guillain-Barré syndrome

Since the start of the global ZIKV epidemic, 23 countries have reported an increase in GBS incidence in parallel with the rising incidence of ZIKV infection. An association between GBS and ZIKV was first established in a case–control study in French Polynesia and many case reports and series have since substantially linked ZIKV and GBS. [2, 12, 20] Although there is no proven causality, the most likely explanation is that ZIKV infection can trigger GBS. ZIKV is not being currently actively being transmitted in Europe, but neurologists may well be confronted with GBS cases following patient travel to areas with ongoing ZIKV transmission GBS is an acute polyradiculoneuropathy and its classic form is characterized by a rapidly progressive symmetrical weakness of muscles in legs and arms with sensory symptoms and reduced tendon reflexes. However, the clinical presentation, disease progression and outcome may vary extensively between patients, complicating the diagnosis and treatment. Several variants of GBS have been identified, including the pure motor form, the paraparetic variant and the Miller Fisher Syndrome (MFS), characterized by ophthalmoplegia, ataxia and areflexia. Two-thirds of GBS cases report symptoms of an infective disease in the month before disease onset, and several pathogens have been associated with GBS, including *Campylobacter jejuni*, cytomegalovirus, hepatitis E virus, *Mycoplasma pneumoniae* and Epstein Barr virus. ZIKV is the latest pathogen to be added to this list. The current paradigm is that the preceding infection triggers an immune-response that causes nerve injury. This has been best described in cases with preceding *C. jejuni* infection in which there is carbohydrate mimicry between lipo-oligosaccharides on *C. jejuni* and gangliosides on human peripheral nerves and cross-reactive antibodies to these structures.[21] Some antibodies are associated with variants or subtypes of GBS, reflecting in part the distribution of gangliosides in peripheral nerves. For instance, the ganglioside GM1 is predominantly present on axons and the presence of antibodies to GM1 is associated with the pure motor and axonal form of GBS. [22] It is not yet clear how ZIKV (and viral infections in general)

can lead to GBS and no particular autoantibody biomarkers that aid in diagnosis have been identified. ZIKV might cause nerve damage by direct infection, as some patients reported onset of GBS shortly following or even concurrent with infective symptoms and many patients with GBS have ZIKV RNA in cerebrospinal fluid (CSF) or serum, indicating ongoing infection.[23] However, many other reported cases have not had these features, and a recent mouse model study showed resistance of the peripheral nervous system to infection by ZIKV, indicating that an immune-mediated disease mechanism is more likely.[24].

Box 3: GBS in a returning traveler

A 60-year-old women that had returned to the Netherlands from Suriname 10 days prior presented with diarrhoea, low-grade fever, nausea and vomiting, and an unsteady gait. Over 4 days, she developed bilateral facial palsy, symmetrical paresis of all limbs and distal hypoesthesia with low to absent tendon reflexes. CSF had a normal cell count and increased protein concentration and electrophysiology showed signs of a demyelinating polyneuropathy. Her urine and blood were positive for ZIKV RNA, and serology for other recent infections associated with GBS was negative. She received a 5-day course of IVIg (0.4 g/L daily). At 1 year follow-up she was limited in her daily activities by fatigue and minor weakness in the legs but was able to walk without aid and had returned to part-time work.

ZIKV does not seem to be associated with a specific GBS phenotype, although most cases have a sensorimotor variant with facial nerve palsy, ventilator insufficiency and a demyelinating pattern on clinical neurophysiology. There have also been more reported cases with paraparesis and intact reflexes than expected based on previous studies although some studies reported a higher amount of paraparetic variants and cases with intact tendon reflexes then you would expect based on previous studies.[12 20] It is not yet clear how to interpret these data. They could be a first indication of a specific clinical phenotype of GBS, but could also reflect the cases being labelled as GBS when in fact they result from CNS pathology. At present, there are no indications that clinicians should deviate from standard diagnostic criteria for GBS in a suspected ZIKV-related GBS case.

Other peripheral nervous system manifestations

There have been sporadic case reports linking other peripheral nerve and neuromuscular diseases to ZIKV infection, including two cases with acute onset of chronic inflammatory demyelinating polyneuropathy (CIDP), three cases of transient polyneuritis and two cases of myasthenia gravis.[25-27] The patients with transient polyneuritis had mild distal sensory and motor symptoms that resolved within 10 days and a positive PCR test for ZIKV in serum and CSF, suggesting a self-limiting direct

peripheral nerve infection by ZIKV. It is not yet clear if these reported cases are mere coincidence or if ZIKV is indeed a causal factor but, in patients with flaccid paralysis following ZIKV infection, clinicians should consider these differential diagnoses. Furthermore, patients diagnosed with GBS after ZIKV need careful follow-up as it may transpire that they have acute-onset CIDP.[27]

Meningitis and Encephalitis

Inflammation of the meninges (meningitis) or brain parenchyma (encephalitis) may result from viral infection of the brain. There has been no robust study associating ZIKV with CNS disease. However, meningoencephalitis in the context of recent ZIKV infection following foreign travel was first reported in early 2016 when, 10 days after returning from a cruise around New Caledonia, a patient who developed focal neurological symptoms and a concurrent rash was found to have ZIKV RT-PCR positive CSF. [28] ZIKV-associated encephalitis is associated with a range of clinical outcomes, from full recovery to death. [29, 30] It is not yet known whether age or a compromised immune system predispose individuals to neurological complications of ZIKV infection. There may be arboviral systemic symptoms at the time of onset of neurological symptoms, encompassing confusion; impairment of memory, attention and processing speed; seizures; and focal motor deficits. Pathological changes on electroencephalography and MRI may be either diffuse or more focal. Currently there are limited data, but by analogy with other flaviviruses, ZIKV might be expected to cause high signal intensities in the thalamus and other basal ganglia.[31]

Myelitis

Myelitis is a spinal cord inflammation that often occurs following infection and may appear in isolation, as part of a spectrum of inflammatory nervous system pathology or in multisystem disease. Patients typically present with rapid onset motor and sensory changes that are usually bilateral and associated with a defined sensory level, as is seen with transverse lesions. Autonomic dysfunction is common and CSF or MRI may give evidence of an inflammatory myelopathy. [32] In contrast, some neurotropic viruses, particularly enteroviruses such as polio and flaviviruses such as Japanese encephalitis virus, directly attack the anterior horn cells of the spinal cord. This causes a longitudinal anterior myelitis on imaging, and a poliomyelitis-like flaccid paralysis clinically.[32, 33]

ZIKV-associated cases of myelitis appear to have elements of both transverse and anterior patterns, including evidence of motor, sensory and autonomic signs, 1 to 2 weeks after systemic symptom onset. Spinal cord lesions on MRI may be anterior

and longitudinally extensive. Clinical improvement has been seen following standard treatment with steroids or plasma exchange. [34-36]

Acute disseminated encephalomyelitis

Acute disseminated encephalomyelitis (ADEM) is a parainfective or postinfective immunological disease associated with multifocal neurological symptoms and encephalopathy, often occurring in young adults and children as is seemingly the case with ZIKV-associated ADEM. The relationship between viral infection and disease onset is heterogeneous among reported cases, occurring between 1 day and 6 weeks after initial illness and there are no known ZIKV-specific clinical or MRI features (**Figure 1**). [37, 38]

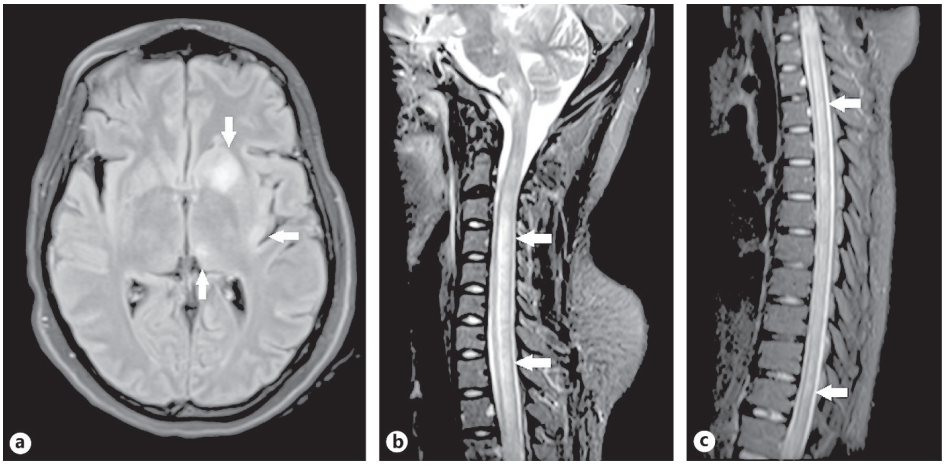


Figure 1: Cranial MRI of ADEM patient A. Axial Flair T2 weighed Cranial MRI showing bilateral assymmetric hyperintensities in a 57 year old female who developed neurological symptoms 15 days after symptoms compatible with an arboviral infection. ZIKV PCR was positive in blood. She was diagnosed with ADEM. B. Coronal Flair T2 weighed MRI of same patient as described in A.

ZIKV Diagnosis in Neurological Disease

Figure 2 gives an algorithm for laboratory investigation of suspected ZIKV infection. Recent ZIKV infection is confirmed through RT-PCR of serum, urine and CSF specimens and ZIKV IgM antibody-capture ELISA (MAC ELISA) of serum and CSF.[39]

Reliable detection of viral RNA is possible for around 14 days in serum and urine. However, as there is a variable window of time between viral infection and neurological symptoms, PCR may well be performed when viral RNA is no longer identifiable.[9]

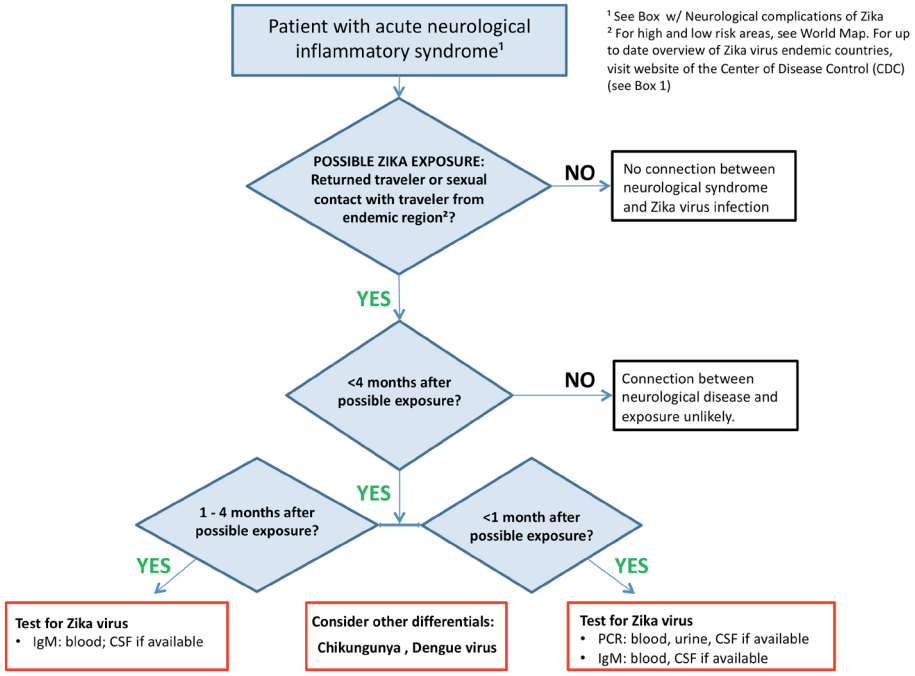


Figure 2: Algorithm for laboratory investigation of suspected ZIKV infection CHIV= chikungunya virus, CSF= cerebrospinal fluid, DENV= dengue virus

ZIKV-specific IgM antibody of serum or CSF can be detected from around 4 days to at least 12 weeks after exposure, although tests have poor specificity due to cross-reactivity with antibodies to other structurally-similar viruses such as DENV.[40] More reliable testing for the presence of antibodies that neutralize the virus is possible with Plaque-reduction neutralization testing (PRNT) but this goes beyond the capability of most hospital laboratories. Detection of virus or antibody in the CSF is more specific for CNS disease caused by ZIKV, than detection in serum, urine or semen only.

Drugs and vaccines against ZIKV

There is no specific antiviral treatment available for ZIKV infection. Management of systemic symptoms should be supportive and standard practice should be followed for cases of ZIKV-related neurological disease.

There are several vaccines in development and two have entered phase 1 clinical trials, although their progress is complicated by multiple factors. [41, 42] Most importantly, vaccine safety must be guaranteed in pregnant women as they are the

main population of interest, and the possibility of vaccine-induced GBS should be considered and prevented. It will likely take years before these vaccines are on the market.

CONCLUSION

The rapid emergence of ZIKV as a potential cause of severe neurological disease has significant implications in endemic areas and beyond. Unsuspecting travelers and an abundance of potential vectors have facilitated its prolific spread, as illustrated by the number of imported cases to date and the extent of at-risk areas for future outbreaks. We advise neurologists working in areas without current active transmission of the virus, to consider a preceding ZIKV infection in patients with acute inflammation of the central- or peripheral nervous system returning from areas with ongoing active viral transmission, or with sexual partners who have returned from such areas. There are currently no antiviral drugs available for ZIKV and recommended treatment does not differ from standard practice.

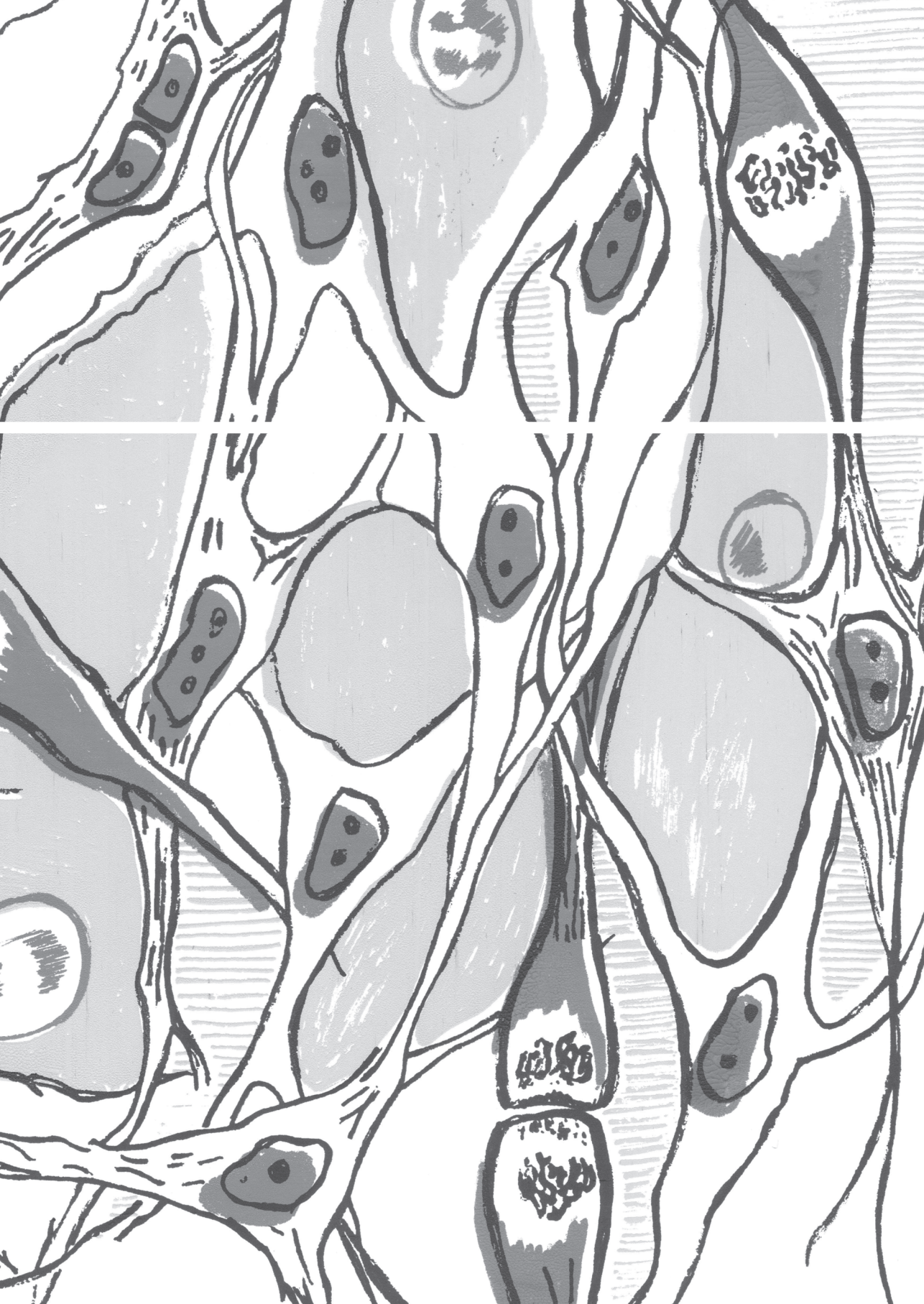
KEY POINTS

- We advise testing for Zika virus in patients with suspected inflammatory neuropathy, unexplained myelitis or meningoencephalitis who have been in an area with local transmission or who have had sexual contact with a confirmed Zika virus case, with or without preceding viral symptoms.
- The recommended treatment in Zika virus-associated neurological disease does not differ from standard practice and there are currently no effective antiviral drugs.
- There are several vaccine candidates against Zika virus in development.

REFERENCES

1. Glaesser D, Kester J, Paulose H, Alizadeh A, Valentin B. Global travel patterns: an overview. *J Travel Med* 2017;24.
2. World Health Organization. Zika situation report 10 March 2017. 2017.
3. Rocklov J, Quam MB, Sudre B, et al. Assessing Seasonal Risks for the Introduction and Mosquito-borne Spread of Zika Virus in Europe. *EBioMedicine* 2016;9:250-256.
4. Teng Y, Bi D, Xie G, et al. Model-informed risk assessment for Zika virus outbreaks in the Asia-Pacific regions. *Journal of Infection* 2017;74:484-491.
5. Massad E, Tan SH, Khan K, Wilder-Smith A. Estimated Zika virus importations to Europe by travellers from Brazil. *Glob Health Action* 2016;9:31669.
6. Gould EA, Solomon T. Pathogenic flaviviruses. *Lancet* 2008;371:500-509.
7. Bogoch, II, Brady OJ, Kraemer MUG, et al. Anticipating the international spread of Zika virus from Brazil. *Lancet* 2016;387:335-336.
8. Spiteri G, Sudre B, Septfons A, Beaute J, On Behalf Of The European Zika Surveillance N. Surveillance of Zika virus infection in the EU/EEA, June 2015 to January 2017. *Euro Surveill* 2017;22.
9. Paz-Bailey G, Rosenberg ES, Doyle K, et al. Persistence of Zika Virus in Body Fluids - Preliminary Report. *N Engl J Med* 2017.
10. Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. *Trans R Soc Trop Med Hyg* 1952;46:509-520.
11. Duffy MR, Chen TH, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med* 2009;360:2536-2543.
12. Cao-Lormeau VM, Blake A, Mons S, et al. Guillain-Barré syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *Lancet* 2016;387:1531-1539.
13. Liu Y, Liu J, Du S, et al. Evolutionary enhancement of Zika virus infectivity in Aedes aegypti mosquitoes. *Nature* 2017;545:482-486.
14. European Centre for Disease Prevention and Control. Rapid Risk Assesment, Zika virus disease epidemic, 4 April 2017. 2017.
15. Center for Disease Control and Prevention. *https://wwwcdcgov/zika/reporting/case-countshtml*.
16. Musso D, Gubler DJ. Zika Virus. *Clin Microbiol Rev* 2016;29:487-524.
17. Mlakar J, Korva M, Tul N, et al. Zika Virus Associated with Microcephaly. *N Engl J Med* 2016;374:951-958.
18. Tang H, Hammack C, Ogden SC, et al. Zika Virus Infects Human Cortical Neural Progenitors and Attenuates Their Growth. *Cell Stem Cell* 2016;18:587-590.
19. Mehta R, Soares CN, Medialdea-Carrera R, et al. The spectrum of neurological disease associated with Zika and chikungunya viruses in adults in Rio de Janeiro, Brazil: A case series. *PLoS Negl Trop Dis* 2018;12:e0006212.
20. Nascimento OJM, da Silva IRF. Guillain-Barre syndrome and Zika virus outbreaks. *Curr Opin Neurol* 2017.
21. Yuki N, Susuki K, Koga M, et al. Carbohydrate mimicry between human ganglioside GM1 and Campylobacter jejuni lipooligosaccharide causes Guillain-Barre syndrome. *Proc Natl Acad Sci U S A* 2004;101:11404-11409.
22. Willison HJ, Yuki N. Peripheral neuropathies and anti-glycolipid antibodies. *Brain* 2002;125:2591-2625.

23. Parra B, Lizarazo J, Jimenez-Arango JA, et al. Guillain-Barre Syndrome Associated with Zika Virus Infection in Colombia. *N Engl J Med* 2016;375:1513-1523.
24. Cumberworth SL, Barrie JA, Cunningham ME, et al. Zika virus tropism and interactions in myelinating neural cell cultures: CNS cells and myelin are preferentially affected. *Acta Neuropathol Commun* 2017;5:50.
25. Nascimento OJM, Frontera JA, Amitrano DA, Bispo de Filippis AM, Da Silva IRF, Group R-G-ZR. Zika virus infection-associated acute transient polyneuritis. *Neurology* 2017;88:2330-2332.
26. Molko N, Simon O, Guyon D, Biron A, Dupont-Rouzeyrol M, Gourinat AC. Zika virus infection and myasthenia gravis: report of 2 cases. *Neurology* 2017;88:1097-1098.
27. Leonhard SE, Munts AG, van der Eijk AA, Jacobs BC. Acute-onset chronic inflammatory demyelinating polyneuropathy after Zika virus infection. *J Neurol Neurosurg Psychiatry* 2018;89:1118-1119.
28. Carteaux G, Maquart M, Bedet A, et al. Zika Virus Associated with Meningoencephalitis. *N Engl J Med* 2016;374:1595-1596.
29. Soares CN, Brasil P, Carrera RM, et al. Fatal encephalitis associated with Zika virus infection in an adult. *J Clin Virol* 2016;83:63-65.
30. Nicastri E, Castilletti C, Balestra P, Galgani S, Ippolito G. Zika Virus Infection in the Central Nervous System and Female Genital Tract. *Emerg Infect Dis* 2016;22:2228-2230.
31. Solomon T. Flavivirus encephalitis. *N Engl J Med* 2004;351:370-378.
32. Kaplin AI, Krishnan C, Deshpande DM, Pardo CA, Kerr DA. Diagnosis and management of acute myelopathies. *Neurologist* 2005;11:2-18.
33. Solomon T, Kneen R, Dung NM, et al. Poliomyelitis-like illness due to Japanese encephalitis virus. *Lancet* 1998;351:1094-1097.
34. da Silva IRF, Frontera JA, Bispo de Filippis AM, Nascimento O, Group R-G-ZR. Neurologic Complications Associated With the Zika Virus in Brazilian Adults. *JAMA Neurol* 2017;74:1190-1198.
35. Mecharles S, Herrmann C, Poullain P, et al. Acute myelitis due to Zika virus infection. *Lancet* 2016;387:1481.
36. Palacios E, Clavijo-Prado C, Ruiz A, Arias Antun A, Julian Duran E. Longitudinal extensive transverse myelitis and Zika virus: A diagnostic challenge in a hospital in Colombia. *Neurologia* 2016.
37. Galliez RM, Spitz M, Rafful PP, et al. Zika Virus Causing Encephalomyelitis Associated With Immunoactivation. *Open Forum Infect Dis* 2016;3:ofw203.
38. Roth W, Tyshkov C, Thakur K, Vargas W. Encephalomyelitis Following Definitive Zika Virus Infection. *Neurol Neuroimmunol Neuroinflamm* 2017;4:e349.
39. Center for Disease Control and Prevention. Guidance for US Laboratories Testing for Zika Virus Infection July 24 2017,. *https://wwwcdcgov/zika/laboratories/lab-guidancehtml* 2017.
40. Safronetz D, Sloan A, Stein DR, et al. Evaluation of 5 Commercially Available Zika Virus Immunoassays. *Emerg Infect Dis* 2017;23:1577-1580.
41. Thomas SJ, L'Azou M, Barrett ADT, Jackson NAC. Fast-Track Zika Vaccine Development — Is It Possible? *New England Journal of Medicine* 2016;375:1212-1216.
42. Durbin A, Wilder-Smith A. An update on Zika vaccine developments. *Expert Rev Vaccines* 2017;16:781-78



Chapter 6

Acute onset chronic inflammatory demyelinating polyneuropathy after Zika virus infection

Sonja E. Leonhard, Alexander G. Munts, Annemiek A. van der Eijk, Bart C. Jacobs

J Neurol Neurosurg Psychiatry. 2018;89(10):1118-9.

CASE DESCRIPTION

In December 2016, a 69-year-old man with a history of hypertension, hypercholesterolaemia and knee operations developed an erythematous pruritic rash on his trunk, cold shivers and swollen hands and feet with paraesthesias and numbness while on holiday in Curaçao. Eight days later he developed pain in his right leg and back, provoked by walking and stretching. This pain slowly increased over the next 5 weeks to the point that it became difficult to walk. He was admitted to the neurology ward of a regional hospital in The Netherlands and neurological examination showed an antalgic gait, hypaesthesia of fingertips and feet and normal muscle strength and tendon reflexes. MRI of the cervical, thoracic and lumbar spine without gadolinium was normal. Eight days after admission he developed a progressive weakness of the legs starting in the right leg. Neurological examination showed a proximal and distal flaccid paraparesis with absent reflexes of the legs and normal reflexes of the arms. Cerebrospinal fluid (CSF) examination 3 days after admission showed a leukocyte count of 1/10 E6/L and a protein level of 620 mg/L. Electrolytes, liver- and kidney function and inflammatory parameters were normal. Nerve conduction studies (NCS) 5 days after admission showed slightly prolonged distal motor latencies of the peroneal and tibial nerves, mildly prolonged F-wave latencies of the ulnar and tibial nerves and a low amplitude of the sural nerve, compatible with a poly(radiculo)neuropathy.

He was diagnosed with Guillain-Barré syndrome (GBS) and a preceding Zika virus (ZIKV) infection was suspected. Blood, urine and CSF samples collected 50 days after onset of infectious symptoms were negative for ZIKV qRT-PCR. Serology was positive for ZIKV IgM and IgG at days 50 and 83 detected by a NS1 based ELISA (Euroimmun, Lübeck, Germany). This was confirmed by neutralizing antibodies against ZIKV in paired testing. Dengue virus serology was negative for IgM and NS1, and weakly positive (1.163/P) for IgG, most likely due to cross reactivity. Serology for other recent infections associated with GBS, including *Campylobacter jejuni, Mycoplasma pneumoniae,* cytomegalovirus, Epstein Barr virus and hepatitis E virus, was negative. The patient improved shortly after treatment with a standard course intravenous immunoglobulin (IVIg) (0.4 mg/kg/day for 5 days). During admission he did not develop upper limb weakness, cranial nerve- or respiratory dysfunction. A second opinion at Erasmus Medical Center confirmed the diagnosis GBS.

Unexpectedly, he deteriorated 6 weeks after start of weakness (**Figure 1**) with increased weakness of all limbs causing inability to walk without aid. He was diagnosed with a treatment-related fluctuation and treated with another course of IVIg,

after which he improved. Eleven weeks after start of weakness, he had a second deterioration with increased weakness of the legs. Repeated NCS were compatible with a demyelinating polyneuropathy, showing decreased motor amplitudes, absent sensory amplitudes, severely prolonged distal motor latencies of the median, ulnar and peroneal nerves, conduction slowing of the median and peroneal nerves and prolonged minimal F-waves latencies of the median and ulnar nerve. The clinical symptoms and NCS fulfilled the diagnostic criteria for definite chronic inflammatory demyelinating polyneuropathy (CIDP).[1] Maintenance therapy was started with biweekly IVIg infusions and he had a good clinical response. At the last follow-up, 41 weeks after onset of weakness, he had a distal sensibility loss with normal strength in all limbs and he was able to walk without aid.

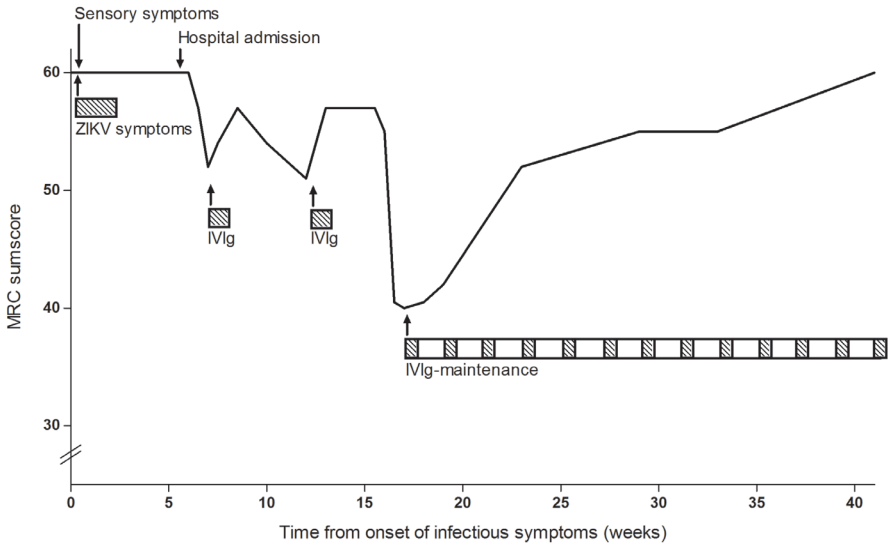


Figure 1. Clinical course and treatment in patient with A-CIDP after Zika virus infection.
MRC-sumscore: sum of score on Medical Research Council scale for muscle weakness of bilateral shoulder abduction, elbow flexion, wrist extension, hip flexion, knee extension, ankle dorsiflexion. IVIg: intravenous immunoglobulin (30 grams/day during 5 days). IVIg-maintenance: intravenous immunoglobulin (30 grams biweekly). ZIKV: Zika virus infection.

DISCUSSION

The ZIKV epidemic in 2015-2016 was followed by a drastic rise in reported GBS cases in 23 countries, including Curaçao.[2] Evidence of an association between ZIKV and GBS has been substantiated by numerous case series and case-control studies. A specific clinical phenotype of ZIKV-related GBS has not emerged from these reports, but

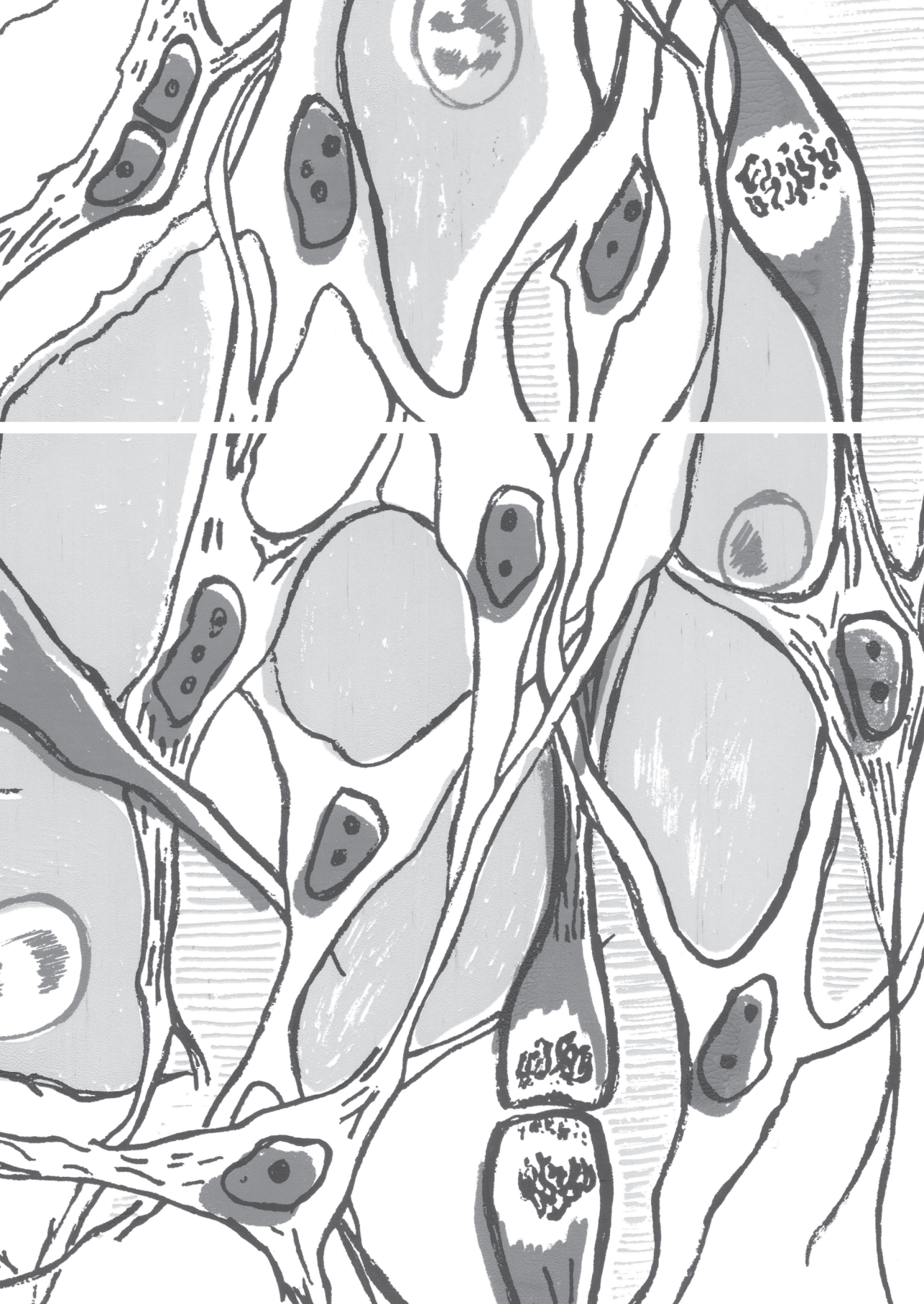
based on the NCS most patients have been classified as the demyelinating variant (acute inflammatory demyelinating polyneuropathy, AIDP).[3] Many other complications of the central- and peripheral nervous system have been reported as well, including myelitis, encephalitis and acute transient polyneuritis.[4] The pathogenesis of these ZIKV associated disorders is unknown but direct infection and immune-mediated injury of nerves may both play a role.

Approximately 5% of patients initially diagnosed as GBS will develop CIDP. Patients with this acute onset CIDP (A-CIDP) often do not have cranial nerve involvement or respiratory failure, but these clinical characteristics lack the specificity to discriminate between GBS and A-CIDP in the acute phase of disease. Moreover, NCS cannot be used to discriminate between AIDP and CIDP in individual patients. A-CIDP is usually diagnosed during follow-up and should be considered when clinical deteriorations occur more than 2 times or beyond 8 weeks from onset, as in the patient described here.[5] Our patient initially fulfilled the current diagnostic criteria for GBS as the progression of weakness was <4 weeks, but the pain and sensory symptoms before the start of weakness may have been a first indication of a more chronic form of neuropathy.

This case indicates that ZIKV infection may trigger a chronic peripheral nervous system disorder, most likely caused by prolonged inflammation, that can present as GBS. The lack of ZIKV genome in blood, urine and CSF further argue against a direct infection and implicates an immune mediated cause of nerve damage in this case. The clinical implication is that all patients initially diagnosed with GBS after ZIKV infection need careful follow-up as they may develop CIDP that can respond well to maintenance treatment with IVIg.

REFERENCES

1. Van den Bergh PY, Hadden RD, Bouche P, et al. European Federation of Neurological Societies/Peripheral Nerve Society guideline on management of chronic inflammatory demyelinating polyradiculoneuropathy: report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society - first revision. *Eur J Neurol* 2010;17:356-363.
2. World Health Organization. Zika situation report 10 March 2017. 2017.
3. Uncini A, Shahrizaila N, Kuwabara S. Zika virus infection and Guillain-Barre syndrome: a review focused on clinical and electrophysiological subtypes. *J Neurol Neurosurg Psychiatry* 2017;88:266-271.
4. Nascimento OJM, da Silva IRF. Guillain-Barre syndrome and Zika virus outbreaks. *Curr Opin Neurol* 2017.
5. Ruts L, Drenthen J, Jacobs BC, van Doorn PA, Dutch GBSSG. Distinguishing acute-onset CIDP from fluctuating Guillain-Barre syndrome: a prospective study. *Neurology* 2010;74:1680-1686.



Chapter 7

Neuromyelitis optica spectrum disorder associated with Zika virus infection

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CASE DESCRIPTION

In September 2017, a healthy 35-year-old Brazilian man with a medical history of dengue fever 18 months prior (confirmed by serology at the time) developed fever, a sore throat, myalgia, and arthralgia. Four days later, he was hospitalized with urinary retention, proximal paraparesis (Medical Research Council [MRC] grade 4), and paresthesias in lower limbs and face. On the first day of hospitalization, sagittal short T1 inversion recovery (STIR) spine MRI showed hyperintense,nonenhancing lesions between T1–T4 and T6–T9 compatible with an acute myelitis (**figure, A**). Brain MRI was normal. Lumbar puncture revealed 1/mm^3 cells, 56 mg/dL protein, 60 mg/dL glucose, and presence of oligoclonal bands. Immunoglobulin G (IgG) index was 0.55. Zika virus (ZIKV) PCR was positive in CSF, blood, urine, and saliva. PCR in the CSF for other viruses, including herpes, dengue (DENV), chikungunya, yellow fever, West Nile, and Saint Louis encephalitis, were all negative. Blood aquaporin-4 IgG (AQP4-IgG) was negative. Rheumatologic disease and other infections, including syphilis, hepatitis, HIV, cytomegalovirus, Epstein-Barr virus, and tuberculosis, were all negative in serologic testing. Methylprednisolone (1 g/d IV for 5 days) was started on the second day of hospitalization. One day later, the patient deteriorated, with increased weakness of the legs (MRC 1), bilateral Babinski sign and ankle clonus, a T2 sensory level, oscillatory vertigo with central pattern nystagmus, constipation, vomiting, somnolence, and discrete hearing loss. Brain MRI 7 days after admission revealed hyperintensity in the pons, superior and middle left cerebellar peduncle, and periependymal lesions on T2-weighted fluid-attenuated inversion recovery (FLAIR) sequences (**figure, B**). At this time, the patient met the criteria[1] for NMOSD with negative AQP4-IgG based on the presence of acute brainstem syndrome with periependymal lesion and acute myelitis with a compatible MRI (intramedullary lesion extending over 3 segments). After corticosteroid therapy, he received IV immunoglobulin for 5 days (2 g/kg total dose) and was discharged with minor sensory impairment and partial recovery of bladder and bowel control, and was able to walk using a walker. The day after discharge, however, the patient returned with visual impairment, dyschromatopsia, afferent pupillary defect, and pain in the right eye (day 27). Another brain MRI showed hyperintense T2 signs of the right optic nerve and an extensive T1 gadolinium-enhancing lesion compromising at least 50% of the nerve length, compatible with optic neuritis (**figure, C**). Another methylprednisolone course was administered, and his visual acuity improved. Maintenance treatment with oral prednisone (60 mg/d) was started. After remaining stable for 1 month, the patient developed tactile and temperature allodynia of both arms (day 63). Sagittal STIR spinal MRI showed hyperintensities at C3 to C6 segments (**figure, D**).

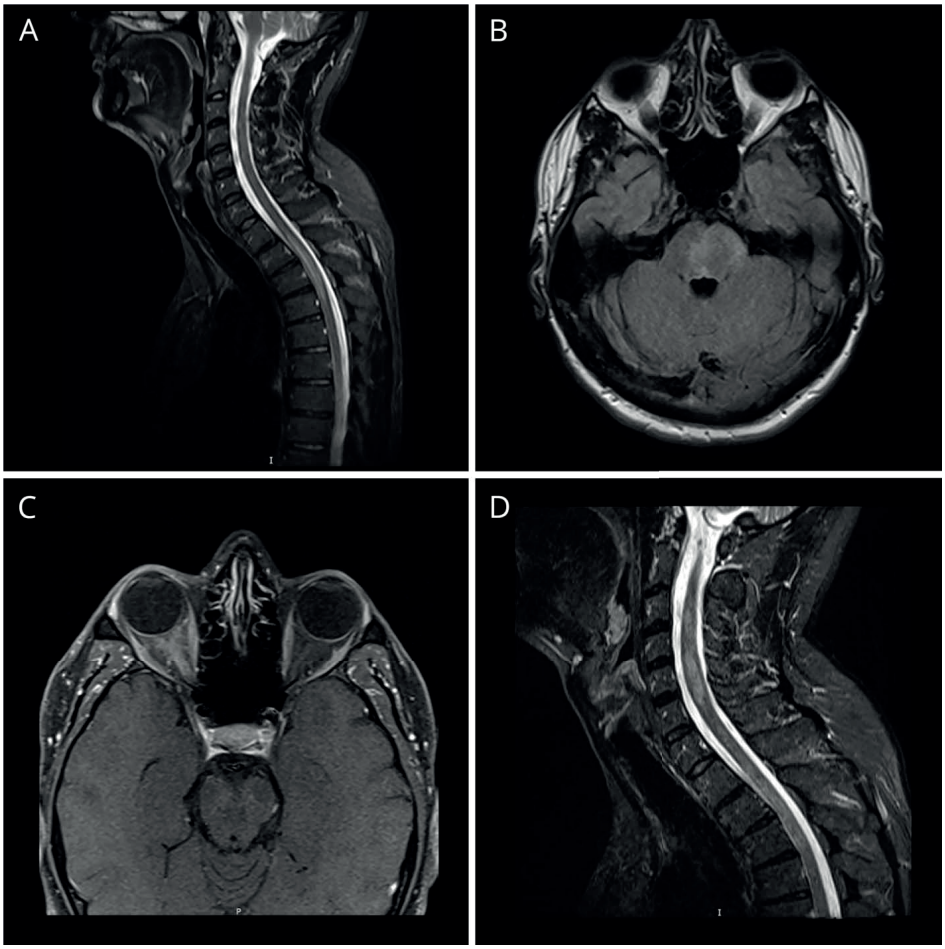


Figure 1. Spine, brain, and orbit MRI of a patient with neuromyelitis optica spectrum disorder

A) Sagittal short T1 inversion recovery (STIR) spine MRI shows hyperintense, nonenhancing lesions in the T1–T4 and T6–T9 segments. (B) Fluid-attenuated inversion recovery axial brain MRI demonstrates hyperintensity in the pons, superior and middle left cerebellar peduncle, as well as periependymal lesion. (C) Axial orbit MRI T1 postcontrast shows extensive gadoliniumenhancing lesion compromising at least 50% of the right nerve length compatible with optic neuritis. (D) Hyperintensities at level C3 to C6 are depicted on sagittal STIR spine MRI.

AQP4-IgG and ZIKV PCR in blood were negative. Anti-MOG was not available for testing. He received a third course of methylprednisolone followed by 5 plasmapheresis sessions, which improved his symptoms. Currently, he has been stable for 6 months using maintenance treatment with azathioprine 2 mg/ kg/d and prednisone 20 mg/d.

DISCUSSION

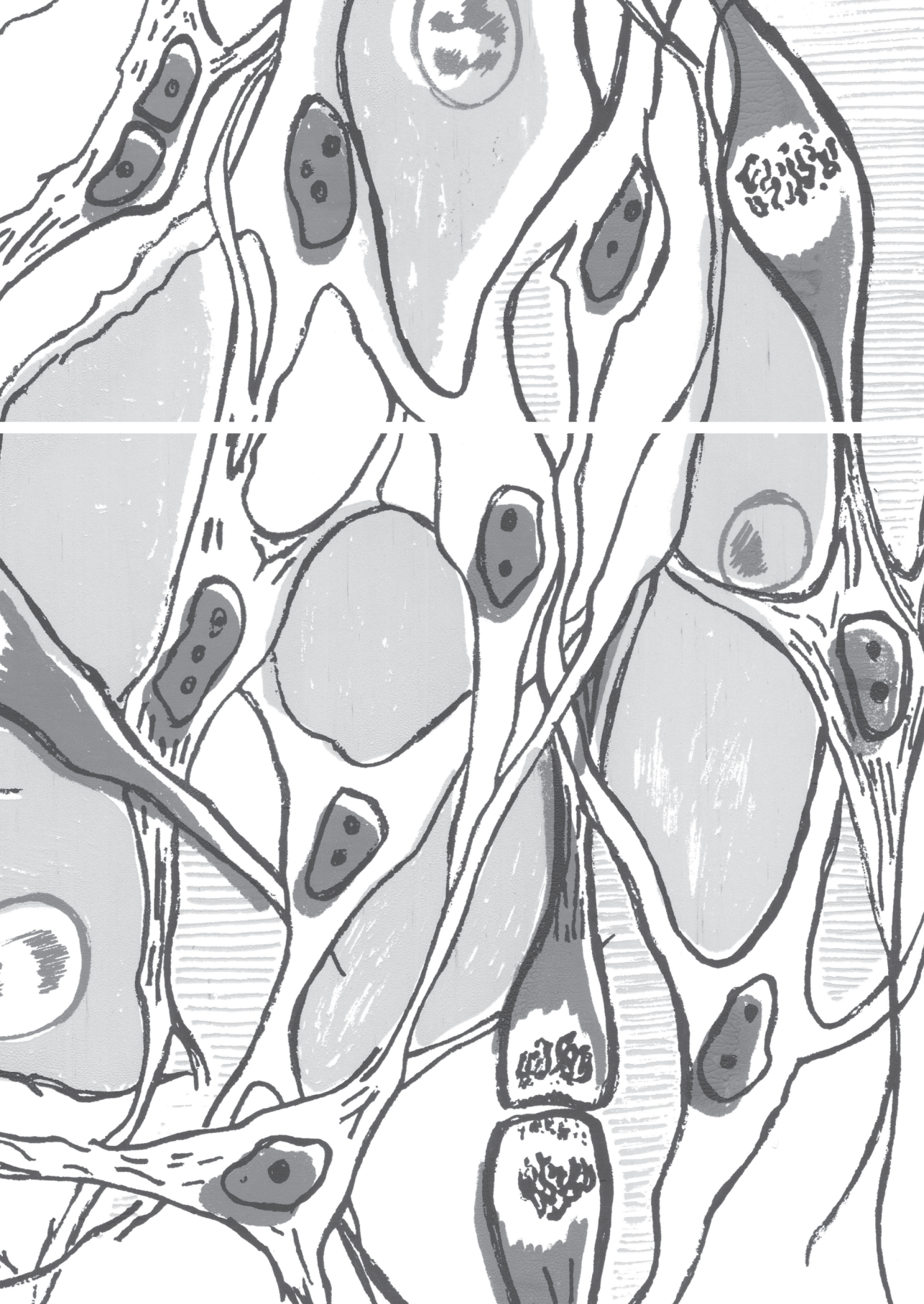
The ZIKV epidemic in 2015 in Latin America was followed by increases in microcephaly and Guillain-Barré Syndrome (GBS) cases, and ZIKV has subsequently been linked to other neurological complications including myelitis, encephalitis and acute disseminated encephalomyelitis.[2, 3] Nevertheless, this is a rare case reported of NMOSD linked to ZIKV infection. How ZIKV can cause or trigger neurological disease is not yet fully understood. In vitro studies and could be due to direct virus neurotropism[4] or immune-mediated processes.[3-6] In this NMOSD case, the high PCR titers in the CSF and other biological materials at onset suggest a direct viral pathogenicity. However, new severe neurologic symptoms occurred when PCR for ZIKV was already negative, suggesting a post- or parainfectious mechanism. Furthermore, the preceding DENV infection, as some studies indicate, could intensify ZIKV neurologic manifestations due to cross-reactive antibodies.[6] The precise pathophysiology of NMOSD and viral infections is yet to be established and further research is required to specifically investigate the relation between ZIKV and NMOSD. Following this case, we recommend neurologists working in areas with ongoing transmission of ZIKV to consider the possibility of ZIKV infection in NMOSD cases, including patients who have not had arboviral symptoms before the onset of neurologic symptoms.[7]

REFERENCES

1. Wingerchuk DM, Banwell B, Bennett JL, et al. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. *Neurology* 2015;85:177-189.
2. da Silva IRF, Frontera JA, Bispo de Filippis AM, Nascimento O, Group R-G-ZR. Neurologic Complications Associated With the Zika Virus in Brazilian Adults. *JAMA Neurol* 2017;74:1190-1198.
3. Bharucha T, Breuer J. Review: A neglected Flavivirus: an update on Zika virus in 2016 and the future direction of research. *Neuropathol Appl Neurobiol* 2016;42:317-325.
4. Tang H, Hammack C, Ogden SC, et al. Zika Virus Infects Human Cortical Neural Progenitors and Attenuates Their Growth. *Cell Stem Cell* 2016;18:587-590.
5. Sellner J, Hemmer B, Muhlau M. The clinical spectrum and immunobiology of parainfectious neuromyelitis optica (Devic) syndromes. *J Autoimmun* 2010;34:371-379.
6. Dejnirattisai W, Supasa P, Wongwiwat W, et al. Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with zika virus. *Nat Immunol* 2016;17:1102-1108.
7. Duffy MR, Chen TH, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med* 2009;360:2536-2543.

Part IV

Guillain-Barré syndrome and other epidemics of infectious disease



Chapter 8

Guillain-Barré syndrome outbreak in Peru 2019 associated with *Campylobacter jejuni* infection

Ana P. Ramos, Mireya A. Cuba, Carlos C. Castañeda, Jose A. Dioses, Martin A. Tipismana, Alejandro Llanos, David R. Cornblath, Dawn Gourlay, Susan K. Halstead, Bart C. Jacobs, Sonja E. Leonhard, Hugh J. Willison, Max Grogl, Mariana Ramos, Jesus D. Rojas, Rina Meza, Daniela Puiu, Rachel M. Sherman, Patricia J. Simner, Steven L. Salzberg, Carlos A. Pardo, Hugo F. Umeres

Neurol Neuroimmunol Neuroinflamm 2021;8:e952.

ABSTRACT

Objective

To identify the clinical phenotypes and infectious triggers in the 2019 Peruvian Guillain-Barré syndrome (GBS) outbreak.

Methods

We prospectively collected clinical and neurophysiological data of GBS patients admitted to a tertiary hospital in Lima, Peru between May and August 2019. Molecular, immunological, and microbiological methods were used to identify causative infectious agents. Sera from 41 controls were compared with cases for antibodies to *Campylobacter jejuni* and gangliosides. Genomic analysis was performed on 4 *C. jejuni* isolates from GBS cases.

Results

The 49 included patients had a median age of 44 years (IQR 30-54), and 28 (57%) were male. Thirty-two (65%) had symptoms of a preceding infection: 24 (49%) diarrhea and 13 (27%) upper respiratory tract infection. The median time from onset of infectious to neurological symptoms was 3 days (IQR 2-9). Eighty percent had a pure motor form of GBS, 21 (43%) had the axonal electrophysiological subtype, and 18% the demyelinating subtype. Evidence of recent *C. jejuni* infection was found in 28/43 (65%). No evidence of recent arbovirus infection was found. Twenty-three cases vs 11 controls (OR: 3.3, IC 95% 1.2-9.2, $p<0.01$) had IgM and/or IgA antibodies against *C. jejuni*. Anti-GM1:phosphatidylserine and/or anti-GT1a:GM1 heteromeric complex antibodies were strongly positive in cases (92.9% sensitivity and 68.3% specificity). Genomic analysis showed the *C. jejuni* strains were closely related and had the Asn51 polymorphism at cst*II* gene.

Conclusions

Our study indicates that the 2019 Peruvian GBS outbreak was associated with *C. jejuni* infection and that the *C jejuni* strains linked to GBS circulate widely in different parts of the world.

INTRODUCTION

Guillain-Barré syndrome (GBS) is an immune-mediated disorder frequently triggered by infections, characterized by an acute flaccid paralysis, accompanied by sensory symptoms and cranial nerve deficits.[1] In recent years, several outbreaks of GBS have been observed globally, including the large outbreaks in Latin-America and the Caribbean during the Zika virus (ZIKV) epidemic,[2–4] and the possibility of an association between severe acute respiratory syndrome coronavirus 2 infection and GBS has been raised.[5] As the Zika virus epidemic transitioned to an endemic phase in the Americas in 2017, 2 major outbreaks of GBS occurred in Peru in 2018 and 2019. The number of reported GBS cases reported increased from 59 in 2017 (incidence of 0.19/100,000) to 262 in 2018 (incidence of 0.81/100,000) and 1,120 in 2019 (incidence of 3.44/100,000).[6] During these outbreaks, the increases in GBS cases were also reported in areas where there is no potential of arboviral transmission such as the highlands of Peru. The outbreaks had a seasonal pattern with the major peaks of incidence between April and July in both years (**Figure 1A**).[6,7] We investigated the causality of these outbreaks, by performing an observational clinical cohort study of adult patients with GBS evaluated at a tertiary university hospital in Lima during the 2019 outbreak.

METHODS

Study population and design

We prospectively evaluated the clinical and laboratory features of patients suspected of GBS at the Hospital Cayetano Heredia (HCH), a university-based tertiary care hospital in Lima, Peru, during the 2019 GBS outbreak (May–August) in Peru. We included all patients who were evaluated by a neurologist and fulfilled the Brighton Collaboration Working Group criteria for diagnosis of GBS with a classification level 1, 2, or 3.[8] Included patients underwent a comprehensive neurological evaluation during the acute and convalescent phase of their illness and were followed-up to 6 months after discharge. Patients with alternative diagnoses or with insufficient data were excluded. Blood, cerebrospinal fluid (CSF), respiratory, and stool samples, when available, were obtained during the acute stage of illness as part of the standard of care to identify potential infectious etiologies. Nerve conduction studies (NCS) and electromyography (EMG) were performed and classified according to the criteria of Hadden et al.[9,10] The clinical and laboratory information was documented using standardized questionnaires of the Neuroviruses Emerging in the Americas Study (NEAS) forms adapted from the International GBS Outcome Study (IGOS). [3,11]

Figure 1A

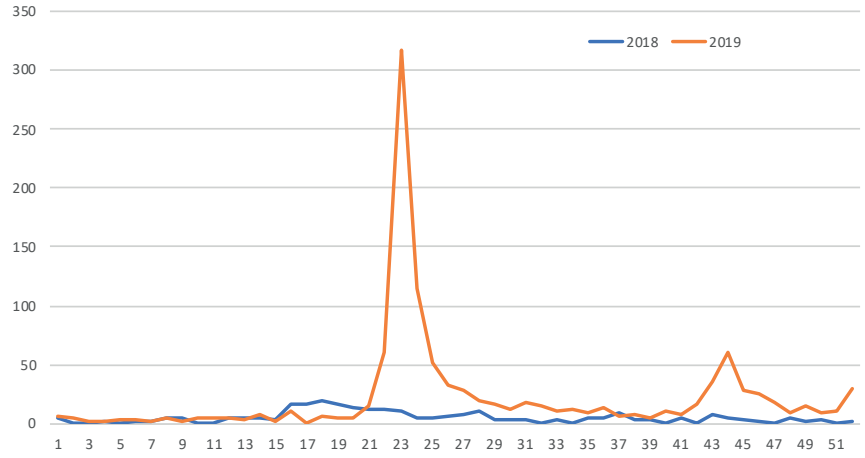


Figure 1B

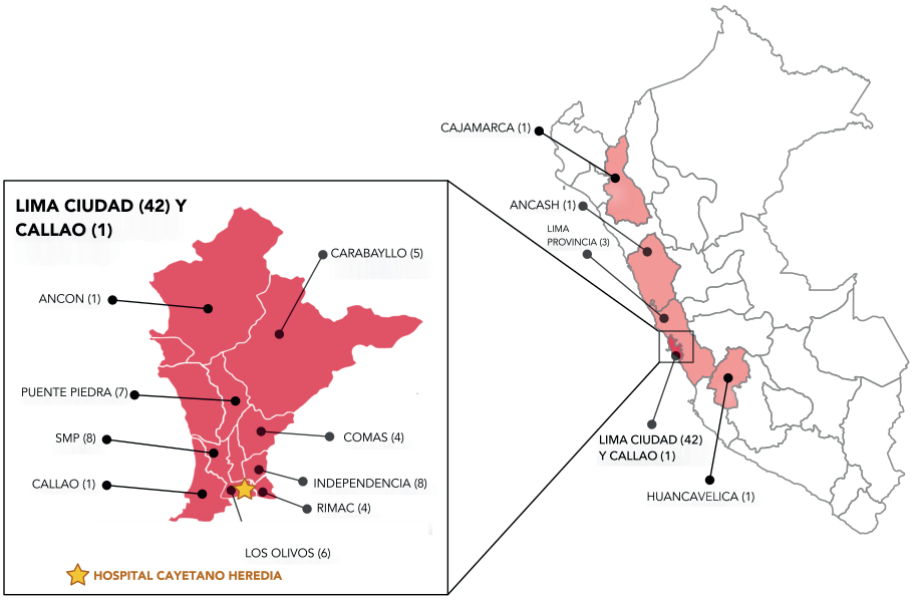


Figure 1. Epidemiologic Profile of 2018–2019 Guillain-Barr´e Syndrome (GBS) Outbreaks in Peru

(A) Epidemiologic curves (2018–2019) of GBS cases in Peru based on Peruvian Ministry of Health data.6 (B) Map of Peru shows regions of origin of GBS cases included in the study. Cajamarca, Huaraz (Ancash), and Huancavelica are cities located in the highlands where no arbovirus transmission was reported.

The onset of GBS and disease nadir were defined as the first day of onset of neurologic symptoms and the most severe clinical weakness, respectively. Pure motor GBS was defined as limb weakness in absence of sensory deficits at neurological examination, and sensorimotor GBS was defined as presence of both limb weakness and sensory deficits. Limb muscle strength was evaluated using Medical Research Council (MRC) sum score.[12] Severity was assessed according to the modified Rankin Scale (mRS),[13] the GBS Disability Score (GBSDS),[14] the modified Erasmus GBS Outcome Score (mEGOS),[15] and the Erasmus GBS Respiratory Insufficiency Score at admission (EGRIS).[16]

Laboratory testing

Hematologic and comprehensive metabolic assessments, including among others sodium and potassium levels, liver and kidney function tests, and HIV serology were performed in all patients at admission. For the investigation of infectious agents, blood, CSF, oropharyngeal swabs, and stool samples were tested at the Naval Medical Research Unit-6 (NAMRU-6) in Lima, Peru. Blood samples were assessed for arboviral infections including ZIKV, dengue virus (DENV), and chikungunya virus (CHIKV) using quantitative real time-PCR.[17] Oropharyngeal swabs were tested for 20 respiratory pathogens using a multiplexed PCR assay (BioFire Diagnostics®, Salt Lake City, USA). Stool swabs in Cary Blair medium were analyzed using a multiplexed PCR assay for gastrointestinal pathogens (BioFire Diagnostics®, Salt Lake City, USA) which included 22 pathogens associated with gastroenteritis, including *Campylobacter* species (jejuni, coli, and upsaliensis), and *Escherichia coli.* Stool samples were cultured for identification and characterization of *E. coli* and *C. jejuni*.[18] Positive culture samples were further characterized molecularly using multiplexed PCR assays for identification of *C. jejuni*[19] and Penner-types.[19,20] *C. jejuni* isolates from stool cultures were sequenced using next generation sequencing techniques, and the genomic assemblies underwent genomic and phylogenetic analysis based on the hypervariable lipo-oligosaccharide (LOS) region. Phylogenetic analysis was based on 83 *C. jejuni* genome assemblies downloaded from NCBI which included all 16 genomes reported to be associated with GBS in the NCBI metadata and 67 additional genomes selected to represent a wide range of the collection locations, dates, and studies available (appendix e-1). These 83 genomes, the *C. jejuni* reference genome (NCTC11168),[21,22] and the 4 genomes of 4 *C. jejuni* isolates assembled from the present study were then used to construct a phylogenic tree from the sequence of the hypervariable LOS biosynthesis gene locus using the Nextstrain-Augur pipeline.[23]

To evaluate the possible association between *C. jejuni* infection and GBS, serum samples from 42 GBS cases were compared with serum samples of 41 controls

for the presence of anti-*C. jejuni* IgA, IgM and IgG antibodies by ELISA following a case-control methodology.[24] Control samples were obtained from subjects from the same or neighboring households of the patients with GBS. The control subjects were evaluated by a neurologist to exclude a history of weakness within the previous year and assessment of neurological status and motor function for documentation of normal neurological status. The presence of anti-*C. jejuni* antibodies was expressed as a ratio of optical density (OD) between a test sample and the cutoff serum sample. A ratio >1.0 for IgM or IgA was considered evidence of a recent *C. jejuni* infection. A concomitant *C. jejuni* infection was established by a positive PCR for *Campylobacter* in a stool sample or by stool culture.

Case-control methodology was also used to study anti-ganglioside immunity using a multiplexed array panel to identify specific anti-ganglioside IgG antibodies. Patients and control sera were screened on microarrays.[25] Glycolipid microarrays consisted of a panel of 16 single glycolipids (GM1, GM2, phosphatidylserine, GM4, GA1, GD1a, GD1b, GT1a, GT1b, GQ1b, GD3, SGPG, LM1, GalNAc-GD1a, GalC and Sulfatide) and 120 heteromeric 1:1 (v:v) complexes printed in duplicate. The presence of anti-glycolipid antibodies was determined using human IgG isotype-specific, fluorescent-conjugated secondary antibodies, the intensity of which was measured on a scale of 0-65535 using a Genepix 4300A (Molecular Devices, USA) microarray scanner. Antibody intensity values were reported as the average of duplicate median fluorescent intensity values per sample. Results were graphically displayed as heat maps using Pearson´s correlation hierarchical clustering (MeV software). The optimal cutoff value for anti-glycolipid IgG antibodies, above baseline levels, was calculated from receiver operating characteristic (ROC) curves using Youden index.

Statistical analysis

The clinical and laboratory findings were described using absolute and relative frequencies. Median and interquartile ranges (IQRs) were reported for quantitative variables. The χ^2 or Fisher exact test, OR, and 95% CIs were used to determine differences between the groups. A p value <0.05 was considered significant. Area under the curve was calculated for each antiglycolipid antibody combination in ROC analysis. Statistical analyses were performed using Stata software, V15.0 (College-Station, TX).

Ethical considerations

This study was reviewed and approved by the HCH Institutional Review Board. All patients (or relatives when patients were incapacitated) and healthy controls (HCs) provided written informed consent.

RESULTS

Clinical features

Fifty-nine patients suspected of GBS were seen between May and August 2019. Ten were excluded: 8 had insufficient data due to transfer to other hospitals during the outbreak, 1 patient had a recent infection with HIV, syphilis and tuberculosis, and 1 patient had only cranial nerve involvement. Of the 49 included patients, 43 were from Lima city and 6 from Northern area and highlands of Peru (**Figure 1B**). The demographic and clinical characteristics of the 49 patients are described in **Table 1**. All patients fulfilled Brighton criteria level 1 (84%) or level 2 (16%). The median age was 44 years (IQR 30–54 years), and 28 (57%) were male. Thirty-two patients (65%) had symptoms of an infection 6 weeks preceding the onset of GBS: 24 (49%) diarrhea and 13 (27%) upper respiratory tract symptoms, and 2 patients (4%) received an influenza vaccine. The median time from onset of infectious to neurologic symptoms was 3 days (IQR 2–9 days), and the time from onset of neurologic symptoms to nadir was 6 days (IQR 3–7 days). At admission, all patients reported limb weakness. Quadriparesis evolving in less than 24 hours from neurological symptom onset was observed in five patients (10%). The median GBSDS at admission was 4 (IQR 3-4), and EGRIS was 3 (IQR 2-4). Fifteen patients (31%) had cranial neuropathy, with the facial nerve most commonly involved. The median MRC sumscore was 42 (IQR 26–50). Most patients (80%) were classified clinically as pure motor GBS. Neurologic examination, treatment, and outcome at nadir and at 6-month follow-up are detailed in **Supplementary Table 1**.

NCS/EMG studies were performed in all patients at a median of 16 days after onset of neurologic symptoms (IQR 10–23 days). Twenty-one patients (43%) had axonal neuropathy (acute motor axonal neuropathy), 9 (18%) demyelinating neuropathy (acute inflammatory demyelinating polyneuropathy), 8 (16%) equivocal, 5 (10%) inexcitable, and 6 (12%) had normal studies. Forty-seven patients (96%) received treatment with IVIg (51%), plasmapheresis (18%), or both (27%). The standard treatment was 5 sessions of plasmapheresis or 0.4 mg/kg/d IVIg for 5 days. IVIg treatment was stopped in 1 patient who developed angioedema during their second session and who died before starting plasmapheresis. Two patients did not receive treatment, one because of lack of treatment availability on admission and one due to initial misdiagnosis. Both patients improved without treatment. Thirteen patients (27%) were admitted to the intensive care unit, 12 (24%) required ventilator support, and 6 (12%) had cardiac dysautonomia. The median hospitalization time was 14 days (IQR 9–23 days). One week after admission, the median mEGOS was 5 (IQR 2–9). Most patients improved as indicated by mRS score at 6-month follow-up (median 2, IQR 1–2) compared with

Table 1. Demographic and clinical characteristics of patients with GBS

Characteristics	N=49
Age– (years)	44 (30-54)
Male sex	28 (57)
General symptoms before the onset of GBS (last 6 weeks)	32 (65)
Diarrhea	24 (49)
Upper respiratory symptoms [a]	13 (27)
Fever	5 (10)
Headache	3 (6)
Arthralgia	4 (8)
None	17 (35)
Time from onset of infectious symptoms to admission (days) [b]	7 (7-14)
Time from onset of infectious symptoms to GBS onset (days)[b]	3 (2-9)
Time from onset of GBS symptoms to admission (days)	4 (3-6)
Time from onset of GBS symptoms to nadir (days)	6 (3-7)
GBS Disability Score at admission	4 (3-4)
Erasmus GBS Respiratory Insufficiency Score at admission	3 (2-4)
Admission to ICU	13 (27)
Mechanical ventilation	12 (24)
Autonomic dysfunction	6 (12)
Duration of hospitalization (days)	14 (9-23)
Brighton criteria for GBS diagnosis	
Level 1	41 (84)
Level 2	8 (16)
GBS Clinical Variant	
Pure motor	39 (80)
Sensorimotor	6 (12)
Pharyngeal-cervical-brachial	2 (4)
Miller Fisher syndrome	1 (2)
Bickerstaff brainstem encephalitis	1 (2)
Cerebrospinal fluid analysis	48 (98)
Time from onset neurological symptoms to CSF sampling (days)	5 (4-7)
White-cell count (cells/mm3)	0 (0-1)
Total protein (mg/dL)	33 (16-58)
Increased protein level[c]	14 (29)
Time from GBS symptoms onset to EMG (days)	16 (10-23)
NCS/EMG results and subtype	
AMAN	21 (43)
AIDP	9 (18)
Inexcitable	5 (10)
Equivocal	8 (16)
Normal	6 (12)

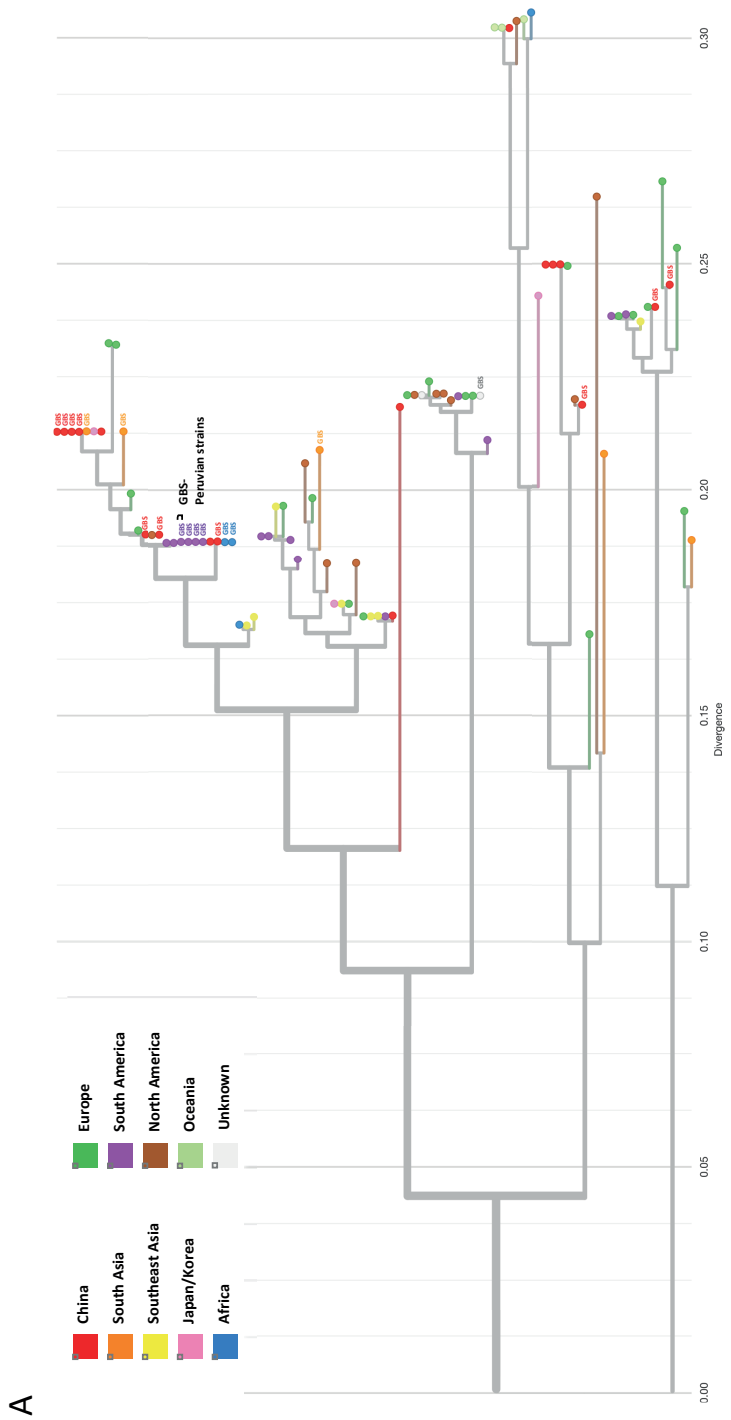
Abbreviations: AIDP = acute inflammatory demyelinating polyneuropathy; AMAN = acute motor axonal neuropathy; GBS = Guillain-Barr´e syndrome; ICU = intensive care unit; NCS = nerve conduction study. Data are presented as n/N (%) or median (interquartile range). [a] Six patients had both diarrhea and upper respiratory symptoms. [b] Based on 32 patients with a history of preceding general symptoms. [c] Increased protein level is defined as >52 mg/dL. The percentage is based on 48 CSF samples.

Table 2. Laboratory studies

A. Investigation of infectious agents in GBS cases			
Test/target		**n /N (%)**	
qRT-PCR (serum)			
Zika virus		0/20 (0)	
Dengue virus[a]		0/26 (0)	
Chikungunya virus		0/22 (0)	
HIV (ELISA)		1/49 (2)	
Respiratory Film Array® (oropharyngeal swab)[b]			
Rhinovirus/Enterovirus		3/19 (16)	
Influenza A		0/19 (0)	
Mycoplasma pneumoniae		0/19 (0)	
Gastrointestinal Film Array® (stool)		24/37 (65)[c]	
Campylobacter sp[d]		14/37 (38)	
E. coli		16/37 (43)	
Stool culture		11/37 (30)[e]	
C. jejuni HS41		4/37 (11)	
E. coli		10/37 (27)	
B. Serological case-control studies			
Campylobacter jejuni serology	**GBS no. (%)**	**Controls no. (%)**	**OR[f] *(p)***
Patients tested, no. (%)	42 (100)	41 (100)	-
Anti-*C. jejuni* IgG	42(100)	41 (100)	-
Anti-*C. jejuni* IgM or IgA	23 (55)	11 (27)	**3.3 (0.01)**
Anti-*C. jejuni* IgM	19 (45)	11 (27)	2.3 (0.081)
Anti-*C. jejuni* IgA	12 (29)	0	-
Anti-Ganglioside Profile	**GBS no. (%)**	**Controls no. (%)**	**OR *(p)***
Patients tested, no. (%)	42 (100)	41 (100)	-
GalNAc-GD1a -GBS cases	3 (7)	4 (10)	0.7 (0.668)
GM1- GBS cases	4 (10)	1 (2)	4.2 (0.175)
GM1:GT1a -GBS cases	14 (33)	4 (10)	**4.6 (0.009)**
GM1:PS- GBS cases	17 (40)	8 (20)	**2.8 (0.037)**
GT1a- GBS cases	4 (9)	1 (2)	4.2 (0.175)

Abbreviations: CHIKV = chikungunya virus;DENV= dengue virus; PS = phosphatidylserine; qRT = quantitative real time; ZIKV = Zika virus. Bold values in OR (p) column indicate statistical significance ($p < 0.05$).

[a] All samples were tested for DENV-1, DENV-2, DENV-3, and DENV-4. [b] In addition to the listed pathogens detected by the respiratory array assay, other pathogens tested were found negative and those included *Mycoplasma pneumoniae*, adenovirus, coronavirus HKU1, NL63, 229E, and OC43, human metapneumovirus, influenza A, A/H1, A/H3, and A/H1-2009, influenza B, parainfluenza virus 1, 2, 3, and 4, *Bordetella pertussis*, and *Chlamydia pneumoniae*. [c] In addition to the *C jejuni* and *E coli* detected, testing for other bacteria, parasite, and viruses included in the assay were negative, which included bacteria: *Clostridium difficile* (toxin A/B), *Plesiomonas shigelloides*, *Salmonella*, *Yersinia enterocolitica*, *Vibrio* (*parahaemolyticus, vulnificus*, and *cholerae*), diarrheagenic *E coli*/*Shigella*, enteroaggregative *E coli* (EAEC), enteropathogenic *E coli* (EPEC), enterotoxigenic *E coli* (ETEC), Shiga-like toxin-producing *E coli* (STEC), *E coli* O157, and *Shigella*/enteroinvasive *E coli* (EIEC). Parasites: cryptosporidium, *Cyclospora cayetanensis*, *Entamoeba histolytica*, and *Giardia lamblia*. Viruses: adenovirus F 40/41, astrovirus, norovirus GI/GII, rotavirus A, and sapovirus (I, II, IV, and V). [d] Six patients had coinfection of both Campylobacter sp and E coli. [e] *Campylobacter* (*jejuni, coli,* and *upsaliensis*). [f] Three patients had positive culture for both *C jejuni* HS41 and *E coli*.


A
China
South Asia
Southeast Asia
Japan/Korea
Africa
Europe
South America
North America
Oceania
Unknown
GBS-
Peruvian strains
0.00
0.05
0.10
0.15
0.20
0.25
0.30
Divergence

B

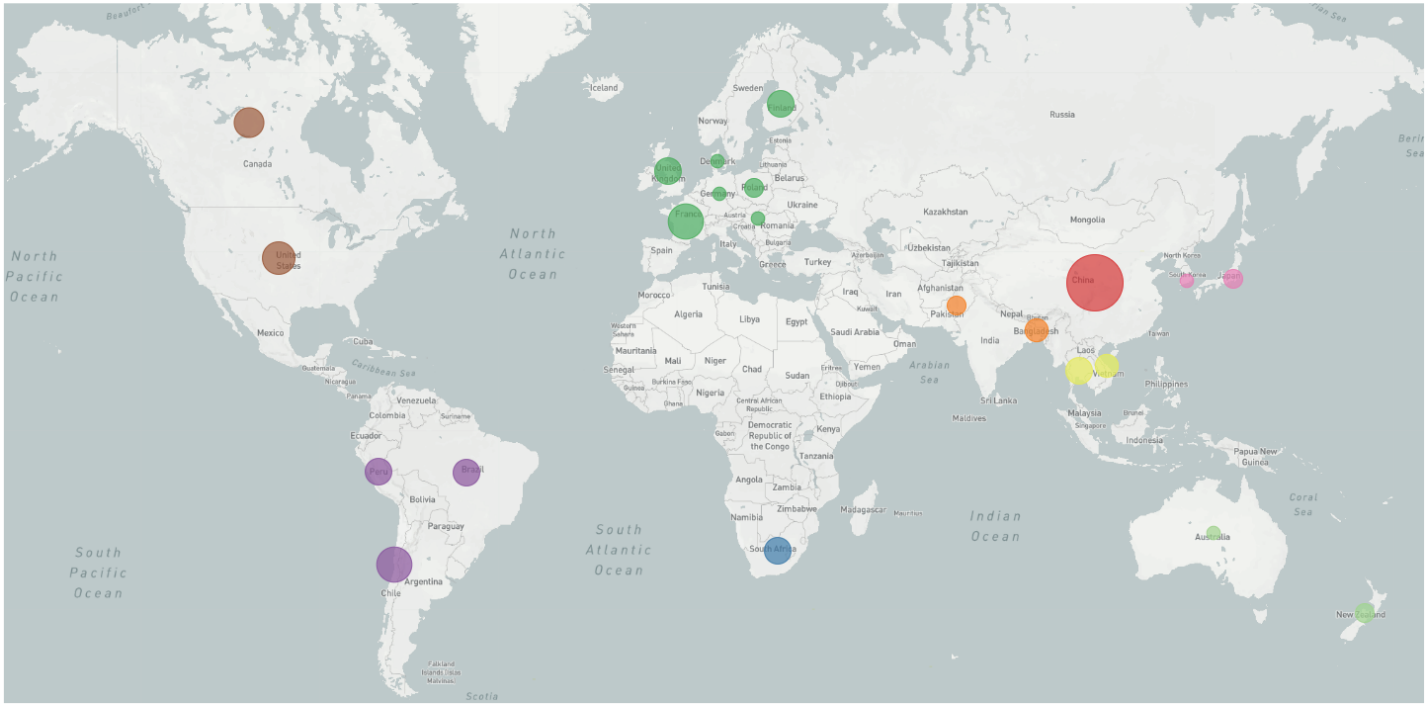
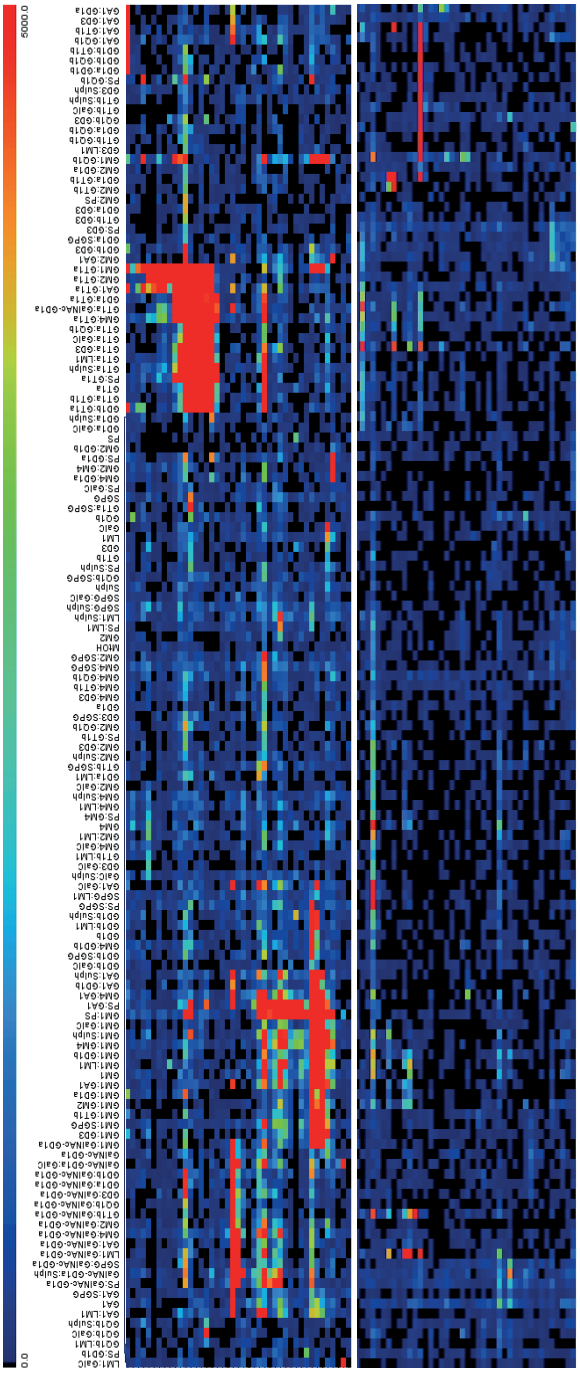


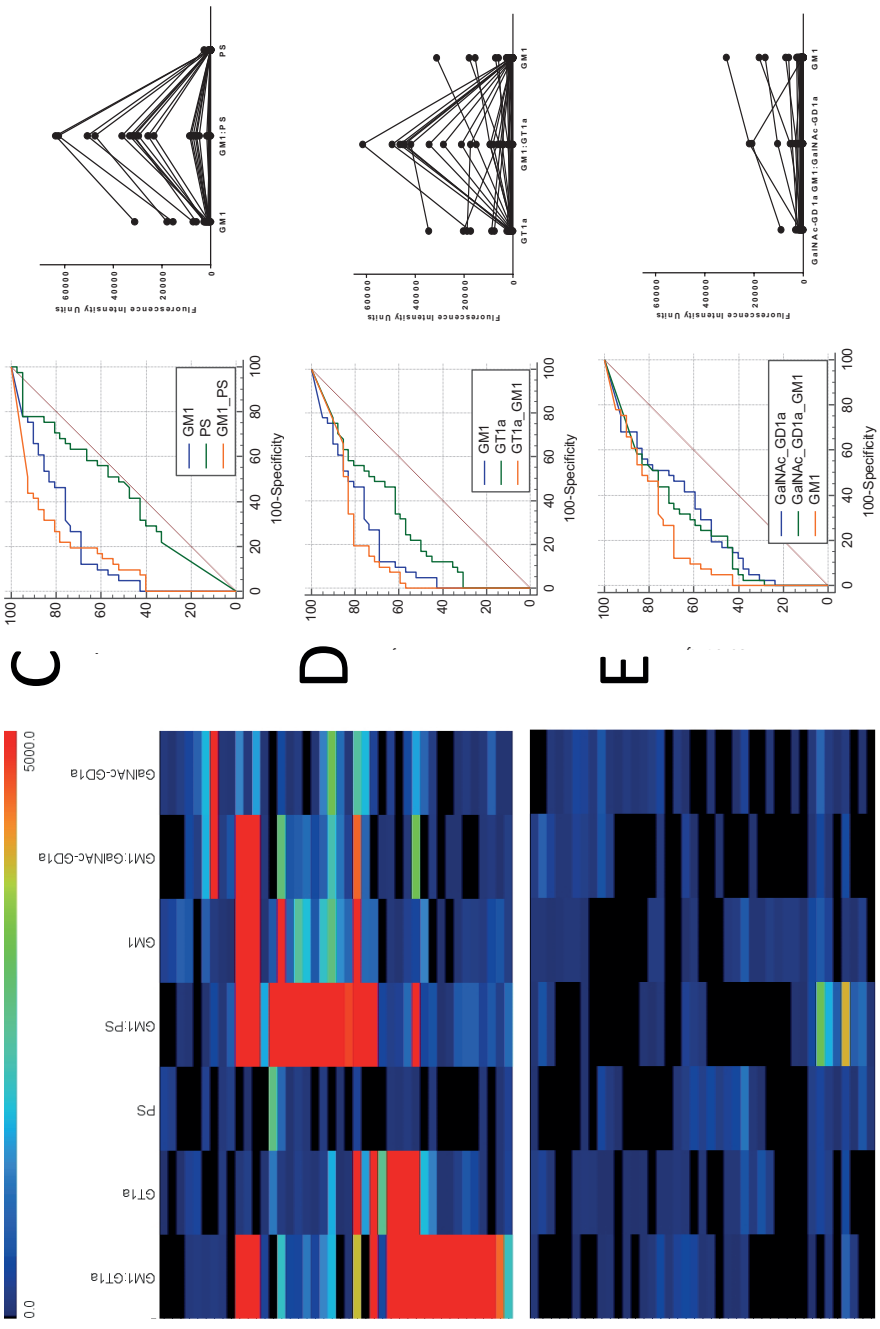
Figure 2. Phylogenetic Tree and Phylogenetic Map of *C. jejuni* Isolates in Guillain-Barré Syndrome

(A) Phylogenetic tree built from the lipo-oligosaccharide region of 88 C jejuni species using the Nextstrain pipeline, colored by region. The 4 Peruvian strain assemblies are indicated. The tree was built via alignment to the ICDCCJ07001 strain and then rooted by inferring ancestral states.

(B) Map distribution of strains depicted in the phylogenetic tree. Strains are colored by region of collection, which was available for all but 2 genomes, the *C. jejuni* reference, NCTC11168, and 1 GBS-associated strain, G1, which had no listed collection location. The size of the circles within countries on the associated world map is proportional to how many samples are included from that country.



A


B
5000.0
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GalNAc-GD1a
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GM1
GM1:PS
Ps
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100-Specificity
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D
GM1
GT1a
GT1a_GM1
100-Specificity
Fluorescence Intensity Units
GT1a
GM1:GT1a
GM1
E
GalNAc_GD1a
GalNAc_GD1a_GM1
GM1
100-Specificity
Fluorescence Intensity Units
GalNAc-GD1a
GM1:GalNAc-GD1a
GM1

Figure 4. Antiganglioside Antibody Binding Profile in Peruvian GBS Cases
Graphical displays of GBS and healthy control (HC) serum IgG antiganglioside antibody binding. (A) Heat maps illustrating the IgG binding intensity to 3 single glycolipids and 4 heteromeric complex antigen targets in GBS cases (upper map, n = 42) and HC sera (lower map, n = 41). Each horizontal row refers to the IgG binding reactivity of an individual GBS or HC serum sample, and each vertical row refers to each of the 7 targets displayed. The rainbow bar denotes the intensity scale of IgG binding from low (blue) to high (red) intensity. Two patterns of reactivity are greatly amplified by presenting glycolipids/lipids targets as heteromeric complexes compared with binding to each target alone: GM1:GT1a complex (first column *) and GM1:PS complex (fourth column **). Note that these 2 patterns of heteromeric complex reactivity do not substantially overlap within any 1 patient, being mutually exclusive. (B) An illustrative receiver operating characteristic (ROC) curve comparing the sensitivity and specificity of GT1a and GM1 as single glycolipids with the GT1a:GM1 heteromeric complex. The highest sensitivity (81%) and specificity (80.5%) are seen with the GM1:GT1a complex. (C) IgG reactivity values of each individual patient are plotted for the same 3 antigen targets (GM1, GT1a, and GM1:GT1a complex) subjected to ROC analysis in panel B. Greatly enhanced binding intensity to the GT1a:GM1 heteromeric complex compared with the sum of the single glycolipid antigens is present in most samples. PS =phosphatidylserine.

nadir (median 4, IQR 4–5). Four patients (8%) died. The most common sequela after 6 months was neuropathic pain (69%) (**Supplementary Table 1**).

Laboratory testing

Hematologic and biochemical testing at admission were normal in all cases. CSF examination was performed in 48/49 patients at a median of 5 days (IQR 4–7 days) after start of neurologic symptoms. All patients had normal cell counts (median 0, IQR 0–1), and 14 (29%) had an increased protein level (>52 mg/dL) (**Table 1**). Laboratory results for infectious agents and antiganglioside profiles are described in **Table 2**. One patient with known HIV infection was HIV positive. Twenty (41%) patients underwent testing for ZIKV, 26 (53%) for DENV, and 22 (45%) for CHIKV, and all were negative. Nineteen patients (39%) underwent testing by Film Array respiratory panel, and 5 (26%) were positive for common respiratory viruses not known to be associated with GBS (**Table 2**).

In 43 patients (88%), biosamples were available for *C. jejuni* infection testing with either molecular or serologic assays or stool cultures (**Supplementary Table 2**). In 23/42 (55%) patients, anti-*C. jejuni* IgM and/or IgA antibodies were found, of whom 9 also tested positive for *Campylobacter sp* PCR in stool. In contrast, only 11 of 41 (27%) control subjects had evidence of anti-*C. jejuni* IgM or IgA (OR:3.3, IC95% 1.2-9.2, $p<0.01$) (**Table 2**).

The PCR-based gastrointestinal panel showed that 14/37 (38%) patients had evidence of *Campylobacter sp* genome. Stool cultures from 4 of these patients grew bacteria, which were confirmed as *C. jejuni* by immunologic and molecular assays (**Table 2, Supplementary Table 2**). Penner molecular typing indicated that these isolates were all HS41 capsule type. Genomic analysis showed that these strains were clonal,

sequence type (ST) ST2993, with class A LOS biosynthesis locus, a pathogenicity island that contains genes with the potential to generate LOS that mimic human gangliosides. Phylogenetic analysis showed that of the 20 GBS-associated *C. jejuni* genomes, 15, including the 4 isolates from this study, have LOS regions fairly closely related to one another and to other strains of *C. jejuni* associated with GBS isolated in China and Africa (**Figures 2 A and B**). Sample collection regions do not appear to define clades, with strains from countries with numerous samples spread throughout the tree. All 4 *C. jejuni* isolates from our study had the Asn51 polymorphism at cstII gene (**Figure e-1**, links.lww.com/NXI/A404) based on the alignments to ICDCCJ07001 indicating the capability to synthesize both alpha 2–3 and alpha 2–8 sialic acid linkages on their LOS core oligosaccharide.[26,27] The genomes of these 4 *C. jejuni* isolates were deposited at NCBI within BioProject PRJNA643291 (accession numbers SAMN15508151, SAMN15508152, SAMN15508153, and SAMN15508154, ncbi.nlm.nih.gov/bioproject/PRJNA643291).

Combining serologic assay and stool PCR, 28/43 patients (65%) had evidence of recent *C. jejuni* infection (**Table 2, Supplementary Table 2**). Of interest, these 28 patients did not significantly differ in the time to nadir, clinical variants, or electrophysiologic subtypes to the 15 patients without evidence of a recent *C. jejuni* infection (**Supplementary Table 2**). Patients with evidence of a recent *C. jejuni* infection had a higher percentage of preceding gastrointestinal symptoms, although this was not significant (43% vs 27%, p = 0.69). Other preceding infectious symptoms were also not significantly different.

Antiganglioside IgG antibodies of differing specificities were detected in a high proportion of cases compared with HCs (**Supplementary Figure 2**). Summarizing this overview heatmap, 2 broad populations of IgG antibodies were dominantly present in this cohort: those reactive with GM1 alone or in complexes and those reactive with GT1a, alone or in complexes (**Table 2, Figure 3**). A smaller number of samples contained antibodies to GalNAc-GD1a alone or in complexes. Antibodies to other gangliosides including GM2, GD1b, GD1a, and GT1b and to myelin glycolipids including SGPG, LM1 and GalC were either very infrequently or not observed. Ganglioside antigens were probed as single molecules and when in heteromeric complex (1:1 ratio) with one other ganglioside or lipid. This use of complexes is known to enhance antiganglioside antibody signals in a proportion of serum samples.[28] To identify the enhanced binding intensities resulting from complexes, samples were probed against GM1 and GT1a in complex with other lipids (**Figure 3A**). Results were then analyzed and displayed using ROC curve analysis in which the true and false-positive rates are calculated at various threshold settings to generate sensitiv-

ity and specificity data for the assay. ROC data for the major targets are shown in **Figure 3 B and C** and **Supplementary Figure 2B and C**. Using this approach, GM1 ganglioside in a 1:1 heteromeric complex ratio with PS or GT1a ganglioside proved to be the most significant diagnostic marker. When GM1 was in complex with PS, antibodies to the GM1:PS complex returned a sensitivity 78.6% and a specificity of 78.0% for GBS (**Supplementary Figure 2B**). When GM1 was in complex with GT1a, antibodies to the GM1:GT1a complex returned a sensitivity of 81.0% and a specificity of 80.5% for GBS (**Figure 4B**). The enhancing effect, as manifested by an increase in fluorescence intensity units, of GM1 in complex with GT1a vs either antigen alone is shown in **Figure 4C**. In contrast, the GM1:GalNAc-GD1a complex did not enhance reactivity with either glycolipid alone (**Supplementary Figure 2C**). When selecting the GM1:PS and GT1a:GM1 complex antigen targets as biomarkers of GBS, 92.9% of patients had IgG antibodies to one or both of these glycolipid complexes compared with 31.7% of HCs.

DISCUSSION

In the aftermath of the large ZIKV epidemic in Latin America, two large seasonal outbreaks of GBS were seen in Peru, one in 2018 and one in 2019.[6,29] Our study, describing a large cohort of patients and controls during the 2019 GBS outbreak in Lima, demonstrates that this outbreak was associated with *C. jejuni* infection, a diarrheal bacterium that is the most common trigger of GBS world-wide. As the outbreak of GBS in 2018 occurred in the same period of the year and affected the same regions of the country (**Figure 1B**), this outbreak was likely related to *C. jejuni* as well. Because stricter public health measures were instituted in Peru, after the first COVID-19 case in March 2020, GBS incidence decreased to less than 0.27/100,000.[6]

We found evidence of recent *C. jejuni* infection in 28/43 patients (65%) of whom 9 were positive for *Campylobacter sp* PCR in stool. Other preceding infections which have previously been associated with GBS, including *Mycoplasma pneumoniae*, DENV, CHIKV and ZIKV, were negative in all tested cases. Recent *C. jejuni* infection was significantly more likely to occur in GBS cases (23/42, 55%) compared with controls (11/41, 27%, OR: 3.3, $p< 0.01$). Of interest, the proportion of controls with a recent *C. jejuni* infection was high (27%), which may be indicative of an ongoing outbreak of *C. jejuni*, although our study was not designed to investigate this. This high percentage may also be in part due to overmatching of cases and controls or a high prevalence of *C. jejuni* in Peru, as has been indicated by previous serosurveillance studies.[30,31] Notably, the vast majority of *C. jejuni* infections, even when bearing ganglioside

mimics in their LOS, manifest as uncomplicated enteritis and are not associated with the development of GBS. Genomic analysis of *C. jejuni* isolates showed that they have closely related LOS regions to one another and to previously described GBS associated *C. jejuni* genomes from China and Africa reported in the past 2 decades, suggesting that these strains were introduced or reemergent infections from an endemic reservoir rather than being new emergent strains.[32–34]

Besides the laboratory evidence, the clinical and electrophysiologic profile is typical for *C. jejuni*–associated GBS as described in previous studies.[9,35,36] The majority of cases had a preceding diarrheal illness, followed by an early-onset, rapidly progressive pure motor axonal GBS. This profile is in contrast to the clinical profile that has been reported in association with ZIKV or COVID-19, where most patients have facial palsy, sensory and motor deficits, and a demyelinating electrophysiologic subtype.[37,38]

However, there was not a uniform relationship between *C. jejuni* serotype and clinical, electrodiagnostic, and antiganglioside profile. This may be due to methodological factors that prevent unambiguous case definition and ascertainment. For example, CSF examination and electrodiagnostic studies are not always sensitive diagnostic tools in GBS, especially when done early in the disease course. This may have resulted in only 29% of patients having an increased protein level in CSF, or inaccurate classification of electrophysiologic studies as axonal or demyelinating.[8,39] The time between onset of systemic and neurologic symptoms (median 3 days, IQR 2–9 days) was also shorter than expected based on previous studies, which may be due to the wide range of the incubation period of *C. jejuni* (1–10 days); patients only reporting symptoms when they become severe; or the presence of a parainfectious rather than postinfectious mechanism, as previously reported in ZIKV-related GBS.[3,40] Another surprising finding was the high percentage (27%) of cases with diarrhea in the group without evidence of a recent *C. jejuni* infection.This may due to the presence of other infections able to trigger GBS that may lead to gastrointestinal symptoms or low sensitivity of the standard serologic testing method for recent *C. jejuni* infection (presence of IgM antibody) in a population.

The main limitation of our study is that we were not able to perform complete laboratory studies in all patients and controls as the study was conducted in the context of an emerging outbreak. We were able to exclude other preceding infections, including arboviruses, in 53% of cases and completed the serologic case-control study in 86% of cases. It is unlikely that different results would have been obtained had all subjects been tested.

In conclusion, we showed that *C. jejuni*, and not ZIKV as was initially thought, was the infectious driver of the 2019 GBS outbreak in Peru, and the clinical, electrophysiologic, and immunologic profile was consistent with *C. jejuni*–related GBS. The *C. jejuni* strains were likely introduced or reemergent infections from an endemic reservoir and not new emergent strains. This finding has global relevance as it indicates that the *C. jejuni* strains linked to GBS circulate widely in different parts of the world. This shows that researchers should remain aware of *C. jejuni* as a trigger for GBS when investigating the association between other infections, including COVID-19, and GBS. Reinforcing public health measures, including setting up campylobacteriosis and GBS surveillance, to rapidly identify new epidemics, pathologic strains, and sources of transmission should be encouraged to prevent future outbreaks.[30,31,46]

SUPPLEMENTARY MATERIAL:

For **Appendix e-1** see: links.lww.com/NXI/A403

For **Supplementary Figure 1** see: links.lww.com/NXI/A404

For **Supplementary Figure 2** see: http://links.lww.com/NXI/A405

For **Supplementary Table 1** see: links.lww.com/NXI/A406

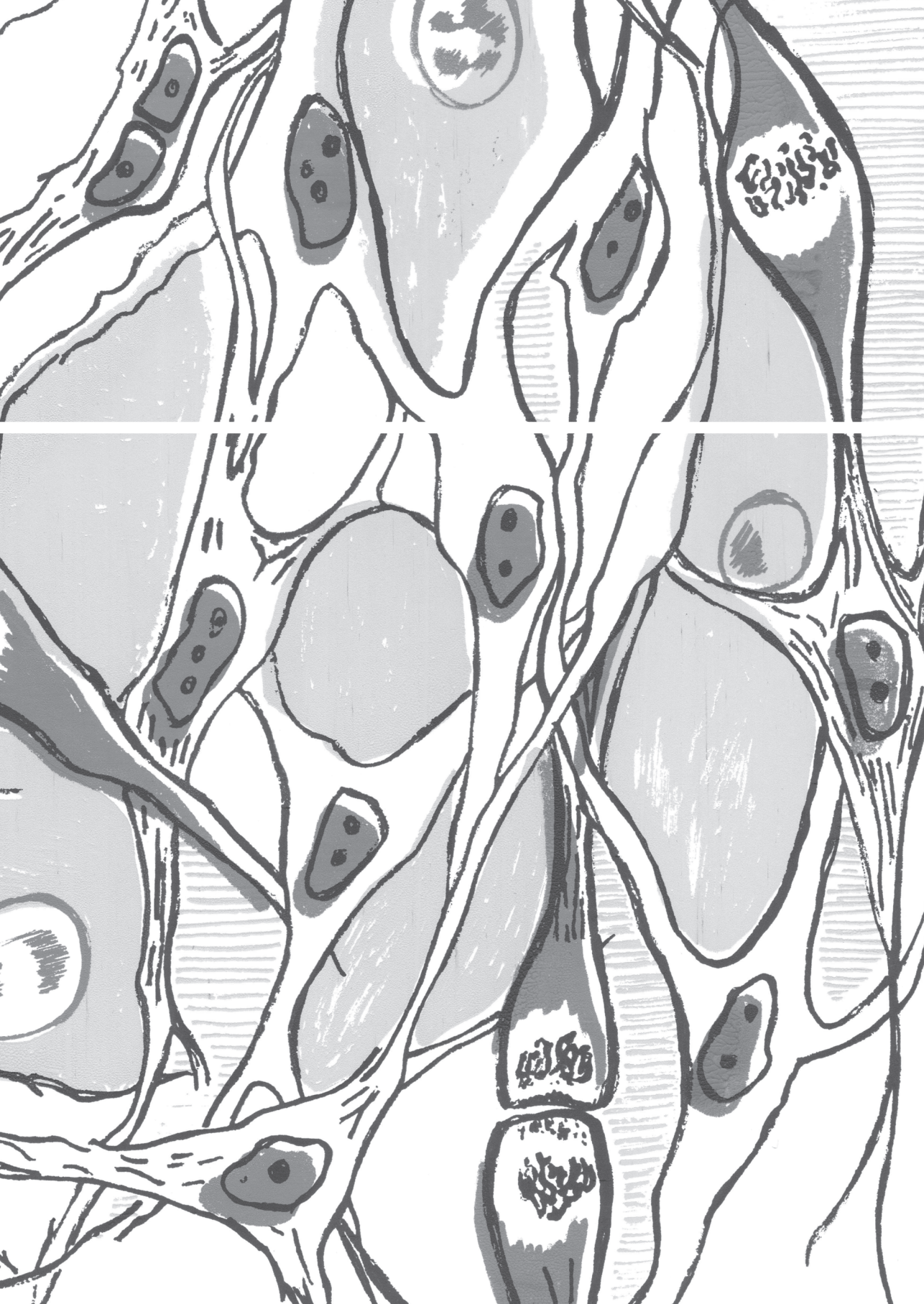
For **Supplementary Table 2** see: links.lww.com/NXI/A407

REFERENCES

1. Willison HJ, Jacobs BC, Van Doorn PA. Guillain-Barré Syndrome.*Lancet* 2016;388:717-727.
2. WHO. Guillain–Barré syndrome. Available at: www.who.int/csr/don/archive/disease/guillain-barre-syndrome/en/. Accessed May 31, 2020.
3. Parra B, Lizarazo J, Jiménez-Arango JA, et al. Guillain-Barré syndrome associated with Zika virus infection in Colombia. *N Engl J Med* 2016;375(16):1513-1523.
4. Styczynski AR, Malta JMAS, Krow-Lucal ER, et al. Increased rates of Guillain-Barré syndrome associated with Zika virus outbreak in the Salvador metropolitan area, Brazil. *PLoS Negl Trop Dis* 2017;11(8):1-13.
5. Gigli GL, Bax F, Marini A, et al. Guillain-Barré syndrome in the COVID-19 era: just an occasional cluster? *J Neurol* 2020;1:3. Letter.
6. Centro Nacional de Epidemiología Prevención y Control de Enfermedades. Situación de Guillain Barre: Perú a la SE 02 - 2020. Available at: www.dge.gob.pe/portal/index.php?option=com_content&view=article&id=654. Accessed May 31, 2020.
7. Centro Nacional de Epidemiología, Prevención y Control de Enfermedades. Reporte de Análisis del comportamiento epidemiologico. Available at: www.dge.gob.pe/salasituacional/sala/index/SALA_VIGILA/141. Accessed June 3, 2020.
8. Sejvar JJ, Kohl KS, Gidudu J, et al. Guillain-Barré syndrome and Fisher syndrome: Case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* 2011;29(3):599-612.
9. Hadden RDM, Cornblath DR, Hughes RAC, et al. Electrophysiological classification of Guillain-Barre syndrome: Clinical associations and outcome. *Ann Neurol* 1998;44(5):780-788.
10. Ho TW, Mishu B, Li CY, et al. Guillain-Barré syndrome in Northern China relationship to Campylobacter jejuni infection and anti-glycolipid antibodies. *Brain* 1995;118(3):597-605.
11. Jacobs BC, van den Berg B, Verboon C, et al. International Guillain-Barré Syndrome Outcome Study: protocol of a prospective observational cohort study on clinical and biological predictors of disease course and outcome in Guillain-Barré syndrome. *J Peripher Nerv Syst* 2017;22(2):68-76.
12. Kleyweg RP, van der Meché FGA, Schmitz PIM. Interobserver Agreement in the Assessment of Muscle Strength Guillain- Barre Syndrome. *Muscle Nerv* 1991;14(November):1103-1109.
13. Van Swieten JC, Koudstaal PJ, Visser MC, Schouten H, Van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke* 1988;19(5):604-607.
14. Hughes RAC, Newsom-Davis JM, Perkin GD, Pierce JM. Controlled Trial of Prednisolone in Acute Polyneuropathy. *Lancet* 1978;312(8093):750-753.
15. Walgaard C, Lingsma HF, Ruts L, Van Doorn PA, Steyerberg EW, Jacobs BC. Early recognition of poor prognosis in Guillain-Barré syndrome. *Neurology* 2011;76(11):968-975.
16. Walgaard C, Lingsma HF, Ruts L, et al. Prediction of respiratory insufficiency in Guillain-Barré syndrome. *Ann Neurol* 2010;67(6):781-787.
17. Sadon N, Delers A, Jarman RG, et al. A new quantitative RT-PCR method for sensitive detection of dengue virus in serum samples. *J Virol Methods* 2008;153(1):1-6.

18. Guion CE, Ochoa TJ, Walker CM, Barletta F, Cleary TG. Detection of diarrheagenic Escherichia coli by use of melting-curve analysis and real-time multiplex PCR. *J Clin Microbiol* 2008;46(5):1752-1757.
19. Klena JD, Parker CT, Knibb K, et al. Differentiation of Campylobacter coli, Campylobacter jejuni, Campylobacter lari, and Campylobacter upsaliensis by a multiplex PCR developed from the nucleotide sequence of the lipid A gene lpxA. *J Clin Microbio*l 2004;42(12):5549-5557.
20. Poly F, Serichantalergs O, Kuroiwa J, et al. Updated campylobacter jejuni capsule PCR multiplex typing system and its application to clinical isolates from south and southeast Asia. *PLoS One* 2015;10(12):1-17.
21. Parkhill J, Wren BW, Mungall K, et al. The genome sequence of the food-borne pathogen Campylobacter jejuni reveals hypervariable sequences. *Nature* 2000;403(6770):665-668.
22. Gundogdu O, Bentley SD, Holden MT, Parkhill J, Dorrell N, Wren BW. Re-annotation and re-analysis of the Campylobacter jejuni NCTC11168 genome sequence. *BMC Genomics* 2007;8:1-8.
23. Hadfield J, Megill C, Bell SM, et al. NextStrain: Real-time tracking of pathogen evolution. *Bioinformatics.* 2018;34(23):4121-4123.
24. Ang CW, Krogfelt K, Herbrink P, et al. Validation of an ELISA for the diagnosis of recent Campylobacter infections in Guillain-Barré and reactive arthritis patients. *Clin Microbiol Infect* 2007;13(9):915-922.
25. Halstead SK, Kalna G, Islam MB, et al. Microarray screening of Guillain-Barré syndrome sera for antibodies to glycolipid complexes. *Neurol Neuroimmunol NeuroInflamm* 2016;3(6):1-9.
26. Gilbert M, Karwaski MF, Bernatchez S, et al. The genetic bases for the variation in the lipo-oligosaccharide of the mucosal pathogen, Campylobacter jejuni. Biosynthesis of sialylated ganglioside mimics in the core oligosaccharide. *J Biol Chem* 2002;277(1):327-337.
27. Godschalk PCR, Heikema AP, Gilbert M, et al. The crucial role of Campylobacter jejuni genes in anti-ganglioside antibody induction in Guillain-Barré syndrome. *J Clin Invest* 2004;114(11):1659-1665.
28. Rinaldi S, Brennan KM, Kalna G, et al. Antibodies to heteromeric glycolipid complexes in guillain-barré syndrome. *PLoS One* 2013;8(12):1-13.
29. Díaz-Soto S, Chavez K, Chaca A, Alanya J, Tirado-Hurtado I. Outbreak of Guillain-Barré syndrome in Peru. *eNeurologicalSci* 2019;14:89-90. Letter.
30. Fernández H. Campylobacter y campylobacteriosis: Una mirada desde América del Sur. *Rev Peru Med Exp Salud Publica.* 2011;28(1):121-148.
31. Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, Man SM. Global epidemiology of campylobacter infection. *Clin Microbiol Rev* 2015;28(3):687-720.
32. Zhang M, Li Q, He L, et al. Association study between an outbreak of Guillain-barre syndrome in jilin, China, and preceding campylobacter jejuni infection. *Foodborne Pathog Dis* 2010;7(8):913-919.
33. Prendergast MM, Lastovica AJ, Moran AP. Lipopolysaccharides from campylobacter jejuni O:41 strains associated with guillain-barre syndrome exhibit mimicry of GM1 ganglioside. *Infect Immun* 1998;66(8):3649-3755.

34. Nachamkin I, Liu J, Li M, et al. Campylobacter jejuni from patients with Guillain-Barré syndrome preferentially expresses a GD1a-like epitope. *Infect Immun* 2002;70(9):5299-5303.
35. Rees JH, Soudain SE, Gregson NA, Hughes R. Campylobacter jejuni infection and Guillain-Barré syndrome. *N Engl J Med* 1995;333:1374–1379.
36. Ye Y, Zhu D, Wang K, et al. Clinical and electrophysiological features of the 2007 Guillain-Barré syndrome epidemic in northeast China. *Muscle Nerve* 2010;42:311–314.
37. Leonhard SE, Bresani-Salvi CC, Lyra Batista JD, et al. Guillain-Barré syndrome related to Zika virus infection: A systematic review and meta-analysis of the clinical and electrophysiological phenotype. *PLoS Negl Trop Dis* 2020;14(4):1-24.
38. Dalakas MC. Guillain-Barré syndrome: the first documented COVID-19-triggered autoimmune neurologic disease: more to come with myositis in the offing. *Neurol Neuroimmunol Neuroinflammation* 2020;7:e781. doi: 10.1212/NXI.0000000000000781.
39. Uncini A, Ippoliti L, Shahrizaila N, Sekiguchi Y, Kuwabara S. Optimizing the electrodiagnostic accuracy in Guillain-Barré syndrome subtypes: criteria sets and sparse linear discriminant analysis. *Clin Neurophysiol* 2017;128:1176–1183.
40. WHO. Campylobacter. Available at: who.int/news-room/fact-sheets/detail/campylobacter. Accessed June 8, 2020.
41. Hadden RDM, Karch H, Hartung HP, et al. Preceding infections, immune factors, and outcome in Guillain-Barré syndrome. *Neurology* 2001;56:758–765.
42. Ogawa G, Kaida KI, Kuwahara M, Kimura F, Kamakura K, Kusunoki S. An antibody to the GM1/GalNAc-GD1a complex correlates with development of pure motor Guillain-Barré syndrome with reversible conduction failure. *J Neuroimmunol* 2013;254(1-2):141-145.
43. Ogawara K, Kuwabara S, Mori M, Hattori T, Koga M, Yuki N. Axonal Guillain-Barré syndrome: relation to anti-ganglioside antibodies and Campylobacter jejuni infection in Japan. *Ann Neurol* 2000;48:624–631
44. Sekiguchi Y, Uncini A, Yuki N, et al. Antiganglioside antibodies are associated with axonal Guillain-Barré syndrome: A Japanese-Italian collaborative study. *J Neurol Neurosurg Psychiatry* 2012;83(1):23-28.
45. Zhang M, Gilbert M, Yuki N, et al. Association of anti-GT1a antibodies with an outbreak of Guillain-Barré syndrome and analysis of ganglioside mimicry in an associated campylobacter jejuni strain. *PLoS One* 2015;10(7):1-13.
46. Leonhard SE, Cornblath DR, Endtz HP, Sejvar JJ, Jacobs BC. Guillain-Barré syndrome in times of pandemics. *J Neurol Neurosurg Psychiatry* (in-press 2020).



Chapter 9

Guillain-Barré syndrome after SARS-CoV-2 infection in an international prospective cohort study

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ABSTRACT

In the wake of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic, an increasing number of patients with neurological disorders including the Guillain-Barré syndrome have been reported following this infection. It remains unclear however if these cases are coincidental or not, as most publications were case-reports or small regional retrospective cohort studies.

The International GBS Outcome Study is an ongoing prospective observational cohort study enrolling patients with Guillain-Barré syndrome within 2 weeks from onset of weakness. Data from patients included in this study, between the 30th of January 2020 and 30th of May 2020, were used to investigate clinical and laboratory signs of a preceding or concurrent SARS-CoV-2 infection and to describe the associated clinical phenotype and disease course. Patients were classified according to the SARS-CoV-2 case definitions of the European Centre for Disease Prevention and Control and laboratory recommendations of the World Health Organization.

Forty-nine patients with Guillain-Barré syndrome were included, of whom eight (16%) had a confirmed and three (6%) a probable SARS-CoV-2 infection. Nine of these 11 patients had no serological evidence of other recent preceding infections associated with Guillain-Barré syndrome, whereas two had serological evidence of a recent *Campylobacter jejuni* infection. Patients with a confirmed or probable SARS-CoV-2 infection frequently had a sensorimotor variant 8/11 (73%) and facial palsy 7/11 (64%). The eight patients who underwent electrophysiological examination all had a demyelinating subtype, which was more prevalent than the other patients included in the same time window (14/30 (47%), P=0.012) as well as historical region and age matched controls included in the International GBS Outcome Study before the pandemic (23/44 (52%), P=0.016). The median time from the onset of infection to neurological symptoms was 16 days (IQR 12-22).

Patients with SARS-CoV-2 infection shared uniform neurological features, similar to those previously described in other post-viral Guillain-Barré syndrome patients. The frequency (22%) of a preceding SARS-CoV-2 infection in our study population was higher than estimates of the contemporaneous background prevalence of SARS-CoV-2, which may be a result of recruitment bias during the pandemic, but could also indicate that Guillain-Barré syndrome may rarely follow a recent SARS-CoV-2 infection. Consistent with previous studies, we found no increase in patient recruitment during the pandemic for our ongoing International GBS Outcome Study compared to previous years, making a strong relationship of Guillain-Barré syndrome

with SARS-CoV-2 unlikely. A case-control study is required to determine if there is a causative link or not.

INTRODUCTION

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic has affected the entire world population, either by direct infection or through its social and economic consequences. The severity and impact of this outbreak prompted the World Health Organization (WHO) to declare SARS-CoV-2 a Public Health Emergency of International Concern on January 30th 2020.[1] Besides the well-known severe respiratory signs, both central and peripheral neurological complications have been reported.[2-4] Potential pathophysiological mechanisms for these complications may be either direct viral invasion, indirect damage as a result of the inflammatory response (para-infectious, post-infectious), or hypercoagulability in case of cardiovascular complications.[5] One of the reported neurological disorders is Guillain-Barré syndrome (GBS), an inflammatory polyradiculoneuropathy characterized by rapidly progressive weakness and sensory signs, usually preceded by an infectious trigger.[6] Several pathogens have previously been associated with GBS and outbreaks of these infections may lead to an increased incidence of GBS as seen during the Zika virus pandemic in 2015-2016.[7-9] The clinical phenotype, electrophysiological subtype and disease course of GBS are heterogeneous and may be influenced by the type of preceding infection as a result of differences in antigenic targets. In some of these clinical variants specific antibody responses against gangliosides could be found. For example, a preceding infection with *Campylobacter jejuni* is associated with antibodies against GM1 and GD1a, and a pure motor axonal variant with a more severe disease course and poor outcome.[10]

Since the beginning of the recent pandemic, over 90 GBS patients with a possible relation to SARS-CoV-2 have been reported.[11-14] However, whether SARS-CoV-2 is another potential infectious trigger or whether the reported cases are coincidental is still unclear. In the current study, we identified GBS cases with a preceding SARS-CoV-2 infection, based on clinical and laboratory features, during the first months of the pandemic within the framework of the International GBS Outcome Study (IGOS), an ongoing prospective observational cohort study which started in 2012.[15] We described in detail the clinical phenotype, electrophysiological subtype, and disease course of these patients.

MATERIALS AND METHODS

Study design and patients

Data from all GBS patients included in IGOS from January 30th until May 30th 2020 were used for this study. IGOS is an international multicenter prospective observational cohort study in which all GBS patients can be included within 2 weeks from the onset of symptoms, independent of the disease severity or clinical variant. Data and biological samples are collected according to a predefined protocol.[15] As the first wave of the SARS-CoV-2 pandemic may have caused delays in hospital referral and study inclusion, we allowed 4 weeks from symptom onset for the inclusion of GBS cases. Information on the acute phase of GBS was collected retrospectively in these patients. Patients needed to fulfill the diagnostic criteria for GBS (National Institute of Neurological Disorders and Stroke) or its clinical variants.[16, 17] Patients with an alternative diagnosis were excluded.

Patient recruitment rates of IGOS from the previous 3 years were compared with the recruitment rate during the first months of the pandemic. Because patient inclusion depends, among other factors, on whether or not study sites are actively recruiting patients, we also looked at inclusion rates in selected countries (China, Italy, Switzerland and The Netherlands) with stable inclusion rates of >10 patients/year in the past years.

Data collection and case definitions

Clinical characteristics

Comprehensive data on demographics, symptoms of preceding infections, comorbidities, clinical presentation of GBS, cerebrospinal fluid (CSF) examination, nerve conduction studies (NCS), treatment, disease progression, and clinical course were collected prospectively at fixed time points.[15] Clinical parameters have been defined in the original IGOS protocol and are described in previous publications.[15, 18] We interpreted data until a maximum follow-up of 13 weeks. Data collected after 13 weeks will be used for future studies. The clinical variant of GBS was identified by the local investigator at week 2 and, if missing, at week 1 or entry. Disease severity was expressed using the GBS disability score (0-6): 0=healthy, 1=minor symptoms but capable of running, 2=able to walk 10 meter without assistance but unable to run, 3=able to walk 10 meter with help, 4=bedridden or chair bound, 5=requiring assisted ventilation for at least part of the day, 6=dead.[19] Severe GBS was defined as a GBS disability score at nadir ≥ 3, similar to previous studies.[20] For patients with week 13 missing who were able to walk independently at week 8 or week 4, this previous visit was used to determine the GBS disability score at week 13. The elec-

trophysiological subtype was determined according to the Hadden classification, by using the raw data of the first NCS.[21] If the raw NCS data were missing, we used the subtype defined by the local investigator.

SARS-CoV-2 suspicion

Additional information regarding the clinical suspicion of SARS-CoV-2 infection was collected with a structured questionnaire, which contained questions on preceding symptoms, laboratory and radiological results, serological evidence of other recent infections, and complications of SARS-CoV-2.

Investigators were asked to test the included patients for SARS-CoV-2 by PCR (on oro/nasopharyngeal, respiratory, or fecal material) and/or serology (IgM and IgG) in the local hospitals. Dutch patients with a probable or confirmed SARS-CoV-2 infection and available material were tested for SARS-CoV-2 serology (Wantai SARS-CoV-2 total Ig and IgM ELISA from Beijing Wantai Biological Pharmacy Enterprise Co., Ltd) at the Erasmus MC University Medical Center.[22]

Patients were classified according to the SARS-CoV-2 case definitions of the European Centre for Disease Prevention and Control (**Box 1**).[23] Patients were classified as 'possible' if they had at least one clinical sign of SARS-CoV-2 infection, 'probable' if they had abnormalities on radiological imaging suspicious for SARS-CoV-2 infection, or if they had both clinical signs and an epidemiological link, and 'confirmed' if there was laboratory confirmation of SARS-CoV-2 infection. Laboratory confirmation was based on the WHO recommendations and defined as a positive PCR for SARS-CoV-2 or positive serology on repeated samples.[24, 25] Radiological findings suspicious for SARS-CoV-2 infection on CT-thorax consisted of bilateral infiltrates, uni- or bilateral ground-glass opacities, multifocal consolidation, or bilateral interstitial abnormalities.[26]

In the main analysis, we focused on the clinical phenotype and subtype of GBS patients with a confirmed/probable SARS-CoV-2 infection and compared these patients with the other patients that were included in the same time window (possible and no suspicion combined). We chose this comparison because the patients in the possible group had non-specific symptoms that are also common in respiratory tract infections caused by other pathogens. We also performed three additional analyses. In the first analysis, we aimed to investigate whether the clinical phenotype and subtype of SARS-CoV-2 confirmed/probable cases was specific for SARS-CoV-2 and compared their neurological features with historical control patients matched for region and age (+/- 15 years) that were included in IGOS before the pandemic (2012-2017). In the other two additional analyses, we compared the clinical GBS

Box 1: Case definitions SARS-CoV-2 infection based on the ECDC criteria and WHO laboratory recommendations

Clinical criteria
Any person with at least one of the following symptoms:
- cough
- fever
- shortness of breath
- sudden onset of anosmia, ageusia or dysgeusia

Diagnostic imaging criteria
Radiological evidence showing lesions compatible with COVID-19a

Laboratory criteria
Detection of SARS-CoV-2 nucleic acid in a clinical specimen OR positive serology on repeated serum samplesb

Epidemiological criteria
At least one of the following two epidemiological links:
- close contact with a confirmed COVID-19 case in the 14 days prior to onset of symptoms
- having been a resident or a staff member, in the 14 days prior to onset of symptoms, in a residential institution for vulnerable people where ongoing COVID-19 transmission has been confirmed

Case classification
Possible case: Any person meeting the clinical criteria
Probable case: Any person meeting the clinical criteria with an epidemiological link OR Any person meeting the diagnostic imaging criteria
Confirmed case: Any person meeting the laboratory criteria

Adapted from: European Centre for Disease Prevention and Control. Case definition for coronavirus disease 2019 (COVID-19), as of 29 May 2020.
[a] 'Radiological evidence compatible with COVID-19' was defined as the presence of bilateral infiltrates, uni- or bilateral ground-glass opacities, multifocal consolidation, or bilateral interstitial abnormalities on CT-thorax.
[b] 'Positive serology on repeated serum samples' was added to the laboratory criteria, as described in the WHO recommendations for laboratory testing.
Abbreviations: ECDC = European Centre for Disease Prevention and Control, SARS-CoV-2 = Severe Acute Respiratory Syndrome Coronavirus 2, WHO = World Health Organization.

phenotype and disease course of the three subgroups of SARS-CoV-2 suspicion separately (confirmed/probable vs. possible vs. no suspicion) and excluded the possible patients (confirmed/probable vs. no suspicion) as some of them may have had a recent SARS-CoV-2 infection. The additional analyses are provided in the **Supplementary Material**. Significant findings and discrepancies between these analyses and the main analysis are described in the main text.

Other preceding infections

SARS-CoV-2 probable and confirmed patients were tested locally for other preceding infections associated with GBS including: *C. jejuni, Mycoplasma pneumoniae*, Epstein-Barr virus (EBV), Cytomegalovirus (CMV) and Hepatitis E virus (HEV), when possible. Test results were defined as positive, negative, or inconclusive based on definitions of the local laboratory. In general, evidence of a recent infection was defined via IgM positivity for *M. pneumoniae* and HEV, IgM or IgA positivity for *C. jejuni*, IgM positivity

with negative IgG or IgG with low avidity for CMV, and VCA IgM positivity with negative EBNA IgG for EBV. See the **Supplementary Material** for a more detailed description of the interpretation of the test results.

Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics 25. Variables were described using medians (interquartile range) and numbers (percentage). To compare variables between subgroups, a Mann-Whitney U test or Kruskal-Wallis test was used for numerical variables and a Chi-square test or Fisher's exact test for categorical variables. A two-sided *P*-value of <0.05 was considered significant. For the comparison of the SARS-CoV-2 confirmed/probable cases with historical control patients included in IGOS before the pandemic, we used a 1:7 ratio and the cases and matched controls were analyzed as two independent groups. In the additional analysis where three subgroups were compared, a Bonferroni correction was used to correct for multiple testing. Therefore, we divided the significance level of 0.05 by the number of possible tests (3 groups = 3 pairwise comparisons), so *P*-values <0.017 were considered to be significant for this analysis.

Ethical approval

IGOS was approved by the institutional review boards of the Erasmus MC University Medical Center (MEC-2011-477) and all participating international local site institutes. Written informed consent was obtained from each patient.

RESULTS

Patient inclusion and classification

Fifty-two GBS patients were enrolled in IGOS from January 30th - May 30th 2020 (**Figure 1**). Three patients were excluded from analysis because of an alternative diagnosis: one patient had myelitis (and a PCR confirmed SARS-CoV-2 infection), one polyradiculopathy due to a B-cell lymphoma, and one thiamine deficiency.

The 49 remaining patients were included in China (*n*=6), Denmark (*n*=1), France (*n*=3), Greece (*n*=1), Italy (*n*=7), Japan (*n*=2), The Netherlands (*n*=12), Spain (*n*=2), Switzerland (*n*=14), and United Kingdom (*n*=1). We did not see an increase in the inclusion rate of IGOS during the pandemic compared to the previous 3 years (**Figure 2**). When focusing on selected regions (e.g. Switzerland, The Netherlands, China) with more stable inclusion rates, only Switzerland had an increase in patient inclusion in April 2020 (six patients vs. an average of one to three inclusions per month in the year before the pandemic).

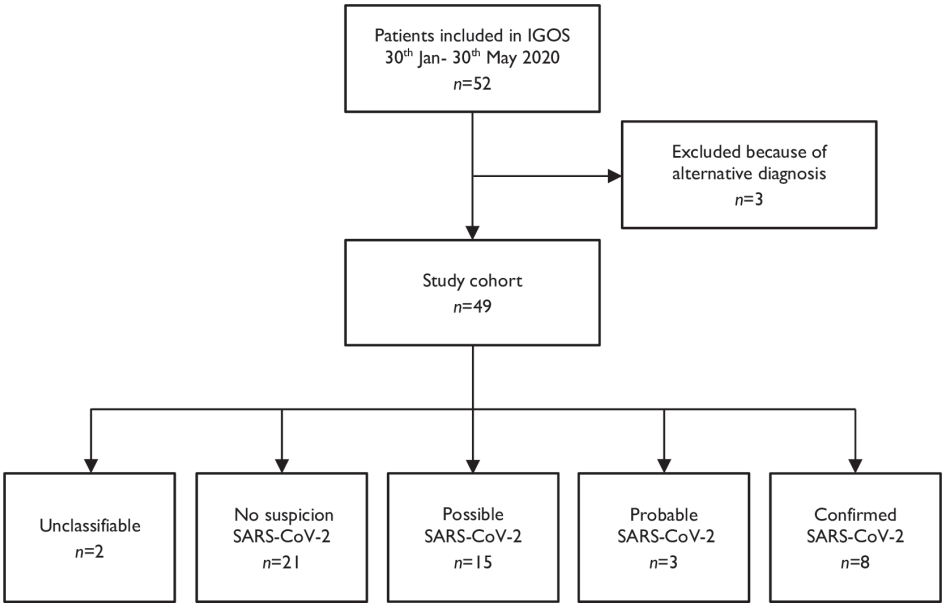

Figure 1: Patient inclusion and SARS-CoV-2 case classification

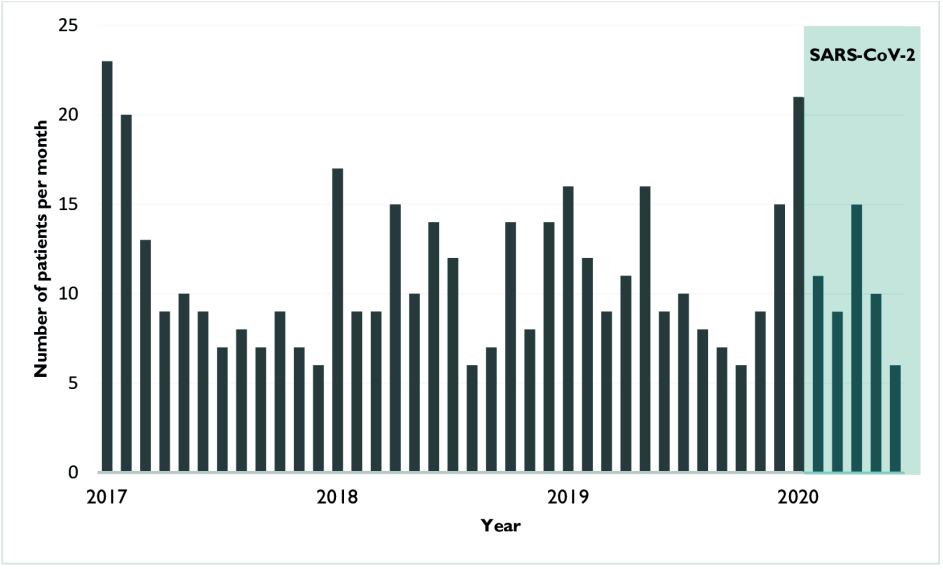

Figure 2: Patient inclusion in IGOS between 2017 and 2020
Patient inclusion within IGOS per month from 2017 until 2020. Fluctuations in inclusion rate can be explained by countries that started or stopped recruiting patients.

Forty-eight questionnaires assessing clinical suspicion for SARS-CoV-2 infection were completed (response rate of 98%). Based on the ECDC case definitions, two

patients (4%) were unclassifiable; 21 (43%) did not have suspicion for SARS-CoV-2; 15 (31%) were classified as possible; three (6%) as probable; and eight (16%) as confirmed cases.

In the next paragraphs, we focus on the clinical features and disease course of GBS patients with a probable or confirmed SARS-CoV-2 infection (*n*=11) and compare them to the other GBS cases included in the same time period with a possible SARS-CoV-2 infection or without SARS-CoV-2 suspicion (*n*=36).

Clinical GBS phenotype in relation to SARS-CoV-2 suspicion

Table 1 summarizes the clinical characteristics of the total cohort and compares the patients with a confirmed/probable SARS-CoV-2 infection to those without. Two patients had an unclassifiable SARS-CoV-2 status and were therefore excluded from this comparison. A more detailed overview of the clinical features of the confirmed and probable SARS-CoV-2 infected patients is shown in **Table 2**.

The median age of the total cohort was 56 years (IQR 37-67). The patients with a confirmed/probable SARS-CoV-2 infection patients were significantly older than the remaining patients (63 years (IQR 60-67) vs. 53 years (IQR 32-66), *P*=0.035). The median time from onset of weakness until study entry was 5 days (IQR 3-10). Three patients entered the study between 2 and 4 weeks after onset of neurological symptoms, due to a delay in hospital admission or a delay in informed consent due to the pandemic. Preceding respiratory symptoms and fever occurred more frequently in patients with a confirmed/probable SARS-CoV-2, which was expected as the classification according to the ECDC case definitions is partly based on such symptoms.

The majority of SARS-CoV-2 confirmed/probable patients had a sensorimotor GBS variant (8/11, 73%), although Miller Fisher GBS overlap syndrome (2/11, 18%) and an ataxic variant (1/11, 9%) were also reported. All patients with a confirmed/probable SARS-CoV-2 infection had a severe form of GBS (GBS disability score at nadir ≥ 3). Common early neurological features were: facial weakness in 7/11 (64%), sensory deficits in 9/11 (82%), and autonomic dysfunction in 7/11 (64%), although not significantly different compared to the other patients.

Electrophysiological examination was performed in 39/49 (80%) patients, with raw data available in 37 (including all patients with a confirmed/probable SARS-CoV-2 infection). The data for these 37 patients were independently assessed and classified according to the Hadden classification. For the other two patients, the classification of the local investigator was used. All confirmed and probable SARS-CoV-2 patients

who underwent nerve conduction study (NCS) had a demyelinating subtype, which was more frequent than in the other GBS patients (8/8 (100%) vs. 14/30 (47%), *P*=0.012). After excluding the patients from Asia (*n*=8), this association was still statistically significant (*P*=0.047). Both SARS-CoV-2 confirmed/probable cases and the other patients underwent extensive electrophysiological examination as approximately four motor and three sensory nerves were examined in both groups.

In 42/49 (86%) patients, a spinal tap was performed with a median leukocyte count of 2 cells/µL (IQR 1-3) and protein level of 1.01 g/L (IQR 0.49-1.55). CSF examination was performed in 9/11 (82%) confirmed/probable SARS-CoV-2 patients of whom only one had an increased (>5 cell/µL) leukocyte count of 20 cells/µL. This patient was negative for SARS-CoV-2 PCR in CSF and other diagnoses (e.g. myelitis, infectious causes) were excluded after extensive investigation. The demyelinating features on NCS further confirmed the diagnosis of GBS in this patient. All SARS-CoV-2 confirmed/probable patients received immunomodulatory treatment: 10/11 (91%) intravenous immunoglobulins and 1/11 (9%) plasma exchange. None of them received additional treatment with steroids. Treatment did not differ between subgroups. Antiganglioside antibodies were tested in two SARS-CoV-2 infected patients and were negative in both.

Based on the Brighton Collaboration criteria, 6/11 SARS-CoV-2 confirmed/probable patients had a Level 1 certainty of GBS, 2/11 a Level 2, 2/11 a Level 3 (no CSF and NCS performed due to SARS-CoV-2 restrictions), and 1/11 a Level 4 (ataxic variant). Notably, one patient (Level 1) also had cervical myelitis with two short-segment foci on spinal cord MRI, one lateral and one central, which did not fully explain all of the observed neurological features such as facial palsy. The CSF of this patient showed a normal leukocyte count (3 cells/µL) and increased protein level (1.11 g/L) with a negative SARS-CoV-2 PCR in CSF. Electrophysiological examination in this patient showed a sensorimotor demyelinating polyneuropathy, which further supported the diagnosis of GBS.

Features of SARS-CoV-2 infection

All 11 GBS patients with a confirmed and probable SARS-CoV-2 developed neurological symptoms between March 22nd and April 24th. These patients were included in Italy (*n*=2), The Netherlands (*n*=4), Spain (*n*=1), Switzerland (*n*=3), and the United Kingdom (*n*=1) (**Table 2**). Three of them have been described in previously published case-reports and another two were included and analyzed in a retrospective cohort study.[13, 27, 28] Common preceding infectious symptoms were fever 10/11 (91%), cough 7/11 (64%), and dyspnea 5/11 (45%). Other reported symptoms were diarrhea 3/11

Table 1: GBS patient characteristics in relation to SARS-CoV-2 infection

		SARS-CoV-2 confirmed/ probable[a]		
	Total (*n*=49)	**Yes (*n*=11)**	**No (*n*=36)**	***P*-value**
Demographics				
Median age (IQR)	56 (37-67)	63 (60-67)	53 (32-66)	0.035*
Males/females (ratio)	31/18 (1.7)	7/4 (1.8)	23/13 (1.8)	0.99
Preceding symptoms (%)				
Fever	22/48 (46)	10 (91)	12 (33)	0.001*
Respiratory[b]	14/47 (30)	9 (82)	5 (14)	<0.001*
Gastro-intestinal	14 (29)	3 (27)	11 (31)	0.84
None	17 (35) *n*=48	1 (9)	16 (44)	0.039*
Days before onset GBS (IQR)	13 (6-22) *n*=31	16 (12-22) *n*=10	12 (5-23) *n*=20	0.40
Clinical GBS variant (%)				
Sensorimotor	35/47 (75)	8 (73)	27/34 (79)	0.69
Pure motor	3/47 (6)	0 (0)	3/34 (9)	0.57
MFS	2/47 (4)	0 (0)	1/34 (3)	-
MFS-GBS overlap syndrome	3/47 (6)	2 (18)	1/34 (3)	0.14
Ataxic	4/47 (9)	1 (9)	2/34 (6)	0.71
Neurological deficits at entry[c]				
Cranial nerve involvement (%)	16/47 (34)	5 (46)	10/34 (29)	0.46
Oculomotor	6 (13)	1 (9)	4 (12)	0.81
Facial	12 (26)	4 (36)	8 (24)	0.45
Bulbar	10 (21)	3 (27)	6 (17)	0.67
Median MRC sum score (IQR)	52 (41-60) *n*=45	51 (22-54)	51 (41-59) *n*=32	0.58
Tetraparesis (%)	30 (67)	8 (73)	22 (69)	0.73
Paraparesis (%)	3 (7)	0 (0)	3 (9)	0.57
Sensory deficits (%)	32/45 (71)	9 (82)	21/32 (66)	0.46
Pain (%)	22/48 (46)	3 (27)	18/35 (51)	0.16
Ataxia(%)	15/37 (41)	3/9 (33)	11/27 (41)	0.69
Autonomic dysfunction[d] (%)	11/47 (23)	4 (36)	7/34 (21)	0.42
Days onset GBS–entry (IQR)	5 (3-10) *n*=48	9 (3-11)	5 (2-9) *n*=35	0.25
Clinical severity of GBS				
Lowest MRC sum score (IQR)	47 (33-56) *n*=46	44 (2-52) *n*=11	46 (34-55) *n*=33	0.39
Highest GBS disability score (%)				
0-2	8/47 (17)	0 (0)	8/34 (24)	0.17
3-4	30/47 (64)	7 (64)	21/34 (62)	0.91
5	9/47 (19)	4 (36)	5/34 (15)	0.19
Cerebrospinal fluid				
Leukocyte count (IQR)	2 (1-3) *n*=42	1 (1-3) *n*=9	2 (1-4) *n*=31	0.50
Protein level (g/L) (IQR)	1.01 (0.49-1.55)	1.50 (0.85-1.87)	0.80 (0.45-1.51)	0.16

		SARS-CoV-2 confirmed/ probable[a]		
	Total (*n*=49)	Yes (*n*=11)	No (*n*=36)	*P*-value
Elevated : >0.45g/L (%)	32 (76)	8 (89)	23 (74)	0.65
Days onset GBS–spinal tap (IQR)	4 (2-8)	4 (2-9)	4 (2-9)	0.69
Electrophysiological subtype (%)				
Demyelinating	23/39 (59)	8/8 (100)	14/30 (47)	0.012*
Axonal	3/39 (8)	0 (0)	3/30 (10)	0.59
Equivocal	13/39 (33)	0 (0)	13/30 (43)	0.034*
Treatment (%)				
Intravenous immunoglobulins	39/47 (83)	10/11 (91)	28/34 (82)	0.66
Plasma exchange	3/47 (6)	1/11 (9)	2/34 (6)	0.71
Corticosteroids[e]	2/47 (4)	0 (0)	2/34 (6)	-
None	5/47 (11)	0 (0)	4/34 (12)	0.56

*Significant values (*P*<0.05)
Results were given as median (25th-75th percentile) or as counts (percentage).
[a] Classification by the ECDC case definitions. Unclassifiable patients (*n*=2) are not shown.
[b] Respiratory symptoms included cough and or dyspnea.
[c] Parameters that could not be examined, were coded as missing values.
[d] Autonomic dysfunction included disturbances in blood pressure and cardiac, gastro-intestinal or bladder dysfunction.
[e] Additional to intravenous immunoglobulins.
Abbreviations: IQR=interquartile range; MFS=Miller Fisher syndrome; MRC sum score=sum of Medical Research Council scores for muscle groups for shoulder abduction, elbow flexion, wrist extension, hip flexion, knee extension and ankle dorsiflexion of both limbs

(27%), fatigue 3/11 (27%), anosmia 3/11 (27%) and ageusia 2/11 (18%). The median time between preceding infectious symptoms and GBS symptoms was 16 days (IQR 12-22), which did not significantly differ when compared with the patients without a probable or confirmed SARS-CoV-2 infection (**Table 1**).

PCR SARS-CoV-2 testing was performed in 26/49 (53%) patients with a median time of 14 (IQR 5-28) days after onset of infectious symptoms. One patient tested positive for SARS-CoV-2 with PCR during rehabilitation 2 months after onset of GBS symptoms, and was therefore not included in the SARS-CoV-2 confirmed/probable group. Of the eight patients with a confirmed SARS-CoV-2 infection, seven had a positive PCR on an oro/nasopharyngeal swab during the acute phase of GBS or in the 3 weeks before, and in one a recent infection was confirmed by paired serology. All three patients with a probable SARS-CoV-2 infection had suspicious findings on CT-thorax in the setting of a negative PCR: in one serology was not performed, one had positive IgM and positive total Ig, and one had a positive IgG, but these were not confirmed in a paired test. SARS-CoV-2 PCR in CSF was performed in 4/8 patients who had a positive oro/nasopharyngeal swab for SARS-CoV-2 and was negative in all four. A CT-thorax

was performed in 8/11 (73%) of the confirmed/probable cases, seven of whom had abnormalities suspicious for SARS-CoV-2 infection. Four had a bilateral interstitial pneumonia and three bilateral or unilateral ground glass opacities.

At hospital admission, 8/11 (73%) confirmed/probable SARS-CoV-2 patients had increased inflammatory markers (C-reactive protein and/or erythrocyte sedimentation rate). Other frequent laboratory abnormalities were increased liver enzymes (aspartate aminotransferase and/or alanine transaminase) 9/10 (90%), lymphocytopenia 4/10 (40%), increased lactate dehydrogenase 4/8 (50%), and increased ferritin and creatinine kinase in 2/3 (67%). Pneumonia was the most common complication of SARS-CoV-2 infection and was present in 8/11 (73%) patients.

Additionally, one patient suffered from acute respiratory distress syndrome and sepsis, one from pulmonary embolism and myocardial infarction, one from sepsis and atrial fibrillation, and one had a cervical myelitis.

Two SARS-CoV-2 confirmed/probable patients had serological evidence of a recent *C. jejuni* infection. One patient had a positive PCR for CMV on respiratory material with a negative IgM antibody response, indicating a reactivation of CMV.

Clinical course and short-term outcome of GBS patients with SARS-CoV-2 infection

Twenty-eight (57%) patients had a follow-up duration of 8 weeks or longer. Three (7%) patients died (**Table 3**): two from SARS-CoV-2 pneumonia or related complications (pulmonary embolism), and one SARS-CoV-2 negative patient died from a *Pseudomonas aeruginosa* pneumonia. Of the SARS-CoV-2 confirmed/probable patients, 6/11 (55%) needed to be admitted to the intensive care unit (ICU), and 4/11 (36%) required mechanical ventilation. All patients admitted to the ICU had CT thorax abnormalities and complications related to SARS-CoV-2 infection (pneumonia, acute respiratory distress syndrome, sepsis, pulmonary embolism), and five had a severe tetraparesis, of whom four with cranial nerve involvement. Three of the six patients admitted to the ICU also underwent NCS and had a demyelinating GBS subtype. GBS disability score and Medical Research Council (MRC) sum score at week 4 and week 13 did not differ between subgroups.

Additional analyses of the SARS-CoV-2 cases

In the previous analysis, we compared the clinical GBS phenotype of the patients with a confirmed/probable SARS-CoV-2 infection to the patients with no/possible SARS-CoV-2 suspicion that were included in the same time window. Additional

Table 2: Characteristics GBS patients with a confirmed or probable SARS-CoV-2 infection

	1	2	3	4	5	6	7	8	9	10	11
Date onset GBS	March 22nd	March 29th	March 26th	April 8th	April 8th	April 7th	April 8th	April 9th	April 24th	April 17th	April 18th
Country	IT	IT	CH	CH	CH	NL	NL	NL	NL	UK	ES
Age (years)	80	78	52	63	61	67	50	63	60	64	66
Sex	Male	Male	Female	Female	Female	Male	Male	Male	Female	Male	Male
Preceding symptoms											
Fever	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Respiratory[a]	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Gastro-intestinal	No	No	No	No	Yes	No	No	Yes	No	Yes	No
Days before onset GBS	4	24	13	13	21	Undefined	28	12	18	12	21
Neurological deficits[b]											
CNI	IX,X	No	No	No	VII,IX,X	III,VII	VII	VII	VII	III,VII,IX,X	VII
Lowest MRC sum[c]	2	60	22	60	49	0	48	0	44	40	52
Sensory deficits	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Ataxia	No	Yes	No	Yes	No	No	Yes	No	No	Yes	No
Auto. dysfunction	No	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No
Clinical GBS variant	Senso-rimotor	Ataxic	Senso-rimotor	Senso-rimotor	Senso-rimotor	Senso-rimotor	MFS-GBS	Senso-rimotor	Senso-rimotor	MFS-GBS	Senso-rimotor
Clinical severity of GBS											
Highest GBS-DS	6	3	5	4	4	6	4	5	4	4	3
ICU admission	Yes	No	Yes	No	Yes	Yes	No	Yes	No	Yes	No
Needed MV	Yes	No	Yes	No	No	Yes	No	Yes	No	No	No

	1	2	3	4	5	6	7	8	9	10	11
Cerebrospinal fluid											
Leukocytes (cells/μL)	1	20	3	2	1	N.t.	1	N.t.	3	0	1
Protein level (g/L)	1,75	1.06	5,91	0,39	1,5	N.t.	0,64	N.t.	1,11	1,8	1,93
PCR SARS-CoV-2	N.t.	Neg.	Neg.	N.t.	N.t.	N.t.	Neg.	N.t.	Neg.	N.t.	N.t.
NCS subtype	AIDP	AIDP	AIDP	AIDP	AIDP	N.t.	AIDP	N.t.	AIDP	N.t.	AIDP
Brighton classification	Level 1	Level 4	Level 1	Level 2	Level 1	Level 3	Level 1	Level 3	Level 1	Level 2	Level 1
SARS-CoV-2 suspicion	Probable	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed	Probable	Confirmed	Probable	Confirmed
PCR (times tested)	Neg. (4x)	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Neg. (5x)	Pos.	Neg. (2x)	Neg. (2x)
Serology[d]	N.t.	N.t.	IgM+ IgG+	IgM+ IgG-	N.t.	IgM+ Ig+	IgM+ Ig+	IgM+ Ig+	IgM- Ig+	IgG+	IgM+IgG+
CT-thorax suspected	Yes	Yes	Yes	N.t.	Yes	Yes	N.t.	Yes	N.t.	Yes	No
Complications	Pneumo, ARDS, sepsis	Pneumo.	Pneumo.	Pneumo.	Pneumo.	Pneumo, PE, cardial	None	Pneumo, sepsis, cardial	Cervical myelitis	Pneumo.	None
Other infections											
Positive	-	CJE	CJE	-	-	-	-	-	-	-	-
Negative[e]	CJE/MP/ EBV/CMV/ HEV	MP/EBV/ CMV/HEV	MP/EBV/ CMV/HEV	CJE/MP/ EBV/CMV/ HEV	CJE/EBV/ CMV	CJE/MP/ EBV/CMV/ HEV	CJE/MP/ EBV/CMV/ HEV	CJE/MP/ EBV/CMV/ HEV	CJE/MP/ EBV/CMV/ HEV	CJE/MP/ EBV/CMV/ HEV	CJE, EBV, CMV[d]

[a] Respiratory symptoms included cough and or dyspnea. [b] Neurological deficits during acute phase GBS (from study entry till 4 weeks). [c] Lowest MRC sum score observed during study period, which is not necessarily equal to the MRC sum score at disease nadir. [d] Patient 11 was tested repeatedly and therefore classified as a confirmed case: first sample IgG positive, IgM not tested; second sample IgM positive, IgG not tested. Patient 8 was tested only once for SARS-CoV-2 serology, and therefore not classified as a confirmed case. [e] Patient 5,6 and 8 were tested on serum samples obtained after treatment with intravenous immunoglobulins, because no pre-treatment material was available. Abbreviations: Auto. dysfunction=autonomic dysfunction; AIDP=acute inflammatory demyelinating polyradiculoneuropathy; CH=Switzerland; CJE=Campylobacter jejuni; CMV=Cytomegalovirus; CNI=cranial nerve involvement; EBV=Epstein-Barr virus; ES=Spain; GBS-DS=GBS disability score; HEV=Hepatitis E virus; ICU=intensive care unit; IT=Italy; MRC sum=sum of Medical Research Council scores for muscle groups for shoulder abduction, elbow flexion, wrist extension, hip flexion, knee extension and ankle dorsiflexion of both limbs; MFS=Miller Fisher syndrome; MFS-GBS=MFS-GBS overlap, MP=Mycoplasma pneumoniae; MV=mechanical ventilation; NCS=nerve conduction study; Neg=negative; NL=Netherlands; N.t.=not tested; PE=pulmonary embolism; Pneumo=pneumonia; Pos=positive; UK=United Kingdom.

analyses on the GBS phenotype and disease course of the SARS-CoV-2 confirmed/probable cases are provided in the **Supplementary Material**. Compared to historical control patients included in IGOS before the pandemic (between 2012 and 2017), matched for region and age (**Supplementary Table 1**), SARS-CoV-2 confirmed/probable patients had significantly more often a demyelinating NCS subtype (8/8 (100%) vs. 23/44 (52%), *P*=0.016) and a higher CSF protein level (1.50 g/L (IQR 0.85-1.87) vs. 0.65 g/L (0.4 – 1.11), *P*=0.014). The timing of the lumbar puncture after onset of weakness was similar with a median of 4 days (IQR 2-9) vs. 5 days (IQR 2-7) (*P*=0.47). Other clinical features, including sex, clinical GBS variant, neurological deficits at study entry, time between onset GBS and study entry, MRC sum score and GBS disability score at nadir, and CSF leukocyte count, did not significantly differ between the SARS-CoV-2 cases and historical controls.

Patients with a confirmed/probable SARS-CoV-2 infection had significantly more often a demyelinating NCS subtype compared to the other subgroups of SARS-CoV-2 suspicion, also after excluding the possible patients (**Supplementary Table 2**). No other significant differences between subgroups were found (**Supplementary Table 2 and 3**).

DISCUSSION

In this international prospective cohort study, 22% of the GBS patients included during the first 4 months of the pandemic had a preceding SARS-CoV-2 infection. Eight (16%) had a confirmed and three (6%) a probable infection. These patients were all ≥ 50 years of age and had a demyelinating electrophysiological subtype. The most common GBS variant in this group was the sensorimotor (73%), and patients frequently had facial palsy (64%). All GBS patients with a SARS-CoV-2 infection had a severe form of GBS as none of them could walk independently at nadir (GBS disability score ≥ 3). We cannot determine whether their recovery was worse compared to the other GBS patients as patient numbers were small and their disease course may have also been affected by the severity and complications of SARS-CoV-2 infection.

Similar phenotypic features were found in previously published SARS-CoV-2 related cases, in which a sensorimotor and demyelinating GBS was predominant, although some variants, such as MFS and axonal subtypes, have been reported as well.[11, 29-31] Our study cohort also contained two confirmed/probable SARS-CoV-2 infected patients with MFS-GBS overlap syndrome, but no patients with a pure motor variant or an axonal electrophysiological subtype. A sensorimotor demyelinating GBS with

facial palsy has also been described in relation to other viral triggers of GBS, including CMV, Zika virus, HEV and varicella zoster virus.[7, 32-34] This is therefore the expected clinical and electrophysiological phenotype in virus-related GBS, although its presence in the vast majority of SARS-CoV-2 infected GBS patients does not provide evidence of a causal effect. Should SARS-CoV-2 indeed be able to trigger GBS, our data are consistent with a post-infectious disease mechanism rather than direct viral invasion, as the time between onset of SARS-CoV-2 symptoms and GBS ranged from 2-3.5 weeks, none of the tested patients were positive for SARS-CoV-2 PCR in the CSF, and all but one patient had a normal leukocyte count in the CSF. Our findings are consistent with the other published SARS-CoV-2-related GBS case-reports, from which in only the first published case the possibility of direct viral invasion was hypothesized.[12]

When comparing the GBS features of SARS-CoV-2 confirmed/probable patients with the other patients that were included in our study during the same time window we found no significant differences, except for a higher age and a higher frequency of a demyelinating subtype in the SARS-CoV-2 confirmed/probable cases. The latter might be partially explained by the fact that the Asian patients in our cohort were all in group with no/possible SARS-CoV-2 suspicion and all GBS patients with confirmed/probable SARS-CoV-2 infection came from Europe, where the sensorimotor (demyelinating) variant is generally the most common subtype.[18] However, this finding remained significant after excluding the patients from Asia and also cannot be explained by the timing of NCS during disease course or extensiveness of NCS, which were equal in both groups. This suggests that a demyelinating NCS might be a specific feature for GBS following a SARS-CoV-2 infection, although this is no proof for an association, and is supported by the fact that also compared to historical matched control patients included in IGOS before the pandemic, a demyelinating subtype was more frequent in the GBS patients with a confirmed/probable infection. On the other hand, an equivocal subtype, which signifies a group in which the distinction between demyelinating and axonal cannot be accurately made, was more common in the patients without a confirmed/probable SARS-CoV-2 infection, and could therefore have caused a lower proportion of demyelinating variant in this group.

Other preceding infections associated with GBS were tested in all SARS-CoV-2 probable/confirmed cases and were absent in 9/11 (82%). Two SARS-CoV-2 confirmed patients had serological evidence of a recent *C. jejuni* infection. In addition, one patient had a positive CMV PCR in respiratory material with a negative IgM antibody response, which is a common finding in patients with respiratory illness

and is considered to be a sign of re-activation rather than a primary infection.[35] The *C. jejuni* infection could have played a role in the induction of GBS, in which concurrent infection with SARS-CoV-2 may or may not have been contributory. Alternatively, it may have been a coincident infection not related to the induction of GBS, or a false-positive result due to polyclonal B-cell bystander activation during the cytokine storm induced by SARS-CoV-2. Furthermore, previous studies reported that approximately one-third of the GBS patients have no known infectious trigger, either symptomatic or serological.[6] Either way, these findings show the importance of testing for other infections that are known to trigger GBS when trying to establish the relation between emerging infectious diseases and GBS. Previous SARS-CoV-2 related GBS case reports often did not perform such serological testing.[12, 30, 31]

In accordance with the Brighton Collaboration criteria, 8/11 GBS patients with a confirmed/probable SARS-CoV-2 infection reached a Level 1 or 2 diagnostic certainty.[17] We considered the presence of an ICU-related (critical illness) polyneuropathy unlikely because of 5/6 patients admitted to the ICU had cranial nerve involvement. Interestingly, one patient with a confirmed SARS-CoV-2 infection had both GBS with facial weakness, limb weakness and a demyelinating NCS and a myelitis with sensory level and urinary/defecation disturbances and a cervical myelopathy. The combination of GBS and myelitis has previously been described in relation to Zika virus.[36] Another patient with myelitis after SARS-CoV-2 infection was initially included in this study, but ultimately excluded because the diagnosis of GBS was subsequently refuted. Other cases of myelitis following SARS-CoV-2 infection have also been published in the past year.[37] This underlines the importance of being aware of myelitis as potential mimic of GBS.

All GBS patients with a confirmed or probable SARS-CoV-2 infection in our cohort were included in Europe over a period of 1 month, late March to late April, which matches the peak of the first wave of the pandemic in Europe. Whether the prevalence of SARS-CoV-2 infection (22%) in our cohort can be solely explained by a large SARS-CoV-2 infection rate in the community, or whether this indicates that SARS-CoV-2 increases the risk of GBS cannot be established in this study. Accurate estimations of the local community infection rates of SARS-CoV-2 infection during these 4 months of the pandemic are lacking, because asymptomatic patients were likely to be missed and because the prevalence varied greatly within short time periods depending on country, city and even neighborhood. Seroprevalence studies in Europe (Spain >60,000 patients and Switzerland >2,500 patients) showed infection rates in the general population of 5-10%, of whom one-third were asymptomatic.[38, 39] This percentage is probably an underestimation of the actual community prevalence, be-

cause 10% of patients with a positive PCR in this study did not (yet) have detectable antibodies.[38] Sensitivity and specificity of antibody testing is strongly dependent on the type and timing of the test.[22, 40] On the other hand, the prevalence of SARS-CoV-2 infection in our cohort could also have been underestimated, because not all patients have been systematically tested by PCR or serology and samples were often collected days to weeks after the start of the infectious symptoms, which could have led to false-negative results. However, the nasopharyngeal swabs of 'negatively tested patients' were not collected at later times than samples of 'positively tested patients'.

Previous studies reported that the incidence of GBS can increase due to vaccine programs or infectious disease epidemics. In 1976, a sevenfold increase of GBS cases was noticed in the USA during the national H1N1 swine flu vaccination program.[41] This association resulted in a more active monitoring of the occurrence of GBS during vaccine safety studies, such as during the 2009 H1N1 flu vaccination program, and the publication of diagnostic criteria for GBS for vaccine safety studies by the Brighton Collaboration in 2011.[17] So far, other studies on the relation between influenza vaccines and GBS have either shown no association or an increase in risk of only one GBS case per 1 million vaccinees.[42] Another example is the Zika virus pandemic of 2015-2016, when two GBS cases per 10,000 Zika virus cases were reported, with an estimated serological community prevalence of Zika virus of 49%, which led to a 1-6 times increase in incidence of GBS.[43, 44] Based on the previously mentioned serological estimated population prevalence of SARS-CoV-2 infection we would also have expected an increase in the incidence of GBS during the first months of the pandemic. In the IGOS study, we did not see such an increase in the total inclusion rate (**Figure 2**). However, we cannot accurately estimate the global GBS incidence based on these data because IGOS is not designed as a surveillance study. The sample size of our study represents only 0.001% of the expected global GBS cases during the time period of 4 months (~30.000 cases). The inclusion rate has fluctuated over the past years, as it is dependent on many factors, including the number of centers actively recruiting patients over time. During the SARS-CoV-2 pandemic, inclusion rate could have been decreased due to SARS-CoV-2 restrictions and general public health measures, or biased due to scientific interest in a possible association between GBS and SARS-CoV-2 infection (referral bias). The latter could have led to an overestimation of the SARS-CoV-2 prevalence in our cohort. When focusing on the patient recruitment in selected regions a small increase in average patient inclusion per month was seen in April 2020 in Switzerland, but not seen in three other regions (Netherlands, China and Italy).

Several other recently published studies have investigated the relationship between SARS-CoV-2 and GBS, reaching varying conclusions. A retrospective multicenter study in two SARS-CoV-2 hotspot regions in Italy found an increase in GBS incidence in March and April 2020 (30 patients) compared to those same months in 2019 (17 patients).[13] From these data they concluded that the incidence rate of GBS is 47.9 cases per 100.000 SARS-CoV-2 infections. However, this is likely to be an overestimation due to underestimation of the total number of SARS-CoV-2 infections.[13] A retrospective case-control study among patients in Spanish emergency departments also found that during March and April 2020 patients with SARS-CoV-2 infection were 6 times more likely to develop GBS compared to patients without a SARS-CoV-2 infection.[45] However, in this study the total number of GBS cases was in fact lower than in the same period in the preceding year.[45] Although these findings suggest a possible relationship, they cannot in themselves establish causation. Both studies had small patient numbers, took place in a short period of time, and have numerous potential confounders. An epidemiological study based on the National Immunoglobulin Database in the UK found a reduction in the incidence of GBS during the SARS-CoV-2 pandemic.[46] And although this could be explained in part by a reduced exposure to other bacterial or viral pathogens due to the SARS-CoV-2 restrictions, namely social distancing, there was no correlation between the regional incidences of GBS and SARS-CoV-2. In the same study, a subset of 47 patients with GBS was described, representing less than 25% of the total 219 GBS cases logged with the National Immunoglobulin Database over the same period, of which 25/47 (53%) patients had a confirmed/probable SARS-CoV-2 infection. No phenotypic differences were found when compared to the remaining 22 non-SARS-CoV-2 controls. In April and May 2020, a total of 25 cases of GBS in London were logged with the national database, by which time an estimated 1.5 million Londoners had already made a serological response to SARS-CoV-2. Assuming all 25 cases were SARS-CoV-2 related, the maximum risk could therefore be calculated at 1 case of GBS for every 60,000 SARS-CoV-2 infections.[46] Should the incidence rate calculated in the Italian study (47.9 cases per 100.000 SARS-CoV-2 infections) be correct, there should have been approximately 718 cases of GBS in London in these 2 months. From these observations, the authors concluded that a causative relationship between SARS-CoV-2 and GBS was unlikely. Based on these findings and given the fact that in our study no increase in inclusion rate was found, it seems that the risk of developing GBS following SARS-CoV-2 infection is either non-existent or at most small, and considerably lower when compared with, for example, *C. jejuni* or Zika virus. Results from a recently published national registry in Singapore showed a decrease in hospital admissions of GBS patients during the pandemic as well.[47]

IGOS is a prospective cohort study and the inclusion of patients is dependent on the efforts of the local investigator, and can be selective. Our study should therefore be regarded a case series with the advantage of having a multicenter and prospective collection of data according to a predefined standard protocol. In addition, we used predefined criteria for a confirmed or probable SARS-CoV-2 infection and were able to exclude other infections in a subgroup of patients. Our study also has several important limitations. First, the study design was not appropriate to establish causation or to determine an association between GBS and SARS-CoV-2 in absence of a matched control group of patients without GBS. Second, as a consequence of the SARS-CoV-2 pandemic, clinical follow-up was limited, and most laboratory tests were performed locally as samples could not be transported to the coordinating center at the Erasmus MC University Medical Center Rotterdam on short notice due to travel restrictions. This led to non-uniform testing of biological samples for SARS-CoV-2 serology and other preceding infections. Since three samples were collected post-immunoglobulin treatment, we were not able to completely rule out a recent EBV and CMV infection in these patients. Third, we used the ECDC case definitions for SARS-CoV-2 infection to classify our patients, although this classification system was developed for clinical purposes. We chose to focus on the GBS patients with a confirmed/probable SARS-CoV-2 infection, because 10/15 (67%) of the patients with a possible infection were not tested for SARS-CoV-2 by PCR, and in 12/15 (80%) no CT-thorax was done, leading to considerable diagnostic uncertainty in this patient population. Although the WHO criteria are stricter, we decided against this criteria set as CT abnormalities are not included, which we considered a valuable diagnostic tool in our cohort. Lastly, patient subgroups were small and the follow-up was short, making interpretation of findings worthy of some caution.

In conclusion, we were able to identify a confirmed or probable preceding SARS-CoV-2 infection in 11 (22%) GBS patients during the first months of the pandemic in the context of a large, international, prospective cohort study. These patients shared similar features, as they frequently had a sensorimotor phenotype with facial palsy and significantly more often had a demyelinating subtype compared with both the other patients included in the same time window as well as historical control patients. In line with other studies, we did not find an increase in inclusion rate in IGOS, suggesting that a strong association between SARS-CoV-2 and GBS is unlikely. Nevertheless, we cannot exclude that SARS-CoV-2 may be an occasional trigger for GBS. Since our study was not designed to quantify a causative link between GBS and SARS-CoV-2, an unbiased multicenter international case-control study is needed to determine whether there is an association or not.

SUPPLEMENTARY MATERIAL

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REFERENCES

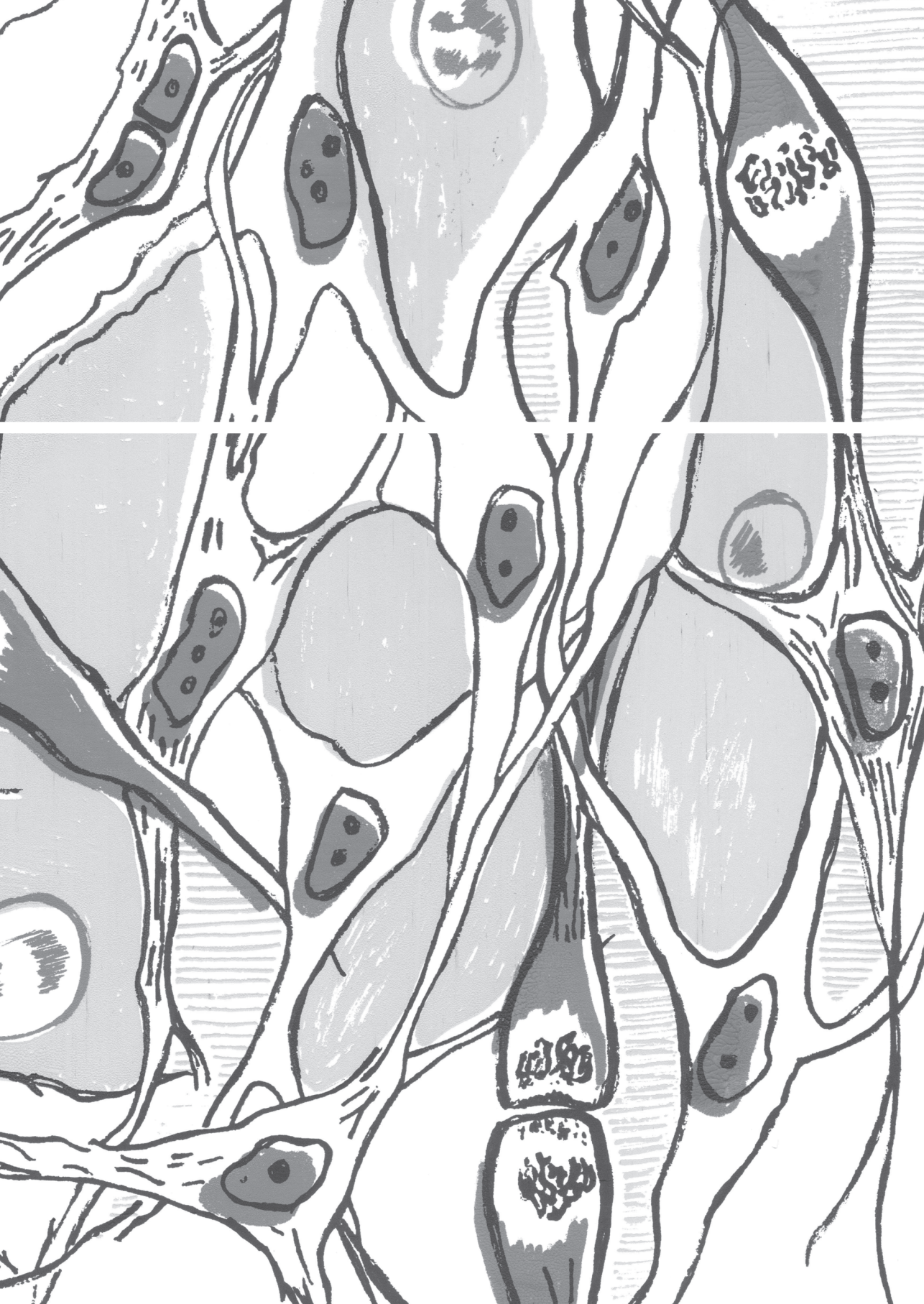
1. World Health Organization. Novel Coronavirus (2019-nCoV) Situation Report - 11 31 January 2020. Report No.: 11.
2. Mao L, Jin H, Wang M, et al. Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. *JAMA Neurol* 2020;77:1-9.
3. Ghannam M, Alshaer Q, Al-Chalabi M, Zakarna L, Robertson J, Manousakis G. Neurological involvement of coronavirus disease 2019: a systematic review. *J Neurol* 2020:1-19.
4. Guidon AC, Amato AA. COVID-19 and neuromuscular disorders. *Neurology* 2020;94:959-969.
5. Ellul MA, Benjamin L, Singh B, et al. Neurological associations of COVID-19. *Lancet Neurol* 2020;19:767-783.
6. Willison HJ, Jacobs BC, van Doorn PA. Guillain-Barré syndrome. *Lancet* 2016;388:717-727.
7. Leonhard SE, Bresani-Salvi CC, Lyra Batista JD, et al. Guillain-Barré syndrome related to Zika virus infection: A systematic review and meta-analysis of the clinical and electrophysiological phenotype. *PLoS Negl Trop Dis* 2020;14:e0008264.
8. Parra B, Lizarazo J, Jiménez-Arango JA, et al. Guillain-Barré Syndrome Associated with Zika Virus Infection in Colombia. *N Engl J Med* 2016;375:1513-1523.
9. Cao-Lormeau VM, Blake A, Mons S, et al. Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *Lancet* 2016;387:1531-1539.
10. Jacobs BC, van Doorn PA, Schmitz PI, et al. Campylobacter jejuni infections and anti-GM1 antibodies in Guillain-Barre syndrome. *Ann Neurol* 1996;40:181-187.
11. De Sanctis P, Doneddu PE, Viganò L, Selmi C, Nobile-Orazio E. Guillain Barré Syndrome associated with SARS-CoV-2 infection. A Systematic Review. *Eur J Neurol* 2020.
12. Zhao H, Shen D, Zhou H, Liu J, Chen S. Guillain-Barre syndrome associated with SARS-CoV-2 infection: causality or coincidence? *Lancet Neurol* 2020;19:383-384.
13. Filosto M, Cotti Piccinelli S, Gazzina S, et al. Guillain-Barre syndrome and COVID-19: an observational multicentre study from two Italian hotspot regions. *J Neurol Neurosurg Psychiatry* 2020.
14. Hasan I, Saif-Ur-Rahman KM, Hayat S, et al. Guillain-Barre syndrome associated with SARS-CoV-2 infection: A systematic review and individual participant data meta-analysis. *J Peripher Nerv Syst* 2020;25:335-343.
15. Jacobs BC, van den Berg B, Verboon C, et al. International Guillain-Barré Syndrome Outcome Study: protocol of a prospective observational cohort study on clinical and biological predictors of disease course and outcome in Guillain-Barré syndrome. *J Peripher Nerv Syst* 2017;22:68-76.
16. Asbury AK, Cornblath DR. Assessment of current diagnostic criteria for Guillain-Barré syndrome. *Ann Neurol* 1990;27 Suppl:S21-24.
17. Sejvar JJ, Kohl KS, Gidudu J, et al. Guillain-Barré syndrome and Fisher syndrome: case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* 2011;29:599-612.
18. Doets AY, Verboon C, van den Berg B, et al. Regional variation of Guillain-Barre syndrome. *Brain* 2018;141:2866-2877.
19. Hughes RA, Newsom-Davis JM, Perkin GD, Pierce JM. Controlled trial prednisolone in acute polyneuropathy. *Lancet* 1978;2:750-753.

20. van Koningsveld R, Steyerberg EW, Hughes RA, Swan AV, van Doorn PA, Jacobs BC. A clinical prognostic scoring system for Guillain-Barré syndrome. *Lancet Neurol* 2007;6:589-594.
21. Hadden RD, Cornblath DR, Hughes RA, et al. Electrophysiological classification of Guillain-Barré syndrome: clinical associations and outcome. Plasma Exchange/Sandoglobulin Guillain-Barré Syndrome Trial Group. *Ann Neurol* 1998;44:780-788.
22. GeurtsvanKessel CH, Okba NMA, Igloi Z, et al. An evaluation of COVID-19 serological assays informs future diagnostics and exposure assessment. *Nat Commun* 2020;11:3436.
23. European Centre for Disease Prevention and Control. Case definition for coronavirus disease 2019 (COVID-19), as of 29 May 2020 [online]. Available at: https://www.ecdc.europa.eu/en/covid-19/surveillance/case-definition.
24. World Health Organization. Laboratory testing for coronavirus disease (COVID-19) in suspected human cases [online]. Available at: https://www.who.int/publications/i/item/10665-331501.
25. World Health Organization. Use of laboratory methods for SARS diagnosis [online]. Available at: https://www.who.int/csr/sars/labmethods/en/#lab).
26. Ye Z, Zhang Y, Wang Y, Huang Z, Song B. Chest CT manifestations of new coronavirus disease 2019 (COVID-19): a pictorial review. *Eur Radiol* 2020;30:4381-4389.
27. Lascano AM, Epiney JB, Coen M, et al. SARS-CoV-2 and Guillain-Barré syndrome: AIDP variant with favorable outcome. *Eur J Neurol* 2020.
28. Kilinc D, van de Pasch S, Doets AY, Jacobs BC, van Vliet J, Garssen MPJ. Guillain-Barré syndrome after SARS-CoV-2 infection. *Eur J Neurol* 2020.
29. Uncini A, Vallat JM, Jacobs BC. Guillain-Barré syndrome in SARS-CoV-2 infection: an instant systematic review of the first six months of pandemic. *J Neurol Neurosurg Psychiatry* 2020;91:1105-1110.
30. Gutiérrez-Ortiz C, Méndez A, Rodrigo-Rey S, et al. Miller Fisher Syndrome and polyneuritis cranialis in COVID-19. *Neurology* 2020.
31. Toscano G, Palmerini F, Ravaglia S, et al. Guillain-Barré Syndrome Associated with SARS-CoV-2. *N Engl J Med* 2020;382:2574-2576.
32. Orlikowski D, Porcher R, Sivadon-Tardy V, et al. Guillain-Barre syndrome following primary cytomegalovirus infection: a prospective cohort study. *Clin Infect Dis* 2011;52:837-844.
33. Islam B, Islam Z, GeurtsvanKessel CH, et al. Guillain-Barre syndrome following varicella-zoster virus infection. *Eur J Clin Microbiol Infect Dis* 2018;37:511-518.
34. van den Berg B, van der Eijk AA, Pas SD, et al. Guillain-Barré syndrome associated with preceding hepatitis E virus infection. *Neurology* 2014;82:491-497.
35. Limaye AP, Kirby KA, Rubenfeld GD, et al. Cytomegalovirus reactivation in critically ill immunocompetent patients. *Jama* 2008;300:413-422.
36. Román GC, Anaya JM, Mancera-Páez Ó, Pardo-Turriago R, Rodríguez Y. Concurrent Guillain-Barré syndrome, transverse myelitis and encephalitis post-Zika: A case report and review of the pathogenic role of multiple arboviral immunity. *J Neurol Sci* 2019;396:84-85.
37. Paterson RW, Brown RL, Benjamin L, et al. The emerging spectrum of COVID-19 neurology: clinical, radiological and laboratory findings. *Brain* 2020;143:3104-3120.
38. Pollán M, Pérez-Gómez B, Pastor-Barriuso R, et al. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. *Lancet* 2020.

39. Stringhini S, Wisniak A, Piumatti G, et al. Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Geneva, Switzerland (SEROCoV-POP): a population-based study. *Lancet* 2020;396:313-319.
40. Long QX, Liu BZ, Deng HJ, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med* 2020;26:845-848.
41. Schonberger LB, Bregman DJ, Sullivan-Bolyai JZ, et al. Guillain-Barre syndrome following vaccination in the National Influenza Immunization Program, United States, 1976--1977. *Am J Epidemiol* 1979;110:105-123.
42. Sejvar JJ, Pfeifer D, Schonberger LB. Guillain-barré syndrome following influenza vaccination: causal or coincidental? *Curr Infect Dis Rep* 2011;13:387-398.
43. Mier YT-RL, Delorey MJ, Sejvar JJ, Johansson MA. Guillain-Barré syndrome risk among individuals infected with Zika virus: a multi-country assessment. *BMC Med* 2018;16:67.
44. Aubry M, Teissier A, Huart M, et al. Zika Virus Seroprevalence, French Polynesia, 2014-2015. *Emerg Infect Dis* 2017;23:669-672.
45. Fragiel M, Miró Ò, Llorens P, et al. Incidence, clinical, risk factors and outcomes of Guillain-Barré in Covid-19. *Ann Neurol* 2020.
46. Keddie S, Pakpoor J, Mousele C, et al. Epidemiological and cohort study finds no association between COVID-19 and Guillain-Barré syndrome. *Brain* 2020.
47. Umapathi T, Er B, Koh JS, Goh YH, Chua L. Guillain-Barré syndrome decreases in Singapore during the COVID-19 pandemic. *J Peripher Nerv Syst* 2021.

Part V

Improving global research, diagnosis and management of Guillain-Barré syndrome



Chapter 10

Diagnosis and treatment of Guillain-Barré syndrome during the Zika virus epidemic in Brazil: a national survey study

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ABSTRACT

Background and aims

The Zika virus (ZIKV) epidemic in Brazil in 2015-2016 was followed by an increase in the incidence of patients with Guillain-Barré syndrome (GBS). With this national survey study, we aimed to gain a better understanding of how neurologists in Brazil are currently diagnosing and treating patients with GBS, and how this increase in incidence has impacted the management of the disease.

Methods

The questionnaire consisted of 52 questions covering: personal profile of the neurologist, practice of managing GBS during and outside of the ZIKV epidemic, and limitations in managing GBS. All 3264 neurologists that were member of the Brazilian Academy of Neurology at the time of the study were invited to participate.

Results

The questionnaire was fully answered by 171 (5%) neurologists. Sixty-one percent of neurologists noticed an increase in patients with GBS during the ZIKV epidemic, and 30% experienced an increase in problems in managing GBS during this time. The most important limitations in the diagnosis and management of GBS included the availability of nerve conduction studies (NCS), beds in the Intensive Care Unit (ICU) and referral to rehabilitation centers. Most neurologists did not use a protocol for treating patients with GBS and the treatment practice varied.

Interpretation

Increasing availability of NCS and beds in the ICU and rehabilitation centers, and the implementation of (inter)national guidelines, are critical in supporting Brazilian neurologist in their management of GBS, and are especially important in preparing for future outbreaks.

INTRODUCTION

GBS is the most common acute paralytic neuropathy worldwide, with a global incidence of approximately 1-2 per 100,000 person-years.[1] GBS typically presents as progressive weakness and sensory signs, starting in the distal legs and progressing to the arms and facial muscles.[2] Disease progression is rapid and often severe, with approximately 20% of patients requiring mechanical ventilation due to involvement of respiratory muscles.[2] Treatment for GBS generally consists of multidisciplinary supportive medical care and immunotherapy. Both intravenously administered immunoglobulin (IVIg) and plasma exchange are proven effective therapies for GBS.[3]

GBS is an immune-mediated neuropathy and in most cases presumed to be triggered by specific types of infections.[2] Several pathogens have been associated with GBS in case-control studies.[4] Most recently, infection with Zika virus (ZIKV) was associated with GBS, when incidence peaked during the outbreaks of ZIKV in Latin America in 2015-2016.[5, 6] Brazil was one of the countries most severely affected by the ZIKV epidemic, with approximately 370,000 cumulative ZIKV cases (suspected or confirmed) reported by the World Health Organization and Ministry of Health between December 2015 and January 2018.[7] The actual incidence is likely to be even higher, as cases may have gone underreported, considering ZIKV usually causes a mild and uncomplicated or subclinical infection. The number of reported cases and the incidence in Brazil was highest in the Northeast, Southeast and Center-West regions (**Figure 1**).[8]

It is unknown how neurologists in Brazil are currently managing GBS and if the ZIKV epidemic has impacted the diagnosis and treatment of GBS patients. Apart from a protocol mainly directed to guide clinicians in decisions on therapy, there are currently no detailed national Brazilian guidelines for the diagnosis and management of GBS, and at the time of the survey no international guidelines were available.[9] This may complicate the management of the syndrome, especially because clinical presentation and disease progression can differ extensively between patients and specific diagnostic or prognostic markers for GBS are not yet available. Furthermore, it is unknown if neurologists experience limitations in the availability of diagnostic tools, treatment or care for GBS, and if the increase of GBS patients during the ZIKV epidemic effected such availability.

To gain a better understanding of the current clinical practice in the management of GBS in Brazil and the impact of the ZIKV epidemic, we have conducted a national survey study among Brazilian neurologists. With this survey, we identified limita-

tions in the diagnosis and management of GBS in Brazil, both during and outside of outbreak periods. This information can help in developing strategies to improve the clinical practice of GBS in Brazil, and to prepare for future outbreaks of ZIKV or other pathogens that may trigger GBS.

METHODS

Ethical Approval

This study was approved by the Ethical Review Board of the Ribeirão Preto Medical School of the University of São Paulo (FMRP-USP) and the National Ethical Research Commission of Brazil (Comissão Nacional de Ética em Pesquisa, CONEP).

Questionnaire

The questionnaire was developed by SEL, RMC, FdAAG and BCJ, and was reviewed for consistency, readability, completeness, and question sequencing by three independent GBS experts. Questions were drafted in English and translated to Brazilian Portuguese by an annotated translation agency. The questionnaire consists of 52 questions, with 41 multiple choice and 11 open-ended questions, covering several topics, including: personal profile of the neurologist, their practice of managing GBS during and outside of the ZIKV epidemic and limitations they experience in managing GBS. The questionnaire was distributed via an online platform (Limesurvey®) that guarantees anonymous and secure data storage and is approved by the Erasmus University Medical Center for the conduction of survey studies.

Study Population

All the neurologists that were member of the Brazilian Academy of Neurology (Academia Brasileira de Neurologia) were approached through the Academy to participate in the survey study. They were contacted via e-mail, containing a link to the online Limesurvey® platform. The first invitation was sent in February 2019 and participants had a total of 70 days to answer the questionnaire. Five reminders were sent during that time.

Analysis

Statistical analysis of multiple-choice questions was done using IBM SPSS Statistics 25® and included descriptive statistics and comparative analyses (Chi square, Fisher's exact test). Two researchers (SEL, RMC) independently grouped open-ended questions into categories. Discrepancies in interpretation were discussed to reach consensus.

RESULTS

A total of 3264 neurologists were member of the Brazilian Academy of Neurology at the start of the survey and were invited to participate in the study. Of this group, 254 (8%) answered the questionnaire, and of these responses, 171 (5%) were complete. For the analysis, only fully completed questionnaires were used.

Profile of the neurologists

The profile of the responding neurologists is described in **Table 1**. The responders are well-varied regarding age, field of interest, and employment in the private versus public sector. The majority of neurologists work in one hospital (49%), some in two (36%) and a few in three (15%). Most neurologists work in hospitals in the city of São Paulo (11%), Rio de Janeiro (9%), Ribeirão Preto (6%), Belo Horizonte (6%) and Curitiba (5%). Corresponding to this, responders most frequently work in the Southeast region of Brazil (54%), followed by the South (18%), Northeast (17%), Center-Wester (8%) and North (3%). (**Figure 2**)

Diagnosis

The clinical practice in diagnosis and treatment of GBS is shown in **Table 2**. Criteria that were used for diagnosing GBS included the criteria developed by the National Institute of Neurologic Diseases and Stroke (NINDS) (1978, revised in 1990) and by the Brighton Collaboration (2010).[11-13] Fifteen percent of the neurologists indicated they used other criteria or no specific criteria.

According to most neurologists, cerebrospinal fluid (CSF) testing was (almost) always indicated for diagnosing GBS, but only 4% (almost) always tested CSF in suspected GBS cases. This discrepancy may be explained in part by practical limitations in the opportunity to examine CSF, which were experienced sometimes or frequently by 17% of neurologists. (Fig 3) These limitations included the availability of laboratory testing (71%), personnel (33%), equipment (17%) and high costs of the procedure (17%).

Nerve conduction studies (NCS) were available at the hospital of 57% of neurologists. Fifteen percent of the neurologists that indicated NCS were not available at their hospital did not or could not always refer the patient to a dedicated clinic. NCS were frequently or (almost) always indicated in the diagnosis of GBS according to 77% of neurologists, but fewer neurologists (66%) frequently or (almost) always made use of this diagnostic tool. (**Figure 3**) This may be explained by limitations in NCS, that were frequently or (almost) always present in 36% of the responders,

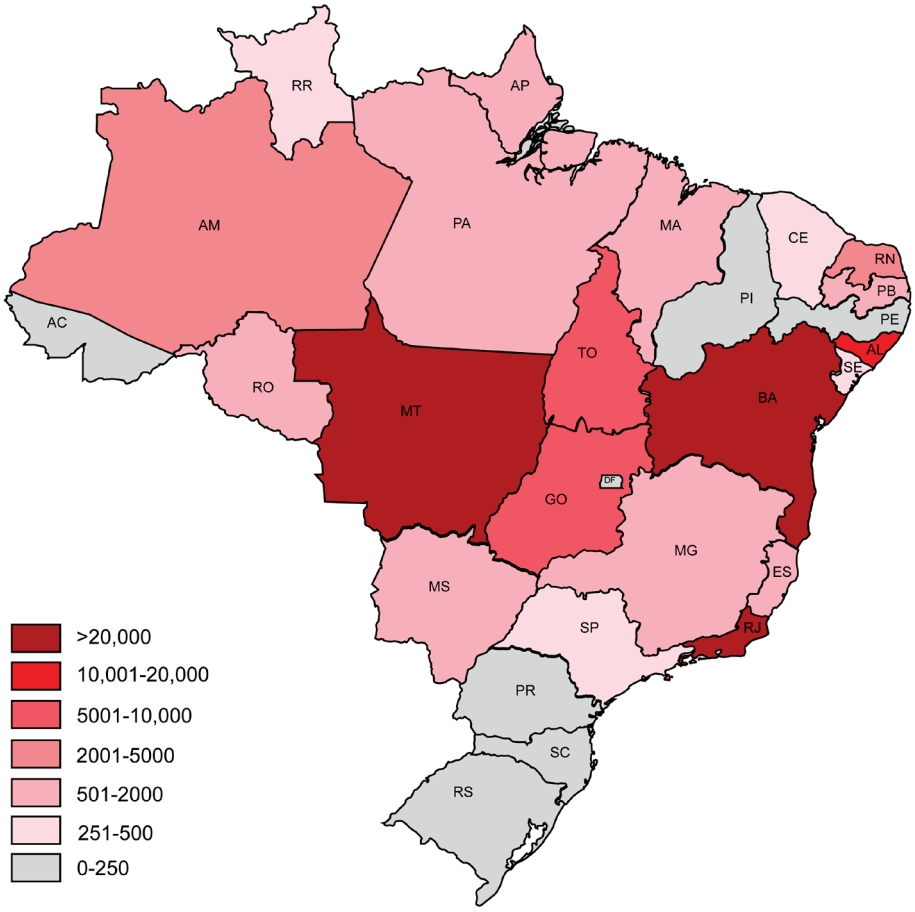


Figure 1. Number of reported suspected Zika virus cases per state in Brazil, 2016
This figure displays the number of reported suspected ZIKV cases in 2016 per state in Brazil, as published by the Brazilian Ministry of Health in in 2017.[10] Not all cases were laboratory confirmed, and other arbovirus infections, were often not excluded. Brazil is divided into 27 states and five regions. The five regions are: North (AC=Acre, AP=Amapá, AM=Amazonas, PA=Pará, RO=Rondônia, RR=Roraima, TO=Tocatins), Northeast (AL=Alagoas, BA=Bahía, CE=Ceará, MA=Maranhão, PB=Paraíba, PE=Pernambuco, PI=Piauí, RN=Rio Grande do Norte, SE=Sergipe), Center-West (GO=Goiás, MT=Mato Grosso, MS=Mato Grosso do Sul, DF=Distrito Federal), Southeast (ES=Espírito Santo, MG=Minas Gerais, RJ= Rio de Janeiro, SP=São Paulo) and South (PR=Paraná, RS=Rio Grande do Sul, SC=Santa Catarina).

and included limited availability of personnel (65%), equipment (57%), high costs of the procedure (24%), and transportation issues (7%). Any limitations in CSF and NCS were experienced more often by neurologists working in public hospitals, versus those only working in private hospitals, and those working in the Northeast and Center-West versus other regions, although this was only significantly different for CSF examination (p=0.04, respectively p<0.001).

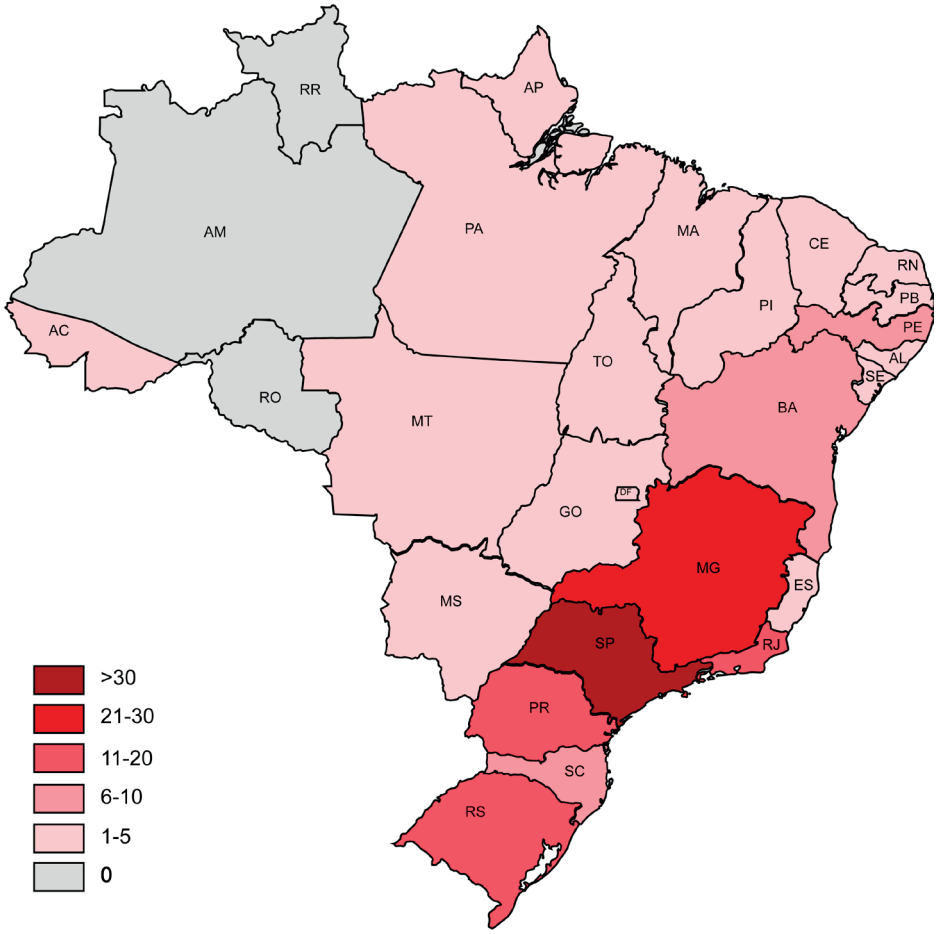


Figure 2. Geographic distribution of responding neurologists (N=171)
This figure displays the number of responding neurologists per state in Brazil.

Treatment and Care

Most neurologists did not use a specific protocol to treat GBS patients. Of the 64 neurologists who indicated to use a specific protocol, only seven provided details of this protocol. These protocols included the Protocolo Clínico e Diretrizes Terapêuticas (PCDT), an expert opinion protocol that is approved by the Ministry of Health of Brazil; the American Academy of Neurology (AAN) Guideline on immunotherapy for GBS; and the BMJ Best Practice guideline on GBS.[9]

When asked what they consider to be the best treatment for GBS, 60% of neurologists answered that IVIg and plasmapheresis are equally effective, followed by 35% who considered IVIg to be the best treatment. However, IVIg was the standard treatment

Table 1: Profile of responding neurologists (N=171)

Age	40 (34-49)
Male: Female (ratio)	96:75 (1.28)
Years practicing as neurologist	10 (5-20)
Field of specialization or interest	
General neurology	103 (64)
Neuromuscular disorders	60 (37)
Neuro-immunology	42 (26)
Vascular disorders	31 (19)
Movement disorders	30 (19)
Epilepsy	27 (17)
Neurodegenerative	26 (16)
Pediatric neurology	12 (7)
Neuro-oncology	5 (3)
Number of newly diagnosed GBS cases per year	
0	4 (2)
1-5	98 (57)
6-10	50 (29)
11-20	14 (8)
>20	5 (3)
Affiliation in public and/or private hospital	
Only public	49/156 (31)
Only private	64/156 (41)
Public and private	43/156 (28)

Data are displayed as n/N (%), median (IQR) or n:n (ratio). For questions with multiple answer format, percentages do not add up to 100. GBS= Guillain-Barré syndrome.

for GBS in the vast majority of responders. According to 48% of neurologists, starting treatment is indicated in all GBS patients, regardless of clinical presentation, severity or progression. When asked what the maximum time period was after the start of neurologic symptoms that they would consider starting treatment in GBS patients, most indicated to start treatment within one month (49%) or two weeks (23%), and 11% of neurologists did not have any restrictions.

Although the preferred treatment was (almost) always available for most responders, for 11% treatment was available only sometimes, infrequently, very rarely or even never. (**Figure 4**) When the preferred treatment was not available, alternative treatment most often consisted of plasmapheresis or IV corticosteroids. Reasons for limited availability of the preferred treatment included high costs (55%), limited access to IVIg within the public health system (33%) and staff or logistics-related issues (38%).

Table 2. Clinical practice of GBS diagnosis and treatment

Diagnostic criteria used	
NINDS	71 (42)
Brighton Collaboration	98 (58)
Other or no specific/published criteria[a]	29 (15)
Treatment protocol used	64/168 (38)
Treatment indication	
All GBS patients are treated	81/171 (48)
Specific treatment indication[b]	
Rapid disease progression	80/90 (89)
Inability to walk independently (any distance)	69/90 (77)
Inability to walk independently for 10m	13/90 (14)
(Imminent) respiratory insufficiency	76/90 (84)
Swallowing dysfunction	72/90 (80)
Severe autonomic dysfunction	72/90 (80)
Standard treatment (first line)	
IVIg	162 (95)
PE	3 (2)
IVIG and IV corticosteroids (combination)	4 (2)
IVIg or PE	2 (1)
Alternative treatment[c] (second line)	28/54 (52)
PE	12/29 (41)
IV corticosteroids	6/29 (21)
Other[d]	7/29 (24)
No response to treatment	
Switch to other treatment	106 (62)
Repeat treatment	67 (39)
No additional treatment	13 (8)
Start corticosteroids	7 (4)
Other[e]	7 (4)
Indication ICU admission[b]	
Inability to walk independently (for any distance)	42 (25)
Inability to walk independently for ≥10m	8 (5)
(Imminent) respiratory insufficiency	163 (95)
Rapid disease progression	142 (83)
Swallowing dysfunction	117 (68)
Severe autonomic dysfunction	147 (86)
Other[f]	3 (2)

Data are displayed as n/N (%) or median (IQR). For questions with multiple answer format, percentages do not add up to 100. NINDS=National Institute of Neurologic Diseases and Stroke[12, 13], Brighton=Brighton Collaboration Criteria[11], IVIg=intravenous immunoglobulin, IV=intravenous, PE=plasma-exchange. ICU=Intensive Care Unit.

[a]Protocolo Clínico e Diretrizes Terapêuticas (n=5), American Academy of Neurology Guideline on immunotherapy for GBS(n=1), BMJ Best Practice guideline for GBS (n=1). [b]Multiple answers were possible. Answer option 'Inability to walk for any distance' was considered mutually exclusive for 'Inability to walk for 10m'. [c]Only neurologists that indicated that the preferred treatment was not always available were asked this question. [d]PE or corticosteroids (n=3), PE or IVIg (n=1), referral to other hospital (n=1), non-pharmaceutical support (n=2). [e]Start (intensive) rehabilitation (n=2), depends on the individual patient (n=2), re-evaluation of diagnosis (n=3) [f]All acute GBS cases (n=2), clinical complications (n=1).

If a patient does not respond to treatment, 51% of neurologists would switch to another treatment (e.g. plasmapheresis if first treatment was IVIg or vice-versa), 27% would repeat the same treatment, and for 12% both repeating and switching therapy were an option. Of the responders that would repeat treatment, 48% would repeat for a maximum of two times, and 12% had no restrictions in how often they would repeat treatment. Neurologists who indicated to have expertise in neuro-immunology or neuromuscular diseases were more likely to repeat treatment (p=0.02), and less likely to switch treatment (p<0.001) compared to other neurologists. Treatment practice did not significantly differ between neurologists who had more experience (>5 years) or who saw more (≥5) GBS patients yearly.

Although an ICU was available in the hospital of 96% of neurologists, 55% experienced limitations in transferring GBS patients to the ICU. (**Figure 4**) A limited amount of beds at the ICU was the main problem, indicated by 98% of the responders.

A rehabilitation program was available in the hospital of 77% of the responders. If present, the program included physical therapy (100%), speech therapy (86%), psychosocial support (60%) and occupational therapy (39%). Referral to a rehabilitation unit at discharge was common, although a quarter of neurologists indicated that this was done only sometimes, infrequently, or never or very rarely. Limitations in referring patients to a rehabilitation unit were experienced by the majority of responders, of which 36% experienced this frequently or (almost) always. (**Figure 4**) The most important limitations were a lack of available beds (54%), no rehabilitation center in the region (25%), and limited accessibility of rehabilitation for patients in the public health sector (28%), including delay due to administrative procedures.

Neurologists working in the public sector more frequently experienced any limitations in intensive care (p=0.03) and referral to a rehabilitation unit (p=0.03) compared to those only working in the private sector. Any limitations in treatment and ICU availability were more frequent in northern states, although this did not statistically differ between regions.

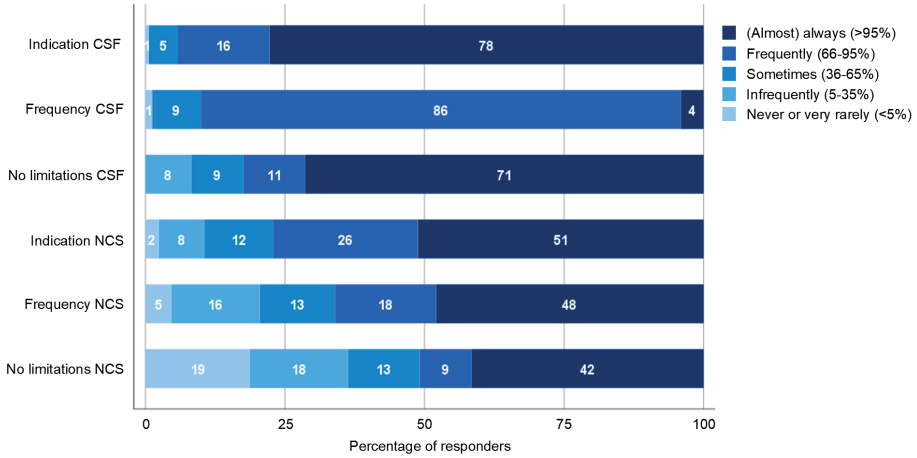


Figure 3. Diagnosis: indication, frequency and availability of CSF and NCS
This figure displays how often neurologists considered CSF or NCS to be indicated in the diagnosis of GBS ('indication'), how often they used these diagnostics tools ('frequency'), and how often they encountered limitations in using these diagnostics ('no limitations').

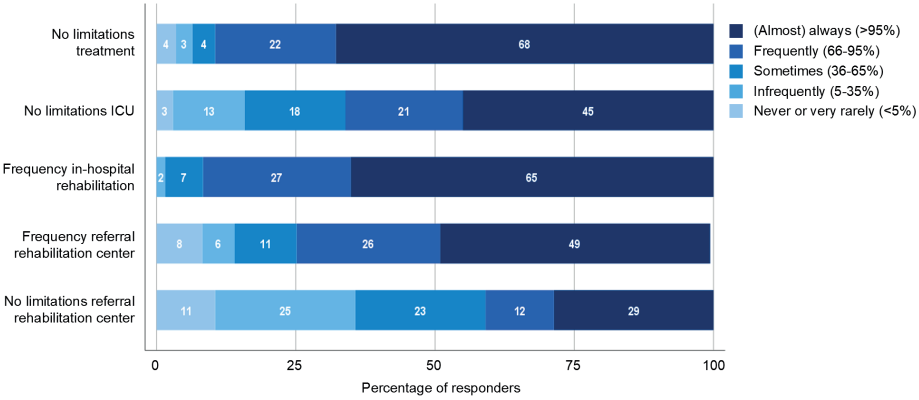


Figure 4. Management: frequency and availability of treatment, ICU and rehabilitation
This figure shows how often neurologists encountered limitations in the availability of the best treatment for GBS, ICU admission and referral to a rehabilitation unit ('no limitations'), and how often patients received in-hospital rehabilitation and were referred to a rehabilitation unit ('frequency'). For the variable 'frequency referral to rehabilitation center', one responder used the answer option 'other'.

GBS during the Zika virus epidemic

During the ZIKV epidemic in Brazil, 61% of neurologists observed an increase in admissions of patients with GBS in their hospital and 30% of these neurologists experienced an increase in problems in the management of GBS patients. These increased problems included limitations in the opportunity to perform NCS (68%) and CSF examination (27%), availability of beds at the hospital (32%) and the ICU

(59%), and availability of treatment (41%). An increase in GBS patients during the ZIKV epidemic was observed most often by neurologists working in the Northeast of Brazil, and an increase in patients or problems in the management were less frequent in the southern regions ($p<0.05$). (**Figure 5**)

At the time of answering the questionnaire, 59% of neurologists tested for ZIKV in (selected) patients with GBS. Of these neurologists, 74% tested for ZIKV PCR, 73% for ZIKV IgM and only three neurologists indicated to use a plaque-reduction neutralization test.

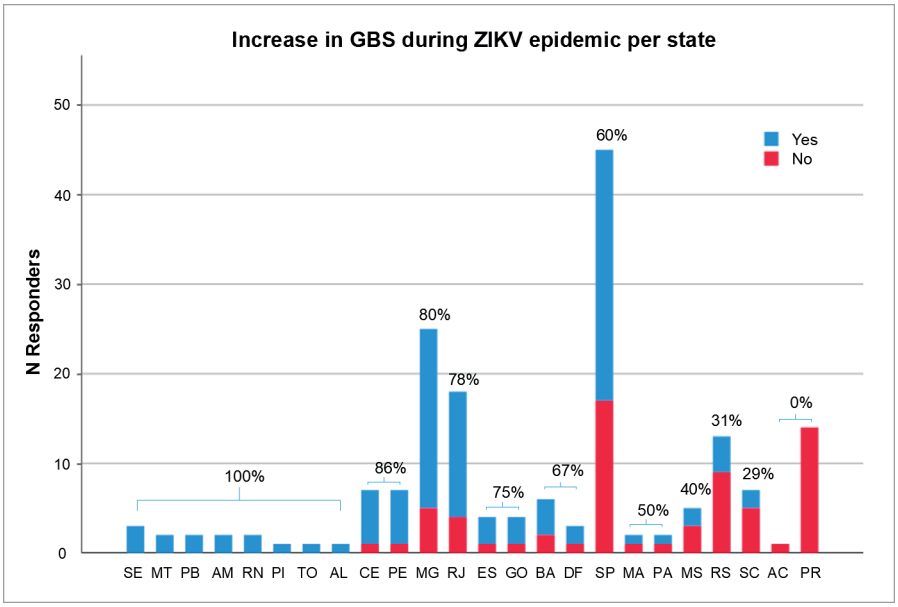


Figure 5. Increase in GBS during ZIKV epidemic displayed per state
Increase in GBS patients during ZIKV epidemic in Brazil (2015-2016) as perceived by the responding neurologists displayed as number of responders per state, with % perceiving increase per state.

DISCUSSION

Neurologists in Brazil often experience limitations in the opportunity to conduct NCS and to refer to the ICU and to rehabilitation centers when confronted with patients with GBS. Most neurologists saw an increase in GBS patients during the ZIKV epidemic, and one third of these neurologists also experienced an increase in problems in managing GBS patients during that time. The treatment practice of GBS of neurologists in Brazil is comparable to treatment practice found in other regions,

and indicates that international guidelines are necessary, especially to help deciding when to start and when to repeat treatment in patients with GBS.[14]

Limitations in the diagnosis or treatment of GBS were experienced frequently. Any limitations in NCS were experienced by 60%, in ICU care by 55%, and in referring patients to a rehabilitation center by approximately 30% of neurologists. Especially the lack of availability of sufficient intensive care for GBS patients is worrying, as this may directly affect morbidity and even mortality of these patients. Limitations were generally more frequently experienced by neurologists working in the Northeast and Center-West of Brazil, which corresponds to a lower socio-economic status in these regions, as reflected by regional contribution to the gross domestic product (GDP).[15] Both neurologists working at the public and private sector experienced these limitations, but they were more frequent in the public health system. So although the public health system in Brazil (Sistema Único de Saúde, SUS) provides universal health coverage for all inhabitants of the country, including access to adequate treatment and care for GBS patients, our data indicate that in practice, access is not guaranteed and often delayed, and that a lack of availability of equipment, treatment, and beds in the ICU and rehabilitation centers occur frequently.

During the ZIKV outbreak in Brazil, about 60% of neurologists experienced an increased frequency of GBS patients admitted to their hospitals, about one-third of whom also experienced an increase in problems managing GBS. Increase in patients with GBS was experienced most frequently in the North, Northeast and Center-West of Brazil, reflecting the areas that were subject to the highest incidence of ZIKV during the outbreak in 2016.[10] Limitations in availability of NCS and ICU admission were again the biggest issues. Furthermore, only 3% of neurologists indicated to use plaque-reduction neutralization test in ZIKV, which is a laboratory test that helps to differentiate between a recent ZIKV and dengue virus infection. Due to cross-reactivity, ZIKV and dengue virus can be difficult to tell apart based on serology, and implementation of this test can be crucial in correctly diagnosing patients with ZIKV, especially when PCR results are negative.[16, 17] Lack of usage of this test suggests that identification of ZIKV-related GBS cases may not be optimal.

Most neurologists do not use a specific protocol for treating GBS patients, and in some situations the treatment practice varies between neurologists or deviates from available evidence from treatment trials. First, of the 29 neurologists that use an alternative treatment when the preferred treatment (IVIg or plasmapheresis) for GBS is not available, about 20% use IV corticosteroids, although studies have proven that corticosteroids are not effective in treating GBS and may even have a negative

effect on outcome.[3] Second, about half of neurologists switch treatment and about a third repeat treatment in patients that do not (sufficiently) respond to therapy, although no evidence exists that this is effective. Third, about half of neurologists start treatment in all patients with GBS, regardless of their clinical condition, although effectivity of plasmapheresis and IVIg has not been sufficiently studied in patients still able to walk.[18, 19] This treatment practice is also common outside of Brazil, and can likely be explained by the lack of evidence and the absence of international guidelines for treatment in these situations.[14]

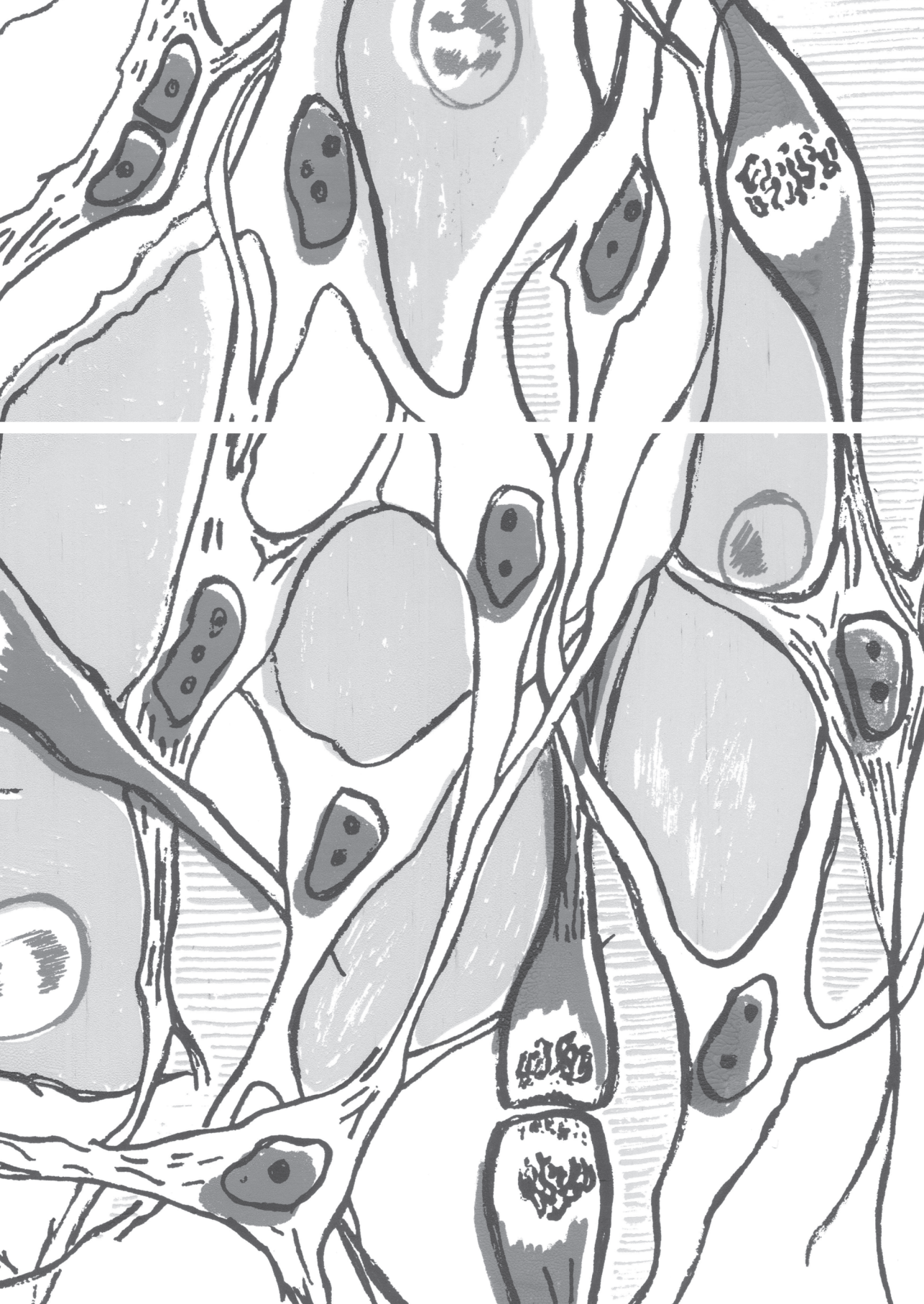
This study has several limitations. First, the percentage of responding neurologists was limited, and may be biased towards specific neurologists, for instance those working in the neuromuscular field, in academic hospitals, or in certain regions. Second, the results presented reflect the estimates and opinions of neurologists, that may deviate from the actual practice.

CONCLUSION

Increasing international migration of humans and vectors pose threats of new epidemics of ZIKV or other arbovirus infections, potentially related to GBS, resulting eventual upsurge of GBS incidence in near future.[20] Our survey identified several critical limitations in the current practice of managing GBS in Brazil and can direct the development of strategies to improve this. Most importantly, the lack of availability of NCS, intensive care management and rehabilitation of GBS should guide redistribution of available funding from the Brazilian government or (inter)national non-profit organizations. Furthermore, treatment practice of GBS is variable and few neurologists use guidelines in when treating patients with GBS. Increasing the visibility of the existing national expert opinion protocol for the management of GBS (PCDT), or implementation of a recently published expert-based international guideline for the management of GBS may help to provide such guidance.[9, 21]

REFERENCES

1. Sejvar JJ, Baughman AL, Wise M, Morgan OW. Population incidence of Guillain-Barré syndrome: a systematic review and meta-analysis. *Neuroepidemiology* 2011;36:123-133.
2. Willison HJ, Jacobs BC, van Doorn PA. Guillain-Barré syndrome. *Lancet* 2016;388:717-727.
3. Hughes RA, Swan AV, Raphael JC, Annane D, van Koningsveld R, van Doorn PA. Immunotherapy for Guillain-Barré syndrome: a systematic review. *Brain* 2007;130:2245-2257.
4. Jacobs BC, Rothbarth PH, van der Meche FG, et al. The spectrum of antecedent infections in Guillain-Barré syndrome: a case-control study. *Neurology* 1998;51:1110-1115.
5. World Health Organization. Zika situation report 5 February 2016. *World Health Organization* 2016:https://www.who.int/emergencies/zika-virus/situation-report/5-february-2016/en/.
6. Parra B, Lizarazo J, Jimenez-Arango JA, et al. Guillain-Barré syndrome associated with Zika virus infection in Colombia. *N Engl J Med* 2016;375:1513-1523.
7. Pan American Health Organization. Zika suspected and confirmed cases reported by countries and territories in the Americas Cumulative cases, 2015-2017. Updated as of 04 January 2018.
8. Pan American Health Organization. Epidemiological Report Brazil. September 2017.
9. Protocolos Clínicos e Diretrizes Terapêuticas - PCDT Sindrome de Guillain-Barré. 2015:http://www.saude.gov.br/protocolos-e-diretrizes.
10. Secretaria de Vigilância em Saúde Ministério da Saúde. Boletim Epidemiológico, Monitoramento dos casos de dengue, febre de chikungunya e febre pelo vírus Zika até a Semana Epidemiológica 52, 2016 [Epidemiologic Bulletin, Monitoring of dengue, chikungunya and Zika virus cases until epidemiologic week 52, 2016]. *Boletim Epidemiológico* 2017;48:http://www.saude.gov.br/boletins-epidemiologicos.
11. Sejvar JJ, Kohl KS, Gidudu J, et al. Guillain-Barré syndrome and Fisher syndrome: case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* 2011;29:599-612.
12. Asbury AK, Arnason, B.G.W., Karp, H.R., McFarlin, D.E. Criteria for diagnosis of Guillain-Barré syndrome. *Ann Neurol* 1978;3:565-566.
13. Asbury AK, Cornblath DR. Assessment of current diagnostic criteria for Guillain-Barré syndrome. *Ann Neurol* 1990;27 Suppl:S21-24.
14. Verboon C, Doets AY, Galassi G, et al. Current treatment practice of Guillain-Barre syndrome. *Neurology* 2019;93:e59-e76.
15. Organisation for Economic Co-operation and Development (OECD). 2019:https://data.oecd.org/brazil.htm.
16. Abushouk AI, Negida A, Ahmed H. An updated review of Zika virus. *Journal of Clinical Virology* 2016;84:53-58.
17. Waggoner JJ, Pinsky BA. Zika Virus: Diagnostics for an Emerging Pandemic Threat. *J Clin Microbiol* 2016;54:860-867.
18. Chevret S. Plasma exchange for Guillain-Barré syndrome. *Cochrane Database Syst Rev* 2017.
19. Hughes RA, Swan AV, van Doorn PA. Intravenous immunoglobulin for Guillain-Barré syndrome. *Cochrane Database Syst Rev* 2014:CD002063.
20. Díaz-Soto S, Chavez K, Chaca A, Alanya J, Tirado-Hurtado I. Outbreak of Guillain-Barre syndrome in Peru. *eNeurologicalSci* 2019;14:89-90.
21. Leonhard SE, Mandarakas MR, Gondim FAA, et al. Diagnosis and management of Guillain-Barre syndrome in ten steps. *Nat Rev Neurol* 2019.



Chapter 11

Guillain–Barré syndrome in low and middle income countries: challenges and prospects

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ABSTRACT

The epidemiology, clinical characteristics, management and outcome of Guillain–Barré syndrome (GBS) differ between low and middle income countries (LMIC) and high income countries (HIC). At present, limited data are available on GBS in LMIC and the true incidence of GBS in many LMIC remains unknown. Increased understanding of GBS in LMIC is needed because poor hygiene and high exposure to infections render populations in LMIC vulnerable to GBS outbreaks. Furthermore, insufficient diagnostic and health-care facilities in LMIC contribute to delayed diagnosis in patients with severe presentations of GBS. In addition, the lack of national clinical guidelines and absence of affordable, effective treatments contribute to worse outcomes and higher mortality in LMIC than HIC. Systematic population-based surveillance studies, cohort and case–control studies are required to understand the incidence and risk factors for GBS. Novel, targeted and cost-effective treatment strategies need to be developed in the context of health system challenges in LMIC. To ensure integrative rehabilitation services in LMIC, existing prognostic models must be validated, and responsive outcome measures that are cross-culturally applicable must be developed. Therefore, fundamental and applied research to improve the clinical management of GBS in LMIC should become a critical focus of future research programmes.

INTRODUCTION

Guillain–Barré syndrome (GBS) is an immune-mediated polyradiculoneuropathy with an acute onset that affects 100,000 people worldwide annually[1-3]. GBS is characterized by rapidly progressive ascending weakness that initially affects the limbs and can eventually affect the cranial and respiratory muscles. Several infectious agents have been identified as triggers for the development of GBS, and clusters of this disease can be associated with outbreaks such as the Zika virus epidemic[4-6]. The severity of GBS is highly variable, ranging from mild distal limb weakness to complete paralysis, respiratory failure and even death. Several variants of GBS have been defined on the basis of their clinical presentation, including a pure motor variant, paraparetic variants and Miller Fisher syndrome (MFS)[7, 8], which is characterized by the clinical triad of ophthalmoplegia, ataxia and areflexia[7]. Several subtypes of GBS have also been identified on the basis of electrophysiological features[1-3], including acute inflammatory demyelinating polyneuropathy (AIDP) and acute motor axonal neuropathy (AMAN)[2, 9, 10]. Patients with AIDP usually have the classic sensorimotor variant of GBS, whereas those with AMAN typically have the pure motor variant[8]. In some patients with axonal GBS, both sensory and motor fibres are affected; this variant is termed acute motor and sensory axonal neuropathy (AMSAN) and is sometimes considered to be a severe variant of AMAN[2]. Plasma exchange and intravenous immunoglobulin infusions are equally effective therapies for all variants of GBS[2-4, 11].

Considerable variation between countries and/or regions is evident in the epidemiology, subtypes and management of GBS[12]. These differences are thought to be related to environmental and economic factors as well as to health awareness and behaviour. Poor hygiene and sanitation, unsafe drinking water and frequent exposure to pathogens render the populations in low income and middle income countries (LMIC) — defined by the World Bank as having an annual gross national income per capita <3,995 USD[13] — highly vulnerable to outbreaks of infectious diseases that are capable of triggering GBS[14, 15]. For example, outbreaks of GBS in Northern China (2007) and Mexico (2011) were due to increases in the incidence of *Campylobacter jejuni* infection[16, 17]. Variations in the incidence and outcomes of GBS can also be partly explained by income per capita[12, 18]. Resource limitations in LMIC, including the limited availability of electrodiagnostic machines, hospital and intensive care unit (ICU) beds and rehabilitation clinics, can hamper the diagnosis and care of patients with GBS[5]. In addition, the lack of national guidelines (in most LMIC) and the high cost of treatment facilities complicate the management of patients with GBS versus their counterparts in high income countries (HIC) — defined according to World

Bank criteria as having an annual gross national income per capita ≥3,995 USD[13], which represents the upper middle and high income categories combined[19-23].

Although the number of studies of GBS in LMIC is increasing, the majority of GBS studies conducted to date have focused on HIC and we are not aware of any prior published reviews focusing on LMIC. Accordingly, this Review aims to provide an overview of GBS in LMIC and to compare the epidemiology, clinical presentation, subtypes and management of GBS in LMIC and HIC. We identify specific challenges related to the diagnosis, treatment and management of patients with GBS in LMIC and explore the prospects for future research and policy.

Epidemiology

Most studies on the incidence of GBS have been performed in populations from HIC; only a few include populations from LMIC (**Table 1**). The reported incidence of GBS ranges from 0.16 to 3.0 cases per 100,000 persons/year[24, 25]; this considerable variation could, in part, be related to geographical location (**Figure 1**). For instance, an incidence of ~0.40 cases per 100,000 persons/year was reported in Brazil, 0.84-1.91 cases per 100,000 persons/year in Europe and North America and 2·1-3.0 cases per 100,000 persons/year were reported in Iran, Curaçao and Bangladesh[2, 24-28]. As well as the factors already mentioned, some of this variation could be due to methodological differences between studies and the lack of robust, systematic population-based studies in certain countries[24].

Most studies from Europe and North America were performed between 1980 and 2000 and the incidence of GBS in these regions has remained stable across the majority of this period (1.0–1.8 cases per 100,000 persons/year), suggesting a consistent exposure to infectious triggers[25]. Seasonal fluctuations in the incidence of GBS also vary by geographical area. One large meta-analysis showed that the incidence of GBS increases in winter (January–March) in Western, Middle Eastern and Far Eastern countries, but decreases during January–March in the Indian subcontinent and Latin America[30]. The increased incidence of GBS during winter in some countries is thought to be due to the increased incidence of respiratory tract infections caused by *Mycoplasma pneumoniae* or *Haemophilus influenzae*[31, 32]. By contrast, an increase in the incidence of GBS has been observed during summer in Northern China and Bangladesh, which is thought to be associated with an increased frequency of preceding diarrhoea[3, 16, 33]. In these countries, the high temperatures and humidity of the summer season favour bacterial growth and are an important determinant of the burden of bacterial diarrhoea[34, 35].

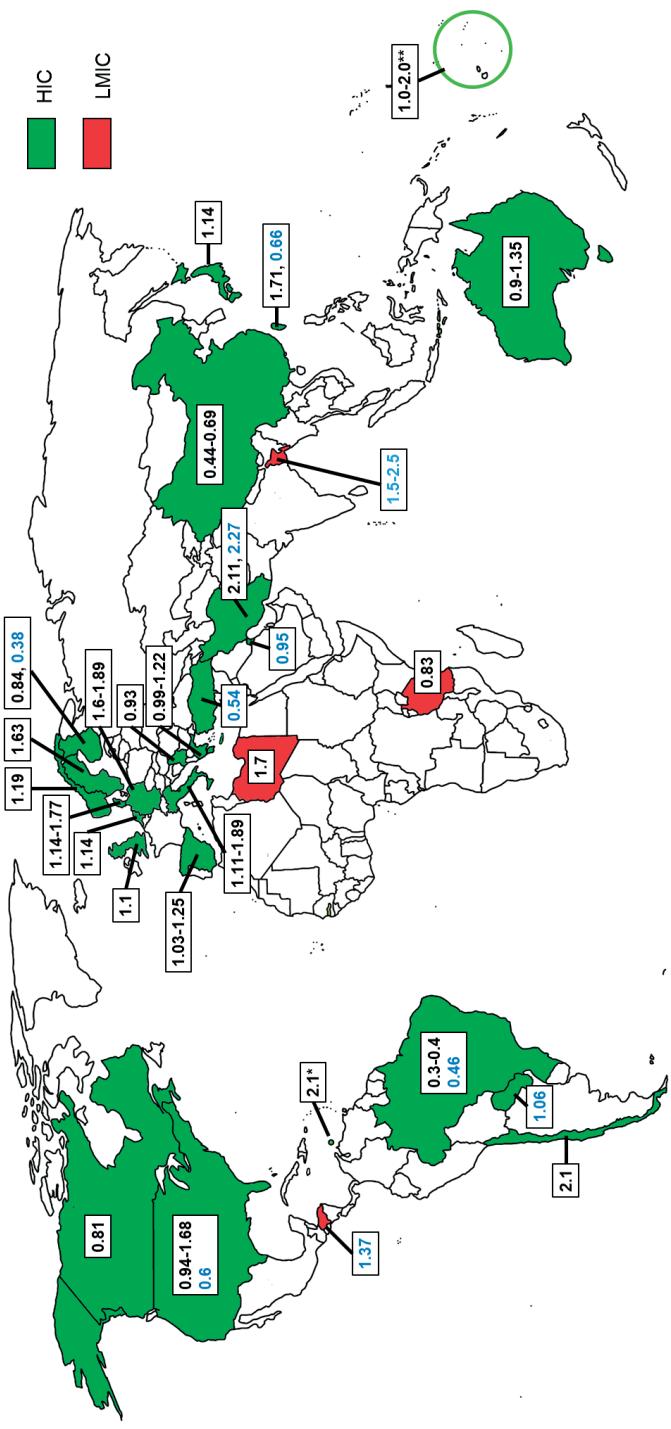

Figure 1. Reported incidence rates of GBS in high income countries (HIC) and low and middle-income countries (LMIC)
According to World Bank definitions, LMIC are those with an annual gross national income per capita <3,995 USD, whereas upper middle and high income countries combined (HIC in this Review) have an annual gross national income per capita of ≥3,995 USD[13].

Table 1. Reviewed publications on GBS by region

Region	Country	Included studies (*n*)	Study design (*n* GBS cases per study)
LMIC			
East Asia and Pacific	Indonesia	1	Retrospective (28)
Middle East and North Africa	Egypt	4	Clinical trial (41); cohort (50, 50);case–control (133)
	Morocco	1	Clinical trial (41)
South Asia	India	14	National surveillance programme (79); clinical trial (37, 12); cohort (328, 140, 102, 70[a]); case–control (80); retrospective (1,166, 273, 173, 90); case reports (2, 1)
	Bangladesh	10	Clinical trial (20); cohort (693, 506, 407, 344, 300, 300, 151); case–control (418, 100)
	Pakistan	3	Retrospective (216, 175, 87)
	Nepal	1	Retrospective (31)
Sub-Saharan Africa	Ethiopia	1	Retrospective (95)
	Kenya	1	Retrospective (54)
	Nigeria	1	Cohort (34)
	Tanzania	1	Retrospective (115)
	Sudan	1	Case report (10)
	Zimbabwe	1	Cohort (32)
HIC			
East Asia and Pacific	Australia	2	Cohort (76); retrospective (46)
	China	6	Cohort (541, 170, 166); retrospective (72); case–control (150, 32)
	Taiwan	3	National surveillance programme (5,998, 5,469); retrospective (96)
	Japan	2	Cohort (97); retrospective (40)
	French Polynesia	2	Case–control (42); national surveillance programme (9)
	Thailand	2	Retrospective (30); case report (1)
	Korea	1	National surveillance programme (48)
	Singapore	1	Retrospective (31)
	New Zealand	1	National surveillance programme (2,056)
Europe and Central Asia	Netherlands	4	Clinical trial (388[b], 85); retrospective (67, 36)
	Denmark	1	National surveillance programme (2,319)
	Germany	1	Retrospective (34)
	Italy	1	Cohort (96)
	Norway	1	Cohort (52)
	Spain	1	Retrospective (106)
	UK	1	Retrospective (110)

Region	Country	Included studies (*n*)	Study design (*n* GBS cases per study)
Latin America and Caribbean	Brazil	5	National survey[c]; cohort (206, 149); case–control (41); case report (1)
	Puerto Rico	2	National surveillance programme (56); cohort (123)
	Colombia	1	Cohort (68)
	Curaçao	1	Retrospective (49)
	Mexico	1	National surveillance programme (467)
Middle East and North Africa	Iraq	1	National surveillance programme (2,611)
	Saudi Arabia	1	Retrospective (49)
North America	USA	1	Case–control (26)
South Asia	Sri Lanka	2	Case report (1, 1)

We mainly selected papers published after1990[29], but we did not exclude commonly referenced and highly regarded older publications. [a]Data were collected prospectively and subjected to retrospective review. [b]Data were collected from two randomized controlled trials and one pilot study: a multinational study (*n*=10); worldwide data, reviews and expert opinion (*n*=30). [c]Survey responses from Brazilian neurologists (no patients with GBS were included in the survey). *n*, number.

Almost all reports document a higher incidence of GBS in men than women (~1.5:1.0), including those from LMIC such as Bangladesh, India, Taiwan, Pakistan, Egypt, Morocco, Ethiopia, Tanzania, and Kenya[3, 4, 9, 14, 23, 27, 36-44]. Most studies indicate that the incidence of GBS increases with age, although the age distribution of cases in each country or region is influenced by the demographics of the background population and the number of people in each age-group at risk of developing GBS. Thus, in Europe and North America, which have ageing populations, GBS occurs most frequently among people aged 50–80 years (2.0–4.0 cases per 100,000 persons/year)[2, 24, 25]. By contrast, studies from Asia (Bangladesh, China, India), South America (Brazil) and sub-Saharan Africa (Ethiopia, Tanzania), which are not affected by population ageing, suggest that GBS occurs most frequently in people aged 21–35 years[12, 39, 41, 45, 46]. In LMIC, where *Campylobacter* infections are endemic, infections are predominantly seen in children and rates of *Campylobacter*-related illness and infection ratios decrease with age[47]. Age can also influence the risk of developing infections that trigger GBS and is an important prognostic factor in individuals with GBS.

The clinical presentation and severity of GBS vary geographically (**Table 2**). In Europe and North America, ~90–95% of patients with GBS have AIDP and the rest have AMAN or AMSAN[7, 9, 12]. The proportion of patients with AMAN or AMSAN is considerably higher (30–65%) in several countries in Latin America, the Caribbean (Curaçao, Mexico, Argentina) and Asia (China, Japan, Bangladesh), although in many of these

Table 2: Clinical features and outcome of GBS by region

Region	Country	Antecedent events (%)	Severity	Subtype (%)	Treatment (%)	Mortality (%)	Ref
Worldwide							
Europe, America and parts of Asia	NA	Adults: 22–53 RTI, 6–26 GE; children: 50–70 RTI, 7–14 GE	Mean MRC-SS at entry 48-49; GBS-DS >2 at nadir 76%	NR	IVIg or PE 87–93	2–10	9, 10, 12, 25, 54
Europe and North America	NA	NR	NR	90–95 AIDP, 5 axonal	NR	3–7	10, 49
LMIC							
Middle East and North Africa	Egypt	24 RTI, 8 gastroenteritis	GBS-DS >2 at admission 76%	76 AIDP, 8 axonal	NR	16	44
	Morocco	51 RTI, 32 gastroenteritis	NR	81 AIDP, 19 axonal	NR		37
South Asia	Bangladesh	18–19 RTI, 36–50 gastroenteritis	Mean MRC-SS at entry 22; GBS-DS >2 at nadir 93%	22–32 AIDP, 53–67 axonal	IVIg or PE 14, supportive care 86	14	12, 14, 22, 55, 56
	India	35–65 RTI, 23–47 gastroenteritis	GBS-DS >2 at admission 76%	57–64 AIDP, 23–41 axonal	NR	4–12	42, 57-59
	Nepal	29 RTI, 3.2 gastroenteritis	NR	19 AIDP, 19 axonal	NR	6	60
	Pakistan	35 RTI, 18 gastroenteritis	NR	46–63 AIDP, 31–34 axonal	NR	8	43, 61
Sub-Saharan Africa	Ethiopia	NR	NR	55 AIDP, 19 axonal	NR	25	41
	Tanzania	NR	NR	NR	NR	15	39
HIC							

Region	Country	Antecedent events (%)	Severity	Subtype (%)	Treatment (%)	Mortality (%)	Ref
East Asia and Pacific	China	24–63 RTI, 7–13 gastroenteritis	Mean GBS-DS at admission 2.57; GBS-DS at nadir 3.15; GBS-DS >2 at nadir 55%	34–57 AIDP; 22–29 axonal	NR	2–8	[53, 62, 63]
	Taiwan	65 RTI, 4 gastroenteritis	NR	80 AIDP, 6% axonal	NR	2–5	[38, 40]
	Korea	11 RTI, 2 gastroenteritis	GBS-DS >2 at nadir 75%	NR	IVIg or PE 81, supportive care 19	2	[64]
	Australia	NR	NR	54 AIDP, 4 axonal	NR	NR	[65]
	Japan [50, 6650, 12250, 12250, 12249, 122]	NR	NR	34 AIDP, 45 axonal	IVIg or PE 90	NR	[50, 66]
Europe and Central Asia	Netherlands	41 RTI, 40 gastroenteritis	NR	60 AIDP, 4 axonal	IVIg or PE 91	2	[19, 67]
	Spain	38 RTI, 27 gastroenteritis	GBS-DS >2 at admission 50%	83 AIDP, 8 axonal	IVIg or PE 86	2	[19, 67]
Latin America & the Caribbean	Brazil	56 RTI, 8 gastroenteritis	NR	82 AIDP, 18 axonal	NR	5	[45]
	Colombia	NR	Median MRC-SS at admission 40; median GBS-DS at nadir 4	78 AIDP, 2 axonal	NR	4	[4]

countries (including Japan) AIDP remains the most frequent variant[14, 36, 45, 48-50]. In the countries and regions where AMAN is the predominant variant, the frequency of AIDP is 22–46%[9]. MFS seems to be more common among patients with GBS from eastern Asia; as an example, 20–26% of patients in Taiwan, Singapore and Japan have MFS, a much higher proportion than in the rest of the world (5–10%)[7, 9, 51]. The high prevalence of AMAN, AMSAN and MFS in Asia might be related to the increased frequency of *C. jejuni* infection in this region[7, 9, 14]. Other infections such as *H. influenza* have also been linked to MFS in Asia[52]. In countries such as Bangladesh and China, where AMAN is more frequent than it is in Europe and North America, approximately 80% of patients present with severe GBS (GBS disability score >2) compared with 40–60% of patients from Europe and North America, where the AIDP subtype is most prevalent[12, 53].

Pathogenesis

Overall, GBS is considered to be the consequence of a preceding infection that triggers an immune response that is responsible for the demyelination and axonal degeneration of peripheral nerves and nerve roots. Treatment with immunomodulatory agents, such as vaccines or biologic drugs, have also been associated with GBS in rare individuals. Other events, including surgery and malignancy, have been temporally related to GBS; the underlying mechanism of GBS in such individuals is not clear[68-70].

Antecedent infections

Approximately two thirds of patients with GBS report symptoms of an infectious disease within the 4 weeks preceding the onset of weakness[2]. Upper respiratory tract infection is the most common antecedent event and is reported by 22–53% of all patients with GBS in Europe, North America, South America and parts of Asia (Taiwan, Nepal, Pakistan, Japan and Malaysia)[10, 12, 38, 48, 61]. The frequency of antecedent respiratory tract infections is even higher in paediatric patients with GBS (50–70%)[25]. By contrast, in India and Bangladesh, gastroenteritis is the most frequent antecedent event associated with GBS (36–47%)[12, 57].

Worldwide, the most frequently identified infectious agent that triggers GBS is *C. jejuni*, which is an important bacterial cause of gastroenteritis and food poisoning[31, 71]. The reported frequencies of antecedent *C. jejuni* infection in patients with GBS differ between studies as well as between countries or regions; for instance, *C. jejuni* infection is substantially more frequent among patients with GBS from Curaçao, China and Bangladesh (~60-70%) than in those from all other countries (30-32%)[14, 28, 33, 49]. The increased frequency of *C. jejuni* infection in these regions could be explained

by the hygienic infrastructure and environmental or host-related factors, including diet[14,27, 45,63]. *C. jejuni* is an established cause of MFS that is probably more frequent in LMIC[72]. However, other infections might be responsible for triggering MFS in countries were *C. jejuni* is less common.

The reported frequencies of antecedent infections in a given population can change over time. For example, China has undergone rapid socioeconomic development and improvements in health services over the past 50 years. A recent study of GBS in China reported a lower incidence of antecedent *C. jejuni* infection (27% in data from 2013–2017)[63] than had previously been reported (66% in data from 1991–1992)[33]. In addition, the trend towards increased life expectancy in China over a similar time period could have decreased the incidence of *C. jejuni* infections, which are more common in younger individuals. We are not aware of any public health interventions undertaken during this time by the Chinese government aimed specifically at reducing the number of *C. jejuni* infections[63, 73]. However, public health interventions can both reduce the prevalence of *Campylobacter* infections and decrease the incidence of GBS: in response to high rates of *C. jejuni* infection between 1980 and 2006, the New Zealand government introduced a national intervention to reduce contamination with *Campylobacter* spp. in poultry. Within 2 years, the country achieved a 52% decline in campylobacteriosis and a simultaneous 13% reduction in GBS hospital admissions[74]. Whether such infection control interventions are feasible in other countries and regions (such as LMIC) remains to be fully explored.

Other infectious agents that have been detected at higher frequencies in patients with GBS than in the background population are cytomegalovirus (10-13%), Epstein–Barr virus (10%), *Mycoplasma pneumonia* (5%; predominantly in children), hepatitis E virus (5%) and Zika virus[31, 49, 71, 75]. Additionally, some infections that are more frequent in LMIC than in other countries and regions have been associated with GBS in case reports or case series: malaria in India, Sri Lanka and Thailand; HIV infection in sub-Saharan Africa; and dengue virus infection in Southeast Asia and Brazil[76-82]. To our knowledge, no reports have linked these infections to GBS in HIC, and epidemiological or case–control studies are required to confirm whether these infections are truly associated with GBS. During the coronavirus disease 2019 (COVID-19) pandemic, several case reports or case series have indicated a possible association between GBS and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection[83-86]. At the time of writing, most such reports are from Europe, although a small number of case reports are from LMIC (four from India, one from Morocco and one from Sudan)[87-89]. However, an epidemiological study in the UK found no increase in the incidence of GBS during the SARS-CoV-2 pandemic[90]. Further studies

are required to confirm the potential relationship between SARS-CoV-2 infection and GBS.

Immunopathogenesis

The geographic differences in clinical and electrophysiological phenotypes of GBS in LMIC and HIC might be in part caused by differences in the rates of preceding infections that tend to trigger different types of GBS. For example, *C. jejuni* infections lead to the development of predominantly (but not exclusively) the axonal type of GBS[31]. In *C. jejuni*-related GBS, an immune response is triggered owing to molecular mimicry between *C. jejuni* lipo-oligosaccharides and human nerve gangliosides, which results in the production of cross-reactive antibodies that activate complement and damage nerves[2]. The pathophysiological mechanisms leading to GBS after infections other than *C. jejuni* have not yet been clearly defined, but similar mechanisms might also play a part in other bacterial infections related to GBS, such as *M. pneumoniae* and *H. influenza*, although these have been less extensively investigated.

The demyelinating and sensorimotor forms of GBS are usually preceded by infection with viruses, such as cytomegalovirus or Epstein–Barr virus; however, the immunopathogenesis remains to be elucidated[10]. Similarly, the specific components of the Zika virus that trigger the immune response leading to GBS have not yet been clarified[91]. Of the patients with SARS-CoV-2-associated GBS, 77–80% had the demyelinating electrophysiological subtype and ~70% had classic sensorimotor GBS[92, 93]. Whether this is the typical phenotype of SARS-CoV-2-related GBS is presently unclear owing to the limited number of reported cases.

Despite the strong associations between specific infectious agents and GBS, the overall risk of developing GBS after infection is very small; for example, only one in 1,000–5,000 patients with *C. jejuni* infection will develop GBS in the subsequent 2 months. One factor that determines this low risk is the requirement for carbohydrate mimicry (which is not present in all *C. jejuni* strains) to develop the cross-reactive antibody response to gangliosides that evolves into GBS[2, 3, 10]. However, genetic and nutritional factors might also influence the patient's susceptibility to producing such antibodies[94-96]. Poor nutritional status, and specifically malnutrition, alters the dysfunctional immune responses implicated in the pathogenesis of various autoimmune diseases[97]. Immune response activation following an infection has also been associated with genetic polymorphisms. Several studies have reported associations between GBS and polymorphisms in the *TNF* (which encodes tumour necrosis factor) and *MBL2* (which encodes mannose-binding protein C) genes[2, 94-96].

Outbreaks of GBS

Although GBS usually occurs sporadically, several outbreaks of this disease have been linked to epidemics of infectious diseases that can trigger GBS. Surges in GBS cases in China (2007) and Mexico (2011) were linked to epidemics of *C. jejuni* infection, and an outbreak of GBS in Peru in 2018 was associated with an epidemic of enterovirus infection[16, 17, 98]. A link between GBS and Zika virus infection was first reported when a 20-fold increase in GBS cases was described during a Zika virus outbreak in French Polynesia in 2013–2014. Subsequently, the incidence of GBS rose by ~3.2–5.1 times in areas affected by the Zika virus epidemic in Latin America and the Caribbean (2014–2016)[4-6, 17, 75, 99]. However, only ~2 in 10,000 patients infected with Zika virus went on to develop GBS, suggesting that a relatively large outbreak of Zika virus is necessary to increase the incidence of GBS[100].

The origins of emerging infectious diseases correlate positively with specific socioeconomic, environmental and ecological factors, which provide a basis for identifying regions where new infections are most likely to originate (so-called emerging disease hotspots)[101]. Zoonoses from wildlife represent the most important and growing threat of emerging infections to global health, whereas vector-borne diseases are responsible for about 25% of emerging infectious diseases. Hotspots for emerging infectious diseases are more common at lower latitudes where wild animals and arthropod vectors reside, such as sub-Saharan Africa and parts of Asia, which mainly consist of LMIC[101]. Other vector-borne viruses transmitted by the same *Aedes* family of mosquitoes as Zika virus (such as chikungunya and dengue) have also been associated with surges in GBS cases[102, 103]. Therefore, these regions are particularly at risk of new outbreaks of GBS. In response to the Zika virus outbreak, several projects have been set up in Latin America to prevent transmission of vector-borne diseases, including surveillance systems for arboviruses and vector control programmes[104]. Further investment in these projects and their implementation in at-risk areas beyond Latin America could help to reduce the likelihood of future outbreaks of GBS.

International disease surveillance initiatives could also help to identify new outbreaks of GBS. The ongoing acute flaccid paralysis (AFP) surveillance programme—originally devised for the surveillance of poliomyelitis—is a useful early warning signal that flags changes in the prevalence of AFP in children up to 15 years of age. Studies conducted in China and Bangladesh show that GBS is now the predominant cause of AFP among children in this age-group, suggesting that AFP surveillance programmes could be expanded to detect changes in the incidence of GBS. Data from this programme have already been used to calculate crude incidence rates of

GBS among children[26, 105]. Extending the AFP surveillance programme to other age groups, and GBS case ascertainment using the Brighton Collaboration criteria to assess the degree of diagnostic certainty, might help to monitor the incidence of GBS[106].

Diagnosis

Diagnosis of GBS is mainly based on clinical features, supported by cerebrospinal fluid (CSF) examination and nerve conduction studies. The National Institute of Neurological Disorders and Stroke (NINDS) criteria and the Brighton Collaboration criteria are the most commonly used sets of validated diagnostic criteria for GBS[1-3, 19, 29, 54, 56, 62, 107].

Patients with GBS can present with remarkably diverse clinical features. In patients with typical GBS, the key presenting feature is ascending bilateral symmetrical weakness that progresses over a period of 12 h to 28 days before a plateau is reached[1-3, 9, 10]. Most patients develop generalized hyporeflexia or areflexia, although tendon reflexes can be normal or even exaggerated in the initial stages. More than half of patients with GBS develop cranial nerve deficits, including bilateral facial weakness, bulbar weakness or extraocular motor dysfunction. In addition to muscle weakness, patients can also experience sensory disturbances, ataxia, muscle pain or radicular pain and signs of autonomic dysfunction, including blood pressure fluctuations and cardiac arrhythmia[1-3, 12]. This diversity can complicate diagnosis in the early stages of GBS, especially in patients with atypical findings—for instance the ~10% of patients who have normal or brisk deep tendon reflexes and the ~8% of patients who present with only paraparesis[108]. Children with GBS might also present with atypical features such as pain, refusal to walk or an abnormal gait; indeed, GBS is correctly diagnosed at admission in only one-third of affected preschool-aged children[2]. Diagnosis is generally even more challenging in LMIC, where facilities for CSF examination and nerve conduction studies might not be readily available, which leads to multiple referrals of patients and diagnostic delay. In one prospective multinational cohort study, the median interval between the onset of weakness and study entry was 5 days in the Netherlands compared with 10 days in Bangladesh[56]. Studies conducted in Africa have also reported lengthy intervals of up to 19 days between the onset of weakness and hospitalization[39]. This delay could lead to under-reporting of GBS in LMIC, as some patients with severe disease might die before reaching the hospital. Moreover, patients with mild symptoms might not seek treatment or recover before reaching a hospital.

The relationships between *C. jejuni* infection and antibodies against the GM1, GM1b, GD1a, GalNAc-GD1a, and GQ1b gangliosides in patients with GBS are well established[2]. Some studies have suggested an association between the presence of anti-GM2 antibodies and a recent cytomegalovirus or Epstein–Barr virus infection[63, 109]. However, serological tests to detect antiganglioside antibodies are not routinely performed at diagnosis, as negative test findings cannot rule out GBS[2]. Furthermore, most of these serological tests require sophisticated techniques and trained personnel that might not be available in LMICs.

In addition, an extensive list of differential diagnoses might need to be excluded. The differential diagnosis of GBS depends on the clinical presentation and variant of GBS (**Box 1**) and is also likely to differ between countries and regions, owing to local variations in the prevalence of infectious diseases, nutritional deficiencies or intoxications, autoimmune diseases and malignancies. As no region-specific information on the differential diagnosis of GBS was included in published studies, we conducted a small survey (Supplementary Material N.P., S.E.L., Q.D.M. and B.C.J., unpublished work) to obtain insight into this important characteristic. The survey was sent to GBS experts working in LMIC within our network, who were asked in turn to distribute the questionnaire to other neurologists in their networks. In total, 17 neurologists (of whom seven frequently see paediatric patients) and two paediatric neurologists working in LMIC returned the questionnaire. Their responses revealed that the differential diagnosis of GBS is generally comparable in LMIC and HIC, although some important differences were noted. For example, sarcoidosis, Sjögren syndrome, Lambert–Eaton myasthenic syndrome and mitochondrial disease seem to be less frequent diagnoses among patients suspected of GBS in LMIC than in HIC. Other diagnoses, such hypokalemic thyrotoxic periodic paralysis, organophosphate intoxication, botulism, rabies, polio and tetanus, seem to be more frequent in LMIC than in HIC. Furthermore, the infectious causes of transverse myelitis, acute flaccid myelitis and polyradiculoneuritis differ between LMIC and HIC. Lyme borreliosis and enterovirus D68 or A71 infection are rarely seen outside Europe and North America, whereas infections with HIV, HTLV-1 and arthropod-borne viruses—including Zika virus, chikungunya virus and West-Nile virus—are frequently reported in several LMIC. These differences might reflect geographic variation in the spread of arthropod vectors (such as those carrying arboviruses) or in the incidence of infectious diseases. For example, polio and rabies eradication programmes have been more successful in HIC than in LMIC. Other explanations might include resource limitations in LMIC that preclude the diagnosis of complex systemic disorders such as Sjögren syndrome and differences in the ages of the populations at risk.

Box 1. Differential diagnosis of GBS

Infection
- Acute transverse myelitis (associated with HIV, CMV, EBV, VZV, syphilis, TB or diphtheria)
- Acute flaccid myelitis due to infections with arthropod-borne viruses (such as ZIKV, CHIKV, WNV) or other viruses such as rabies, polio[a] and enterovirus D68 or A71
- Poly(radiculo-)neuritis owing to infection with HIV, cytomegalovirus, EBV, VZV, diphtheria or Lyme borreliosis
- Botulism *(Clostridium botulinum)* or tetanus (*Cl. tetani*)
- Myositis caused by influenza virus, HIV, HTLV-1 or enterovirus infection[b]
- Meningitis and/or meningo-encephalitis[b]

Inflammation
- Acute transverse myelitis
- Neuromyelitis optica, myelin oligodendrocyte glycoprotein-antibody associated disorder, sarcoidosis, Sjögren syndrome
- (Acute onset) chronic inflammatory demyelinating polyneuropathy (CIDP)[c]
- Myasthenia gravis
- Lambert–Eaton myasthenic syndrome

Metabolic
- Electrolyte disorders such as hypokalemia or hypokalemic thyrotoxic periodic paralysis (common), hypophosphatemia or hypermagnesemia
- Deficiency of vitamins B1 (associated with beri beri or Wernicke's encephalopathy); B12 (associated with subacute combined degeneration of the spinal cord) and E[c]
- Porphyria
- Diabetic neuropathy and/or drug-induced diabetic neuropathy[c]
- Hyperthyroidism and hypothyroidism
- Copper deficiency

Malignancy
- Leptomeningeal metastases or neurolymphomatosis[c]
- Brainstem or spinal cord tumour[b]

Vascular
- Brainstem or spinal cord stroke[c]
- Vasculitis[c]

Toxins
- Organophosphates (common), lead, thallium, arsenic, diethylene glycol, ethylene glycol, methanol and *N*-hexane
- Ethylalcohol (ethanol) or paraquat poisoning
- Drug-induced (for example by colchicine, chloroquine, emetine or statins
- Snakebite envenomation

Mechanical factors
- Compression of the brainstem or spinal cord[b]
- Cauda equina syndrome

Other
- Functional and/or conversion disorder
- Critical illness polyneuropathy
- Myopathy or acute rhabdomyolysis
- Mitochondrial disease

CMV= cytomegalovirus, EBV=Epstein-Barr virus, VZV=varicella zoster virus, TB= tuberculosis
[a]Polio has been eradicated in most regions, with the exception of several countries in sub-Saharan Africa and Southeast Asia (mostly Pakistan), where sporadic cases can occur. Although this Box mainly focuses on the differential diagnosis of GBS in adults, [b]diagnoses that are more common in children than adults and [c]diagnoses that are less common in children than in adults are indicated.

The differential diagnosis of paediatric GBS differs from that in adults owing to the presence of atypical or non-specific features that complicate the diagnosis, such as meningism or poorly localized pain[110, 111]. Furthermore, vascular causes, vitamin deficiencies, drug-induced myopathy or polyneuropathy and chronic inflammatory demyelinating polyneuropathy occur less frequently in children than in adults[110, 111]. These differences between adults and children in the differential diagnosis of GBS occur in both HIC and LMIC, although (as reported for adults) the infectious causes of conditions that mimic paediatric GBS differ between LMIC and HIC.

Treatment

Management of GBS requires a multidisciplinary approach including supportive medical care and immunotherapy. Intravenous immunoglobulin (0.4 g/kg for 5 days) and plasma exchange (usually five sessions at 200–250 ml/kg) are proven and equally effective treatments for GBS[3, 11, 112]. However, most randomized controlled trials that evaluated the effectiveness of these two treatments for GBS were conducted in populations from HIC. These trials mainly included adult patients who were treated either with intravenous immunoglobulin within 2 weeks or with plasma exchange within 4 weeks after the onset of weakness[11, 112]. Included patients had a GBS disability score ≥3 and the majority had the AIDP subtype of GBS[11, 112]. Therefore, the efficacy of these therapies might differ in LMIC, where AMAN and AMSAN are prevalent and patients usually present to hospital in the later stages of disease than they do in HIC.

Considerable variations in treatment protocols for GBS are observed throughout the world[21]. In general, intravenous immunoglobulin is considered the first choice of treatment as it is easy to administer, widely available and associated with a reduced frequency of adverse effects compared with plasma exchange[11, 113]. Conversely, plasma exchange is less costly than intravenous immunoglobulin and could theoretically be a preferred treatment option for GBS in LMIC[59, 114, 115]. However, in practice, clinicians in LMIC face various limitations and obstacles that are not considered by existing GBS therapeutic studies. For example, owing to the low per capita income and lack of coverage by the national health insurance system in Bangladesh, neither intravenous immunoglobulin (~$12,000–16,000) nor plasma exchange (~$4,500–5,000) are affordable for the majority of patients[18]. Therefore, only 10-12% of patients in Bangladesh receive one of these treatments, even though most patients with GBS in Bangladesh are severely affected. For instance, 93% of patients from Bangladesh were unable to walk independently at nadir (GBS disability score >2) in comparison to 76% of patients in Europe, America or other parts of Asia[12, 18, 22, 55]. This situation underscores the need for low-cost and effective treatment strategies for GBS in LMIC. Small volume plasma exchange (SVPE) is a novel,

relatively low cost (~$500), simple technique for selective removal of plasma, and has been shown to be a safe and feasible treatment for GBS in resource-limited settings such as India and Bangladesh[18, 116]. However, as the efficacy of SVPE has only been shown in a small number of patients, large-scale studies are required before this technique can be implemented in routine clinical practice.

Complement inhibitors are a new focus in the treatment of GBS in HIC. Eculizumab, a humanized monoclonal recombinant antibody against complement factor 5, is currently being studied in the UK and Japan[113,71]. Another humanized antibody against complement factor 3 was shown to be safe and well tolerated in patients with GBS[117], and efficacy trials of this agent are currently ongoing in Europe, America and Asia. Although the high cost of these biologic agents is likely to greatly restrict their use in patients with GBS from LMIC, such drugs might be made available for specific indications within LMIC at affordable price levels in the future; for instance, HIV drugs have been made available to some African countries at much lower prices than in HIC[118]. Moreover, several different phases of efficacy trials for complement factor 3 inhibitors are currently ongoing in patients in Bangladesh, which indicates that research groups in some LMICs are able to conduct treatment trials in accordance with the latest scientific methods and regulatory requirements. We hope that this experience will lead to opportunities to develop affordable treatments for patients with GBS in LMIC in future.

Outcome and prognosis

Admission to the ICU is recommended for patients with GBS who have imminent respiratory insufficiency, severe autonomic dysfunction with cardiovascular instability, severe swallowing dysfunction and/or diminished cough reflex or rapidly progressive weakness[55, 58, 119-121]. However, in LMIC the number of ICU beds are limited and ICU services in private hospitals are too costly (~300–1,200 USD daily) for most patients[83, 122]. A study from Bangladesh found that the absence of ICU support when required was the strongest risk factor for death in patients with GBS[22].

In most studies worldwide, the mortality rate for GBS is 2–10%[9, 10, 54] although disparities are evident between regions. For example, reported mortality rates are 2-7% in Europe and North America[10, 12, 19, 67] 13% in Hong Kong,[43] 14-17% in Bangladesh[12, 14, 22] and 16% in Egypt[123]. Moreover, access to integrative rehabilitation services is limited in LMIC, which can adversely affect the recovery and long-term quality of life of patients with GBS[124]. Across the globe, ~20% of patients with GBS are unable to walk unaided 6 months after disease onset[2, 3, 9, 10, 54, 125] and this rate is higher (30-40%) in countries such as Bangladesh where AMAN predominates and most patients do not

receive immunotherapy[12, 14, 66]. In addition to physical complications, a substantial proportion of patients in HIC experience residual problems, including persistent pain (~35-40%), fatigue (60-80%) and anxiety or depression (6-7%)[2, 65, 126]. No data have been reported on rates of these complications in LMIC. However, as most patients with GBS in LMIC only receive supportive care, these sequelae are also likely to vary between countries and to be worse for patients in LMIC than for those in HIC.

The ability to predict which patients with GBS will develop respiratory insufficiency or have a poor prognosis has been a long-held desire worldwide, as it would enable physicians to take the necessary precautions and provide additional treatment for the patients most at risk[48, 127]. To this end, the Erasmus GBS Respiratory Insufficiency Score (EGRIS) was developed to predict the risk of requiring mechanical ventilation within 1 week and the Erasmus GBS Outcome Score (EGOS) and modified EGOS (mEGOS) were developed to predict the outcomes of patients with GBS at 6 months[48, 121, 127]. However, these tools were derived and validated in cohorts from European countries and might not be applicable worldwide. Indeed, a study from northeast Brazil reported that EGOS was not a good predictive tool in that population[128]. By contrast, both EGRIS and mEGOS can accurately predict GBS outcome in populations from Japan and Malaysia[129, 130]. Therefore, these models might need to be validated or adapted before they can be used in LMIC.

Various measures have been employed to capture outcomes in clinical trials of GBS around the world. Improvement in GBS disability scale scores is the main prognostic variable in the majority of studies. The Rasch-Built Overall Disability Score (RODS), Overall Neuropathy Limitations Scale (ONLS), and Fatigue Severity Scale (FSS) were developed as outcome measures for clinical trials and are used to assess disability, activity limitations and fatigue, respectively, in patients with GBS[131-133]. However, these tools were developed in cohorts of patients with GBS from HIC and the questions might not be culturally appropriate in LMIC.

CONCLUSIONS AND FUTURE PROSPECTS

At present, only limited data are available on GBS in LMIC. Most studies in LMIC were conducted in South Asia (Bangladesh and India) and publications from other LMIC are scarce, especially those from Africa. LMIC are hotspots for many emerging infectious disease outbreaks, some of which have been associated with GBS. Therefore, publications from LMIC are often related to outbreaks of GBS associated with specific antecedent infectious diseases. Owing to the lack of well-designed

epidemiological studies, the true incidence of GBS in many LMIC remains largely unknown. The long intervals between onset of weakness and hospitalization that are frequently observed for patients in LMIC might introduce selection bias at the hospital level, as patients with mild symptoms might not reach the health system and severely affected individuals might die before reaching the hospital. Moreover, diagnostic facilities, health care infrastructure and adequately trained health professionals are frequently lacking in LMIC. The absence of national treatment guidelines and high costs of existing treatments relative to local wages contribute to the worse outcomes and higher mortality rates of GBS in LMIC compared with HIC. Finally, current models for predicting the outcome of GBS might not be valid in LMIC, owing to variations in disease severity, clinical presentation, electrophysiological subtypes and management.

A number of strategies can address these challenges. Firstly, the expansion and improvement of GBS research capacity in LMIC is required. Systematic population-based surveillance and cohort studies that employ accurate standardized case definitions are needed to understand and monitor the incidence and overall burden of GBS. Case–control studies are crucial to identify the risk factors associated with GBS and to detect new antecedent infections that trigger GBS in LMIC. Observational cohort studies are important to define the clinical course of GBS and the factors that influence and predict this course. An example of a cohort study of GBS that is ongoing globally in both LMIC and HIC is the International GBS Outcome Study (IGOS).[96] The standardized trial protocol and web-based data entry system used in this international prospective cohort study are an example of how methodological differences between GBS studies conducted in different regions and countries might be overcome. However, African and Latin American countries and regions are underrepresented in IGOS, and expanding the study to these regions and the long-term sustainability of this global initiative needs to be assured. Nonetheless, IGOS has already highlighted differences in the presentation and outcome of patients with GBS between HIC and LMIC such as Bangladesh, which provide insight into the challenges associated with caring for these patients in LMIC that might facilitate future research[12].

Affordable and cost-effective treatment strategies need to be developed and multinational efficacy trials are required to study and scale up innovative treatment approaches. Several randomized controlled trials, including a safety, feasibility and efficacy trial of SVPE and a phase I (leading to phase II–III) trial of a new investigational drug are currently being conducted in Asia, Europe and the USA. Additional clinical intervention studies of innovative and affordable treatments need to be

designed, taking into account the specific context of the health system challenges in LMIC. A sustainable clinical trial infrastructure including physical health-care facilities and adequately trained health professionals needs to be established to support research into GBS in LMIC; these efforts should include high-quality diagnostic laboratories and training programmes for health-care professionals involved in the management of patients with GBS and in clinical research.

Moreover, existing prognostic models need to be validated and adapted for use in LMIC. Such tools would help clinicians in LMIC to accurately identify the patients most in need of ICU care at an early stage, thereby improving the management of individual patients and increasing the efficiency of ICU services in low-resource settings. Valid, responsive and cross-culturally applicable outcome measures need to be developed to improve our understanding of the long-term outcome of GBS in LMIC and to optimize the management of patients in rehabilitation services. Patients and their caregivers can also contact the GBS|CIDP Foundation International for support (https://www.gbs-cidp.org/).

In conclusion, GBS is an under-reported disease in LMIC, although the limited available evidence suggests that the disease has a more-severe clinical course in LMIC and that affected patients in LMIC have worse outcomes than do their counterparts in HIC. This Review highlights the most important knowledge gaps and provides suggestions and recommendations for future research. Increasing the breadth and quality of fundamental and applied research should become a critical focus to improve the clinical management of GBS in LMIC in the future. More than 100 years after the first description of the syndrome by George Guillain, Jean Alexandre Barré and André Strohl, now is the time to reduce the disease burden of GBS in LMIC.

KEY POINTS

- The considerable regional variation evident in the epidemiology, subtypes and management of GBS can be explained by geography, population demographics, environmental and economic factors.
- Poor hygiene and sanitation along with frequent exposure to pathogens render populations in low and middle income countries (LMIC) prone to outbreaks of infectious diseases that can trigger GBS.
- High rates of adverse outcomes and mortality in LMIC can be explained by insufficient health care infrastructure leading to diagnostic delays, and the lack of available and affordable treatment.

- Owing to differences in disease severity, clinical presentation and patient management between high-income countries (HIC) and LMIC, existing models to predict the outcome of GBS must be validated for LMIC.
- New and low-cost treatment strategies for GBS need to be developed along with improved access to integrative rehabilitation services in LMIC.

SUPPLEMENTARY MATERIAL

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REFERENCES

1. Fokke C, van den Berg B, Drenthen J, Walgaard C, van Doorn PA, Jacobs BC. Diagnosis of Guillain-Barré syndrome and validation of Brighton criteria. *Brain* 2014;137:33-43.
2. Van Den Berg B, Walgaard C, Drenthen J, Fokke C, Jacobs BC, Van Doorn PA. Guillain–Barré syndrome: pathogenesis, diagnosis, treatment and prognosis. *Nature Reviews Neurology* 2014;10:469.
3. Van Doorn PA, Ruts L, Jacobs BC. Clinical features, pathogenesis, and treatment of Guillain-Barré syndrome. *The Lancet Neurology* 2008;7:939-950.
4. Parra B, Lizarazo J, Jiménez-Arango JA, et al. Guillain–Barré syndrome associated with Zika virus infection in Colombia. *New England Journal of Medicine* 2016;375:1513-1523.
5. Leonhard SE, Conde RM, de Assis Aquino Gondim F, Jacobs BC. Diagnosis and treatment of Guillain-Barré syndrome during the Zika virus epidemic in Brazil: A national survey study. *Journal of the Peripheral Nervous System* 2019;24:340-347.
6. Cao-Lormeau V-M, Blake A, Mons S, et al. Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *The Lancet* 2016;387:1531-1539.
7. Dimachkie MM, Barohn RJ. Guillain-Barré syndrome and variants. *Neurologic clinics* 2013;31:491-510.
8. Hosokawa T, Nakajima H, Unoda K, et al. Serial electrophysiological findings in Guillain–Barré syndrome not fulfilling AIDP or AMAN criteria. *Journal of neurology* 2016;263:1709-1718.
9. Yuki N, Hartung H-P. Guillain–Barré syndrome. *New England Journal of Medicine* 2012;366:2294-2304.
10. Willison HJ, Jacobs BC, Van Doorn PA. Guillain-Barré syndrome. *The Lancet* 2016;388:717-727.
11. Hughes RA, Swan AV, Raphaël J-C, Annane D, van Koningsveld R, van Doorn PA. Immunotherapy for Guillain-Barré syndrome: a systematic review. *Brain* 2007;130:2245-2257.
12. Doets AY, Verboon C, Van Den Berg B, et al. Regional variation of Guillain-Barré syndrome. *Brain* 2018;141:2866-2877.
13. Worldbank. World Bank Country and Lending Groups [online]. Available at: https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups
14. Islam Z, Jacobs B, van Belkum A, et al. Axonal variant of Guillain-Barre syndrome associated with Campylobacter infection in Bangladesh. *Neurology* 2010;74:581-587.
15. Pal M, Ayele Y, Hadush M, Panigrahi S, Jadhav VJ. Public health hazards due to unsafe drinking water. *Air Water Borne Dis* 2018;7:2.
16. Zhang M, Li Q, He L, et al. Association study between an outbreak of Guillain-Barre syndrome in Jilin, China, and preceding Campylobacter jejuni infection. *Foodborne pathogens and disease* 2010;7:913-919.
17. Jackson B, Zegarra JA, Lopez-Gatell H, et al. Binational outbreak of Guillain–Barré syndrome associated with Campylobacter jejuni infection, Mexico and USA, 2011. *Epidemiology & Infection* 2014;142:1089-1099.
18. Islam B, Islam Z, Rahman S, et al. Small volume plasma exchange for Guillain-Barré syndrome in resource-limited settings: a phase II safety and feasibility study. *BMJ open* 2018;8:e022862.

19. Roodbol J, de Wit M-CY, van den Berg B, et al. Diagnosis of Guillain–Barré syndrome in children and validation of the Brighton criteria. *Journal of neurology* 2017;264:856-861.
20. Van der Meché F, Van Doorn P, Meulstee J, Jennekens F. Diagnostic and classification criteria for the Guillain-Barré syndrome. *European neurology* 2001;45:133-139.
21. Verboon C, Doets AY, Galassi G, et al. Current treatment practice of Guillain-Barré syndrome. *Neurology* 2019;93:e59-e76.
22. Ishaque T, Islam MB, Ara G, et al. High mortality from Guillain-Barré syndrome in Bangladesh. *Journal of the Peripheral Nervous System* 2017;22:121-126.
23. Nagappa M, Rahul W, Sinha S, et al. Guillain Barre Syndrome in the elderly: Experience from a tertiary-care hospital in India. *Journal of Clinical Neuroscience* 2017;46:45-49.
24. Sejvar JJ, Baughman AL, Wise M, Morgan OW. Population incidence of Guillain-Barré syndrome: a systematic review and meta-analysis. *Neuroepidemiology* 2011;36:123-133.
25. McGrogan A, Madle GC, Seaman HE, De Vries CS. The epidemiology of Guillain-Barré syndrome worldwide. *Neuroepidemiology* 2009;32:150-163.
26. Islam Z, Jacobs BC, Islam MB, Mohammad QD, Diorditsa S, Endtz HP. High incidence of Guillain-Barre syndrome in children, Bangladesh. *Emerging infectious diseases* 2011;17:1317.
27. Hughes RA, Cornblath DR. Guillain-barre syndrome. *The Lancet* 2005;366:1653-1666.
28. Van Koningsveld R, Rico R, Gerstenbluth I, et al. Gastroenteritis-associated Guillain–Barre syndrome on the Caribbean island Curacao. *Neurology* 2001;56:1467-1472.
29. Asbury AK, Cornblath DR. Assessment of current diagnostic criteria for Guillain-Barré syndrome. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society* 1990;27:S21-S24.
30. Webb AJ, Brain SA, Wood R, Rinaldi S, Turner MR. Seasonal variation in Guillain-Barré syndrome: a systematic review, meta-analysis and Oxfordshire cohort study. *Journal of Neurology, Neurosurgery & Psychiatry* 2015;86:1196-1201.
31. Wakerley BR, Yuki N. Infectious and noninfectious triggers in Guillain–Barré syndrome. *Expert review of clinical immunology* 2013;9:627-639.
32. Vellozzi C, Iqbal S, Broder K. Guillain-Barre syndrome, influenza, and influenza vaccination: the epidemiologic evidence. *Clinical infectious diseases* 2014;58:1149-1155.
33. Ho T, Mishu B, Li C, et al. Guillain-Barre syndrome in northern China Relationship to Campylobacter jejuni infection and anti-glycolipid antibodies. *Brain* 1995;118:597-605.
34. Platts-Mills JA, Babji S, Bodhidatta L, et al. Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). *The Lancet Global Health* 2015;3:e564-e575.
35. Larrosa-Haro A, Macias-Rosales R, Sánchez-Ramírez CA, Cortés-López MC, Aguilar-Benavides S. Seasonal variation of enteropathogens in infants and preschoolers with acute diarrhea in western Mexico. *Journal of pediatric gastroenterology and nutrition* 2010;51:534-536.
36. Bahemuka M. Guillain-Barre syndrome in Kenya: a clinical review of 54 patients. *Journal of neurology* 1988;235:418-421.
37. Charra B, Hachimi A, Benslama A, Motaouakkil S. Intravenous immunoglobulin vs plasma exchange in treatment of mechanically ventilated adults with Guillain-Barré syndrome. *Pan African Medical Journal* 2014;18.

38. Cheng BC, Chang WN, Chang CS, et al. Guillain–Barré syndrome in southern Taiwan: clinical features, prognostic factors and therapeutic outcomes. *European journal of neurology* 2003;10:655-662.
39. Howlett W, Vedeler C, Nyland H, Aarli J. Guillain-Barré syndrome in northern Tanzania: a comparison of epidemiological and clinical findings with western Norway. *Acta neurologica scandinavica* 1996;93:44-49.
40. Liou L-S, Chung C-H, Wu Y-T, et al. Epidemiology and prognostic factors of inpatient mortality of Guillain-Barré syndrome: a nationwide population study over 14 years in Asian country. *Journal of the neurological sciences* 2016;369:159-164.
41. Melaku Z, Zenebe G, Bekele A. Guillain-Barré syndrome in Ethiopian patients. *Ethiopian medical journal* 2005;43:21-26.
42. Netto AB, Taly AB, Kulkarni GB, Rao UG, Rao S. Mortality in mechanically ventilated patients of Guillain Barré Syndrome. *Annals of Indian Academy of Neurology* 2011;14:262.
43. Siddiqui SH, Siddiqui TH, Babar MU, Khoja A, Khan S. Outcomes of patients with Guillain Barre Syndrome–Experience from a tertiary care hospital of a developing Asian country and review of regional literature. *Journal of Clinical Neuroscience* 2019;62:195-198.
44. Wierzba TF, Abdel-Messih IA, Bayoumi Gharib SB, et al. Campylobacter infection as a trigger for Guillain-Barre syndrome in Egypt. *PloS one* 2008;3.
45. Dourado M, Felix R, da Silva W, Queiroz J, Jeronimo S. Clinical characteristics of Guillain–Barré syndrome in a tropical country: a Brazilian experience. *Acta neurologica scandinavica* 2012;125:47-53.
46. Meena A, Khadilkar S, Murthy J. Treatment guidelines for Guillain–Barré syndrome. *Annals of Indian Academy of Neurology* 2011;14:S73.
47. Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, Man SM. Global epidemiology of Campylobacter infection. *Clinical microbiology reviews* 2015;28:687-720.
48. van Koningsveld R, Steyerberg EW, Hughes RA, Swan AV, van Doorn PA, Jacobs BC. A clinical prognostic scoring system for Guillain-Barré syndrome. *The Lancet Neurology* 2007;6:589-594.
49. Bae JS, Yuki N, Kuwabara S, et al. Guillain–Barré syndrome in Asia. *J Neurol Neurosurg Psychiatry* 2014;85:907-913.
50. Mitsui Y, Kusunoki S, Arimura K, et al. A multicentre prospective study of Guillain-Barré Syndrome in Japan: a focus on the incidence of subtypes. *Journal of Neurology, Neurosurgery & Psychiatry* 2015;86:110-114.
51. Ng Y, Lo Y, Lim P. Characteristics and acute rehabilitation of Guillain-Barre syndrome in Singapore. *Annals-Academy of Medicine Singapore* 2004;33:314-319.
52. Koga M, Yuki N, Tai T, Hirata K. Miller Fisher syndrome and Haemophilus influenzae infection. *Neurology* 2001;57:686-691.
53. Zhang G, Li Q, Zhang R, Wei X, Wang J, Qin X. Subtypes and prognosis of Guillain-Barré syndrome in southwest China. *PLoS One* 2015;10.
54. Lehmann HC, Hughes RA, Kieseier BC, Hartung HP. Recent developments and future directions in Guillain-Barré syndrome. *Journal of the Peripheral Nervous System* 2012;17:57-70.
55. Islam Z, Papri N, Ara G, et al. Risk factors for respiratory failure in Guillain-Barré syndrome in Bangladesh: a prospective study. *Annals of clinical and translational neurology* 2019;6:324-332.

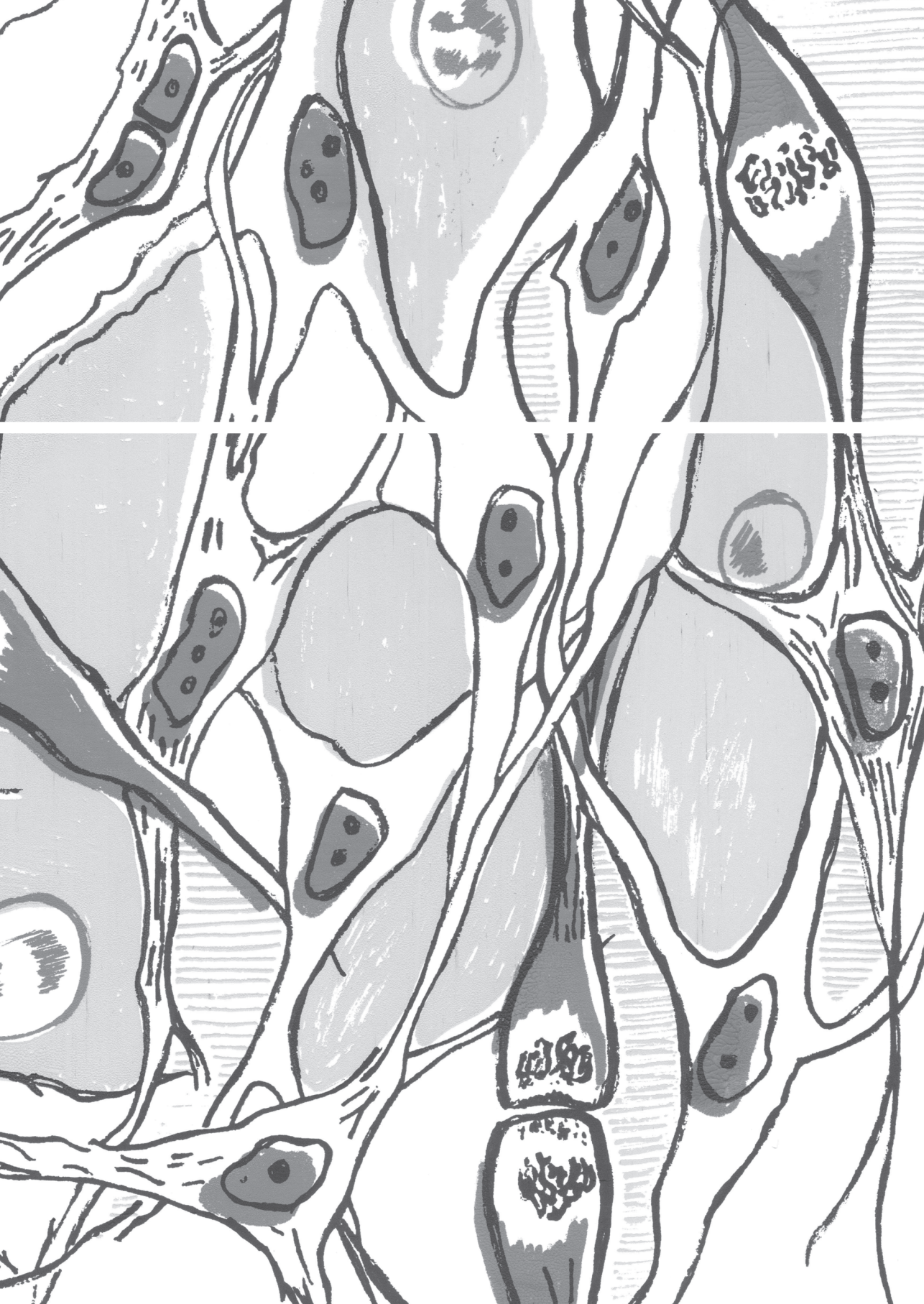
56. Islam MB, Islam Z, Farzana KS, et al. Guillain-Barré syndrome in Bangladesh: validation of Brighton criteria. *Journal of the Peripheral Nervous System* 2016;21:345-351.
57. Sudulagunta SR, Sodalagunta MB, Sepehrar M, et al. Guillain-Barré syndrome: clinical profile and management. *GMS German Medical Science* 2015;13.
58. Kalita J, Ranjan A, Misra U. Outcome of Guillain–Barre syndrome patients with respiratory paralysis. *QJM: An International Journal of Medicine* 2016;109:319-323.
59. Chaudhuri JR, Alladi S, Mridula KR, et al. Clinical outcome of Guillain-Barré syndrome with various treatment methods and cost effectiveness: A study from tertiary care center in South India: Yashoda GBS Registry. *Neurology Asia* 2014;19.
60. Bhagat SK, Sidhant S, Bhatta M, Ghimire A, Shah B. Clinical Profile, Functional Outcome, and Mortality of Guillain-Barre Syndrome: A Five-Year Tertiary Care Experience from Nepal. *Neurology research international* 2019;2019.
61. Shafqat S, Khealani B, Awan F, Abedin S. Guillain–Barré syndrome in Pakistan: similarity of demyelinating and axonal variants. *European journal of neurology* 2006;13:662-665.
62. Zeng Y, Liu Y, Xie Y, Liang J, Xiao Z, Lu Z. Clinical Features and the Validation of the Brighton Criteria in Guillain-Barré Syndrome: Retrospective Analysis of 72 Hospitalized Patients in Three Years. *European Neurology* 2019;81:231-238.
63. Hao Y, Wang W, Jacobs BC, et al. Antecedent infections in Guillain-Barré syndrome: a single-center, prospective study. *Annals of clinical and translational neurology* 2019;6:2510-2517.
64. Park Y-S, Lee K-J, Kim SW, Kim KM, Suh BC. Clinical features of post-vaccination Guillain-Barré syndrome (GBS) in Korea. *Journal of Korean medical science* 2017;32:1154-1159.
65. Khan F, Pallant J, Ng L, Bhasker A. Factors associated with long-term functional outcomes and psychological sequelae in Guillain–Barre syndrome. *Journal of neurology* 2010;257:2024-2031.
66. Hiraga A, Mori M, Ogawara K, et al. Recovery patterns and long term prognosis for axonal Guillain–Barré syndrome. *Journal of Neurology, Neurosurgery & Psychiatry* 2005;76:719-722.
67. González-Suárez I, Sanz-Gallego I, de Rivera FJR, Arpa J. Guillain-Barré syndrome: natural history and prognostic factors: a retrospective review of 106 cases. *BMC neurology* 2013;13:95.
68. Principi N, Esposito S. Vaccine-preventable diseases, vaccines and Guillain-Barre'syndrome. *Vaccine* 2019;37:5544-5550.
69. Rudant J, Dupont A, Mikaeloff Y, Bolgert F, Coste J, Weill A. Surgery and risk of Guillain-Barré syndrome: a French nationwide epidemiologic study. *Neurology* 2018;91:e1220-e1227.
70. Hiew FL, Rajabally YA. Malignancy in Guillain-Barré syndrome: a twelve-year single-center study. *Journal of the Neurological sciences* 2017;375:275-278.
71. Poropatich KO, Walker CLF, Black RE. Quantifying the association between Campylobacter infection and Guillain-Barré syndrome: a systematic review. *Journal of health, population, and nutrition* 2010;28:545.
72. Willison HJ, O'Hanlon GM. The immunopathogenesis of Miller Fisher syndrome. *Journal of neuroimmunology* 1999;100:3-12.
73. Takahashi M, Koga M, Yokoyama K, Yuki N. Epidemiology of Campylobacter jejuni isolated from patients with Guillain-Barré and Fisher syndromes in Japan. *J Clin Microbiol* 2005;43:335-339.

74. Baker MG, Kvalsvig A, Zhang J, Lake R, Sears A, Wilson N. Declining Guillain-Barré syndrome after campylobacteriosis control, New Zealand, 1988–2010. *Emerging infectious diseases* 2012;18:226.
75. Styczynski AR, Malta JM, Krow-Lucal ER, et al. Increased rates of Guillain-Barré syndrome associated with Zika virus outbreak in the Salvador metropolitan area, Brazil. *PLoS neglected tropical diseases* 2017;11:e0005869.
76. Kanjalkar M, Karnad D, Narayana R, Shah P. Guillain-Barre syndrome following malaria. *Journal of Infection* 1999;38:48-50.
77. Sithinamsuwan P, Sinsawaiwong S, Limapichart K. Guillain-Barre's syndrome associated with Plasmodium falciparum malaria: role of plasma exchange. *Journal of the Medical Association of Thailand= Chotmaihet thangphaet* 2001;84:1212-1216.
78. Wijesundere A. Guillain-Barré syndrome in Plasmodium falciparum malaria. *Postgraduate medical journal* 1992;68:376.
79. Thornton CA, Latif AS, Emmanuel JC. Guillain-Barré syndrome associated with human immunodeficiency virus infection in Zimbabwe. *Neurology* 1991;41:812-812.
80. Gupta P, Jain V, Chatterjee S, Agarwal A. Acute inflammatory motor axonopathy associated with dengue fever. *JIACM* 2009;10:58-59.
81. Ralapanawa DMPUK, Kularatne SAM, Jayalath WATA. Guillain–Barre syndrome following dengue fever and literature review. *BMC research notes* 2015;8:729.
82. Santos NQ, Azoubel ACB, Lopes AA, Costa G, Bacellar A. Guillain-Barré syndrome in the course of dengue: case report. *Arquivos de neuro-psiquiatria* 2004;62:144-146.
83. Sedaghat Z, Karimi N. Guillain Barre syndrome associated with COVID-19 infection: a case report. *Journal of Clinical Neuroscience* 2020.
84. Toscano G, Palmerini F, Ravaglia S, et al. Guillain–Barré syndrome associated with SARS-CoV-2. *New England Journal of Medicine* 2020.
85. Zhao H, Shen D, Zhou H, Liu J, Chen S. Guillain-Barré syndrome associated with SARS-CoV-2 infection: causality or coincidence? *The Lancet Neurology* 2020;19:383-384.
86. Camdessanche J-P, Morel J, Pozzetto B, Paul S, Tholance Y, Botelho-Nevers E. COVID-19 may induce Guillain-Barré syndrome. *Revue neurologique* 2020.
87. Nanda S, Handa R, Prasad A, et al. Covid-19 associated Guillain-Barre Syndrome: Contrasting tale of four patients from a tertiary care centre in India. *The American Journal of Emergency Medicine* 2020.
88. Sidig A, Abbasher K, Digna MF, et al. COVID-19 and Guillain-Barre Syndrome-a Case report. 2020.
89. El Otmani H, El Moutawakil B, Rafai M-A, et al. Covid-19 and Guillain-Barré syndrome: More than a coincidence! *Revue neurologique* 2020.
90. Keddie S, Pakpoor J, Mousele C, et al. Epidemiological and cohort study finds no association between COVID-19 and Guillain-Barré syndrome. *medRxiv* 2020.
91. Muñoz LS, Parra B, Pardo CA, Study NEitA. Neurological implications of Zika virus infection in adults. *The Journal of Infectious Diseases* 2017;216:S897-S905.
92. Uncini A, Vallat J-M, Jacobs BC. Guillain-Barré syndrome in SARS-CoV-2 infection: an instant systematic review of the first six months of pandemic. *Journal of Neurology, Neurosurgery & Psychiatry* 2020;91:1105-1110.
93. Abu-Rumeileh S, Abdelhak A, Foschi M, Tumani H, Otto M. Guillain–Barré syndrome spectrum associated with COVID-19: an up-to-date systematic review of 73 cases. *Journal of neurology* 2020:1-38.

94. Blum S, McCombe PA. Genetics of Guillain-Barré syndrome (GBS) and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP): current knowledge and future directions. *Journal of the Peripheral Nervous System* 2014;19:88-103.
95. Caporale CM, Papola F, Fioroni MA, et al. Susceptibility to Guillain–Barré syndrome is associated to polymorphisms of CD1 genes. *Journal of neuroimmunology* 2006;177:112-118.
96. Islam Z, Jahan I, Ahammad RU, Shahnaij M, Nahar S, Mohammad QD. FAS promoter polymorphisms and serum sFas level are associated with increased risk of nerve damage in Bangladeshi patients with Guillain-Barré syndrome. *PloS one* 2018;13.
97. Harbige LS. Nutrition and immunity with emphasis on infection and autoimmune disease. *Nutrition and Health* 1996;10:285-312.
98. Díaz-Soto S, Chavez K, Chaca A, Alanya J, Tirado-Hurtado I. Outbreak of guillain-barre syndrome in Peru. *eNeurologicalSci* 2019;14:89.
99. Dirlikov E, Kniss K, Major C, et al. Guillain-Barre syndrome and healthcare needs during Zika virus transmission, Puerto Rico, 2016. *Emerging infectious diseases* 2017;23:134.
100. Mier-y-Teran-Romero L, Delorey MJ, Sejvar JJ, Johansson MA. Guillain–Barré syndrome risk among individuals infected with Zika virus: a multi-country assessment. *BMC medicine* 2018;16:67.
101. Jones KE, Patel NG, Levy MA, et al. Global trends in emerging infectious diseases. *Nature* 2008;451:990-993.
102. Ferreira MLB, de Brito CAA, de Oliveira França RF, et al. Neurological disease in adults with Zika and chikungunya virus infection in Northeast Brazil: a prospective observational study. *The Lancet Neurology* 2020;19:826-839.
103. Simon O, Billot S, Guyon D, et al. Early Guillain–Barré Syndrome associated with acute dengue fever. *Journal of Clinical Virology* 2016;77:29-31.
104. Lowe R, Barcellos C, Brasil P, et al. The Zika virus epidemic in Brazil: from discovery to future implications. *International journal of environmental research and public health* 2018;15:96.
105. Zhang Y, Wang D, Han H, Li F, Sheng L, Link H. Epidemiological survey of the incidence of Guillain-Barre syndrome in Harbin from 1997 to 1999. *Chin J Clin Rehab* 2004;34:7812-7815.
106. Leonhard SE, Cornblath DR, Endtz HP, Sejvar JJ, Jacobs BC. Guillain-Barré syndrome in times of pandemics. BMJ Publishing Group Ltd, 2020.
107. Mateen FJ, Cornblath DR, Jafari H, et al. Guillain–Barré Syndrome in India: Population-based validation of the Brighton criteria. *Vaccine* 2011;29:9697-9701.
108. Yuki N, Kokubun N, Kuwabara S, et al. Guillain–Barré syndrome associated with normal or exaggerated tendon reflexes. *Journal of neurology* 2012;259:1181-1190.
109. Jacobs B, Rothbarth P, Van der Meché F, et al. The spectrum of antecedent infections in Guillain-Barré syndrome: a case-control study. *Neurology* 1998;51:1110-1115.
110. Roodbol J, De Wit M, Walgaard C, de Hoog M, Catsman-Berrevoets C, Jacobs B. Recognizing Guillain-Barre syndrome in preschool children. *Neurology* 2011;76:807-810.
111. Korinthenberg R, Schessl J, Kirschner J. Clinical presentation and course of childhood Guillain-Barré syndrome: a prospective multicentre study. *Neuropediatrics* 2007;38:10-17.
112. Khan F, Ng L, Amatya B, Brand C, Turner-Stokes L. Multidisciplinary care for Guillain-Barré syndrome. *Eur J Phys Rehabil Med* 2011;47:607-612.
113. Verboon C, van Doorn PA, Jacobs BC. Treatment dilemmas in Guillain-Barré syndrome. *Journal of Neurology, Neurosurgery & Psychiatry* 2017;88:346-352.

114. Willison HJ, Jacobs BC, van Doorn PA. Guillain-Barré syndrome: surveillance and cost of treatment strategies–authors' reply. *The Lancet* 2017;389:253-254.
115. Kishore CK, Vijayabhaskar J, Vishnu Vardhan R, et al. Management of Guillain–Barre syndrome with plasmapheresis or immunoglobulin: our experience from a tertiary care institute in South India. *Renal failure* 2014;36:732-736.
116. Iyer RR, Shah PH, Roy SSK, Suri SKK. Reducing the economic burden in management of Guillain–Barre syndrome using modified plasmapheresis. *Asian journal of transfusion science* 2016;10:118.
117. Islam Z, Papri N, Jahan I, et al. Inhibition of C1q, Initiator of the Classical Complement Cascade, by ANX005 for the Treatment of Guillain-Barré Syndrome: Results from a Phase 1b Study (763). AAN Enterprises, 2020.
118. Lucchini S, Cisse B, Duran S, et al. Decrease in prices of antiretroviral drugs for developing countries: from political "philanthropy" to regulated markets. *Economics of AIDS and access to HIV/Aids care in developing countries Issues and challenges, Sciences Sociales et Sida* 2003:169-211.
119. Leonhard SE, Mandarakas MR, Gondim FA, et al. Diagnosis and management of Guillain–Barré syndrome in ten steps. *Nature Reviews Neurology* 2019;15:671-683.
120. Wu X, Li C, Zhang B, et al. Predictors for mechanical ventilation and short-term prognosis in patients with Guillain-Barré syndrome. *Critical Care* 2015;19.
121. Walgaard C, Lingsma HF, Ruts L, et al. Prediction of respiratory insufficiency in Guillain-Barre syndrome. *Annals of neurology* 2010;67:781-787.
122. ICU facilities scanty at government hospitals of Bangladesh [online]. Available at: https://www.thedailystar.net/frontpage/icu-facilities-scanty-143884.
123. Halawa EF, Ahmed D, Nada MA. Guillain-Barré syndrome as a prominent cause of childhood acute flaccid paralysis in post polio eradication era in Egypt. *european journal of paediatric neurology* 2011;15:241-246.
124. Bright T, Wallace S, Kuper H. A systematic review of access to rehabilitation for people with disabilities in low-and middle-income countries. *International journal of environmental research and public health* 2018;15:2165.
125. Rajabally YA, Uncini A. Outcome and its predictors in Guillain–Barré syndrome. *J Neurol Neurosurg Psychiatry* 2012;83:711-718.
126. Merkies IS, Kieseier BC. Fatigue, pain, anxiety and depression in guillain-barré syndrome and chronic inflammatory demyelinating polyradiculoneuropathy. *European neurology* 2016;75:199-206.
127. Walgaard C, Lingsma H, Ruts L, Van Doorn P, Steyerberg E, Jacobs B. Early recognition of poor prognosis in Guillain-Barre syndrome. *Neurology* 2011;76:968-975.
128. Dourado Júnior ME, Fernandes UT, Ramos ES, et al. Egos has a reduced capacity to predicts GBS prognosis in Northeast Brazil. *Acta Neurologica Scandinavica* 2018;138:459-462.
129. Yamagishi Y, Suzuki H, Sonoo M, et al. Markers for Guillain-Barré syndrome with poor prognosis: a multi-center study. *Journal of the Peripheral Nervous System* 2017;22:433-439.
130. Tan CY, Razali SN, Goh KJ, Shahrizaila N. The utility of Guillain-Barré syndrome prognostic models in Malaysian patients. *Journal of the Peripheral Nervous System* 2019;24:168-173.
131. Graham RC, Hughes R. A modified peripheral neuropathy scale: the Overall Neuropathy Limitations Scale. *Journal of Neurology, Neurosurgery & Psychiatry* 2006;77:973-976.
132. Lunn MP, Van den Bergh PY. Outcome measures in neuromuscular disease: is the world still flat? *Journal of the Peripheral Nervous System* 2015;20:255-259.

133. Van Nes SI, Vanhoutte EK, Faber CG, et al. Improving fatigue assessment in immune-mediated neuropathies: the modified Rasch-built fatigue severity scale. *Journal of the Peripheral Nervous System* 2009;14:268-278.



Chapter 12

Diagnosis and management of Guillain–Barré syndrome in ten steps

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ABSTRACT

Guillain-Barré syndrome (GBS) is a rare, but potentially fatal, immune-mediated disease of the peripheral nerves and nerve roots that is usually triggered by infections. The incidence of GBS can therefore increase during outbreaks of infectious diseases, as was seen during the Zika virus epidemics in 2013 in French Polynesia and 2015 in Latin America. Diagnosis and management of GBS can be complicated as its clinical presentation and disease course are heterogeneous, and no international clinical guidelines are currently available. To support clinicians, especially in the context of an outbreak, we have developed a globally applicable guideline for the diagnosis and management of GBS.

The guideline is based on current literature and expert consensus, and has a ten-step structure to facilitate its use in clinical practice. We first provide an introduction to the diagnostic criteria, clinical variants and differential diagnoses of GBS. The ten steps then cover early recognition and diagnosis of GBS, admission to the intensive care unit, treatment indication and selection, monitoring and treatment of disease progression, prediction of clinical course and outcome, and management of complications and sequelae.

INTRODUCTION

Guillain–Barré syndrome (GBS) is an inflammatory disease of the peripheral nervous system and is the most common cause of acute flaccid paralysis, with an annual global incidence of approximately 1–2 per 100,000 person–years[1]. GBS occurs more frequently in males than in females and the incidence increases with age, although all age groups can be affected[1]. Patients with GBS typically present with weakness and sensory signs in the legs that progress to the arms and cranial muscles, although the clinical presentation of the disease is heterogeneous and several distinct clinical variants exist. Diagnosis of GBS is based on the patient history and neurological, electrophysiological and cerebrospinal fluid (CSF) examinations[2-4]. Other diseases that produce a similar clinical picture to GBS must be ruled out[4]. Electrophysiological studies provide evidence of peripheral nervous system dysfunction and can distinguish between the subtypes of GBS: acute inflammatory demyelinating polyradiculoneuropathy (AIDP), acute motor axonal neuropathy (AMAN) and acute motor sensory axonal neuropathy (AMSAN)[5]. Disease progression can be rapid, and most patients reach their maximum disability within 2 weeks. About 20% of patients develop respiratory failure and require mechanical ventilation. Cardiac arrhythmias and blood pressure instability can occur owing to involvement of the autonomic nervous system[6]. This involvement of the autonomic nervous system contributes to mortality, which is estimated at 3–10% of patients even with the best medical care available[7-9]. After the initial progressive phase, patients reach a plateau phase that can last from days to weeks or months, after which they start to recover, and 60–80% of patients are able to walk independently 6 months after disease onset, with or without treatment[10, 11]. GBS is a monophasic illness, although some patients can deteriorate after first stabilizing or improving on therapy — a phenomenon that is referred to as a treatment-related fluctuation (TRF). Relapses of GBS can occur in 2–5% of patients[10, 12-15].

GBS is thought to be caused by an aberrant immune response to infections that results in damage to peripheral nerves, although the pathogenesis is not fully understood. In a subgroup of patients with GBS, serum antibodies are found against gangliosides, which reside at high densities in the axolemma and other components of the peripheral nerves[16, 17]. Complement activation, infiltration of macrophages and oedema are typical characteristics of affected peripheral nerves and nerve roots in patients with GBS[16].

The incidence of GBS can increase during outbreaks of infectious illnesses that trigger the disease[18]. Most recently, the Zika virus epidemics in French Polynesia in 2013

and in Latin America and the Caribbean in 2015–2016 were linked to an increase in individuals being diagnosed with GBS[19-21].

The Zika virus outbreaks brought to light the lack of globally applicable guidelines for the diagnosis and management of GBS. Such guidelines are necessary because the diagnosis of GBS can be challenging owing to heterogeneity in clinical presentation, an extensive differential diagnosis, and the lack of highly sensitive and specific diagnostic tools or biomarkers. Guidance for the treatment and care of patients with GBS is also needed because disease progression can vary greatly between patients, which complicates an entirely prescriptive approach to management. In addition, treatment options are limited and costly, and many patients experience residual disability and complaints that can be difficult to manage.

Availability of globally applicable clinical guidelines for GBS is especially important as new outbreaks of pathogens that trigger GBS are likely to occur in the future. To generate this globally applicable clinical guideline for GBS, the ten most important steps in the management of GBS, covering diagnosis, treatment, monitoring, prognosis and long-term management, were identified by a group of international GBS experts (**Figure 1**). For each step, recommendations were provided on the basis of evidence from the literature and/or expert opinion, and consensus was sought for each recommendation to finalize the guideline. These recommendations are intended to assist healthcare providers in clinical decision- making; however, the use of the information in this article is voluntary. The authors assume no responsibility for any injury or damage to persons or property arising out of or related to any use of this information, or for any errors or omissions.

METHODS

Following the outbreak of Zika virus and its association with an increase in the incidence of GBS, the European Union-funded Zika Preparedness Latin American Network (ZikaPLAN) was established[22]. Our new guideline was initially prepared by participants of the ZikaPLAN network, comprising GBS experts from the Netherlands (S.E.L., M.R.M. and B.C.J.), Brazil (F.G. and M.E.D.) and the UK (H.J.W.). These members brought specific clinical and research expertise to the guideline from their leading roles in large international projects on GBS (such as the International GBS Outcome Study, IGOS), along with direct experience in managing the large increases in GBS cases in Zika virus-affected regions of Latin America[23]. To develop the preliminary guidelines, a series of in-person meetings were held between lead authors on the

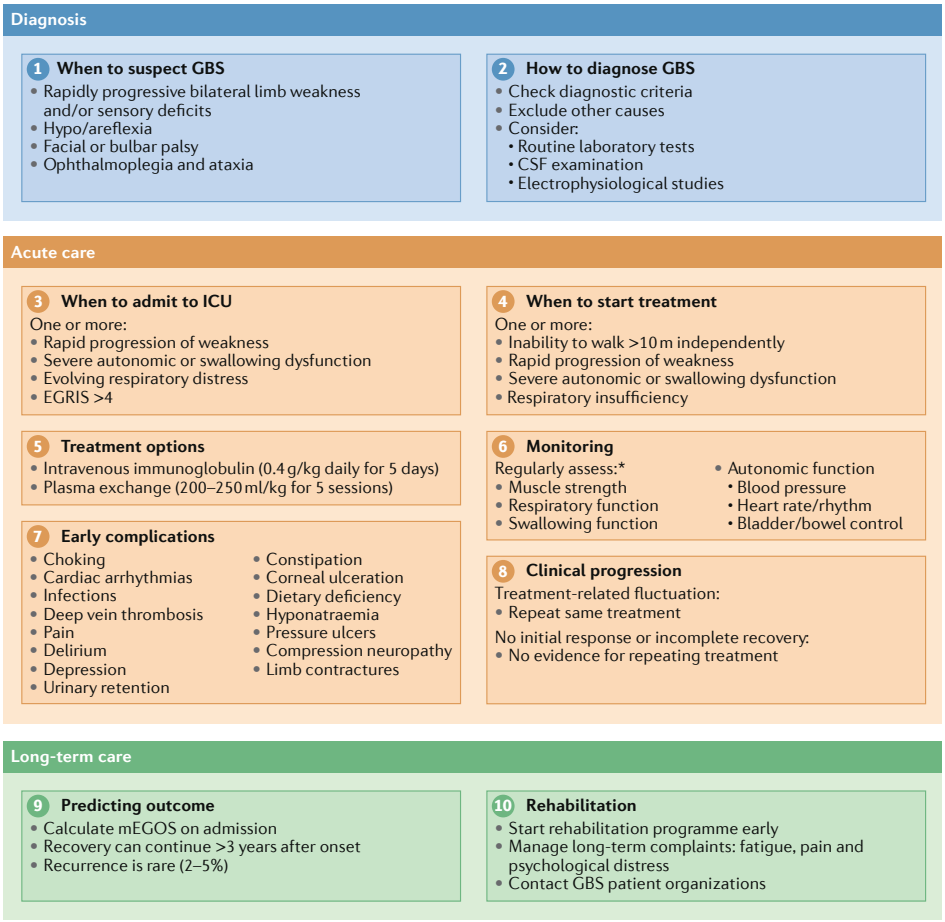

Figure 1. Ten-step approach to the diagnosis and management of Guillain–Barré syndrome. This bullet point summary provides an overview of each of the ten steps described in the guideline. Frequency of monitoring is dependent on the clinical picture, and should be assessed in individual patients. CSF, cerebrospinal fluid; EGRIS, Erasmus GBS Respiratory Insufficiency Score (Box 3); GBS, Guillain-Barré syndrome; ICU, intensive care unit; MCU, medium care unit; mEGOS, modified Erasmus GBS Outcome Score (Supplementary Table 3); MFS, Miller Fisher syndrome.

writing committee (S.E.L., M.R.M., B.C.J. and H.J.W.), along with smaller individual meetings with colleagues in Latin America (S.E.L., F.G. and M.E.D.) and continuous email correspondence to review drafts and receive input. On the basis of their expert opinion and through consensus, this group identified ten of the most important steps in the diagnosis and management of GBS.

For each step, structured literature searches were performed in October 2018 by members of the writing committee (S.E.L and M.R.M), using PubMed and Embase, and the results of these searches provided the basis for the first draft of the guideline.

The main inclusion criterion for the literature searches was any study, trial, review or case report published from 2015 onwards that provided detail on the diagnosis, treatment, management or prognosis of patients with GBS. Publications on the pathogenesis of GBS, or those with a focus on diseases not related to GBS, along with publications written in a language other than English or Dutch were excluded from the review. Key words used in the search strategy included the following Medical Subject Headings (MeSH) terms: "Guillain–Barré syndrome" AND ["diagnosis" OR "therapeutics" OR "treatment outcome" OR "prognosis"]. To obtain literature for more specific topics, additional MeSH terms were combined with primary search keywords, including "intravenous immunoglobulins", "plasma exchange", "intensive care units", "pregnancy", "Miller Fisher syndrome" and "HIV". Following this review of the most recent literature, landmark studies published prior to 2015 were identified for inclusion by the writing committee (S.E.L., M.R.M., B.C.J. and H.J.W.), along with additional papers selected by screening the reference lists of already included manuscripts and consultation with the authors. Where possible, our recommendations regarding treatment were based on systematic reviews. Expert opinion from the authors was sought for recommendations when more limited evidence (for example, cohort studies or case–control studies) was available, for instance on topics regarding the differential diagnosis or rehabilitation of GBS.

In consideration of the global variation in health-care context and clinical variants of GBS, this first draft was subsequently reviewed by an international group of GBS experts from Argentina (R.R.), Australia (E.M.Y.), Bangladesh (B.I.), Brazil (M.L.B.F. and C.S.), China (Y.W.), Colombia (C.A.P.), Japan (S.K.), Malaysia (N.S.), the Netherlands (P.A.D.), Singapore (T.U.), South Africa (K.B.), the USA (D.R.C. and J.J.S.) and the UK (R.A.C.H). In total, seven rounds of review were held to reach a consensus. To consider the perspective of patients with GBS on the management of the disease, the GBS/CIDP Foundation International, a non-profit organization that provides support, education, research funding and advocacy to patients with GBS or chronic inflammatory demyelinating polyneuropathy (CIDP) and their families, reviewed the manuscript and provided comment during the development of the guideline.

To enhance the global usability of these guidelines, we have translated them to Portuguese Spanish, and Chinese (Mandarin). To ensure an accurate translation, we assembled a group of co-authors who are native speakers to coordinate the translation process and review the translations. The translation was done by the ISO certified translating agency Etymax. The manuscript was first translated and this translation was then back-translated to English by a different translator. Both the translation

and the English back-translation were reviewed by the review committee and edited if deemed necessary. See Appendix of this thesis for the published translations.

Step 1: when to suspect GBS

Typical clinical features

GBS should be considered as a diagnosis in patients who have rapidly progressive bilateral weakness of the legs and/or arms, in the absence of central nervous system involvement or other obvious causes. Patients with the classic sensorimotor form of GBS present with distal paraesthesias or sensory loss, accompanied or followed by weakness that starts in the legs and progresses to the arms and cranial muscles. Reflexes are decreased or absent in most patients at presentation and in almost all patients at nadir[10, 24]. Dysautonomia is common and can include blood pressure or heart rate instability, pupillary dysfunction, and bowel or bladder dysfunction[25]. Pain is frequently reported and can be muscular, radicular or neuropathic[26]. Disease onset is acute or subacute, and patients typically reach maximum disability within 2 weeks[11]. In patients who reach maximum disability within 24 h of disease onset or after 4 weeks, alternative diagnoses should be considered[2, 3]. GBS has a monophasic clinical course, although TRFs and relapses occur in a minority of patients[12, 13].

Atypical clinical presentation

GBS can also present in an atypical manner. Weakness and sensory signs, though always bilateral, can be asymmetrical or predominantly proximal or distal, and can start in the legs, the arms or simultaneously in all limbs[6, 26]. Furthermore, severe and diffuse pain or isolated cranial nerve dysfunction can precede the onset of weakness[26]. Young (<6 years) children in particular can present with non-specific or atypical clinical features, such as poorly localized pain, refusal to bear weight, irritability, meningism, or an unsteady gait[27, 28]. Failure to recognize these signs as an early presentation of GBS might cause delay in diagnosis[28]. In a minority of patients with atypical GBS, particularly those with only motor signs (pure motor variant) and an AMAN subtype on electrophysiological examination, normal or even exaggerated reflexes might be observed throughout the disease course[29].

Variants

Some patients have a distinct and persistent clinical variant of GBS that does not progress to the classic pattern of sensory loss and weakness. These variants include weakness without sensory signs (pure motor variant); weakness limited to the cranial nerves (bilateral facial palsy with paraesthesias), upper limbs (pharyngeal–cervical–brachial weakness) or lower limbs (paraparetic variant); and the Miller Fisher syndrome (MFS), which in its full manifestation consists of ophthalmoplegia,

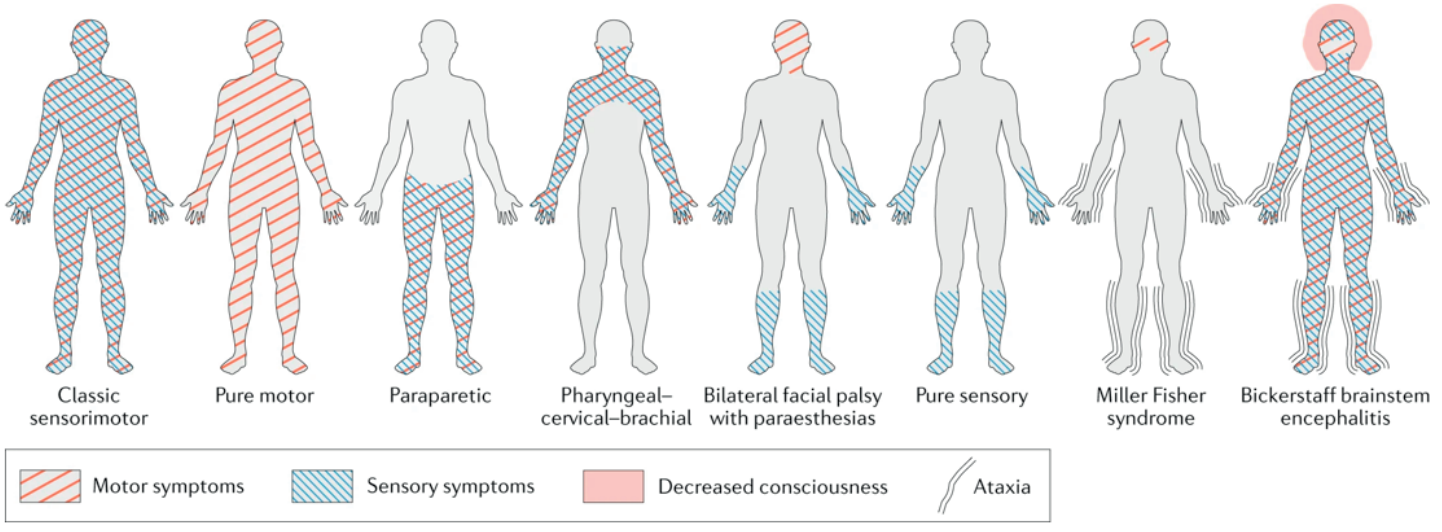

Figure 2. Pattern of symptoms in variants of Guillain–Barré syndrome.
Graphic representation of the pattern of symptoms typically observed in the different clinical variants of Guillain–Barré syndrome (GBS). Symptoms can be purely motor, purely sensory (rare) or a combination of motor and sensory. Ataxia can be present in patients with Miller Fisher syndrome and both decreased consciousness and ataxia can be present in patients with Bickerstaff brainstem encephalitis. Symptoms can be localized to specific regions of the body, and the pattern of symptoms differs between variants of GBS. Although bilateral facial palsy with parathesias, the pure sensory variant and Bickerstaff brainstem encephalitis are included in the GBS spectrum, they do not fulfill the diagnostic criteria for GBS. Adapted with permission from REF.[33], BMJ Publishing Group Limited.

areflexia and ataxia (**Figure 2** and **Table 1**)[6, 30, 31]. In general, GBS variants are rarely 'pure' and often overlap in part with the classic syndrome or show features that are typical of other variant forms[32].

Table 1. Variants of Guillain–Barré syndrome

Variant	Frequency (% of GBS cases)[a]	Clinical features	Refs
Classic sensorimotor GBS[b]	30–85	Rapidly progressive symmetrical weakness and sensory signs with absent or reduced tendon reflexes, usually reaching nadir within 2 weeks	11, 24, 34, 35
Pure motor[c]	5–70	Motor weakness without sensory signs	5, 11, 24
Paraparetic	5–10	Paresis restricted to the legs	10, 24, 35
Pharyngeal–cervical–brachial	<5	Weakness of pharyngeal, cervical and brachial muscles without lower limb weakness	10, 34, 35
Bilateral facial palsy with paraesthesias[d]	<5	Bilateral facial weakness, paraesthesias and reduced reflexes	34-36
Pure sensory[d]	<1	Acute or subacute sensory neuropathy without other deficits	37, 38
Miller Fisher syndrome	5–25	Ophthalmoplegia, ataxia and areflexia. Incomplete forms with isolated ataxia (acute ataxic neuropathy) or ophthalmoplegia (acute ophthalmoplegia) can occur.[31] Overlaps with classical sensorimotor GBS in an estimated 15% of patients	11, 24, 34, 36-39
Bickerstaff brainstem encephalitis[d]	<5	Ophthalmoplegia, ataxia, areflexia, pyramidal tract signs and impaired consciousness, often overlapping with sensorimotor GBS	34, 35

[a]Estimated frequencies, with percentages displayed to the nearest 5% , based on ten (primarily adult) cohort studies in various geographical regions[10, 11, 24, 34-39]. Frequencies differ by region and study, contributing to the variability. Most studies are biased owing to exclusion of some of the variants. [b]The sensorimotor form is seen in an estimated 70% of patients with GBS in Europe and the Americas, and in 30–40% of cases in Asia[11]. [c]The pure motor variant is reported in 5–15% of patients with GBS in most studies, but in 70% cases in Bangladesh[11, 40]. [d]Does not fulfil commonly used diagnostic criteria for GBS, which require the presence of bilateral limb weakness or fulfilment of the criteria for Miller Fisher syndrome[3, 4].

Besides the variants listed above, pure sensory ataxia, Bickerstaff brainstem encephalitis (BBE) and a pure sensory variant are often included in the GBS spectrum because they share clinical or pathophysiological features with GBS. However, the inclusion of these clinical variants is subject to debate as they do not fulfil the diagnostic criteria for GBS (**Box 1**)[2, 3, 31]. The pure sensory variant shares clinical features with the classic sensorimotor form of GBS, with the exception of the presence of motor symptoms and signs[31, 41]; pure sensory ataxia and MFS have overlapping clinical

profiles; and patients with BBE usually present with symptoms resembling MFS and subsequently develop signs of brainstem dysfunction, including impaired consciousness and pyramidal tract signs[30-32, 42-44]. Similar to patients with MFS, individuals with sensory ataxia or BBE can exhibit IgG antibodies to GQ1b or other gangliosides in their serum[30, 42]. However, whether pure sensory GBS, pure sensory ataxia and BBE are variants of GBS and/or an incomplete form of MFS is subject to debate, and careful diagnostic work-up is required when these variants are suspected (Boxes 1 and 2) [31, 41, 43].

Box 1: Diagnostic criteria for Guillain–Barré syndrome

Features required for diagnosis

- Progressive bilateral weakness of arms and legs (initially only legs may be involved)[a]
- Absent or decreased tendon reflexes in affected limbs (at some point in clinical course)[a]

Features that strongly support diagnosis

- Progressive phase lasts from days to 4 weeks (usually <2 weeks)
- Relative symmetry of symptoms and signs
- Relatively mild sensory symptoms and signs (absent in pure motor variant)[a]
- Cranial nerve involvement, especially bilateral facial palsy[a]
- Autonomic dysfunction
- Muscular or radicular back or limb pain[b]
- Increased protein level in cerebrospinal fluid (CSF); normal protein levels do not rule out the diagnosis[b]
- Electrodiagnostic features of motor or sensorimotor neuropathy (normal electrophysiology in the early stages does not rule out the diagnosis)[b]

Features that cast doubt on diagnosis

- Increased numbers of mononuclear or polymorphonuclear cells in CSF (>50×10^6/l)
- Marked, persistent asymmetry of weakness
- Bladder or bowel dysfunction at onset or persistent during disease course[b]
- Severe respiratory dysfunction with limited limb weakness at onset[b]
- Sensory signs with limited weakness at onset[a]
- Fever at onset
- Nadir <24 h[b]
- Sharp sensory level indicating spinal cord injury[a]
- Hyper-reflexia or clonus[b]
- Extensor plantar responses[b]
- Abdominal pain[b]
- Slow progression with limited weakness without respiratory involvement
- Continued progression for >4 weeks after start of symptoms[b]
- Alteration of consciousness (except in Bickerstaff brainstem encephalitis)[b]

This box lists the diagnostic criteria for Guillain–Barré syndrome (GBS) developed by the National Institute of Neurological Disorders and Stroke (NINDS)[3], and subsequently modified in a review paper.[6] We have added some features that cast doubt on the diagnosis, which were not mentioned in the original criteria[2, 3, 6], and have made some adaptations to improve readability. These criteria are not applicable to some of the specific variants of GBS, as described in Table 1.

Minor adaptations were made by the authors to a simplified version of the original NINDS criteria, presented by Willison *et al.*[6] to improve clarity and completeness including: [a]Statements in NINDS criteria that were adapted by authors to improve readability. [b]Additional features, which were not included in the NINDS, to provide a more comprehensive list. Note: For clarity, we have omitted 'Features that rule out the diagnosis' from the original NINDS criteria for this adapted version.

Preceding events

About two-thirds of patients who develop GBS report symptoms of an infection in the 6 weeks preceding the onset of the condition[11]. These infections are thought to trigger the immune response that causes GBS[6]. Six pathogens have been temporally associated with GBS in case–control studies: *Campylobacter jejuni*, cytomegalovirus, hepatitis E virus, *Mycoplasma pneumoniae,* Epstein–Barr virus and Zika virus[18, 20, 45]. It has been suggested that other pathogens are linked to GBS on the basis of evidence from case series or epidemiological studies, but their role in the pathogenesis of GBS is uncertain[46-51]. In general, the absence of an antecedent illness does not exclude a diagnosis of GBS, as putative infections or other immunological stimuli can be subclinical.

Vaccines were first linked to GBS in 1976 when a 7.3-fold increase in risk of GBS was observed among non-military individuals in the USA who had received the 'swine' influenza vaccine [52]. The epidemiological link between other vaccines and GBS has been examined many times since then, but only two further studies showed a relationship between GBS and influenza vaccines[53, 54]. These studies suggested an increase of approximately one additional GBS case per one million vaccinations, which is several orders of magnitude lower than that observed for the 1976 influenza vaccine[55, 56]. No other vaccines have been convincingly linked to GBS[15].

A relationship between administration of immunobiologicals (for example, tumor necrosis factor antagonists, immune checkpoint inhibitors or type I interferons) and GBS has been reported on the basis of case series information and biological plausibility[57]. Other events, including but not limited to surgery and malignancy, have been temporally related to GBS, but these relationships lack a clear biological rationale and the epidemiological evidence is limited.[58, 59]

Step 2: how to diagnose GBS

In the absence of sufficiently sensitive and specific disease biomarkers, the diagnosis of GBS is based on clinical history and examination, and is supported by ancillary investigations such as CSF examination and electrodiagnostic studies. The two most commonly used sets of diagnostic criteria for GBS were developed by the National Institute of Neurological Disorders and Stroke (NINDS) in 1978 (revised in 1990) (Box 1)[2, 3] and the Brighton Collaboration in 2011 (**Supplementary Table 1**)[2-4] Both sets of criteria were designed to investigate the epidemiological association between GBS and vaccinations but have since been used in other clinical studies and trials. We consider the NINDS criteria to be more suited to the clinician as they present the clinical features of typical and atypical forms of GBS, although the criteria from the

Brighton Collaboration are also important, widely used, and can help the clinician to classify cases with (typical) GBS or MFS according to diagnostic certainty. Various differential diagnoses must also be kept in mind when GBS is suspected, and some symptoms should raise suspicion of alternative diagnoses (**Boxes 1 and 2**). The role of ancillary investigations in confirming a GBS diagnosis is described in more detail in the next section.

Laboratory investigations

Laboratory testing is guided by the differential diagnosis in individual patients, but in general all patients with suspected GBS will have complete blood counts and blood tests for glucose, electrolytes, kidney function and liver enzymes. Results of these tests can be used to exclude other causes of acute flaccid paralysis, such as infections or metabolic or electrolyte dysfunctions (**Box 2**). Further specific tests may be carried out with the aim of excluding other diseases that can mimic GBS (**Box 2**). Testing for preceding infections does not usually contribute to the diagnosis of GBS, but can provide important epidemiological information during outbreaks of infectious diseases, as was seen in previous outbreaks of Zika virus and *C. jejuni* infection[19, 60] The diagnostic value of measuring serum levels of anti-ganglioside antibodies is limited and assay-dependent. A positive test result can be helpful, especially when the diagnosis is in doubt, but a negative test result does not rule out GBS[61]. Anti-GQ1b antibodies are found in up to 90% of patients with MFS[17, 62] and therefore have greater diagnostic value in patients with suspected MFS than in patients with classic GBS or other variants. When GBS is suspected, we advise not to wait for antibody test results before starting treatment.

Cerebrospinal fluid examination

CSF examination is mainly used to rule out causes of weakness other than GBS and should be performed during the initial evaluation of the patient. The classic finding in GBS is the combination of an elevated CSF protein level and a normal CSF cell count (known as albumino-cytological dissociation)[63]. However, protein levels are normal in 30–50% of patients in the first week after disease onset and 10–30% of patients in the second week[10, 11, 24, 64]. Therefore, normal CSF protein levels do not rule out a diagnosis of GBS. Marked pleocytosis (>50 cells μl^{-1}) suggests other pathologies, such as leptomeningeal malignancy or infectious or inflammatory diseases of the spinal cord or nerve roots. Mild pleocytosis (10–50 cells μl^{-1}), though compatible with GBS, should still prompt clinicians to consider alternative diagnoses, such as infectious causes of polyradiculitis (**Box 2**)[10, 11].

Box 2. Differential diagnosis of Guillain–Barré syndrome

Central nervous system
- Inflammation or infection of the brainstem (for example, sarcoidosis, Sjögren syndrome, neuromyelitis optica or myelin oligodendrocyte glycoprotein antibody-associated disorder)[a]
- Inflammation or infection of the spinal cord (for example, sarcoidosis, Sjögren syndrome or acute transverse myelitis)
- Malignancy (for example, leptomeningeal metastases or neurolymphomatosis)
- Compression of brainstem or spinal cord
- Brainstem stroke
- Vitamin deficiency (for example, Wernicke encephalopathy[a], caused by deficiency of vitamin B1, or subacute combined degeneration of the spinal cord, caused by deficiency of vitamin B12)

Anterior horn cells
- Acute flaccid myelitis (for example, as a result of polio, enterovirus D68 or A71, WNV, JEV or rabies virus)

Nerve roots
- Infection (for example, Lyme disease, CMV, HIV,EBV, VZV)
- Compression
- Leptomeningeal malignancy

Peripheral nerves
- Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP)
- Metabolic or electrolyte disorders (for example, hypoglycaemia, hypothyroidism, porphyria or copper deficiency)
- Vitamin deficiency (for example, deficiency of vitamin B1 (also known as beriberi), B12 or E)
- Toxins (for example, drugs, alcohol, vitamin B6, lead, thallium, arsenic, organophosphate, ethylene glycol, diethylene glycol, methanol or N-hexane)
- Critical illness polyneuropathy
- Neuralgic amyotrophy
- Vasculitis
- Infection (for example, diphtheria or HIV)

Neuromuscular junction
- Myasthenia gravis
- Lambert–Eaton myasthenic syndrome
- Neurotoxins (for example, botulism, tetanus, tick paralysis or snakebite envenomation)
- Organophosphate intoxication

Muscles
- Metabolic or electrolyte disorders (for example, hypokalaemia, thyrotoxic hypokalaemic periodic paralysis, hypomagnesaemia or hypophosphataemia)
- Inflammatory myositis
- Acute rhabdomyolysis
- Drug-induced toxic myopathy (for example, induced by colchicine, chloroquine, emetine or statins)
- Mitochondrial disease

Other
- Conversion or functional disorder

The differential diagnosis of Guillain–Barré syndrome is broad and highly dependent on the clinical features of the individual patient. Here, we present an overview of the most important differential diagnoses, categorized by location in the nervous system.

WNV=West Nile virus, JEV=Japanese encephalitis virus, CMV=cytomegalovirus, EBV=Epstein–Barr virus, VZV=varicella zoster virus. [a]Differential diagnosis for Bickerstaff brainstem encephalitis.

Electrodiagnostic studies

Electrodiagnostic studies are not required to diagnose GBS. However, we recommend that these studies are performed wherever possible, as they are helpful in supporting the diagnosis, particularly in patients with an atypical presentation. In general, electrophysiological examination in patients with GBS will reveal a sensorimotor polyradiculoneuropathy or polyneuropathy, indicated by reduced conduction velocities, reduced sensory and motor evoked amplitudes, abnormal temporal dispersion and/or partial motor conduction blocks[6, 65]. Typical for GBS is a 'sural sparing pattern' in which the sural sensory nerve action potential is normal while the median and ulnar sensory nerve action potentials are abnormal or even absent[6, 65]. However, electrophysiological measurements might be normal when performed early in the disease course (within 1 week of symptom onset) or in patients with initially proximal weakness, mild disease, slow progression or clinical variants[66,5,67]. In these patients, a repeat electrodiagnostic study 2–3 weeks later can be helpful. In patients with MFS, results of electrodiagnostic studies are usually normal or demonstrate only a reduced amplitude of sensory nerve action potentials[4, 68].

Electrodiagnostic studies can also differentiate between the three electrophysiological subtypes of classical GBS: AIDP, AMAN, and AMSAN. Several sets of electrodiagnostic criteria exist that aim to classify patients into these different electophysiological subtypes on the basis of the presence of specific electrodiagnostic characteristics in at least two motor nerves. International consensus is yet to be reached on which set of criteria best defines the electrophysiological subtypes[5, 60, 69]. However, about one-third of patients with GBS do not meet any of these criteria and are labelled 'equivocal' or 'inexcitable'. Studies have demonstrated that repeating electrodiagnostic studies 3–8 weeks after disease onset might aid electrodiagnostic classification by allowing classification of cases that were initially unclassifiable, or re- classification of cases that were initially classified as AIDP, AMAN or AMSAN, although this practice is controversial[70-72].

Imaging

MRI is not part of the routine diagnostic evaluation of GBS, but can be helpful, particularly for excluding differential diagnoses such as brainstem infection, stroke, spinal cord or anterior horn cell inflammation, nerve root compression or leptomeningeal malignancy (**Box 2**). The presence of nerve root enhancement on gadolinium-enhanced MRI is a non-specific but sensitive feature of GBS[73] and can support a GBS diagnosis, especially in young children, in whom both clinical and electrophysiological assessment can be challenging[74]. In light of recent outbreaks of acute flaccid myelitis in young children, the clinical presentation of which can

mimic GBS, the potential use of MRI to distinguish between these two diagnoses should be given special attention[75, 76]. However, clinicians should be mindful that nerve root enhancement can be found in a minority of individuals with acute flaccid myelitis[77].

A new potential diagnosic tool in GBS is ultrasound imaging of the peripheral nerves, which has revealed enlarged cervical nerve roots early in the disease course, indicating the importance of spinal root inflammation as an early pathological mechanism[78, 79]. This technique might, therefore, help establish a diagnosis of GBS early in the disease course, although further validation is required.

Step 3: when to admit to the ICU

Reasons to admit patients to the intensive care unit (ICU) include the following: evolving respiratory distress with imminent respiratory insufficiency, severe autonomic cardiovascular dysfunction (for example, arrhythmias or marked variation in blood pressure), severe swallowing dysfunction or diminished cough reflex, and rapid progression of weakness[80, 81]. A state of imminent respiratory insufficiency is defined as clinical signs of respiratory distress, including breathlessness at rest or during talking, inability to count to 15 in a single breath, use of accessory respiratory muscles, increased respiratory or heart rate, vital capacity of <15–20 ml/kg or <1 l, or abnormal arterial blood gas or pulse oximetry measurements.

As up to 22% of patients with GBS require mechanical ventilation within the first week of admission, patients at risk of respiratory failure must be identified as early as possible[82]. The Erasmus GBS Respiratory Insufficiency Score (EGRIS) prognostic tool was developed for this purpose and calculates the probability (1–90%) that a patient will require ventilation within 1 week of assessment (**Box 3**)[82].

Risk factors for prolonged mechanical ventilation include the inability to lift the arms from the bed at 1 week after intubation, and an axonal subtype or unexcitable nerves in electrophysiological studies[83]. Early tracheostomy should be considered in patients who have these risk factors.

Step 4: when to start treatment

Immunomodulatory therapy should be started if patients are unable to walk independently for 10 m[84, 85]. Evidence on treatment efficacy in patients who can still walk independently is limited, but treatment should be considered especially if these patients display rapidly progressive weakness or other severe symptoms such as autonomic dysfunction, bulbar failure or respiratory insufficiency[86-88]. Clinical

Box 3: Erasmus GBS Respiratory Insufficiency Score

Measure	*Categories*	*Score*
Days between onset of weakness and hospital admission	>7 days	0
	4–7 days	1
	≤3 days	2
Facial and/or bulbar weakness at hospital admission	Absent	0
	Present	1
MRC sum score at hospital admission	60–51	0
	50–41	1
	40–31	2
	30–21	3
	≤20	4
EGRIS	NA	0–7

NA, not applicable. Adapted from Table 2 in REF.[61].
The Erasmus Guillain–Barré syndrome (GBS) respiratory insufficiency score (EGRIS) calculates the probability that a patient with GBS will require mechanical ventilation within 1 week of assessment and is based on three key measures. Each measure is categorized and assigned an individual score; the sum of these scores gives an overall EGRIS for that patient (between 0 and 7). An EGRIS of 0–2 indicates a low risk of mechanical intervention (4%), 3–4 indicates an intermediate risk (24%) and ≥5 indicates a high risk (65%). This model is based on a Dutch population of patients with GBS (aged >6 years) and has not yet been validated internationally. Therefore, it may not be applicable in other age groups or populations. The Medical Research Council (MRC) sum score is the sum of the score on the MRC scale for: muscle weakness of bilateral shoulder abduction, elbow flexion, wrist extension, hip flexion, knee extension, and ankle dorsiflexion. The MRC score ranges between 0 and 60, with 60 indicating no weakness and 0 complete paralysis.

trials have demonstrated a treatment effect for intravenous immunoglobulin (IVIg) when started within 2 weeks of the onset of weakness and for plasma exchange when started within 4 weeks[84, 85]. Beyond these time periods, evidence on efficacy is lacking.

Step 5: treatment options

Treatment strategies

IVIg (0.4 g per kg body weight daily for 5 days) and plasma exchange (200–250 ml plasma per kg body weight in five sessions) are equally effective treatments for GBS[84, 88]. IVIg and plasma exchange carry comparable risks of adverse events, although early studies showed that plasma exchange was more likely than IVIg to be discontinued[84, 89]. As IVIg is also easier to administer and generally more widely available than plasma exchange, it is usually the treatment of choice. Besides IVIg and plasma exchange, no other procedures or drugs have been proven effective in the treatment of GBS. Although corticosteroids would be expected to be beneficial in reducing inflammation and, therefore, disease progression in GBS, eight randomized controlled trials on the efficacy of corticosteroids for GBS showed no significant benefit, and treatment with oral corticosteroids was even shown to have a negative

effect on outcome[90]. Furthermore, plasma exchange followed by IVIg is no more effective than either treatment alone and insufficient evidence is available for the efficacy of add-on treatment with intravenous methylprednisolone in IVIg-treated patients[90, 91]. In clinical settings where resources are limited, small-volume plasma exchange might be an economical and relatively safe alternative to conventional plasma exchange, but this approach cannot be recommended for general use until its efficacy has been established in further trials[92].

Antimicrobial or antiviral treatment can be considered in patients with GBS who have an ongoing infection; however, preceding infections have usually resolved before the onset of weakness.

Specific patient groups

GBS variants

Patients with pure MFS tend to have a relatively mild disease course, and most recover completely without treatment within 6 months[93]. Therefore, treatment is generally not recommended in this patient group, but patients should be monitored closely because a subgroup can develop limb weakness, bulbar or facial palsy, or respiratory failure[32, 88]. The severity of the disease course of BBE justifies treatment with IVIg or plasma exchange, although evidence for the efficacy of treatment in this context is limited[42, 93]. For the other clinical variants, no evidence regarding treatment is currently available, although many experts will administer IVIg or plasma exchange[94].

Pregnant women

Neither IVIg nor plasma exchange is contraindicated during pregnancy. However, as plasma exchange requires additional considerations and monitoring, IVIg might be preferred[95-97].

Children

There is no indication that it is necessary to deviate from standard adult practice when treating children with GBS[84, 86, 98]. Evidence on the relative efficacies of plasma exchange and IVIg in children is limited[98]. However, as plasma exchange is only available in centres that are experienced with its use and seems to produce greater discomfort and higher rates of complications than IVIg in children, IVIg is usually the first-line therapy for children with GBS [99]. Although some paediatric centres administer IVIg as 2 g per kg (body weight) over 2 days, rather than the standard adult regimen of 2 g per kg (body weight) over 5 days, one study indicated that TRFs

were more frequent with a 2 day regimen (5 of 23 children) than with the 5 day regimen (0 of 23 children)[86].

Step 6: monitoring disease progression

Regular assessment is required to monitor disease progression and the occurrence of complications. First, routine measurement of respiratory function is advised, as not all patients with respiratory insufficiency will have clinical signs of dyspnoea. These respiratory measurements can include usage of accessory respiratory muscles, counting during expiration of one full-capacity inspiratory breath (a single breath count of ≤19 predicts requirement for mechanical ventilation), vital capacity, and maximum inspiratory and expiratory pressure[81, 100]. Clinicians should consider using the '20/30/40 rule', whereby the patient is deemed at risk of respiratory failure if the vital capacity is <20 ml/kg, the maximum inspiratory pressure is <30 cm H_2O or the maximum expiratory pressure is <40 cm H_2O[101]. Second, muscle strength in the neck, arms and legs should be assessed using the Medical Research Council grading scale or a similar scale, and functional disability should be assessed on the GBS disability scale (**Supplementary Table 2**) — a widely used tool for documenting GBS disease course[102]. Third, patients should be monitored for swallowing and coughing difficulties. Last, autonomic dysfunction should be assessed via electrocardiography and monitoring of heart rate, blood pressure, and bowel and bladder function.

The nature and frequency of monitoring depends on the rate of deterioration, the presence or absence of autonomic dysfunction, the phase of the disease and the health-care setting, and should be carefully assessed in each individual patient. Up to two-thirds of the deaths of patients with GBS occur during the recovery phase and are mostly caused by cardiovascular and respiratory dysfunction[6, 7, 11]. We therefore advise clinicians to stay alert during this phase and monitor the patient for potential arrhythmias, blood pressure shifts or respiratory distress caused by mucus plugs. This monitoring is especially important in patients who have recently left the ICU and in those with cardiovascular risk factors.

Step 7: managing early complications

Complications in GBS can cause severe morbidity and death[103]. Some of these complications, including pressure ulcers, hospital-acquired infections (for example, pneumonia or urinary tract infections) and deep vein thrombosis, can occur in any hospitalized bed-bound patient, and standard-practice preventive measures and treatment are recommended. Other complications are more specific to GBS, for example, the inability to swallow safely in patients with bulbar palsy; corneal ulceration in patients with facial palsy; and limb contractures, ossification and pressure

palsies in patients with limb weakness (**Table 2**). Pain, hallucinations, anxiety and depression are also frequent in GBS, and caregivers should specifically ask patients whether they are experiencing these symptoms, especially if patients have limited communication abilities and/or are in the ICU. Recognition and adequate treatment of psychological symptoms and pain at an early stage is important because these symptoms can have a major impact on the wellbeing of patients. Caregivers should also be aware that patients with GBS, even those with complete paralysis, usually have intact consciousness, vision and hearing. It is important, therefore, to be mindful of what is said at the bedside, and to explain the nature of procedures to patients to reduce anxiety. Adequate management of complications is best undertaken by a multidisciplinary team, which might include nurses, physiotherapists, rehabilitation specialists, occupational therapists, speech therapists and dietitians.

Table 2. Important complications of Guillain–Barré syndrome

Complication	When to be alert
Choking	Bulbar palsy
Cardiac arrhythmias	All patients
Hospital-acquired infections (e.g. pneumonia, sepsis or urinary tract infection)	Bulbar and facial palsy, immobility, bladder dysfunction, mechanical ventilation
Pain and tactile allodynia	Limited communication
Delirium	Limited communication
Depression	Limited communication
Urinary retention	All patients
Constipation	Immobility
Corneal ulceration	Facial palsy
Dietary deficiency	Bulbar and facial palsy
Hyponatraemia	All patients
Pressure ulcers	Immobility
Compression neuropathy	Immobility
Limb contractures and ossifications	Severe weakness for prolonged period of time

Most of these complications can occur in any patient with GBS, at any time, but the second column shows when they are most likely to occur and/or when to be especially alert.[80]

Step 8: managing clinical progression

Insufficient response to treatment

About 40% of patients treated with standard doses of plasma exchange or IVIg do not improve in the first 4 weeks following treatment [88, 90]. Such disease progression does not imply that the treatment is ineffective, as progression might have been worse without therapy[6]. Clinicians may consider repeating the treatment or changing to an alternative treatment, but at present no evidence exists that this approach will

improve the outcome[104,105]. A clinical trial investigating the effect of administering a second IVIg dose is ongoing[106].

Treatment-related fluctuations

TRFs are observed in 6–10% of patients with GBS and are defined as disease progression occurring within 2 months following an initial treatment-induced clinical improvement or stabilization[12, 13]. TRFs should be distinguished from clinical progression without any initial response to treatment. The general view is that a TRF indicates that the treatment effect has worn off while the inflammatory phase of the disease is still ongoing. Therefore, patients with GBS who display TRFs might benefit from further treatment, and repeating the full course of IVIg or plasma exchange in these patients is a common practice, although evidence to support this approach is lacking[88].

CIDP

In ~5% of patients with GBS, repeated clinical relapses suggest a more chronic disease process, and the diagnosis is changed to acute-onset chronic inflammatory demyelinating polyneuropathy (CIDP)[12]. Acute onset CIDP typically presents with three or more TRFs and/or clinical deterioration ≥8 weeks after disease onset[12].

Step 9: predicting outcome

Most patients with GBS, even those who were tetraplegic at nadir or required mechanical ventilation for a long period of time, show extensive recovery, especially in the first year after disease onset[11, 107]. About 80% of patients with GBS regain the ability to walk independently at 6 months after disease onset.[11] The probability of regaining walking ability can be calculated in individual patients using the modified Erasmus GBS outcome score (mEGOS) prognostic tool (**Supplementary Table 3**)[108].

Despite the generally positive prospects for patients with GBS, death occurs in 3–10% of cases, most commonly owing to cardiovascular and respiratory complications, which can occur in both the acute and the recovery phase[7-9]. Risk factors for mortality include advanced age and severe disease at onset[7]. Long-term residual complaints are also common and can include neuropathic pain, weakness and fatigue[109-111]. However, recovery from these complaints may still occur >5 years after disease onset[111].

Recurrent episodes of GBS are rare, affecting 2–5% of patients, but this percentage is still higher than the lifetime risk of GBS in the general population (0.1%)[14, 15]. Many vaccines carry a warning about GBS, although prior GBS is not a strict contraindi-

cation for vaccination. Discussion with experts might be useful for patients who were diagnosed with GBS <1 year before a planned vaccination or who previously developed GBS shortly after receiving the same vaccination. In these patients, the benefits of vaccination for specific illnesses (for example, influenza in elderly individuals) must be weighed against the small and possibly only theoretical risk of a recurrent GBS episode[14].

Step 10: planning rehabilitation

Patients with GBS can experience a range of long-term residual problems, including incomplete recovery of motor and sensory function, as well as fatigue, pain and psychological distress[111]. Before the patient is discharged, these possible long-term effects of GBS should be considered and managed[112, 113].

Physical function

Arranging a rehabilitation programme with a rehabilitation specialist, physiotherapist and occupational therapist is a crucial step towards recovery. Programmes should aim to reduce disability in the early stages of recovery and later to restore motor and sensory function and physical condition to pre-disease levels[114]. Exercise programmes for patients with GBS, which include range-of-motion exercises, stationary cycling, and walking and strength training, have been shown to improve physical fitness, walking ability and independence in activities of daily living[114]. However, the intensity of exercise must be closely monitored as overwork can cause fatigue[114].

Fatigue

Fatigue, unrelated to residual motor deficits, is found in 60–80% of patients with GBS and is often one of the most disabling complaints[115, 116]. Other causes should be considered before concluding that fatigue in a patient is a residual complaint of GBS. As with recovery of physical function, a graded, supervised exercise programme has been shown to be useful in reducing fatigue[117].

Pain

Severe pain is reported in at least one-third of patients with GBS one year after disease onset and can persist for >10 years[14, 26]. Chronic pain in GBS is characterized by muscle pain in the lower back and limbs, painful paraesthesias, arthralgia, and radicular pain. Although the pathogenesis of this pain is not fully understood, muscle pain and arthralgia might be attributable to immobility, and neuropathic pain might be caused by regeneration of, or persistent damage to, small nerve fi-

bres[26]. Management strategies include encouraging mobilization and administering drugs for neuropathic or nociceptive pain[112].

Psychological distress

Rapid loss of physical function, often in previously healthy individuals, can be severely traumatic and may cause anxiety and/or depression. Early recognition and management of psychological distress is important in patients with GBS, especially as mental status can influence physical recovery and vice versa[118]; referral to a psychologist or psychiatrist might be beneficial for some patients[118]. Providing accurate information to patients on the relatively good chance of recovery and low recurrence risk (2–5%) can help reduce their fear[11, 14]. Connecting patients with others who have had GBS can also help guide them through the rehabilitation process. The GBS/CIDP Foundation International, — the international patient association for GBS — and other national organizations can help establish these networks.

CONCLUSIONS

GBS can be a complex disorder to diagnose and manage, as the clinical presentation is heterogeneous and the prognosis varies widely between patients. Managing GBS can be especially challenging during outbreaks triggered by infectious disease, as was most recently seen during the Zika virus epidemic. In the absence of an international clinical guideline for GBS, we have developed this consensus guideline for the diagnosis and management of GBS. This guideline was developed by a team of clinical neurologists from around the world and is designed for general applicability in all clinical environments, irrespective of specialist capabilities or availability of resources. The step-by-step design was used to focus attention on the most important issues in GBS and to make the guideline easy to use in clinical practice.

As the field of GBS research develops, and ongoing studies aim to improve diagnostics, treatment and prognostic modelling, this guideline will need to be updated regularly. For example, ultrasound imaging of the peripheral nerves is emerging as a potential diagnostic tool and might require further comment in future versions of this guideline. In relation to treatment, the efficacy of complement inhibitors, IgG-cleaving enzymes and a second course of IVIg is being investigated[78,119, 120]. Little is known about how to measure and predict long-term outcome in patients with GBS, and validation studies of known prognostic models (for example, mEGOS and EGRIS) and research into new outcome measures are needed. We intend to seek

feedback on this guideline and provide updates based on results from ongoing studies and future research.

To further improve the worldwide management of GBS, we aim to use this consensus report as a basis for the development of online information resources, training material and teaching courses. These resources will be directed towards health-care workers, including clinical neurologists, as well as patients with GBS and their relatives.

KEY POINTS

- Classic GBS is an acute-onset ascending sensorimotor neuropathy, but the disease can present atypically or as a clinical variant.
- Abnormal results in electrophysiological studies and a combination of an increased protein level and normal cell count in in cerebrospinal fluid (CSF) are classic features of GBS, but patients with GBS can have normal results in both tests, especially early in the disease course.
- Respiratory function should be monitored in all patients as respiratory failure can occur without symptoms of dyspnoea.
- Intravenous immunoglobulin and plasma exchange are equally effective in treating GBS; no other treatments have been proven to be effective.
- The efficacy of repeat treatment in patients who have shown insufficient clinical response is uncertain; nevertheless, this practice is common in patients who show deterioration after an initial treatment response.
- Clinical improvement is usually most extensive in the first year after disease onset and can continue for > 5 years.

Supplementary material

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REFERENCES

1. Sejvar JJ, Baughman AL, Wise M, Morgan OW. Population incidence of Guillain-Barré syndrome: a systematic review and meta-analysis. *Neuroepidemiology* 2011;36:123-133.
2. Asbury AK, Arnason, B.G.W., Karp, H.R., McFarlin, D.E. Criteria for diagnosis of Guillain-Barré syndrome. *Ann Neurol* 1978;3:565-566.
3. Asbury AK, Cornblath DR. Assessment of current diagnostic criteria for Guillain-Barré syndrome. *Ann Neurol* 1990;27 Suppl:S21-24.
4. Sejvar JJ, Kohl KS, Gidudu J, et al. Guillain-Barré syndrome and Fisher syndrome: case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* 2011;29:599-612.
5. Hadden RD, Cornblath DR, Hughes RA, et al. Electrophysiological classification of Guillain-Barré syndrome: clinical associations and outcome. *Ann Neurol* 1998;44:780-788.
6. Willison HJ, Jacobs BC, van Doorn PA. Guillain-Barré syndrome. *Lancet* 2016;388:717-727.
7. Van den Berg B, Bunschoten C, van Doorn PA, Jacobs BC. Mortality in Guillain-Barré syndrome. *Neurology* 2013;80:1650-1654.
8. Dominguez-Moreno R, Tolosa-Tort P, Patino-Tamez A, et al. Mortalidad asociada al diagnostico de sindrome de Guillain-Barré en adultos ingresados en instituciones del sistema sanitario Mexicano [Mortality associated with a diagnosis of Guillain-Barré syndrome in adults of Mexican health institutions] *Rev Neurol* 2014;58:4-10.
9. Dourado ME, Felix RH, da Silva WK, Queiroz JW, Jeronimo SM. Clinical characteristics of Guillain-Barre syndrome in a tropical country: a Brazilian experience. *Acta Neurol Scand* 2012;125:47-53.
10. Fokke C, van den Berg B, Drenthen J, Walgaard C, van Doorn PA, Jacobs BC. Diagnosis of Guillain-Barré syndrome and validation of Brighton criteria. *Brain* 2014;137:33-43.
11. Doets AY, Verboon C, van den Berg B, et al. Regional variation of Guillain-Barré syndrome. *Brain* 2018;141:2866-2877.
12. Ruts L, Drenthen J, Jacobs BC, van Doorn PA, Dutch Guillain-Barré syndrome Study Group. Distinguishing acute-onset CIDP from fluctuating Guillain-Barré syndrome: a prospective study. *Neurology* 2010;74:1680-1686.
13. Kleyweg RP, van der Meche FG. Treatment related fluctuations in Guillain-Barré syndrome after high-dose immunoglobulins or plasma-exchange. *J Neurol Neurosurg Psychiatry* 1991;54:957-960.
14. Kuitwaard K, Bos-Eyssen ME, Blomkwist-Markens PH, van Doorn PA. Recurrences, vaccinations and long-term symptoms in GBS and CIDP. *J Peripher Nerv Syst* 2009;14:310-315.
15. Principi N, Esposito S. Vaccine-preventable diseases, vaccines and Guillain-Barré syndrome. *Vaccine* 2018.
16. Yuki N. Infectious origins of, and molecular mimicry in, Guillain-Barre and Fisher syndromes. *Lancet Infect Dis* 2001;1:29-37.
17. Yoshikawa K, Kuwahara M, Morikawa M, et al. Varied antibody reactivities and clinical relevance in anti-GQ1b antibody-related diseases. *Neurol Neuroimmunol Neuroinflamm* 2018;5:e501.
18. Jacobs BC, Rothbarth PH, van der Meche FG, et al. The spectrum of antecedent infections in Guillain-Barré syndrome: a case-control study. *Neurology* 1998;51:1110-1115.

19. World Health Organization. Zika situation report 5 February 2016. *World Health Organization* 2016:https://www.who.int/emergencies/zika-virus/situation-report/5-february-2016/en/.
20. Cao-Lormeau VM, Blake A, Mons S, et al. Guillain-Barre Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *Lancet* 2016;387:1531-1539.
21. Parra B, Lizarazo J, Jimenez-Arango JA, et al. Guillain-Barré syndrome associated with Zika virus infection in Colombia. *N Engl J Med* 2016;375:1513-1523.
22. Wilder-Smith A, Preet R, Renhorn KE, et al. ZikaPLAN: Zika Preparedness Latin American Network. *Glob Health Action* 2017;10:1398485.
23. Jacobs BC, van den Berg B, Verboon C, et al. International Guillain-Barre Syndrome Outcome Study: protocol of a prospective observational cohort study on clinical and biological predictors of disease course and outcome in Guillain-Barre syndrome. *J Peripher Nerv Syst* 2017;22:68-76.
24. Ropper AHW, E.F.M; Truax, B.T. Guillain-Barré Syndrome: F.A. Davis Company, 1991: 155-160.
25. Van den Berg B, Walgaard C, Drenthen J, Fokke C, Jacobs BC, van Doorn PA. Guillain-Barré syndrome: pathogenesis, diagnosis, treatment and prognosis. *Nat Rev Neurol* 2014;10:469-482.
26. Ruts L, Drenthen J, Jongen JL, et al. Pain in Guillain-Barré syndrome: a long-term follow-up study. *Neurology* 2010;75:1439-1447.
27. Korinthenberg R, Schessl J, Kirschner J. Clinical presentation and course of childhood Guillain-Barré syndrome: a prospective multicentre study. *Neuropediatrics* 2007;38:10-17.
28. Roodbol J, de Wit MC, Walgaard C, de Hoog M, Catsman-Berrevoets CE, Jacobs BC. Recognizing Guillain-Barré syndrome in preschool children. *Neurology* 2011;76:807-810.
29. Yuki N, Kokubun N, Kuwabara S, et al. Guillain-Barré syndrome associated with normal or exaggerated tendon reflexes. *J Neurol* 2012;259:1181-1190.
30. Ito M, Kuwabara S, Odaka M, et al. Bickerstaff's brainstem encephalitis and Fisher syndrome form a continuous spectrum: clinical analysis of 581 cases. *J Neurol* 2008;255:674-682.
31. Wakerley BR, Uncini A, Yuki N, et al. Guillain–Barré and Miller Fisher syndromes—new diagnostic classification. *Nat Rev Neurol* 2014;10:537.
32. Sekiguchi Y, Mori M, Misawa S, et al. How often and when Fisher syndrome is overlapped by Guillain-Barré syndrome or Bickerstaff brainstem encephalitis? *Eur J Neurol* 2016;23:1058-1063.
33. Wakerley BR, Yuki N. Mimics and chameleons in Guillain-Barré and Miller Fisher syndromes. *Pract Neurol* 2015;15:90-99.
34. Wakerley BR, Kokubun N, Funakoshi K, Nagashima T, Hirata K, Yuki N. Clinical classification of 103 Japanese patients with Guillain-Barre syndrome. *J Neurol Sci* 2016;369:43-47.
35. Hiew FL, Ramlan R, Viswanathan S, Puvanarajah S. Guillain-Barré Syndrome, variants & forms fruste: Reclassification with new criteria. *Clin Neurol Neurosurg* 2017;158:114-118.
36. Blum S, Reddel S, Spies J, McCombe P. Clinical features of patients with Guillain-Barré syndrome at seven hospitals on the East Coast of Australia. *J Peripher Nerv Syst* 2013;18:316-320.

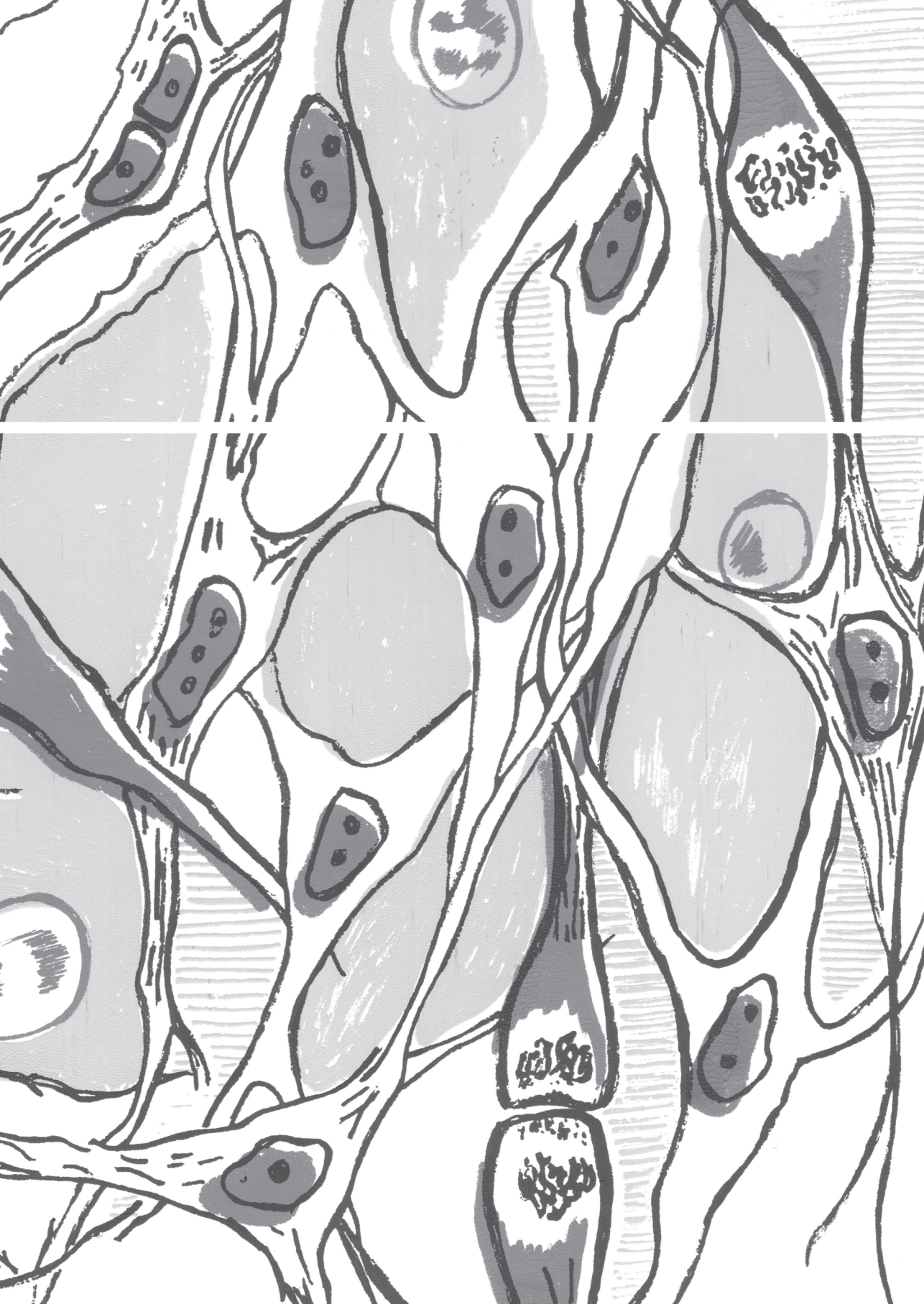
37. Peric S, Milosevic V, Berisavac I, et al. Clinical and epidemiological features of Guillain-Barré syndrome in the Western Balkans. *J Peripher Nerv Syst* 2014;19:317-321.
38. Zhang G, Li Q, Zhang R, Wei X, Wang J, Qin X. Subtypes and Prognosis of Guillain-Barré Syndrome in Southwest China. *PLoS ONE* 2015;10:e0133520.
39. Mitsui Y, Kusunoki S, Arimura K, et al. A multicentre prospective study of Guillain-Barré Syndrome in Japan: a focus on the incidence of subtypes. *J Neurol Neurosurg Psychiatry* 2015;86:110.
40. Ishaque T, Islam MB, Ara G, et al. High mortality from Guillain-Barre syndrome in Bangladesh. *J Peripher Nerv Syst* 2017;22:121-126.
41. Uncini A, Yuki N. Sensory Guillain-Barré syndrome and related disorders: an attempt at systematization. *Muscle Nerve* 2012;45:464-470.
42. Odaka M, Yuki N, Yamada M, et al. Bickerstaff's brainstem encephalitis: clinical features of 62 cases and a subgroup associated with Guillain-Barré syndrome. *Brain* 2003;126:2279-2290.
43. Ito M, Matsuno K, Sakumoto Y, Hirata K, Yuki N. Ataxic Guillain-Barré syndrome and acute sensory ataxic neuropathy form a continuous spectrum. *J Neurol Neurosurg Psychiatry* 2011;82:294-299.
44. Graus F, Titulaer MJ, Balu R, et al. A clinical approach to diagnosis of autoimmune encephalitis. *Lancet Neurol* 2016;15:391-404.
45. Van den Berg B, van der Eijk AA, Pas SD, et al. Guillain-Barré syndrome associated with preceding hepatitis E virus infection. *Neurology* 2014;82:491-497.
46. Vellozzi C, Iqbal S, Broder K. Guillain-Barré syndrome, influenza, and influenza vaccination: the epidemiologic evidence. *Clin Infect Dis* 2014;58:1149-1155.
47. Thornton CA, Latif AS, Emmanuel JC. Guillain-Barré syndrome associated with human immunodeficiency virus infection in Zimbabwe. *Neurology* 1991;41:812-815.
48. Islam B, Islam Z, GeurtsvanKessel CH, et al. Guillain-Barre syndrome following varicella-zoster virus infection. *Eur J Clin Microbiol Infect Dis* 2018;37:511-518.
49. Carod-Artal FJ, Wichmann O, Farrar J, Gascon J. Neurological complications of dengue virus infection. *Lancet Neurol* 2013;12:906-919.
50. Wielanek AC, Monredon JD, Amrani ME, Roger JC, Serveaux JP. Guillain-Barre syndrome complicating a Chikungunya virus infection. *Neurology* 2007;69:2105-2107.
51. Cornblath DR, McArthur JC, Kennedy PG, Witte AS, Griffin JW. Inflammatory demyelinating peripheral neuropathies associated with human T-cell lymphotropic virus type III infection. *Ann Neurol* 1987;21:32-40.
52. Schonberger LB, Bregman DJ, Sullivan-Bolyai JZ, et al. Guillain-Barré syndrome following vaccination in the National Influenza Immunization Program, United States, 1976-1977. *Am J Epidemiol* 1979;110:105-123.
53. Burwen DR, Ball R, Bryan WW, et al. Evaluation of Guillain-Barré syndrome among recipients of influenza vaccine in 2000 and 2001. *Am J Prev Med* 2010;39:296-304.
54. Kaplan JE, Katona P, Hurwitz ES, Schonberger LB. Guillain-Barré syndrome in the United States, 1979-1980 and 1980-1981: lack of an association with influenza vaccination. *JAMA* 1982;248:698-700.
55. Lasky T, Terracciano GJ, Magder L, et al. The Guillain-Barré syndrome and the 1992-1993 and 1993-1994 influenza vaccines. *N Engl J Med* 1998;339:1797-1802.
56. Juurlink DN, Stukel TA, Kwong J, et al. Guillain-Barré syndrome after influenza vaccination in adults: a population-based study. *Arch Intern Med* 2006;166:2217-2221.

57. Kao JC, Brickshawana A, Liewluck T. Neuromuscular Complications of Programmed Cell Death-1 (PD-1) Inhibitors. *Curr Neurol Neurosci Rep* 2018;18:63.
58. Hiew FL, Rajabally YA. Malignancy in Guillain-Barré syndrome: A twelve-year single-center study. *Journal of the Neurological Sciences* 2017;375:275-278.
59. Rudant J, Dupont A, Mikaeloff Y, Bolgert F, Coste J, Weill A. Surgery and risk of Guillain-Barré syndrome. *A French nationwide epidemiologic study* 2018;91:e1220-e1227.
60. Ho TW, Mishu B, Li CY, et al. Guillain-Barre syndrome in northern China. Relationship to Campylobacter jejuni infection and anti-glycolipid antibodies. *Brain* 1995;118 (Pt 3):597-605.
61. Kuijf ML, van Doorn PA, Tio-Gillen AP, et al. Diagnostic value of anti-GM1 ganglioside serology and validation of the INCAT-ELISA. *J Neurol Sci* 2005;239:37-44.
62. Uchibori A, Gyohda A, Chiba A. Ca(2+)-dependent anti-GQ1b antibody in GQ1b-seronegative Fisher syndrome and related disorders. *J Neuroimmunol* 2016;298:172-177.
63. Guillain G. Sur un syndrome de radiculo-nevrite avec hyperalbuminose du liquode cephalo-rachidien sans reaction cellulaire: remarques sur les caracteres cliniques et graphiques des reflexes tendineux [Radiculoneuritis syndrome with hyperalbuminosis of cerebrospinal fluid without cellular reaction. Notes on clinical features and graphs of tendon reflexes]. *Bell Mem Soc Med Paris* 1916;40:1462-1470.
64. Wong AH, Umapathi T, Nishimoto Y, Wang YZ, Chan YC, Yuki N. Cytoalbuminologic dissociation in Asian patients with Guillain-Barré and Miller Fisher syndromes. *J Peripher Nerv Syst* 2015;20:47-51.
65. Vucic S, Cairns KD, Black KR, Chong PS, Cros D. Neurophysiologic findings in early acute inflammatory demyelinating polyradiculoneuropathy. *Clin Neurophysiol* 2004;115:2329-2335.
66. Meulstee J, van der Meche F, Dutch Guillain-Barré Study Group. Electrodiagnostic criteria for polyneuropathy and demyelination: application in 135 patients with Guillain-Barré syndrome. *J Neurol Neurosurg Psychiatry* 1995;59:482-486.
67. Berciano J, Sedano MJ, Pelayo-Negro AL, et al. Proximal nerve lesions in early Guillain-Barré syndrome: implications for pathogenesis and disease classification. *J Neurol* 2017;264:221-236.
68. Kuwabara S, Sekiguchi Y, Misawa S. Electrophysiology in Fisher syndrome. *Clin Neurophysiol* 2017;128:215-219.
69. Rajabally YA, Durand MC, Mitchell J, Orlikowski D, Nicolas G. Electrophysiological diagnosis of Guillain-Barré syndrome subtype: could a single study suffice? *J Neurol Neurosurg Psychiatry* 2015;86:115-119.
70. Uncini A, Kuwabara S. The electrodiagnosis of Guillain-Barré syndrome subtypes: Where do we stand? *Clin Neurophysiol* 2018;129:2586-2593.
71. Van den Bergh PYK, Pieret F, Woodard JL, et al. Guillain-Barré syndrome subtype diagnosis: A prospective multicentric European study. *Muscle Nerve* 2018.
72. Uncini A, Kuwabara S. Electrodiagnostic criteria for Guillain-Barré syndrome: a critical revision and the need for an update. *Clin Neurophysiol* 2012;123:1487-1495.
73. Gorson KC, Ropper AH, Muriello MA, Blair R. Prospective evaluation of MRI lumbosacral nerve root enhancement in acute Guillain-Barré syndrome. *Neurology* 1996;47:813-817.
74. Yikilmaz A, Doganay S, Gumus H, Per H, Kumandas S, Coskun A. Magnetic resonance imaging of childhood Guillain-Barré syndrome. *Childs Nerv Syst* 2010;26:1103-1108.

75. Elrick MJ, Gordon-Lipkin E, Crawford TO, et al. Clinical subpopulations in a sample of North American children diagnosed with Acute Flaccid Myelitis, 2012-2016. *JAMA Pediatr* 2018.
76. Hopkins SE, Elrick MJ, Messacar K. Acute Flaccid Myelitis - Keys to diagnosis, questions about treatment, and future directions. *JAMA Pediatr* 2018;173:117-118.
77. Maloney JA, Mirsky DM, Messacar K, Dominguez SR, Schreiner T, Stence NV. MRI findings in children with acute flaccid paralysis and cranial nerve dysfunction occurring during the 2014 enterovirus D68 outbreak. *AJNR Am J Neuroradiol* 2015;36:245-250.
78. Gallardo E, Sedano MJ, Orizaola P, et al. Spinal nerve involvement in early Guillain-Barré syndrome: a clinico-electrophysiological, ultrasonographic and pathological study. *Clin Neurophysiol* 2015;126:810-819.
79. Razali SNO, Arumugam T, Yuki N, Rozalli FI, Goh KJ, Shahrizaila N. Serial peripheral nerve ultrasound in Guillain-Barré syndrome. *Clin Neurophysiol* 2016;127:1652-1656.
80. Vereninging Spierziekten Nederland, Nederlandse Vereniging van Revalidatieartsen, [Dutch Association of Muscular Diseases, Dutch Society of Rehabilitation Specialists]. Multidisciplinaire richtlijn Guillain-Barré syndroom [Multidisciplinary guideline Guillain-Barré syndrome]: Libertas, Bunnik, 2011.
81. Mehta S. Neuromuscular disease causing acute respiratory failure. *Respir Care* 2006;51:1016-1021; discussion 1021-1013.
82. Walgaard C, Lingsma HF, Ruts L, et al. Prediction of respiratory insufficiency in Guillain-Barre syndrome. *Ann Neurol* 2010;67:781-787.
83. Walgaard C, Lingsma HF, van Doorn PA, van der Jagt M, Steyerberg EW, Jacobs BC. Tracheostomy or not: Prediction of prolonged mechanical ventilation in Guillain–Barré Syndrome. *Neurocrit Care* 2017;26:6-13.
84. Hughes RA, Swan AV, van Doorn PA. Intravenous immunoglobulin for Guillain-Barré syndrome. *Cochrane Database Syst Rev* 2014:CD002063.
85. Chevret S. Plasma exchange for Guillain-Barré syndrome. *Cochrane Database Syst Rev* 2017.
86. Korinthenberg R, Schessl J, Kirschner J, Monting JS. Intravenously administered immunoglobulin in the treatment of childhood Guillain-Barré syndrome: a randomized trial. *Pediatrics* 2005;116:8-14.
87. The French Cooperative Group on Plasma Exchange in Guillain-Barré Syndrome. Appropriate number of plasma exchanges in Guillain-Barré syndrome. *Ann Neurol* 1997;41:298-306.
88. Verboon C, van Doorn PA, Jacobs BC. Treatment dilemmas in Guillain-Barré syndrome. *J Neurol Neurosurg Psychiatry* 2017;88:346-352.
89. Raphael JC, Chevret S, Hughes RA, Annane D. Plasma exchange for Guillain-Barré syndrome. *Cochrane Database Syst Rev* 2012:CD001798.
90. Hughes RA, Swan AV, Raphael JC, Annane D, van Koningsveld R, van Doorn PA. Immunotherapy for Guillain-Barré syndrome: a systematic review. *Brain* 2007;130:2245-2257.
91. Van Koningsveld R, Schmitz PI, Meche FG, et al. Effect of methylprednisolone when added to standard treatment with intravenous immunoglobulin for Guillain-Barré syndrome: randomised trial. *Lancet* 2004;363:192-196.
92. Islam MB, Islam Z, Rahman S, et al. Small volume plasma exchange for Guillain-Barré syndrome in resource poor settings: a safety and feasibility study. *Pilot Feasibility Stud* 2017;3:40.

93. Overell JR, Hseih S-T, Odaka M, Yuki N, Willison HJ. Treatment for Fisher syndrome, Bickerstaff's brainstem encephalitis and related disorders. *Cochrane Database Syst Rev* 2007.
94. Verboon C, Doets AY, Galassi G, et al. Current treatment practice of Guillain-Barré syndrome. *Neurology* 2019:[In press].
95. Tomimatsu T, Sugihara M, Nagai T, Sunada Y, Kimura T, Shimoya K. Guillain-Barré syndrome after trivalent influenza vaccination during pregnancy. *Eur J Obstet Gynecol Reprod Biol* 2016;201:225-226.
96. Pacheco LD, Saad AF, Hankins GD, Chiosi G, Saade G. Guillain-Barré Syndrome in pregnancy. *Obstet Gynecol* 2016;128:1105-1110.
97. Branch DW, Porter TF, Paidas MJ, Belfort MA, Gonik B. Obstetric uses of intravenous immunoglobulin: successes, failures, and promises. *J Allergy Clin Immunol* 2001;108:S133-138.
98. El-Bayoumi MA, El-Refaey AM, Abdelkader AM, El-Assmy MM, Alwakeel AA, El-Tahan HM. Comparison of intravenous immunoglobulin and plasma exchange in treatment of mechanically ventilated children with Guillain-Barré syndrome: a randomized study. *Crit Care* 2011;15:R164.
99. Michon B, Moghrabi A, Winikoff R, et al. Complications of apheresis in children. *Transfusion* 2007;47:1837-1842.
100. Kannan Kanikannan MA, Durga P, Venigalla NK, Kandadai RM, Jabeen SA, Borgohain R. Simple bedside predictors of mechanical ventilation in patients with Guillain-Barre syndrome. *Journal of Critical Care* 2014;29:219-223.
101. Lawn ND, Fletcher DD, Henderson RD, Wolter TD, Wijdicks EF. Anticipating mechanical ventilation in Guillain-Barré syndrome. *Arch Neurol* 2001;58:893-898.
102. Hughes RAC, Newsom-Davis JM, Perkin GD, Pierce JM. Controlled trial of prednisolone in acute polyneuropathy. *Lancet* 1978;312:750-753.
103. Dhar R, Stitt L, Hahn AF. The morbidity and outcome of patients with Guillain-Barré syndrome admitted to the intensive care unit. *J Neurol Sci* 2008;264:121-128.
104. Oczko-Walker M, Manousakis G, Wang S, Malter JS, Waclawik AJ. Plasma exchange after initial intravenous immunoglobulin treatment in Guillain-Barré syndrome: critical reassessment of effectiveness and cost-efficiency. *J Clin Neuromuscul Dis* 2010;12:55-61.
105. Farcas P, Avnun L, Frisher S, Herishanu YO, Wirguin I. Efficacy of repeated intravenous immunoglobulin in severe unresponsive Guillain-Barré syndrome. *Lancet* 1997;350:1747.
106. Walgaard C, Jacobs BC, Lingsma HF, et al. Second IVIg course in Guillain-Barré syndrome patients with poor prognosis (SID-GBS trial): Protocol for a double-blind randomized, placebo-controlled clinical trial. *J Peripher Nerv Syst* 2018;23:210-215.
107. Van den Berg B, Storm EF, Garssen MJP, Blomkwist-Markens PH, Jacobs BC. Clinical outcome of Guillain-Barré syndrome after prolonged mechanical ventilation. *J Neurol Neurosurg Psychiatry* 2018.
108. Walgaard C, Lingsma HF, Ruts L, van Doorn PA, Steyerberg EW, Jacobs BC. Early recognition of poor prognosis in Guillain-Barre syndrome. *Neurology* 2011;76:968-975.
109. Soysal A, Aysal F, Caliskan B, et al. Clinico-electrophysiological findings and prognosis of Guillain-Barré syndrome-10 years' experience. *Acta Neurol Scand* 2011;123:181-186.
110. Bersano A, Carpo M, Allaria S, Franciotta D, Citterio A, Nobile-Orazio E. Long term disability and social status change after Guillain-Barré syndrome. *J Neurol* 2006;253:214-218.

111. Forsberg A, Press R, Holmqvist LW. Residual disability 10 years after falling ill in Guillain-Barré syndrome: a prospective follow-up study. *J Neurol Sci* 2012;317:74-79.
112. Hughes RA, Wijdicks EF, Benson E, et al. Supportive care for patients with Guillain-Barré syndrome. *Arch Neurol* 2005;62:1194-1198.
113. Davidson I, Wilson C, Walton T, Brissenden S. Physiotherapy and Guillain-Barré syndrome: results of a national survey. *Physiotherapy* 2009;95:157-163.
114. Simatos Arsenault N, Vincent P-O, Yu BHS, Bastien R, Sweeney A. Influence of exercise on patients with Guillain-Barré syndrome: a systematic review. *Physiother Can* 2016;68:367-376.
115. Garssen MP, Van Koningsveld R, Van Doorn PA. Residual fatigue is independent of antecedent events and disease severity in Guillain-Barré syndrome. *J Neurol* 2006;253:1143-1146.
116. Merkies IS, Schmitz PI, Samijn JP, van der Meche FG, van Doorn PA, European Inflammatory Neuropathy Cause and Treatment (INCAT) Group. Fatigue in immune-mediated polyneuropathies. *Neurology* 1999;53:1648-1654.
117. Garssen MPJ, Bussmann JBJ, Schmitz PIM, et al. Physical training and fatigue, fitness, and quality of life in Guillain-Barré syndrome and CIDP. *Neurology* 2004;63:2393-2395.
118. Bernsen RA, de Jager AE, Kuijer W, van der Meche FG, Suurmeijer TP. Psychosocial dysfunction in the first year after Guillain-Barré syndrome. *Muscle Nerve* 2010;41:533-539.
119. Wang Y, Shi Q, Lv H, et al. IgG-degrading enzyme of Streptococcus pyogenes (IdeS) prevents disease progression and facilitates improvement in a rabbit model of Guillain-Barré syndrome. *Exp Neurol* 2017;291:134-140.
120. Misawa S, Kuwabara S, Sato Y, et al. Safety and efficacy of eculizumab in Guillain-Barré syndrome: a multicentre, double-blind, randomised phase 2 trial. *Lancet Neurol* 2018;17:519-529.
121. Guillain-Barré Syndrome Steroid Trial Group. Double-blind trial of intravenous methylprednisolone in Guillain-Barré syndrome. *Lancet* 1993;341:586-590.
122. Yamagishi Y, Suzuki H, Sonoo M, et al. Markers for Guillain-Barré syndrome with poor prognosis: a multi-center study. *J Peripher Nerv Syst* 2017;22:433-439.



Chapter 13

Guillain-Barré syndrome in times of pandemics

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BACKGROUND

In the past decade the world confronted several pandemics of emerging infectious diseases including Zika virus and most recently SARS-CoV-2 virus. One of the neurological complications reported in relation to these infectious diseases is the Guillain-Barré syndrome (GBS), a rapidly progressive immune-mediated polyradiculoneuropathy that can cause paresis in all limbs, cranial, and respiratory muscles.[1-3] Approximately 20% require admission at an Intensive Care Unit (ICU), and 2-12% die, depending on the care available.[4]

In the past, research responses investigating a possible link between GBS and outbreaks of infectious diseases or vaccines have been delayed. This is problematic as health care institutions need to be able to prepare for increased incidences in patients with GBS, and public health personnel need to identify any possible mitigating factors. History now seems to repeat itself when case reports of SARS-CoV-2-related GBS are mounting, and disquiet over a possible association increases. As threats of epidemics of emerging infectious diseases persist, this is the time to learn from the past and to advance our response to future outbreaks in terms of research and management of GBS.

CHALLENGES AND PROSPECTS IN RESEARCH PREPAREDNESS

The first aims when studying a possible link between an infectious agent and GBS are to determine if a true association exists and to determine the impact in terms of frequency and severity. During an outbreak, observational cohorts are set up rapidly by clinicians, some of whom may lack experience in diagnosing and managing GBS due to the need to quickly mobilize personnel. These studies are often done at a single center and not harmonized with GBS research from other centers, which can result in missing out of important clinical information.

How can one ensure a high-quality study within the limited time frame afforded by an infectious disease epidemic? Many hurdles must be overcome before recruitment can be started, and accurate and sufficient data collection is complex. Here we list the most important hurdles and provide suggestions on how to deal with them.

Study design: surveillance and case-control studies

To determine the impact in terms of frequency, a reliable and international surveillance platform for GBS incidence during and between epidemics, either active or passive, should be in place to define the background incidence and to detect an increase in cases. A surveillance system for acute flaccid paralysis (AFP) in children under the age of 15 was set up to eradicate polio and is operative globally (http://polioeradication.org/). The international community may benefit from introducing AFP surveillance for all ages or for GBS specifically.

To determine an association between GBS and an infectious agent, a cohort study with a case-control design is necessary. A predefined research protocol should be developed that is feasible in different health care infrastructures and easy to activate and use, to ensure a high quality study within a limited timeframe. Critical requirements for the study include clear case-definitions for GBS and the collection of data on the clinical and electrophysiological phenotype, as this can be associated with a specific infectious agent and may provide evidence of an association. To study the impact for patients, outcome of at least 6-12 months with validated outcome measures should be recorded.

Such a protocol would be supported by a network of neurologists, such as the Inflammatory Neuropathy Consortium of the Peripheral Nerve Society, and can be based on the protocol of the International GBS Outcome Study (IGOS), that is running in 19 countries and is also used by other research groups.[5, 6] Existing networks, such as The Global Health Network could help to make the existence of such a protocol widely known.[7] Inspiration can be drawn from large international research consortia on infectious diseases, such as the International Severe Acute Respiratory and emerging Infection Consortium (ISARIC) and the Platform for European Preparedness Against (Re-)emerging Epidemics (PREPARE) that assure and prepare an agile research response to outbreak-prone infectious diseases (https://www.prepare-europe.eu/; https://isaric.tghn.org/).

Funding application and ethical permission

The time between application and receipt of funding and between submission and acceptance by an ethical review board is usually several months.[8, 9] This sequential process therefore often leads to significant delays. A recent example is the Zika virus pandemic that peaked at the beginning of 2016 when the World Health Organization declared it a Public Health Emergency of International Concern. By the time the Zika virus research consortia could initiate their work with funding from the

European Union in October 2016, the peak of the epidemic had passed, and most participating researchers still needed to go through ethical approval.[10] (**Figure 1**)

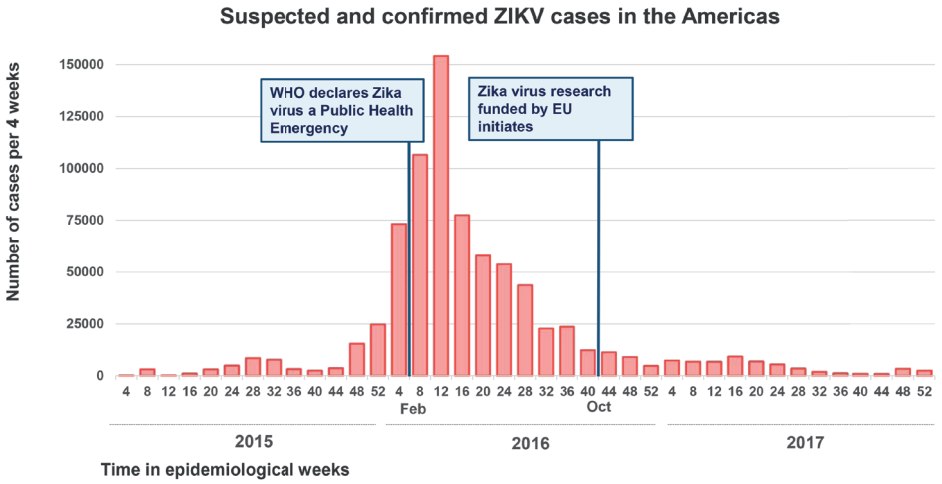


Figure 1 Suspected and confirmed ZIKV cases reported in the Americas by the WHO between January 2015 and December 2017, displayed per 4 epidemiological weeks.[11]
In October 2016 the ZikaPLAN Consortium was able to initiate with funding from the EU as part of the Horizon 2020 program.[10] ZIKV= Zika virus, WHO= World Health Organization, PHEIC= Public Health Emergency of International Concern, EU= European Union

Fortunately, there are already initiatives in place to accelerate the process of grant application and ethical review during an outbreak. The Global Research Collaboration for Infectious Disease Preparedness (GloPID-R) joins together major public and private research funding organizations to facilitate the mobilization of resources and the immediate start of critical research in an outbreak situation (https://www.glopid-r.org/).[12, 13] Legal, ethical, logistical, and administrative barriers that delay a research response at the peak of a health crisis could be addressed by making funding available during interpandemic periods, which can be used to develop standardized study protocols and research networks, with an additional budget to support infrastructure when the next outbreak occurs.

The idea of a 'central' or 'universal' Institutional Review Board (IRB) in which institutional review could be fast-tracked in situations of emergent infectious diseases has recently been launched by the National Institutes of Health, and in some jurisdictions events of public health emergency can bypass complete IRB approval, thus shortening the time to implementation.[14, 15]

Collecting and sharing data and biosamples

As GBS is a rare disease (1-2 per 100.000 per year), a multi-center, or even multi-national approach is generally necessary to capture a sufficient number of cases to provide evidence of an association and describe the clinical phenotype.[16] Setting up a multi-center study is time-consuming, and increasingly complex privacy regulations further restrict the sharing of data and biosamples between institutions. Operational consortia allow for the continued multi-center collection of data and samples during an epidemic, although sharing of biosamples often still requires material transfer agreements. Having pre-approved protocols and agreements ready for use upfront could accelerate this process.

CHALLENGES AND OPPORTUNITIES IN DIAGNOSIS AND MANAGEMENT

In case of a sudden increase in GBS patients, clinicians with limited expertise in GBS may need to manage these patients, and availability of facilities and resources may run out. We expect limitations mainly in ICU beds and rehabilitation care, as this was also reported during the Zika virus outbreak in Brazil.[17] These limitations are especially important in low-resource countries that often have suboptimal or malfunctional health care systems, a lack of health professionals, and are hot spots for outbreaks of emerging infectious diseases.[18] Here we provide recommendations on how to safeguard good quality diagnosis and management of GBS during a pandemic.

Guideline for management of GBS

Diagnosis, treatment and monitoring of GBS can be complicated as patients may present with non-specific symptoms and vary with respect to clinical severity, treatment response and outcome.[19] Furthermore, there are several diseases that can be difficult to distinguish from GBS, such as critical illness neuropathy, which is now especially important as many patients are admitted to the ICU for extended periods of time due to COVID-19. Recently a 10-step evidence-based consensus guideline for GBS was developed in response to the Zika virus outbreak.[20] This guideline was designed to be compact and easy-to-use and applicable in all health care settings. An online version of the guideline is supported by The Global Health Network (https://rede.tghn.org/gbs-flowchart-sample/introduction-gbs/). Its use may help improve the management of GBS during an outbreak.

Availability of resources

The two proven effective therapeutics for GBS, intravenous immunoglobulin (IVIg) and plasmapheresis, are expensive, and unaffordable for many patients in low-resource countries. Furthermore, demand for IVIg has tripled in the past decades, and shortages may occur in times of crisis.[21, 22] New and affordable treatment options for GBS are therefore warranted. A pilot study on small volume plasma exchange showed potential, but the therapeutic efficacy needs to be determined.[23]

The COVID-19 pandemic has made it apparent that upscaling availability of ICU beds is necessary to prepare for future outbreaks of infectious diseases that cause acute respiratory distress. Prediction models for respiratory failure in GBS patients, such as the Erasmus GBS Respiratory Insufficiency Score, may further relieve pressure from ICU facilities but need to be validated in non-Western countries.[24] Now that more patients are recovering from COVID-19, lack of caretakers and beds in rehabilitation units is also increasingly becoming a problem.[25] Upscaling availability is imperative to cope with this new wave of patients and will also be of use in a future outbreak of GBS.

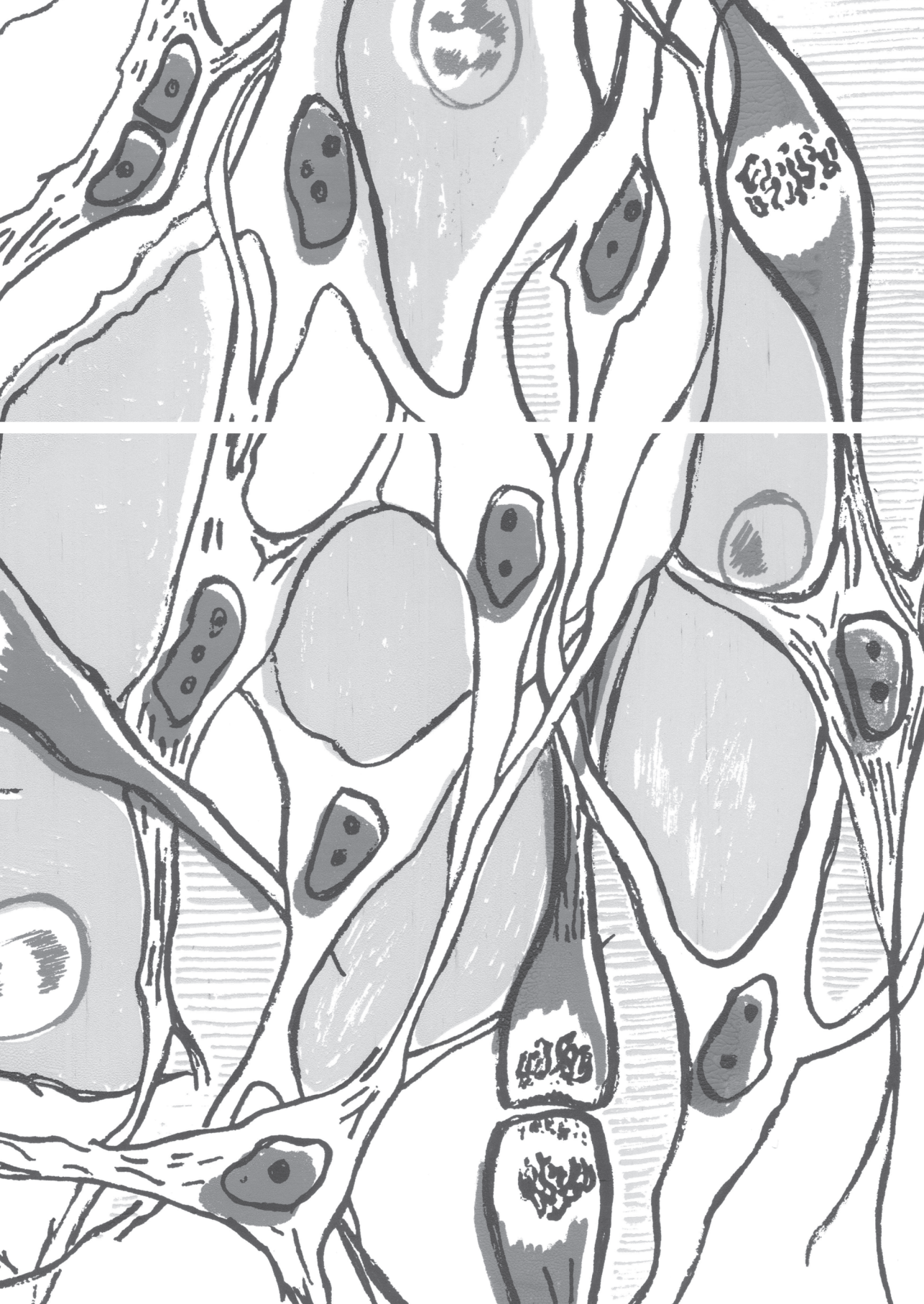
CONCLUSION

In the past decade, multiple pandemics of infectious diseases have been linked to increased incidence of GBS. Epidemics will continue to occur, and it is vital to advance preparedness in research and clinical management of GBS in an outbreak setting.

REFERENCES

1. Zhao H, Shen D, Zhou H, Liu J, Chen S. Guillain-Barre syndrome associated with SARS-CoV-2 infection: causality or coincidence? *Lancet Neurol* 2020.
2. Styczynski AR, Malta J, Krow-Lucal ER, et al. Increased rates of Guillain-Barré syndrome associated with Zika virus outbreak in the Salvador metropolitan area, Brazil. *PLoS Negl Trop Dis* 2017;11:e0005869.
3. Toscano G, Palmerini F, Ravaglia S, et al. Guillain-Barre Syndrome Associated with SARS-CoV-2. *N Engl J Med* 2020.
4. Doets AY, Verboon C, van den Berg B, et al. Regional variation of Guillain-Barré syndrome. *Brain* 2018;141:2866-2877.
5. Muñoz LS, Barreras P, Lizarazo J, et al. Neuroviruses Emerging in the Americas Study (NEAS): The Colombian experience during the 2016 outbreak of Zika virus infection (N4.002). *Neurology* 2017;88:N4.002.
6. International GBS Outcome Study Consortium. International GBS Outcome Study Consortium website [online]. Available at: www.gbsstudies.erasmusmc.nl.
7. Jacobs BC, van den Berg B, Verboon C, et al. International Guillain-Barre Syndrome Outcome Study: protocol of a prospective observational cohort study on clinical and biological predictors of disease course and outcome in Guillain-Barre syndrome. *J Peripher Nerv Syst* 2017;22:68-76.
8. Christie DR, Gabriel GS, Dear K. Adverse effects of a multicentre system for ethics approval on the progress of a prospective multicentre trial of cancer treatment: how many patients die waiting? *Intern Med J* 2007;37:680-686.
9. Schnitzbauer AA, Lamby PE, Mutzbauer I, Zuelke C, Schlitt HJ, Geissler EK. Procedures for ethical review for clinical trials within the EU. *Bmj* 2009;338:b1893.
10. Wilder-Smith A, Preet R, Renhorn KE, et al. ZikaPLAN: Zika Preparedness Latin American Network. *Glob Health Action* 2017;10:1398485.
11. Pan American Health Organization. PLISA Health Information Platform for the Americas, Cumulative Zika Virus Disease Cases [online]. Available at: https://www.paho.org/data/index.php/en/?option=com_content&view=article&id=524:zika-weekly-en&Itemid=352.
12. Gobat N, Amuasi J, Yazdanpanah Y, et al. Advancing preparedness for clinical research during infectious disease epidemics. *ERJ Open Res* 2019;5.
13. World Health Organization. World experts and funders set priorities for COVID-19 research. 2020.
14. Schopper D, Dawson A, Upshur R, et al. Innovations in research ethics governance in humanitarian settings. *BMC Medical Ethics* 2015;16:10.
15. Scott AM, Kolstoe S, Ploem MCC, Hammatt Z, Glasziou P. Exempting low-risk health and medical research from ethics reviews: comparing Australia, the United Kingdom, the United States and the Netherlands. *Health Res Policy Syst* 2020;18:11.
16. Sejvar JJ, Baughman AL, Wise M, Morgan OW. Population incidence of Guillain-Barré syndrome: a systematic review and meta-analysis. *Neuroepidemiology* 2011;36:123-133.
17. Leonhard SE, Conde RM, de Assis Aquino Gondim F, Jacobs BC. Diagnosis and treatment of Guillain-Barre syndrome during the Zika virus epidemic in Brazil: A national survey study. *J Peripher Nerv Syst* 2019;24:340-347.
18. Jones KE, Patel NG, Levy MA, et al. Global trends in emerging infectious diseases. *Nature* 2008;451:990-993.

19. Verboon C, van Doorn PA, Jacobs BC. Treatment dilemmas in Guillain-Barré syndrome. *J Neurol Neurosurg Psychiatry* 2017;88:346-352.
20. Leonhard SE, Mandarakas MR, Gondim FAA, et al. Diagnosis and management of Guillain–Barré syndrome in ten steps. *Nature Reviews Neurology* 2019.
21. Edington HJ, Sutton KS, Bennett C, Chandrakasan S, Sterner-Allison J, Castellino SM. Dealing with a critical national shortage-Approaches to triaging immune globulin supply in pediatric hematology and oncology. *Pediatr Blood Cancer* 2020:e28260.
22. Kerr J, Quinti I, Eibl M, et al. Is dosing of therapeutic immunoglobulins optimal? A review of a three-decade long debate in europe. *Front Immunol* 2014;5:629.
23. Islam MB, Islam Z, Rahman S, et al. Small volume plasma exchange for Guillain-Barré syndrome in resource poor settings: a safety and feasibility study. *Pilot Feasibility Stud* 2017;3:40.
24. Walgaard C, Lingsma HF, Ruts L, et al. Prediction of respiratory insufficiency in Guillain-Barré syndrome. *Ann Neurol* 2010;67:781-787.
25. Boldrini P, Bernetti A, Fiore P, Committee SE, affairs SCfi. Impact of COVID-19 outbreak on rehabilitation services and Physical and Rehabilitation Medicine (PRM) physicians' activities in Italy. An official document of the Italian PRM Society (SIMFER). *Eur J Phys Rehabil Med* 2020.



Discussion

In this thesis the role of infectious diseases in the development of GBS during endemic and epidemic phases of transmission was investigated. In the first part of the thesis the global distribution of infections known to trigger GBS and their association with clinical and electrophysiological subtypes and disease severity was studied. In the second and third part GBS during the Zika virus epidemic and the spectrum of neurological disease associated with Zika virus were investigated. In the fourth part GBS during other epidemics of infectious disease, including SARS-CoV-2, was studied. And in the final part ways were explored to improve the international management of GBS to advance our response to future outbreaks. Here I discuss the key findings of the previous chapters.

PART I. PRECEDING INFECTIONS IN GUILLAIN-BARRÉ SYNDROME

GBS is thought to be caused by an aberrant immune response to an infectious agent. GBS has a diverse spectrum of clinical phenotypes and outcome, and this heterogeneity is considered to be due to differences in the underlying pathophysiology, determining the types and parts of the nerves affected and the severity of nerve damage. The type of preceding infection is hypothesized to play an important role in the pathophysiology and disease diversity. Previous studies have shown that clinical phenotypes and outcome differ among GBS patients across the world, which was believed to be due in part to differences in the distribution of infections across regions.[1] We aimed to gain a better understanding of the global distribution of infections and the association with clinical variants and outcome of GBS. We used data collected in the International GBS Outcome Study (IGOS): the largest international cohort study on GBS, with patients included from 20 countries across Asia, Europe, North- and South America and Africa. For the study the first 1000 included patients with available biosamples (N=768) were tested for a recent infection with five pathogens that have been associated with GBS in previous studies: *Campylobacter jejuni*, hepatitis E virus (HEV), *Mycoplasma pneumoniae,* cytomegalovirus (CMV), and Epstein-Barr virus (EBV) (**Chapter 1**).

Global distribution of infections

C. jejuni was the most common preceding infection in GBS across the studied countries, occurring in 30% of patients, followed by *M. pneumoniae* in 10%, CMV in 4%, HEV in 3%, and EBV in 1%. The distribution of infections was similar across continents. This is remarkable, as previous studies have indicated that *C. jejuni* infection is more common in several Asian countries, compared to Europe and the Americas,

and this has been suggested to be the cause of regional variation in the clinical and electrophysiological phenotype and severity of GBS.[1-3] Patients with a recent *C. jejuni* infection from Asia did have a significantly higher rate of the pure motor variant and axonal electrophysiological subtype compared to patients from Europe or the Americas. This suggests that, instead of differences in the distribution of infections, differences in the strength of the association between the infection and the phenotype of GBS may partly explain the geographical variation. This may be caused by regional variation in bacterial strains, host susceptibility, or patient selection or -management.[1, 4] A substantial proportion of patients (59%) had no laboratory evidence of a recent infection with the tested pathogens. It may be that part of the cases were falsely tested negative, as we were not able to perform PCR or culture and may have missed cases that did not (yet) mount a serological response. Nevertheless, I believe that it is most likely that many of these patients had a recent infection with other pathogens than were investigated in the study. Although GBS has also been reported after non-infectious events, including surgery or vaccination, such events were reported in only a minority of cases in our cohort.

Clinical phenotype and prognosis associated with infections

Infections were associated with demographic, clinical and electrophysiological features. Patients with a recent *C. jejuni* infection more often had a pure motor variant and axonal electrophysiological subtype, severe weakness, and a worse prognosis.[3, 5, 6] Patients with a HEV infection were almost all middle-aged males with a severe form of sensorimotor demyelinating GBS. Patients with CMV or EBV generally had a sensorimotor demyelinating GBS and a good long-term prognosis. *M. pneumoniae* infection was associated with younger age and relatively high proportion of the pure motor and MFS variant. In general, patients with a bacterial infection more often had a faster disease progression and a pure motor axonal variant or Miller Fisher syndrome, whereas virus infection was associated with a sensorimotor demyelinating phenotype. In other studies where we described GBS after Zika virus and chikungunya virus (**Part II**) and SARS-CoV-2 virus (**Part V**), the sensorimotor demyelinating phenotype was also most frequently found. This could be due to a different underlying pathophysiology in virus-related compared to bacterium-related GBS. Lipo-oligosaccharides of *C. jejuni* have been demonstrated to mimic gangliosides of the peripheral nerve, and antibodies against *C. jejuni* subsequently cross-react with nerve gangliosides, causing inflammation and nerve damage.[7] Different types of such anti-ganglioside antibodies have been found depending on the preceding infection. Antibodies to GM1 and GD1a are typically found in patients with a preceding *C. jejuni* infection, and antibodies to galactocerebroside (GalC) are associated with a preceding *M. pneumoniae* infection.[8] Anti-ganglioside antibodies

have however not convincingly been demonstrated in GBS patients with a preceding virus infection.[9-12] Although anti-GM2 antibodies have been found in GBS patients with a CMV infection, these were also present in patients with a CMV infection without GBS, and therefore the pathogenicity of this antibody is not clear.[11-13] This suggests that virus-related GBS may be caused by an immune response that does not involve anti-ganglioside antibodies, although it could also be that such antibodies in have simply not yet been found. Other possible pathophysiological mechanisms may include: molecular mimicry between viral and human proteins, instead of glycolipids, or the incorporation of host antigens in the viral envelope.[14-16]

In contrast to some previous studies,[17] we showed that *C. jejuni* is not uniquely associated with a pure motor axonal GBS, and can elicit sensorimotor demyelinating GBS as well.[18] A demyelinating electrophysiological subtype was even found in the majority of *C. jejuni*-positive patients. This discrepancy between our and some previous studies may be due to the limitation of a single nerve conduction study to determine the electrophysiological subtype of GBS, as studies have shown that patterns may change in serial testing.[17, 19] It may also be that *C. jejuni* is able to cause primary demyelination of the peripheral nerves, although it is unclear what the underlying mechanism would be in these cases.

Multiple recent infections

Interestingly, evidence of more than one recent infection was found in 6% of patients. In other studies in this thesis, presence of multiple recent infections has also been found: in 14/72 (29%) patients in **Chapter 3**, a GBS cohort collected during the Zika virus and chikungunya virus epidemic in Northeast Brazil, in 4/49 (8%) in **Chapter 4**, a GBS cohort from Brazil, Argentina and Malaysia, and in 2/49 (4%) patients in **Chapter 9**, a GBS cohort collected during the SARS-CoV-2 pandemic. In previous GBS cohort studies reporting on multiple recent infections, percentages varied between 2 and 17%.[20-25] It is not clear what this finding signifies. Perhaps the presence of several infections causes a more severe immune response that increases the risk of GBS. This 'dual hit hypothesis' may likewise partly explain why some people develop GBS after certain common infections, while others do not.[26] The finding in **Chapter 3**, that patients with a dual infection with Zika and chikungunya had a more severe disease course, may be seen as further evidence for this hypothesis. Another explanation is polyclonal B-cell activation as a response to one preceding infection, leading to false positive results of others.[27, 28] In some patients with more than one recent infection, infectious symptoms or clinical GBS phenotypes were more typical for one of the diagnosed infections, which can be seen as further support for this proposition. This was also seen in a study from

Bangladesh, where 9/18 Zika virus-positive GBS cases also had evidence of a recent *C. jejuni* infection and a clinical phenotype (diarrhea, pure motor variant, axonal electrophysiological subtype) typical for *C. jejuni*.[29] Nevertheless, our study (**Chapter 1**) also showed that presence or type of preceding symptoms of an infection were non-specific in identifying the underlying trigger. For instance, 67/181 (37%) patients that did not report symptoms of an infection had serological evidence of a recent infection, and in 10/43 (23%) patients reporting a vaccination, serological evidence of an infection was found. These findings indicate that broad serological testing is important in defining the most likely infectious trigger in GBS. In light of the ongoing vaccination campaign for SARS-CoV-2, this highlights the importance for clinicians and public health officials of thoroughly investigating other infectious causes in patients developing GBS after receiving a vaccine.[30, 31]

PART II. GUILLAIN-BARRÉ SYNDROME DURING THE ZIKA VIRUS EPIDEMIC

During the Zika virus epidemic in Latin America in 2015-2016, increased incidence of GBS was reported in regions where the Zika virus transmission peaked.[32] Our aims were to determine whether a true association existed and to investigate the clinical features and outcome of GBS related to Zika virus infection.

Association between GBS and Zika virus infection

In **Chapter 2** we presented a systematic literature review and meta-analysis of all reported patients with GBS and evidence of a recent Zika virus infection. Thirty-five studies were included, containing five case-control studies,[33-36, 37] reporting on a total of 601 GBS cases. The case-control studies investigating the Zika virus epidemics in the Pacific island groups of French Polynesia and New Caledonia (2013-2014) and in Latin America (2015-2016), all found evidence of an association between Zika and GBS.[33-36] In the study from French Polynesia, GBS incidence increased more than tenfold compared to the preceding 5 years, and the risk of GBS was estimated at 0.24 per 1000 Zika virus infections.[33] A case-control study from Singapore, investigating a smaller Zika virus outbreak in 2016 (455 cases reported to national surveillance) did not find an association between GBS and Zika.[37] At least three more case-control studies published after our systematic review established an association between Zika and GBS.[38-40] Further evidence of an association was provided by our observational cohort of GBS patients collected during the Zika virus epidemic in Northeast Brazil (December 2014-February 2017) (**Chapter 3**). We found an increase in GBS patients admitted to the study hospital compared to previous years, and 39 (55%)

of 71 included patients had evidence of a recent Zika virus infection. Evidence of both a recent Zika and chikungunya virus infection was found in 14 (20%) cases, and another 8 (11%) had evidence of a chikungunya mono-infection, probably reflecting the overlap of the Zika virus epidemic (end 2014-2016) and chikungunya virus epidemic (2016) in the region. Just one patient had a recent dengue virus infection.

When the number of Zika virus cases declined, we wondered whether this virus was also a driver of GBS beyond phases of epidemic transmission. We investigated this in the IGOS-Zika case-control study (**Chapter 4**) that was conducted in Brazil, Argentina and Malaysia after the peak of the Zika virus pandemic, between January 2017 and December 2019. The study was based on the IGOS protocol, with the additional collection of two hospital-based time, age- and sex-matched controls for every case. All cases and controls were tested for serological evidence of a recent infection with Zika virus, chikungunya virus, dengue virus, *C. jejuni*, *M. pneumoniae*, CMV, EBV and HEV. Of the 49 included patients, two (4%) had evidence of a recent chikungunya virus infection, two (4%) of a recent dengue virus infection, and none of a recent Zika virus infection. The patients with a dengue virus infection also had evidence of other recent infections (CMV and *C. jejuni*). Although no evidence of a recent arbovirus infection was found in the 35 controls, the study was underpowered to find significant differences. Considering results from a previous meta-analysis which estimated the overall risk of reported GBS at 2.0 (95% CI 0.5-4.5) per 10,000 Zika virus cases, it makes sense that during smaller outbreaks, such as the one reported in Singapore, or during endemic phases with low transmission, no association with GBS is found.[37, 41] This estimated risk is comparable to the risk of GBS after *C. jejuni* infection (±2.5-6.5 cases per 10,000 infections),[42] but magnitudes higher than the annual global incidence of GBS (±1–2 cases per 100,000 person–years), indicating the potential of Zika virus to cause large outbreaks of GBS during epidemics.

Clinical phenotype of GBS after Zika virus

A short time between onset of neurological signs after Zika virus symptoms in early publications, in combination with the neurotrophic potential of Zika, initially led to the suggestion that Zika virus-related GBS was, in contrast to GBS after other infections, not a post-infectious disease, but caused by a direct infection of the peripheral nerves or through a para-infectious mechanism.[43, 44] In the 35 studies included in our systematic review (**Chapter 2**), the median time between onset of neurological symptoms after the first symptoms of infection (5-12 days) was similar to GBS after other infections in **Chapter 1** (10 days) and **Chapter 4** (7 days), and in other previous publications.[5, 45, 46] Considering the estimated 1–2 week incubation period of Zika virus, the latency between the initial infection and GBS was more than a week for

most cases.[47] Furthermore, the lack of an increased cerebrospinal fluid (CSF) cell count in any of the studies in the systematic review, or in any of the cases in the cohort from Northeast Brazil, as well as a lack of Zika virus PCR in the CSF in most reported cases, argue against direct infection of the peripheral nerves. This is reinforced by post-mortem findings of a Zika virus-related GBS patient where no evidence of Zika virus was found in nervous tissue.[48] The patients with GBS following Zika virus infection described in **Chapter 2** and **Chapter 3** had a fairly uniform clinical and electrophysiological phenotype: sensorimotor clinical variant and demyelinating electrophysiological subtype, which is similar to the phenotype in other virus-related GBS found in **Chapter 1** and previous studies.[23, 45, 49] In **Chapter 2**, a severe disease course was found in a high proportion of Zika virus-related GBS patients, with ±50% admitted to the ICU, compared to 15-30% in the systematic review.[50, 51] In the cohort from Northeast Brazil (**Chapter 3**), the percentage of patients admitted to the ICU was also high (36%) in patients with evidence of both a recent Zika and chikungunya virus infection, although this percentage was relatively low (4%) in those with a Zika virus mono-infection. Whether this is a reflection of inclusion bias, or indicates that Zika virus can cause a particularly severe form of GBS, is not clear. All these findings combined indicate that Zika virus-related GBS is clinically similar to other virus-related GBS, and likely shares the same pathophysiological mechanism.

The absence of a specific anti-ganglioside antibody signature in all Zika virus-related GBS patients in the studies described in **Chapter 4** and **Chapter 5** is also in line with what has been found in GBS after other virus infections. These findings contradict studies of Zika virus-related GBS patients in French-Polynesia, in whom antibody activity against GD1a was found,[33] and Brazil, where a universal increase in anti-glycolipid antibodies was observed in patients with GBS following Zika virus infection.[52] The discrepancy between our findings and the Brazilian study is likely due to their underestimation of nonspecific binding amongst individuals that we observed in our platforms. Differences in Zika virus strains may explain why findings from the French Polynesian study have not been replicated in any study conducted during the Latin American Zika virus epidemic. Still molecular mimicry and cross-reactive immune responses may play a role in the pathogenesis of Zika virus-related GBS. First, Zika virus may trigger an immune response to other glycolipids or proteins residing at the peripheral nerve. One study showed several sequence homologies between Zika virus and host proteins.[53] Second, Zika virus may trigger a cross-reactive T-cell response to human proteins or glycolipids instead of cross-reactive antibodies. However, whether this homology indeed contributes to pathogenesis should be studied further in animal models.

GBS and other arbovirus infections

In both **Chapter 3** and **Chapter 4** evidence of arbovirus infections other than Zika virus was found in a proportion of cases. Dengue and chikungunya virus have been linked to GBS in previous studies as well, although evidence of an association is limited in comparison to Zika. Two surveillance studies have associated increased incidence of GBS temporally to increased incidence of dengue virus infection,[54, 55] and further case reports and –series have indicated a link between the two.[55-60] Zika and dengue virus are both flaviviruses of the family of *Flaviviridae*, and it can be difficult to distinguish them based on serological studies, as antibodies against dengue virus are able to cross-react with Zika virus particles.[61] A few studies described an association between 'flavivirus infection' or dengue virus neutralizing antibodies and GBS, but in these cases Zika could not be sufficiently distinguished from dengue virus.[38, 39, 54] This is generally not an issue with chikungunya virus as it belongs to a different genus and family (Alphavirus, family *Togaviridae*). Several studies[62-64] linked clusters of GBS cases with outbreaks of chikungunya virus infection, and a case-control study performed in Guadaloupe[65] showed that chikungunya was a risk factor for GBS. Combined with our findings from **Chapter 4** this indicates that besides Zika virus, chikungunya virus is also able to trigger GBS.

PART III. THE SPECTRUM OF NEUROLOGICAL DISEASE ASSOCIATED WITH ZIKA VIRUS INFECTION

Zika virus is able to infect a broad range of central nervous system cells, and -although less effectively- peripheral nervous system cells.[66] The potential of Zika virus to infect fetal neural progenitor cells is evidenced by congenital Zika virus syndrome.[67, 68] And although the mature brain seems less susceptible to neuroinvasion, a large spectrum of neurological disease in adult patients has been linked to Zika virus, as described in **Chapter 5**. In the cohort from Northeast Brazil (**Chapter 3**), patients with other neurological disease besides GBS were also studied and described in a separate publication.[69] In this cohort, a large spectrum of central nervous system disease was found in patients with evidence of a recent Zika virus infection, including (meningo)encephalitis, myelitis, myeloradiculopathy, and acute disseminated encephalomyelitis (ADEM), which have been reported in relation to Zika virus in other studies as well.[70, 71] Nevertheless, evidence of Zika virus was much more common in peripheral nervous system disease, which consisted mostly of GBS, in addition to a few patients with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) or radiculitis. In contrast, in patients with a recent

chikungunya virus infection, central nervous system disorders were more common than peripheral nervous system disorders.

We also described a case with (acute onset) CIDP (**Chapter 6**) and neuromyelitis optica (**Chapter 7**) in relation to Zika virus infection. Although these reported cases do not prove a causal relationship, the neurotrophic and neuroinflammatory capabilities of the virus, the temporal relation, and the exclusion of other potential causes in the described cases provide evidence for such a link. The spectrum of neurological complications covers diseases with a presumed role for a direct infection of the nervous system, and those with immune-mediated disorders. This indicates that both these mechanisms contribute to the disease burden of Zika virus, although the exact pathophysiology has not been fully elucidated for most of these disorders.[72, 73]

PART IV. GBS IN RELATION TO OTHER EPIDEMICS OF INFECTIOUS DISEASE

In this part of the thesis we described two other epidemics in relation to GBS, and reflected on the pitfalls of investigating an association between GBS and infectious diseases.

Outbreak of GBS related to *C. jejuni*

The number of GBS cases reported to the public health system in Peru increased from 59 in 2017 (incidence: 0.19/100,000) to 262 in 2018 (incidence: 0.81/100,000) and 1,120 in 2019 (incidence: 3.44/100,000).[74] In **Chapter 8** we investigated the cause of this large outbreak in a case-control study. Although it was initially thought that the cause of the outbreak was Zika virus, as transmission still occurred at high rates in Latin America during that time, we discovered that in fact *C. jejuni* was the infectious driver. Evidence of recent *C. jejuni* infection was found in 28/43 (65%) cases, and none had evidence of a recent arbovirus infection. The odds ratio of a recent *C. jejuni* infection in cases versus controls was 3.3 (confidence interval 1.2-9.32, $P<0.01$). Patients with a *C. jejuni* infection generally had the clinical (pure motor, axonal GBS) and anti-ganglioside antibody (to GM1, GT1a) profile typical for this infection. Hence, the clinical and electrophysiological phenotype already suggested that *C. jejuni* and not Zika virus was the infectious driver, showing the relevance of the phenotypical features of GBS as a directive in determining the most likely causal infectious agent.

GBS during the SARS-CoV-2 pandemic

At least 90 GBS cases were reported in relation to SARS-CoV-2 between the start of the pandemic (end 2019) and February 2021.[75-78] It was unclear if these cases were co-incidental or signified a true association. To investigate this further, we investigated patients included in IGOS during the first 3 months of the pandemic for clinical and laboratory signs of a recent SARS-CoV-2 infection (**Chapter 9**). Of the 49 included patients, eight (16%) had a confirmed and three (6%) a probable SARS-CoV-2 infection.[79-81] Two of these 11 patients also had serological evidence of a recent *C. jejuni* infection. Patients with a confirmed or probable SARS-CoV-2 infection generally had a sensorimotor demyelinating phenotype of GBS, which is again similar to GBS after other virus infections. No increase in inclusion rate in IGOS was found compared to previous years. The frequency (22%) of a probable/confirmed SARS-CoV-2 infection in the study population was higher than the reported estimated background prevalence at the time (5-10%), although accurate estimates of SARS-CoV-2 incidence in the first months of the pandemic are lacking.[82, 83] While some smaller studies reported an increase in GBS hospital admissions during the pandemic,[76, 84] epidemiological studies from the United Kingdom and Singapore found a decrease in the incidence of GBS when compared to previous years.[85] [86] This could be explained by a reduced exposure to other pathogens due to the governmental measures, such as lockdown, increased hygienic measures, and curfew. Nevertheless, given the immense exposure to SARS-CoV-2 in this period and the lack of an increase in cases, the risk of GBS after COVID-19 would have to be small.[31] Another study from the UK looked at the risk of several neurological complications in the 2 months after the ChAdOx1nCoV-19 (Oxford Astra Zenica) and BNT162b2 (BioNTech Pfizer) vaccine using data collected from all vaccinated people in England.[87] They found an increased risk of 38 excess GBS cases per 10 million ChAdOx1nCoV-19 vaccines and no increased risk in those receiving the BNT162b2 vaccine. They also looked at the people testing positive for SARS-CoV-2 in the same population (before or after receiving the vaccine) and found a much higher risk of 145 excess GBS cases per 10 million. So according to this study, both the vaccine and the infection are associated with a small increase in risk of GBS, but the risk is relatively much higher in infected cases. Combining the results of all these studies, it seems likely that there is an association between SARS-CoV-2 infection and GBS in rare cases.

PART V. IMPROVING GLOBAL RESEARCH, DIAGNOSIS AND MANAGEMENT OF GBS

Impact of the Zika virus epidemic on management of GBS

During the Zika virus epidemic GBS incidence increased up to 20-fold in several countries. To investigate how this impacted the diagnosis and management of these patients, we conducted a survey study among Brazilian neurologists (**Chapter 10**). All 3264 neurologists that were member of the Brazilian Academy of Neurology at the time of the study were invited to participate. The questionnaire was fully answered by 171 (5%) neurologists. During the Zika virus epidemic 61% of neurologists noticed an increase in incidence of GBS, and 30% experienced increased problems in managing these patients. No national protocol for treating GBS was available at the time, and treatment practice varied. The most important limitations in managing GBS included the availability of nerve conduction studies, ICU beds, and beds in the rehabilitation unit. These limitations in several basic aspects of the management of GBS stand in stark contrast to the clinical practice known to us in The Netherlands, and reported in most papers on GBS.[88, 89]

Management of GBS in low- and middle income countries

Following this study we wanted to broaden our scope and gain a better understanding of GBS in low- and middle-income countries in general, and how this compares to high-income countries. We reviewed existing literature on GBS in low- and middle-income countries (**Chapter 11**), and found that the incidence of GBS in these countries is largely unknown. Diagnosis of GBS is often delayed due to late admission to the hospital, and diagnostic tools such as nerve conduction studies are frequently unavailable.[90, 91] Intravenous immunoglobulins or plasma exchange are unaffordable for most and ICU support is not always available for GBS patients in low- and middle-income countries.[92] This contributes to a considerable higher mortality (10-26%),[1, 3, 92-95] compared to Europe and North America (2-7%).[1, 89, 96] This study underscored the need for the expansion of GBS research to low- and middle-income countries, specifically to identify affordable treatment options.[97, 98]

Improving global management of GBS

To aid clinicians world-wide in diagnosing and managing GBS, especially in the context of emerging epidemics, we developed the first international guideline for GBS (**Chapter 12**). The guideline is based on available evidence in literature and consensus of a team of 21 international GBS experts based in North- and South America, Asia, Europe, and Africa. The guideline was developed for general applicability in all clinical environments, irrespective of specialist capabilities or availability of re-

sources, and has a ten step approach to facilitate its use in clinical practice. The ten steps cover all important aspects of the diagnosis, the management during the acute and plateau phase, and the prognosis and outcome. We also took actions to make sure the guideline was widely distributed and implemented in clinical practice. We published with Open Access, translated to Portuguese, Spanish, and Mandarin, and also published the translations in national Brazilian, Argentinian, and Chinese journals (Appendix).[99, 100] Furthermore, an online version of the guideline was developed in collaboration with The Global Health Network, an online research-sharing platform used by ±28 million health workers and researchers from all over the world, including many low- and middle-income countries.[99] Following the success of this online guideline, we received funding to create a webpage fully dedicated to GBS, on which these research items and learning modules are shared.

FUTURE DIRECTIONS

In this thesis we demonstrated that preceding infections in GBS are associated with clinical phenotypes, disease severity and outcome. In all cohorts described in this thesis, patients with GBS following virus infections generally had a sensorimotor demyelinating variant, and no typical anti-ganglioside antibody profile. This suggests that virus infections may elicit a different type of immune response compared to bacterial infections. The testing of anti-ganglioside antibodies in the IGOS 1000 cohort may shed more light on this enigma. We also found that *C. jejuni* infection is associated with a worse outcome, and it should be investigated in future studies whether infection serology is a useful addition to prognostic models that predict the disease course in GBS patients. In the cohorts described in this thesis many patients tested negative for the five infections most commonly associated with GBS (*C. jejuni*, *M. pneumoniae*, HEV, CMV, EBV), and I believe it is most likely that these patients had a recent infection with pathogens that we did not test for. This may include other infections of the herpes family (of which CMV and EBV are part), such as varicella zoster and herpes simplex virus,[101] or other pathogens that have been associated with GBS in some previous studies, including *Haemophilus influenzae*, HIV and influenza virus.[20-22, 25, 102-110] To further investigate the association between GBS and preceding infections, a large cohort of cases and controls should be compared with this broader spectrum of infections. Such studies may also include Zika virus and chikungunya virus, to further investigate whether these infections also play a role in triggering GBS during endemic phases of transmission.

An important limitation of the studies in this thesis is that most described cohorts may not be representative of GBS in the general population, as participating centers were often academic hospitals, and inclusion of patients may be biased towards more complicated or severe cases.[111] Surveillance studies or (inter)national cohort studies where all patients within previously defined regions are included are necessary to determine whether recruitment bias indeed plays a role in our findings. A successful surveillance system for acute flaccid paralysis in children under the age of 15 has been set up to eradicate polio and is operative globally (http://polioeradication.org/). This existing surveillance system could perhaps be expanded to include acute flaccid paralysis surveillance of all ages, or for GBS specifically.

In the past, research responses investigating a link between GBS and outbreaks of infectious disease have been delayed. This is problematic as healthcare institutions need to prepare for a potential increase in incidence of GBS patients. The SARS-CoV-2 pandemic and the disquiet over a possible association with GBS exemplifies the ongoing threat of emerging infectious diseases, and shows the importance of advancing our response to future outbreaks. During the Zika virus epidemic I experienced first-hand how challenging it can be to set up a study in the limited time frame afforded by an infectious disease outbreak. In **Chapter 13** the lessons that I learned from these studies are discussed. As GBS is a rare disease, a multicenter or multinational approach is generally necessary to capture a sufficient number of cases. However, the acquisition of funding and the obtainment ethical permission of all participating centers is time-consuming, and increasingly stringent privacy regulations restrict the sharing of data and biosamples between institutions. By the time we were able to start collecting patients, the peak of the Zika virus epidemic had passed. Legal, logistical and administrative barriers that delay a research response could be addressed in future outbreaks by accelerating the process of grant application or by making funding available during interpandemic periods, which can be used to develop standardized study protocols and research networks. The Global Research Collaboration for Infectious Disease Preparedness is an initiative that aims to accelerate this process by joining together major research funding organizations to facilitate the mobilization of resources and the immediate start of research during an outbreak. (https://www.glopid-r.org). Ethical barriers could be overcome by instituting a 'universal' institutional review board (IRB), which would be applicable to all participating (international) institutions. An initiative for such an IRB has recently been launched by the National Institutes of Health.[112] And finally, operational research networks and preapproved protocols and agreements are important to accelerate the response to future outbreaks of GBS. The continuation of the IGOS Consortium with the addition of a case-control protocol that can

be pre-approved and ready-to-go in all participating centers would be an important contribution to the response to future outbreaks of infectious disease related to GBS.

KEY POINTS

- Distribution of preceding infections in GBS is similar across the world, and therefore other factors play a role in the global variation in clinical features and outcome of GBS.
- GBS after virus infections generally presents as a classic sensorimotor variant and demyelinating subtype.
- Routine serological studies may offer practical information about the prognosis of GBS in clinical practice.
- Evidence of more than one recent infection frequently occurs in GBS patients and may play a role in the underlying pathophysiology.
- Serious limitations in the diagnosis and management of GBS in low- and middle income countries may lead to critical situations during outbreaks of GBS.
- Global research capacity and resource availability for GBS need to be improved to prepare for future outbreaks.

REFERENCES

1. Doets AY, Verboon C, van den Berg B, et al. Regional variation of Guillain-Barré syndrome. Brain 2018;141:2866-2877.
2. Ho TW, Mishu B, Li CY, et al. Guillain-Barré syndrome in northern China. Relationship to Campylobacter jejuni infection and anti-glycolipid antibodies. Brain 1995;118 (Pt 3):597-605.
3. Islam Z, Jacobs BC, van Belkum A, et al. Axonal variant of Guillain-Barré syndrome associated with Campylobacter infection in Bangladesh. Neurology 2010;74:581-587.
4. Zhao Y, Zhu R, Tian D, Liu X. Genetic polymorphisms in Guillain-Barré Syndrome: A field synopsis and systematic meta-analysis. Autoimmunity Reviews 2020;19:102665.
5. Rees JH, Soudain SE, Gregson NA, Hughes RA. Campylobacter jejuni infection and Guillain-Barré syndrome. N Engl J Med 1995;333:1374-1379.
6. Mishu B, Ilyas AA, Koski CL, et al. Serologic evidence of previous Campylobacter jejuni infection in patients with the Guillain-Barré syndrome. Ann Intern Med 1993;118:947-953.
7. Yuki N, Susuki K, Koga M, et al. Carbohydrate mimicry between human ganglioside GM1 and Campylobacter jejuni lipooligosaccharide causes Guillain-Barré syndrome. Proc Natl Acad Sci U S A 2004;101:11404-11409.
8. Kuwahara M, Samukawa M, Ikeda T, et al. Characterization of the neurological diseases associated with Mycoplasma pneumoniae infection and anti-glycolipid antibodies. J Neurol 2017;264:467-475.
9. van den Berg B, van der Eijk AA, Pas SD, et al. Guillain-Barré syndrome associated with preceding hepatitis E virus infection. Neurology 2014;82:491-497.
10. Leonhard SE, Bresani-Salvi CC, Lyra Batista JD, et al. Guillain-Barré syndrome related to Zika virus infection: A systematic review and meta-analysis of the clinical and electrophysiological phenotype. PLoS Negl Trop Dis 2020;14:e0008264.
11. Jacobs BC, van Doorn PA, Groeneveld JH, Tio-Gillen AP, van der Meche FG. Cytomegalovirus infections and anti-GM2 antibodies in Guillain-Barré syndrome. J Neurol Neurosurg Psychiatry 1997;62:641-643.
12. Yuki N, Tagawa Y. Acute cytomegalovirus infection and IgM anti-GM2 antibody. J Neurol Sci 1998;154:14-17.
13. Irie S, Saito T, Nakamura K, et al. Association of anti-GM2 antibodies in Guillain-Barré syndrome with acute cytomegalovirus infection. J Neuroimmunol 1996;68:19-26.
14. A. Nath JRB. Clinical Neurovirology, Second ed: CRC Press, 2020.
15. Oldstone MB. Viruses and autoimmune diseases. Scand J Immunol 1997;46:320-325.
16. Oldstone MB. Molecular mimicry and immune-mediated diseases. FASEB J 1998;12:1255-1265.
17. Kuwabara S, Ogawara K, Misawa S, et al. Does Campylobacter jejuni infection elicit "demyelinating" Guillain-Barré syndrome? Neurology 2004;63:529-533.
18. Drenthen J, Yuki N, Meulstee J, et al. Guillain-Barré syndrome subtypes related to Campylobacter infection. J Neurol Neurosurg Psychiatry 2011;82:300-305.
19. Uncini A, Ippoliti L, Shahrizaila N, Sekiguchi Y, Kuwabara S. Optimizing the electrodiagnostic accuracy in Guillain-Barré syndrome subtypes: Criteria sets and sparse linear discriminant analysis. Clin Neurophysiol 2017;128:1176-1183.

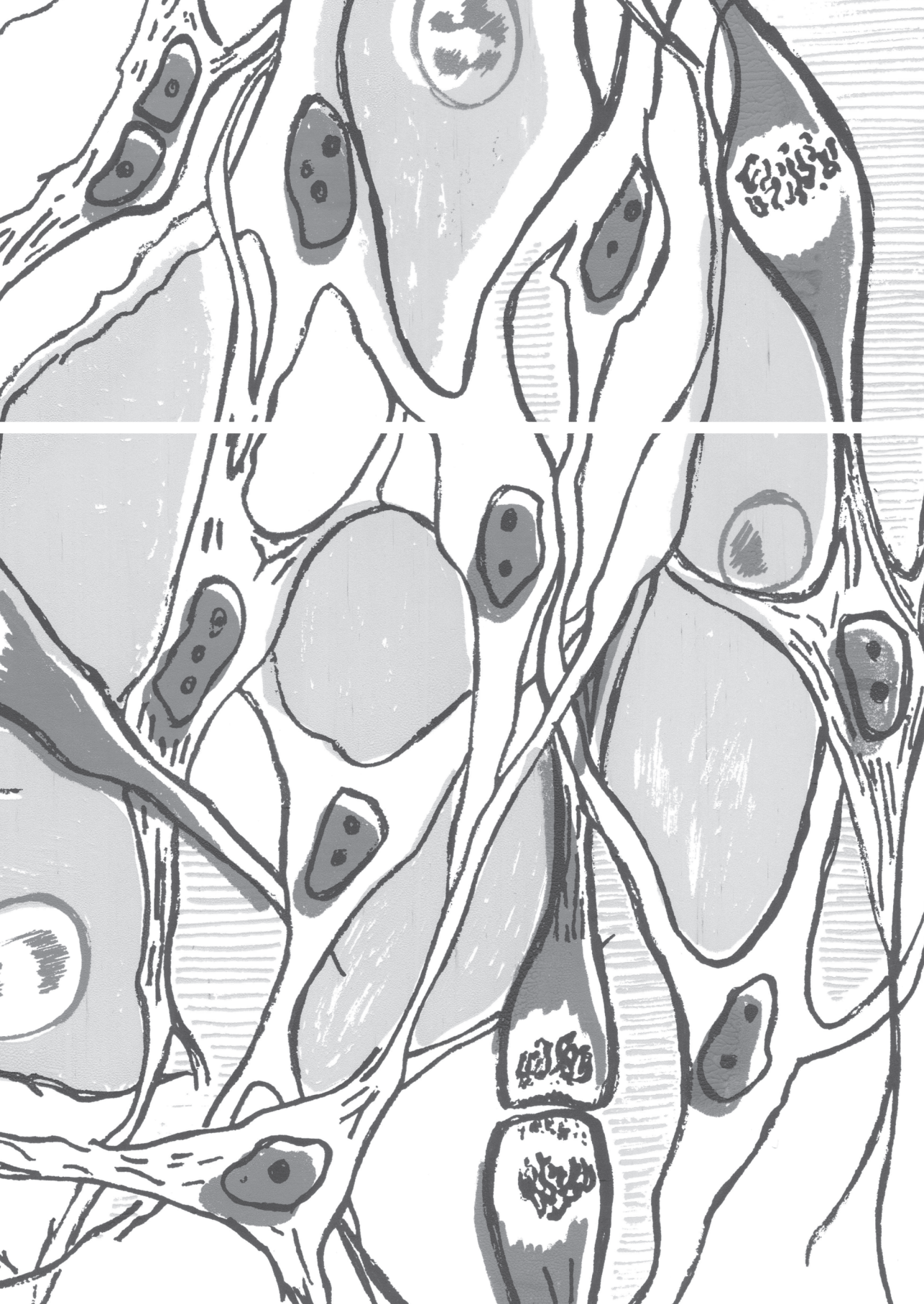
20. Jacobs BC, Rothbarth PH, van der Meche FG, et al. The spectrum of antecedent infections in Guillain-Barré syndrome: a case-control study. Neurology 1998;51:1110-1115.
21. Koga M, Yuki N, Tai T, Hirata K. Miller Fisher syndrome and Haemophilus influenzae infection. Neurology 2001;57:686-691.
22. Boucquey D, Sindic CJ, Lamy M, Delmee M, Tomasi JP, Laterre EC. Clinical and serological studies in a series of 45 patients with Guillain-Barré syndrome. J Neurol Sci 1991;104:56-63.
23. Caudie C, Quittard Pinon A, Taravel D, et al. Preceding infections and anti-ganglioside antibody profiles assessed by a dot immunoassay in 306 French Guillain-Barré syndrome patients. J Neurol 2011;258:1958-1964.
24. Stevens O, Claeys KG, Poesen K, Saegeman V, Van Damme P. Diagnostic Challenges and Clinical Characteristics of Hepatitis E Virus-Associated Guillain-Barré Syndrome. JAMA Neurol 2017;74:26-33.
25. Hao Y, Wang W, Jacobs BC, et al. Antecedent infections in Guillain-Barré syndrome: a single-center, prospective study. Ann Clin Transl Neurol 2019;6:2510-2517.
26. Huizinga R, van den Berg B, van Rijs W, et al. Innate Immunity to Campylobacter jejuni in Guillain-Barré Syndrome. Ann Neurol 2015;78:343-354.
27. Hyams C, Mabayoje DA, Copping R, et al. Serological cross reactivity to CMV and EBV causes problems in the diagnosis of acute hepatitis E virus infection. J Med Virol 2014;86:478-483.
28. Kuijf ML, Samsom JN, van Rijs W, et al. TLR4-Mediated Sensing of Campylobacter jejuni by Dendritic Cells Is Determined by Sialylation. The Journal of Immunology 2010;185:748-755.
29. GeurtsvanKessel CH, Islam Z, Islam MB, et al. Zika virus and Guillain-Barré syndrome in Bangladesh. Ann Clin Transl Neurol 2018;5:606-615.
30. Keddie S, Pakpoor J, Mousele C, et al. Epidemiological and cohort study finds no association between COVID-19 and Guillain-Barré syndrome. Brain 2021;144:682-693.
31. Lunn MP, Cornblath DR, Jacobs BC, et al. COVID-19 vaccine and Guillain-Barré syndrome: let's not leap to associations. Brain 2021;144:357-360.
32. World Health Organization. Zika situation report 10 March 2017. 2017.
33. Cao-Lormeau VM, Blake A, Mons S, et al. Guillain-Barré syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. Lancet 2016;387:1531-1539.
34. Simon O, Acket B, Forfait C, et al. Zika virus outbreak in New Caledonia and Guillain-Barré syndrome: a case-control study. J Neurovirol 2018;24:362-368.
35. Styczynski AR, Malta J, Krow-Lucal ER, et al. Increased rates of Guillain-Barré syndrome associated with Zika virus outbreak in the Salvador metropolitan area, Brazil. PLoS Negl Trop Dis 2017;11:e0005869.
36. Salinas JL, Walteros DM, Styczynski A, et al. Zika virus disease-associated Guillain-Barré syndrome-Barranquilla, Colombia 2015-2016. J Neurol Sci 2017;381:272-277.
37. Umapathi T, Kam YW, Ohnmar O, et al. The 2016 Singapore Zika virus outbreak did not cause a surge in Guillain-Barré syndrome. J Peripher Nerv Syst 2018;23:197-201.
38. Grijalva I, Grajales-Muñiz C, González-Bonilla C, et al. Zika and dengue but not chikungunya are associated with Guillain-Barré syndrome in Mexico: A case-control study. PLoS Negl Trop Dis 2020;14:e0008032.

39. Lynch RM, Mantus G, Encinales L, et al. Augmented Zika and Dengue Neutralizing Antibodies Are Associated With Guillain-Barré Syndrome. J Infect Dis 2019;219:26-30.
40. Dirlikov E, Medina NA, Major CG, et al. Acute Zika Virus Infection as a Risk Factor for Guillain-Barré Syndrome in Puerto Rico. Jama 2017;318:1498-1500.
41. Mier YT-RL, Delorey MJ, Sejvar JJ, Johansson MA. Guillain-Barré syndrome risk among individuals infected with Zika virus: a multi-country assessment. BMC Med 2018;16:67.
42. Sejvar JJ, Baughman AL, Wise M, Morgan OW. Population incidence of Guillain-Barré syndrome: a systematic review and meta-analysis. Neuroepidemiology 2011;36:123-133.
43. Parra B, Lizarazo J, Jimenez-Arango JA, et al. Guillain-Barré syndrome associated with Zika virus infection in Colombia. N Engl J Med 2016;375:1513-1523.
44. Nascimento OJM, Frontera JA, Amitrano DA, Bispo de Filippis AM, Da Silva IRF, Group R-G-ZR. Zika virus infection-associated acute transient polyneuritis. Neurology 2017;88:2330-2332.
45. Orlikowski D, Porcher R, Sivadon-Tardy V, et al. Guillain-Barré syndrome following primary cytomegalovirus infection: a prospective cohort study. Clin Infect Dis 2011;52:837-844.
46. Takahashi M, Koga M, Yokoyama K, Yuki N. Epidemiology of Campylobacter jejuni isolated from patients with Guillain-Barré and Fisher syndromes in Japan. J Clin Microbiol 2005;43:335-339.
47. Fourie T, Grard G, Leparc-Goffart I, Briolant S, Fontaine A. Variability of Zika Virus Incubation Period in Humans. Open Forum Infect Dis 2018;5:ofy261.
48. Dirlikov E, Torres JV, Martines RB, et al. Postmortem Findings in Patient with Guillain-Barré Syndrome and Zika Virus Infection. Emerg Infect Dis 2018;24:114-117.
49. Visser LH, van der Meche FG, Meulstee J, et al. Cytomegalovirus infection and Guillain-Barré syndrome: the clinical, electrophysiologic, and prognostic features. Dutch Guillain-Barré Study Group. Neurology 1996;47:668-673.
50. Gracey DR, McMichan JC, Divertie MB, Howard FM, Jr. Respiratory failure in Guillain-Barré syndrome: a 6-year experience. Mayo Clin Proc 1982;57:742-746.
51. van Leeuwen N, Lingsma HF, Vanrolleghem AM, et al. Hospital Admissions, Transfers and Costs of Guillain-Barré Syndrome. PLOS ONE 2016;11:e0143837.
52. Rivera-Correa J, de Siqueira IC, Mota S, et al. Anti-ganglioside antibodies in patients with Zika virus infection-associated Guillain-Barré Syndrome in Brazil. PLOS Neglected Tropical Diseases 2019;13:e0007695.
53. Lucchese G, Kanduc D. Zika virus and autoimmunity: From microcephaly to Guillain-Barré s yndrome, and beyond. Autoimmunity Reviews 2016;15:801-808.
54. Pastula DM, Khan AS, Sharp TM, et al. Investigation of a Guillain-Barré syndrome cluster in the Republic of Fiji. J Neurol Sci 2017;372:350-355.
55. Suryapranata FST, Ang CW, Chong LL, Murk J-L, Falconi J, Huits RMHG. Epidemiology of Guillain-Barré Syndrome in Aruba. Am J Trop Med Hyg 2016;94:1380-1384.
56. Simon O, Billot S, Guyon D, et al. Early Guillain-Barré Syndrome associated with acute dengue fever. J Clin Virol 2016;77:29-31.
57. Santos NQ, Azoubel AC, Lopes AA, Costa G, Bacellar A. Guillain-Barré syndrome in the course of dengue: case report. Arq Neuropsiquiatr 2004;62:144-146.
58. Fragoso YD, Gomes S, Brooks JB, et al. Guillain-Barré syndrome and dengue fever: report on ten new cases in Brazil. Arq Neuropsiquiatr 2016;74:1039-1040.

59. Umapathi T, Lim CS, Ooi EE, et al. Asymptomatic dengue infection may trigger Guillain-Barré syndrome. J Peripher Nerv Syst 2016;21:375-377.
60. Soares CN, Cabral-Castro M, Oliveira C, et al. Oligosymptomatic dengue infection: a potential cause of Guillain Barré syndrome. Arq Neuropsiquiatr 2008;66:234-237.
61. Langerak T, Mumtaz N, Tolk VI, et al. The possible role of cross-reactive dengue virus antibodies in Zika virus pathogenesis. PLoS Pathog 2019;15:e1007640.
62. Oehler E, Fournier E, Leparc-Goffart I, et al. Increase in cases of Guillain-Barré syndrome during a Chikungunya outbreak, French Polynesia, 2014 to 2015. Euro Surveill 2015;20:30079.
63. Wielanek AC, Monredon JD, Amrani ME, Roger JC, Serveaux JP. Guillain-Barré syndrome complicating a Chikungunya virus infection. Neurology 2007;69:2105-2107.
64. Matos AMB, Maia Carvalho FM, Malta DL, et al. High proportion of Guillain-Barré syndrome associated with chikungunya in Northeast Brazil. Neurol Neuroimmunol Neuroinflamm 2020;7.
65. Stegmann-Planchard S, Gallian P, Tressières B, et al. Chikungunya, a Risk Factor for Guillain-Barré Syndrome. Clinical Infectious Diseases 2019.
66. Cumberworth SL, Barrie JA, Cunningham ME, et al. Zika virus tropism and interactions in myelinating neural cell cultures: CNS cells and myelin are preferentially affected. Acta Neuropathologica Communications 2017;5:50.
67. Mlakar J, Korva M, Tul N, et al. Zika Virus Associated with Microcephaly. New England Journal of Medicine 2016;374:951-958.
68. Tang H, Hammack C, Ogden Sarah C, et al. Zika Virus Infects Human Cortical Neural Progenitors and Attenuates Their Growth. Cell Stem Cell 2016;18:587-590.
69. Brito Ferreira ML, Militao de Albuquerque MFP, de Brito CAA, et al. Neurological disease in adults with Zika and chikungunya virus infection in Northeast Brazil: a prospective observational study. Lancet Neurol 2020;19:826-839.
70. Mehta R, Soares CN, Medialdea-Carrera R, et al. The spectrum of neurological disease associated with Zika and chikungunya viruses in adults in Rio de Janeiro, Brazil: A case series. PLoS Negl Trop Dis 2018;12:e0006212.
71. Brito Ferreira ML, Antunes de Brito CA, Moreira AJP, et al. Guillain-Barré Syndrome, Acute Disseminated Encephalomyelitis and Encephalitis Associated with Zika Virus Infection in Brazil: Detection of Viral RNA and Isolation of Virus during Late Infection. Am J Trop Med Hyg 2017;97:1405-1409.
72. Acosta-Ampudia Y, Monsalve DM, Castillo-Medina LF, et al. Autoimmune Neurological Conditions Associated With Zika Virus Infection. Front Mol Neurosci 2018;11:116.
73. Muñoz LS, Parra B, Pardo CA, Neuroviruses Emerging in the Americas S. Neurological Implications of Zika Virus Infection in Adults. The Journal of infectious diseases 2017;216:S897-S905.
74. Centro Nacional de Epidemiología Prevención y Control de Enfermedades. Situación de Guillain Barré: Perú a la SE 02—2020. July, 2020.
75. De Sanctis P, Doneddu PE, Vigano L, Selmi C, Nobile-Orazio E. Guillain-Barré syndrome associated with SARS-CoV-2 infection. A systematic review. Eur J Neurol 2020;27:2361-2370.
76. Filosto M, Cotti Piccinelli S, Gazzina S, et al. Guillain-Barré syndrome and COVID-19: an observational multicentre study from two Italian hotspot regions. J Neurol Neurosurg Psychiatry 2020.

77. Hasan I, Saif-Ur-Rahman KM, Hayat S, et al. Guillain-Barré syndrome associated with SARS-CoV-2 infection: A systematic review and individual participant data meta-analysis. J Peripher Nerv Syst 2020;25:335-343.
78. Zhao H, Shen D, Zhou H, Liu J, Chen S. Guillain-Barré syndrome associated with SARS-CoV-2 infection: causality or coincidence? Lancet Neurol 2020;19:383-384.
79. European Centre for Disease Prevention and Control. Case definition for coronavirus disease 2019 (COVID-19). 2020.
80. World Health Organization. Laboratory testing for coronavirus disease (COVID-19) in suspected human cases. 2020.
81. World Health Organization. Use of laboratory methods for SARS diagnosis. 2020.
82. Pollán M, Pérez-Gómez B, Pastor-Barriuso R, et al. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. Lancet 2020.
83. Stringhini S, Wisniak A, Piumatti G, et al. Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Geneva, Switzerland (SEROCoV-POP): a population-based study. Lancet 2020;396:313-319.
84. Fragiel M, Miro O, Llorens P, et al. Incidence, clinical, risk factors and outcomes of Guillain-Barré in Covid-19. Ann Neurol 2020.
85. Keddie S, Pakpoor J, Mousele C, et al. Epidemiological and cohort study finds no association between COVID-19 and Guillain-Barré syndrome. Brain 2020.
86. Umapathi T, Er B, Koh JS, Goh YH, Chua L. Guillain-Barré syndrome decreases in Singapore during the COVID-19 pandemic. Journal of the Peripheral Nervous System;n/a.
87. Patone M, Handunnetthi L, Saatci D, et al. Neurological complications after first dose of COVID-19 vaccines and SARS-CoV-2 infection. Nat Med 2021.
88. Head VA, Wakerley BR. Guillain-Barré syndrome in general practice: clinical features suggestive of early diagnosis. Br J Gen Pract 2016;66:218-219.
89. Willison HJ, Jacobs BC, van Doorn PA. Guillain-Barré syndrome. Lancet 2016;388:717-727.
90. Howlett WP, Vedeler CA, Nyland H, Aarli JA. Guillain-Barré syndrome in northern Tanzania: a comparison of epidemiological and clinical findings with western Norway. Acta Neurol Scand 1996;93:44-49.
91. Islam MB, Islam Z, Farzana KS, et al. Guillain-Barré syndrome in Bangladesh: validation of Brighton criteria. J Peripher Nerv Syst 2016;21:345-351.
92. Ishaque T, Islam MB, Ara G, et al. High mortality from Guillain-Barré syndrome in Bangladesh. J Peripher Nerv Syst 2017;22:121-126.
93. Halawa EF, Ahmed D, Nada MA. Guillain-Barré syndrome as a prominent cause of childhood acute flaccid paralysis in post polio eradication era in Egypt. Eur J Paediatr Neurol 2011;15:241-246.
94. Melaku Z, Zenebe G, Bekele A. Guillain-Barré syndrome in Ethiopian patients. Ethiop Med J 2005;43:21-26.
95. Dominguez-Moreno R, Tolosa-Tort P, Patino-Tamez A, et al. Mortality associated with a diagnosis of Guillain-Barré syndrome in adults of Mexican health institutions]. Rev Neurol 2014;58:4-10.
96. González-Suárez I, Sanz-Gallego I, Rodríguez de Rivera FJ, Arpa J. Guillain-Barré syndrome: natural history and prognostic factors: a retrospective review of 106 cases. BMC Neurol 2013;13:95-95.

97. Islam B, Islam Z, Rahman S, et al. Small volume plasma exchange for Guillain-Barré syndrome in resource-limited settings: a phase II safety and feasibility study. BMJ Open 2018;8:e022862.
98. Iyer RR, Shah PH, Roy SS, Suri SK. Reducing the economic burden in management of Guillain-Barré syndrome using modified plasmapheresis. Asian J Transfus Sci 2016;10:118-121.
99. The Global Health Network (TGHN). GBS 10 steps guideline [online]. Available at: https://rede.tghn.org/gbs-flowchart-sample/introduction-gbs/.
100. Leonhard SE, Mandarakas MR, Gondim FAA, et al. Diretrizes Baseadas em Evidências Diagnóstico e manejo da Síndrome de Guillain–Barré em dez etapas. Revista Neurociências 2021;29:1-52.
101. Islam B, Islam Z, GeurtsvanKessel CH, et al. Guillain-Barré syndrome following varicella-zoster virus infection. Eur J Clin Microbiol Infect Dis 2018;37:511-518.
102. Sinha S, Prasad KN, Jain D, Pandey CM, Jha S, Pradhan S. Preceding infections and anti-ganglioside antibodies in patients with Guillain-Barré syndrome: a single centre prospective case-control study. Clin Microbiol Infect 2007;13:334-337.
103. Ju YY, Womersley H, Pritchard J, Gray I, Hughes RA, Gregson NA. Haemophilus influenzae as a possible cause of Guillain-Barré syndrome. J Neuroimmunol 2004;149:160-166.
104. Mori M, Kuwabara S, Miyake M, et al. Haemophilus influenzae infection and Guillain-Barré syndrome. Brain 2000;123 (Pt 10):2171-2178.
105. Nafissi S, Vahabi Z, Sadeghi Ghahar M, Amirzargar AA, Naderi S. The role of cytomegalovirus, Haemophilus influenzae and Epstein Barr virus in Guillain Barré syndrome. Acta Med Iran 2013;51:372-376.
106. Cornblath DR, McArthur JC, Kennedy PG, Witte AS, Griffin JW. Inflammatory demyelinating peripheral neuropathies associated with human T-cell lymphotropic virus type III infection. Ann Neurol 1987;21:32-40.
107. Thornton CA, Latif AS, Emmanuel JC. Guillain-Barré syndrome associated with human immunodeficiency virus infection in Zimbabwe. Neurology 1991;41:812-815.
108. Stowe J, Andrews N, Wise L, Miller E. Investigation of the temporal association of Guillain-Barré syndrome with influenza vaccine and influenzalike illness using the United Kingdom general practice research database. American Journal of Epidemiology 2009;169:382-388.
109. Tam CC, O'Brien SJ, Petersen I, Islam A, Hayward A, Rodrigues LC. Guillain-Barré Syndrome and Preceding Infection with Campylobacter, Influenza and Epstein-Barr Virus in the General Practice Research Database. PLOS ONE 2007;2:e344.
110. Ghaderi S, Gunnes N, Bakken IJ, Magnus P, Trogstad L, Håberg SE. Risk of Guillain-Barré syndrome after exposure to pandemic influenza A(H1N1)pdm09 vaccination or infection: a Norwegian population-based cohort study. European Journal of Epidemiology 2016;31:67-72.
111. Al-Hakem H, Sindrup SH, Andersen H, et al. Guillain-Barré syndrome in Denmark: a population-based study on epidemiology, diagnosis and clinical severity. J Neurol 2019;266:440-449.
112. Schopper D, Dawson A, Upshur R, et al. Innovations in research ethics governance in humanitarian settings. BMC Medical Ethics 2015;16:10.



Summary

Many issues remain unresolved on the role infections play in GBS, including the global differences in distribution of infections, the association with clinical and electrophysiological phenotypes and outcome, and the impact of emerging infectious diseases. In this thesis I intended to close these knowledge gaps and explored ways to improve research, diagnosis, and management of GBS to prepare for future outbreaks.

In the **Introduction**, a summary is given of the clinical features, diagnosis and management of GBS. Furthermore an overview is presented of the literature on the role infections play in the pathogenesis of the syndrome, including an historic overview of outbreaks of GBS linked to infectious diseases.

In **Part I, Chapter 1** the global distribution of infections known to trigger GBS and their association with clinical and electrophysiological subtypes and disease severity was studied in GBS patients that were included in the International GBS Outcome Study (IGOS). IGOS is the largest ongoing international cohort study on GBS, that is operative in over 150 centers across 20 countries. The first 1000 patients included in IGOS were tested for a recent infection with the five pathogens most commonly associated with GBS: *Campylobacter jejuni*, Hepatitis E virus (HEV), *Mycoplasma pneumoniae,* cytomegalovirus (CMV) and Epstein-Barr virus (EBV). We found that *C. jejuni* is world-wide the most common infection in GBS, occurring in 20-30% of cases, and that the proportions of preceding infections are similar across continents. Six percent of patients had evidence of more than one recent infection, of which most had a recent *C. jejuni* and *M. pneumoniae* infection. Infections were associated with, but not specific for, demographic and clinical profiles. Most notably, patients with a *C. jejuni* infection more often had a pure motor axonal form of GBS and patients with EBV or CMV infection almost exclusively had the sensorimotor demyelinating phenotype. *C. jejuni* was associated with more severe weakness and worse long-term outcome. These findings show the association between preceding infections and the clinical presentation, subtype and course of GBS, and indicate that serological testing for preceding infections may provide useful prognostic information in clinical practice.

In **Part II** GBS during the Zika virus epidemic was studied. The clinical phenotype of GBS related to Zika virus infection was investigated in a systematic review and meta-analysis of all published studies on GBS related to Zika virus infection (**Chapter 2**), and in a cohort of 72 GBS patients collected in Northeast Brazil during the Zika and chikungunya virus epidemics (2015-2017) (**Chapter 3**). From both these studies it followed that in patients with a preceding Zika virus infection, the sensorimo-

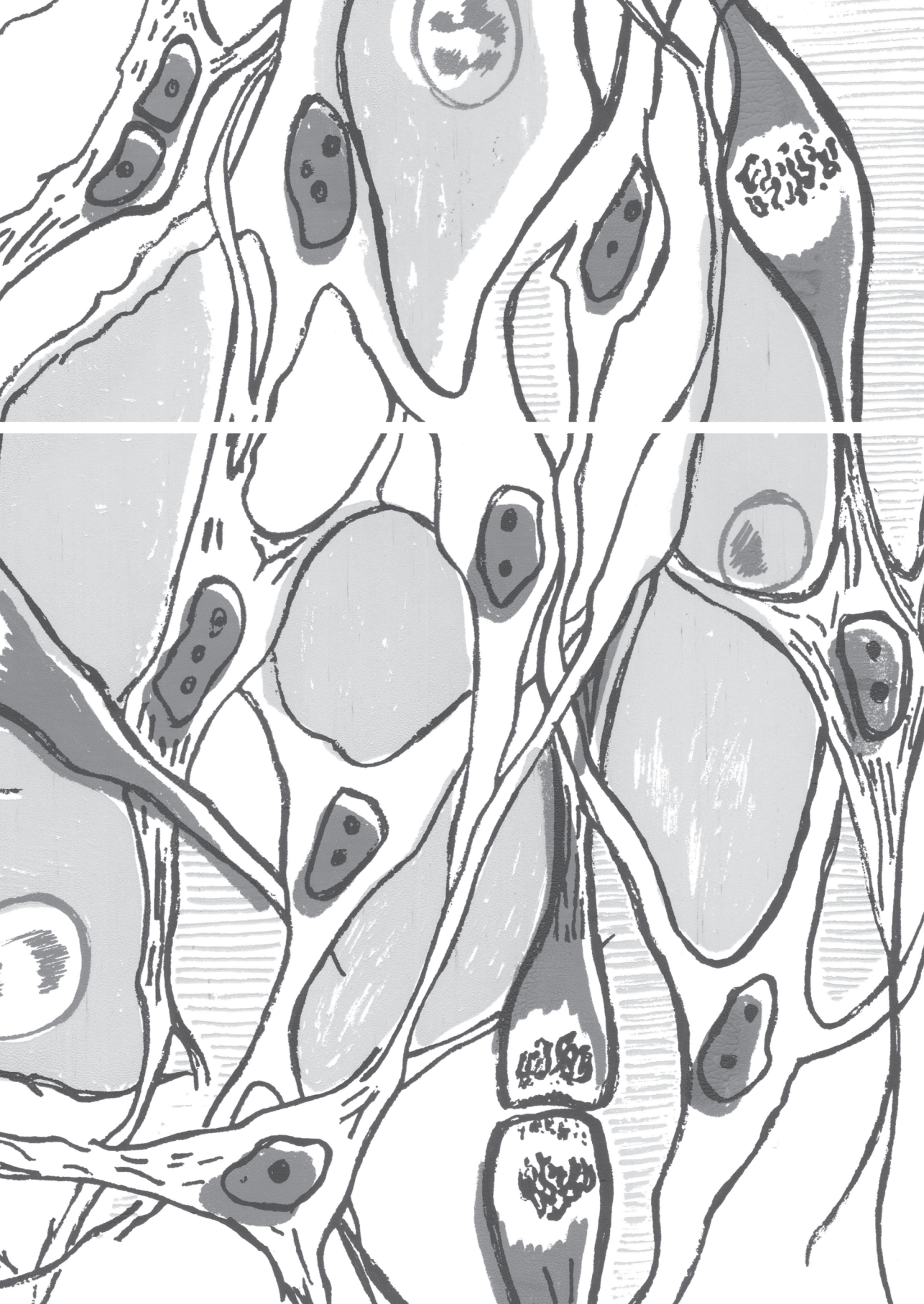
tor demyelinating variant of GBS is typically found. In most patients polymerase chain reaction for Zika virus was negative, there was no increased cell count in the cerebrospinal fluid, and the time between onset of infectious and neurologic symptoms was 1-2 weeks, suggesting a post-infectious disease mechanism. In the systematic review we found that most patients with a preceding Zika virus infection have a severe disease course with high rates of admission to the Intensive Care Unit (ICU). In the cohort from Northeast Brazil 68% of patients had laboratory evidence of a recent arbovirus infection; 25 (52%) Zika, 8 (17%) chikungunya, 1 (2%) dengue, and 14 (29%) Zika and chikungunya virus. Patients with evidence of both chikungunya and Zika virus infection had a more severe disease course and higher rates of ICU admission. No specific serum anti-glycolipid antibody signature was identified in association with arbovirus infection in this cohort. To study whether Zika virus or other arboviruses were also driving GBS outside of epidemic phases of transmission, we conducted a multicenter case-control study of GBS patients collected in Brazil, Argentina, and Malaysia during the endemic phase of Zika virus transmission in these regions (2017-2019) (**Chapter 4**). In total, 49 cases and 35 controls were collected. All cases and controls were tested for serological evidence of a recent infection with Zika virus, chikungunya virus, dengue virus, *C. jejuni*, *M. pneumoniae*, CMV, EBV and HEV. Two of 49 patients (4%) had evidence of a recent chikungunya virus infection, 2/49 (4%) of a recent dengue virus infection, and none of a recent Zika virus infection. The patients with a dengue virus infection also had evidence of other recent infections: one with CMV, and the other with *C. jejuni*. Although no evidence of a recent arbovirus infection was found in the 35 controls, the study was underpowered to find significant differences. Similar to **Chapter 3**, patients with a recent arbovirus infection did not show specific binding of anti-ganglioside antibodies.

In **Part III** the spectrum of neurological diseases associated with Zika virus was explored. In a review article in **Chapter 5** we described the broad range of Zika virus-associated neurological diseases in reported literature, including meningitis, encephalitis, myelitis, and acute disseminated encephalomyelitis. Case reports of chronic inflammatory demyelinating polyradiculoneuropathy (**Chapter 6**) and neuromyelitis optica spectrum disorder (**Chapter 7**) after recent Zika virus infection further illustrate the potential spectrum of associated neurological disease. As this spectrum covers diseases with a presumed role for direct infection as well as immune-mediated disorders, both these pathophysiological mechanisms may contribute to the disease burden of Zika virus.

In **Part IV** two other outbreaks of infectious disease in relation to GBS were studied. In **Chapter 8** a large outbreak of GBS cases in 2019 in Peru was investigated. In this case-control study we found that *C. jejuni*, and not Zika, was the infectious driver behind the outbreak. Evidence of a recent *C. jejuni* infection was found in 28/43 (65%) cases, and no evidence of a recent arbovirus infection was found. The odds ratio of having had a recent *C. jejuni* infection in cases versus controls was 3.3 (confidence interval 1.2-9.32, $p<0.01$). Patients with a recent *C. jejuni* infection generally had a clinical, electrophysiological and anti-glycolipid profile typically associated with this type of infection. Genomic analysis of the *C. jejuni* strains showed they were closely related to previously described GBS-associated genomes from China and Africa and not new emergent strains. In **Chapter 9** the relation between GBS and SARS-CoV-2 virus was investigated in a cohort of GBS patients included in IGOS during the first 4 months of the pandemic. Eleven of 49 (22%) patients had a confirmed or probable SARS-CoV-2 infection. These patients generally had a severe sensorimotor demyelinating variant of GBS. However, no increase in inclusion rate of patients was registered in our ongoing cohort study, nor in larger surveillance studies, suggesting that if SARS-CoV-2 is linked to GBS, the risk is likely to be significantly lower than other known triggers, including Zika virus and *C. jejuni*.

The first two chapters of **Part V** are dedicated to understanding how GBS is managed world-wide; during and outside of outbreak periods, and in both high income countries and low- and middle income countries. We investigated how GBS was diagnosed and managed in Brazil before and during the Zika virus epidemic in a national survey study (**Chapter 10**). Of all 3264 neurologist members of the Brazilian Academy of Neurology, 171 (5%) fully answered the questionnaire. During the Zika virus epidemic, 61% of them noticed an increase in incidence of GBS, and 30% experienced an increase in problems managing these patients. The availability of nerve conduction studies and beds in the ICU and rehabilitation centers were among the most important reported limitations. Most neurologists did not use a standard protocol for treating GBS and treatment practice varied. In a review article in **Chapter 11** we study how diagnosis and management of GBS differs between in low- and middle income countries and high income countries. In low- and middle income countries, patients present to the hospital later in the disease course and more often have a severe pure motor axonal form of GBS, treatment options are often limited, and mortality is higher compared to high income countries. Important knowledge gaps of GBS in low- and middle income countries included: incidence, triggering infections, clinical features, and outcome. The second two chapters of **Part V** incorporate the lessons learnt from the previous chapters to improve the global management and research of GBS. To aid clinicians world-wide in diagnosing

and managing GBS, a clinical guideline for GBS was developed (**Chapter 12**). This guideline is based on current literature and consensus of a team of international experts on GBS and was developed for general applicability in all clinical environments, irrespective of specialist capabilities or availability of resources. To enhance the global application of the guideline, a web-based tool was built and hosted on The Global Health Network, and translations to Portuguese, Spanish and Chinese were published in national journals. I concluded with **Chapter 13** where the question is tackled how one can ensure high-quality research and safeguard diagnosis and management of GBS during an infectious disease pandemic.



Samenvatting

Er zijn nog veel onopgeloste vraagstukken met betrekking tot de rol die infecties spelen bij het ontstaan van GBS. Het is bijvoorbeeld nog niet opgehelderd wat de wereldwijde verschillen zijn in het voorkomen van infecties, de associatie met klinische en elektrofysiologische fenotypes en prognose, en de impact van uitbraken van opkomende infectieziekten. In dit proefschrift heb ik deze kennislacunes willen dichten en heb ik manieren onderzocht om het onderzoek, de diagnostiek en de behandeling van GBS te verbeteren ter voorbereiding op toekomstige uitbraken.

In de **Inleiding** wordt een samenvatting gegeven van de klinische kenmerken, diagnose en behandeling van GBS. Daarnaast wordt een historisch overzicht gegeven van het verband tussen het voorkomen van GBS en (uitbraken van) infectieziekten.

In **Deel I**, **Hoofdstuk 1** is de wereldwijde verspreiding van infecties waarvan bekend is dat ze GBS veroorzaken en hun associatie met klinische en elektrofysiologische subtypes en prognose bestudeerd bij patiënten die zijn geïncludeerd in de International GBS Outcome Study (IGOS). IGOS is de grootste internationale cohortstudie naar GBS, en loopt in meer dan 150 centra in 20 landen. De eerste 1000 patiënten die in IGOS zijn geïncludeerd werden getest op een recente infectie met de vijf pathogenen die in associatiestudies zijn gelinkt aan GBS: *Campylobacter jejuni*, Hepatitis E-virus (HEV), *Mycoplasma pneumoniae*, cytomegalovirus (CMV) en Epstein-Barr-virus (EBV). We ontdekten dat *C. jejuni* wereldwijd de meest voorkomende infectie bij GBS is, in 20-30% van de gevallen, en dat infecties in vergelijkbare proporties voorkomen in Europa, Noord-Amerika, Azië en Zuid-Afrika. Zes procent van de patiënten had tekenen van meer dan één recente infectie, waarvan de meesten een recente infectie met zowel *C. jejuni* en *M. pneumoniae* hadden. Infecties waren geassocieerd met, maar niet specifiek voor, demografische en klinische profielen. Het meest opvallend was dat patiënten met een *C. jejuni* infectie vaker een puur motore axonale vorm van GBS hadden en patiënten met een EBV- of CMV infectie bijna uitsluitend het sensomotorische demyeliniserende fenotype hadden. *C. jejuni* was geassocieerd met ernstigere zwakte en een slechtere lange termijn prognose. Deze bevindingen tonen het verband aan tussen voorgaande infecties en de klinische presentatie, het subtype, en het beloop van GBS, en geven aan dat serologisch onderzoek naar voorgaande infecties nuttige prognostische informatie kan opleveren in de klinische praktijk.

In **Deel II** werd GBS tijdens de zikavirusepidemie bestudeerd. Het klinische fenotype van GBS gerelateerd aan infectie met het zikavirus werd onderzocht in een systematische review en meta-analyse van alle gepubliceerde studies over GBS gerelateerd aan zikavirusinfectie (**Hoofdstuk 2**), en in een cohort van 72 GBS patiënten verzameld in Noordoost-Brazilië tijdens de zika- en chikungunyavirusepidemieën

(2015-2017) (**Hoofdstuk 3**). Uit beide onderzoeken bleek dat bij patiënten met een eerdere zikavirusinfectie meestal de sensomotorische demyeliniserende variant van GBS wordt gevonden. Bij vrijwel alle patiënten was de polymerasekettingreactie (PCR) voor het zikavirus negatief, was er geen verhoogd celgetal in de cerebrospinale vloeistof, en was de tijd tussen het begin van infectieuze en neurologische symptomen 1-2 weken, wat wijst op een post-infectieus ziektemechanisme. In de systematische review vonden we dat de meeste patiënten met een voorafgaande zikavirusinfectie een ernstig ziekteverloop hadden met hoge opnamecijfers op de Intensive Care (IC). In het cohort uit Noordoost-Brazilië had 68% van de patiënten aanwijzingen (serologisch of middels PCR) voor een recente arbovirusinfectie; 25 (52%) zika, 8 (17%) chikungunya, 1 (2%) dengue, en 14 (29%) zika- en chikungunyavirus. Patiënten met aanwijzingen voor zowel een chikungunya- als zikavirusinfectie hadden significant vaker een ernstig ziekteverloop en IC opname. Er werd in dit cohort geen specifiek anti-glycolipide-antistoffenprofiel gevonden bij patiënten met een recente arbovirusinfectie. Om te onderzoeken of het zikavirus ook buiten de epidemische fasen van transmissie een belangrijke trigger voor GBS zijn, hebben we een multicenter case-control onderzoek uitgevoerd bij GBS patiënten in Brazilië, Argentinië en Maleisië tijdens de endemische fase van transmissie van het zikavirus in deze regio's (2017-2019) (**Hoofdstuk 4**). In totaal werden 49 GBS patiënten en 35 controles verzameld. Alle GBS patiënten en controles werden getest op serologisch bewijs van een recente infectie met het zikavirus, chikungunyavirus, denguevirus, *C. jejuni*, *M. pneumoniae*, CMV, EBV en HEV. Twee van de 49 patiënten (4%) hadden tekenen van een recente infectie met het chikungunyavirus, 2/49 (4%) een recente infectie met het denguevirus, en geen een recente infectie met het zikavirus. De patiënten met een denguevirusinfectie hadden ook aanwijzingen voor andere recente infecties: de ene met CMV en de andere met *C. jejuni*. Hoewel er bij de 35 controles geen bewijs van een recente arbovirusinfectie werd gevonden, had het onderzoek onvoldoende power om significante verschillen te vinden. Net als in **Hoofdstuk 3** werd er geen specifiek anti-ganglioside-antistoffenprofiel gevonden bij patiënten met een recente arbovirusinfectie.

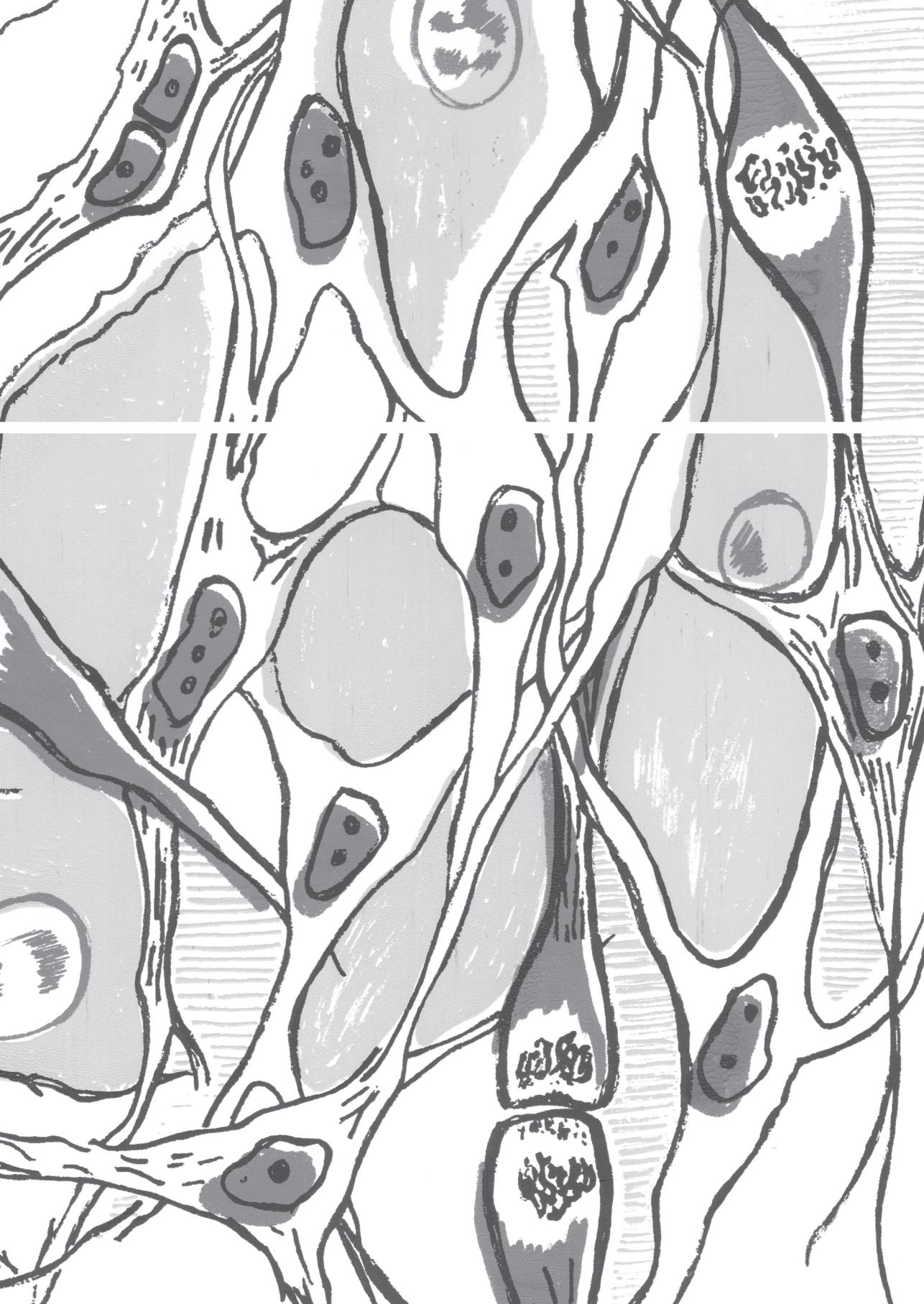
In **Deel III** werd het spectrum van neurologische ziekten geassocieerd met het zikavirus onderzocht. In een overzichtsartikel in **Hoofdstuk 5** hebben we de grote variatie van zikavirus-geassocieerde neurologische aandoeningen beschreven die in de literatuur zijn gerapporteerd, waaronder meningitis, encefalitis, myelitis en acute gedisseminеerde encefalomyelitis. Case reports van chronische inflammatoire demyeliniserende polyradiculoneuropathie (**Hoofdstuk 6**) en neuromyelitis optica spectrum stoornis (**Hoofdstuk 7**) na recente zikavirusinfectie illustreren verder het potentiële spectrum van geassocieerde neurologische aandoeningen. Aangezien dit

spectrum zowel ziekten omvat met een veronderstelde rol voor directe infectie als immuungemedieerde aandoeningen, kunnen beide pathofysiologische mechanismen vermoedelijk bijdragen aan de ziektelast van het zikavirus.

In **Deel IV** werd de relatie tussen twee andere uitbraken van infectieziekten en GBS bestudeerd. In **Hoofdstuk 8** werd een grote toename van het aantal GBS patiënten in 2019 in Peru onderzocht. In deze case-control studie ontdekten we dat *C. jejuni*, en niet zika, de infectieuze aanjager was van de uitbraak. Bewijs van een recente *C. jejuni*-infectie werd gevonden in 28/43 (65%) patiënten en er werd geen bewijs van een recente arbovirusinfectie gevonden. De odds ratio van het hebben van een recente infectie met *C. jejuni* bij patiënten versus controles was 3,3 (betrouwbaarheidsinterval 1,2-9,32, $P<0,01$). Patiënten met een recente *C. jejuni* infectie hadden over het algemeen een klinisch, elektrofysiologisch en anti-glycolipidenprofiel dat doorgaans wordt geassocieerd met dit type infectie. Genomische analyse van de *C. jejuni*-stammen toonde aan dat ze nauw verwant waren met eerder beschreven GBS-geassocieerde genomen uit China en Afrika en niet met nieuwe stammen. In **Hoofdstuk 9** werd de relatie tussen GBS en SARS-CoV-2 onderzocht in een cohort van GBS patiënten die werden geïncludeerd in IGOS gedurende de eerste 4 maanden van de pandemie. Elf van de 49 (22%) patiënten hadden een bevestigde of waarschijnlijke SARS-CoV-2-infectie. Deze patiënten hadden over het algemeen een ernstige sensomotorische demyeliniserende variant van GBS. Er werd echter geen toename van het inclusiepercentage van patiënten geregistreerd in IGOS, noch in grotere surveillancestudies, wat suggereert dat als SARS-CoV-2 is geassocieerd met GBS, het risico waarschijnlijk veel lager is dan bij andere bekende triggers, waaronder zikavirus en *C. jejuni*.

De eerste twee hoofdstukken van **Deel V** hebben als doel een beter begrip te genereren van de wereldwijde diagnostiek en behandeling van GBS; zowel tijdens epidemieën als daarbuiten, en zowel in hoge-inkomenslanden als in lage- en middeninkomenslanden. We hebben onderzocht hoe GBS werd gediagnosticeerd en behandeld in Brazilië voor en tijdens de zikavirusepidemie in een nationale enquêtestudie (**Hoofdstuk 10**). Van alle 3264 neurologen van de Braziliaanse Academie voor Neurologie hebben 171 (5%) de vragenlijst volledig beantwoord. Tijdens de zikavirusepidemie merkte 61% van hen een verhoogde GBS-incidentie op, en 30% ervoer een toename van problemen bij de behandeling van deze patiënten. De beschikbaarheid van zenuwgeleidingsonderzoeken en bedden op de IC en revalidatiecentra behoorden tot de belangrijkste gerapporteerde beperkingen. De meeste neurologen gebruikten geen standaardprotocol voor de behandeling van GBS en de behandelpraktijk varieerde. In een overzichtsartikel in **Hoofdstuk 11** hebben we

bestudeerd hoe de diagnose en behandeling van GBS verschilt tussen lage- en middeninkomenslanden en hoge-inkomenslanden. In lage- en middeninkomenslanden komen patiënten later in het ziekteverloop naar het ziekenhuis en hebben ze vaker een ernstige puur motore axonale vorm van GBS, zijn de behandelingsopties vaak beperkt en is de mortaliteit hoger in vergelijking met landen met een hoog inkomen. Belangrijke hiaten in de kennis van GBS in lage- en middeninkomenslanden waren onder meer: incidentie, uitlokkende infecties, klinische kenmerken en prognose. De laatste twee hoofdstukken van **Deel V** hebben als doel de diagnose en behandeling van- en onderzoek naar GBS te verbeteren. Om clinici over de hele wereld te helpen bij het diagnosticeren en behandelen van GBS, is een klinische richtlijn ontwikkeld (**Hoofdstuk 12**). Deze richtlijn is gebaseerd op de huidige literatuur en consensus van een team van internationale GBS experts en is ontwikkeld voor algemene toepasbaarheid in alle klinische settings, ongeacht specialistische capaciteiten of beschikbaarheid van middelen. Om de wereldwijde toepassing van de richtlijn te verbeteren, werd een website gebouwd en gehost op The Global Health Network, en werden vertalingen naar het Portugees, Spaans en Chinees gepubliceerd in nationale tijdschriften. Ik sloot af met **Hoofdstuk 13** waar de vraag wordt behandeld hoe men kan zorgen voor kwalitatief hoogstaand onderzoek en de diagnose en behandeling van GBS kan waarborgen tijdens een pandemie.

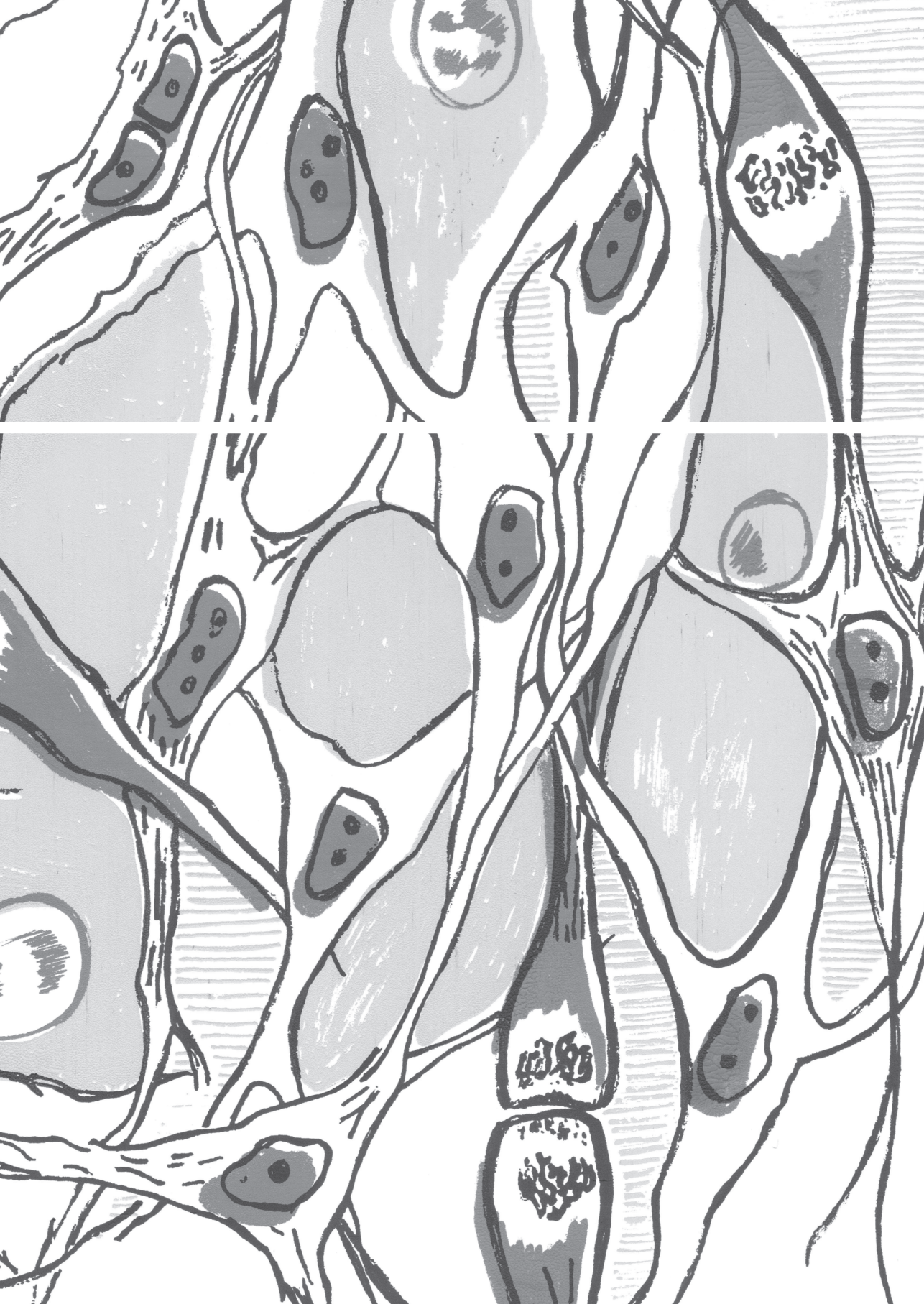


About the author

Sonja Leonhard was born on January 30th 1988 in Amsterdam, The Netherlands. She graduated from the Vossius Gymnasium in Amsterdam in 2006 and subsequently started studying Philosophy at the University of Amsterdam. After obtaining her Bachelor Degree in 2009, she studied Medicine at the Vrije Universiteit in Amsterdam. She completed her Research internship and Master thesis at the Academic Medical Centre in Amsterdam on the topic of Neurosarcoidosis and published two papers under supervision of Prof. Dr. D. van der Beek and Dr. M.C. Brouwer. She obtained her Medical Degree with Honours (Cum Laude) in 2015.

Between 2015 and 2016 she worked as a Neurology resident (ANIOS) at the OLVG Oost Hospital in Amsterdam. In December 2016 she started her PhD trajectory at the Neurology Department of the Erasmus MC in Rotterdam under supervision of Prof. Dr. B. C. Jacobs and Prof. Dr. H. J. Willison. She participated in several initiatives dedicated to increase the scientific outreach of her study results, including writing an international guideline for Guillain-Barré syndrome and translating this to Spanish, Portuguese, and Chinese (Mandarin), setting up and managing a platform on Guillain-Barré syndrome on The Global Health Network and serving as a Board Member of the International Outreach Committee of the Peripheral Nerve Society. She was selected as a participant of the Arts/Science Academy Honours Programme of the Koninklijke Nederlandse Akademie van Wetenschappen in 2018 where she could combine her interest in both art and science.

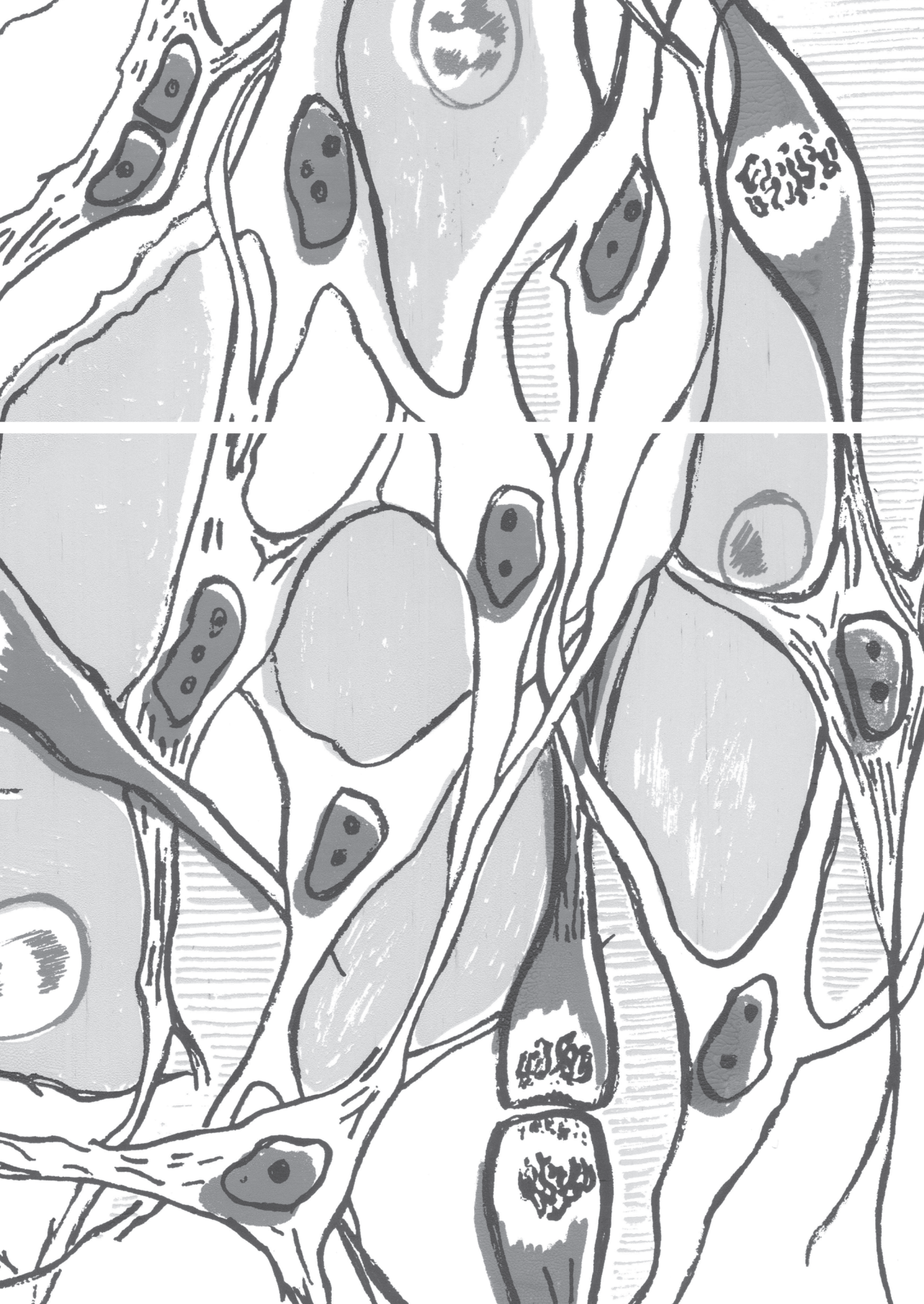
She has started her residency at the Clinical Microbiology Department at the Erasmus MC in June 2021 where she hopes to continue to join her interest in infectious diseases and neurology. In her pastime she enjoys reading and has been a proud member of a feminist book club for over a decade. She lives with her girlfriend Katrein in Rotterdam.



Publications

1. Luijten LWG, **Leonhard SE**, van der Eijk AA, et al. Guillain-Barré syndrome after SARS-CoV-2 infection in an international prospective cohort study. *Brain.* 2021.
2. **Leonhard SE**, Mandarakas MR, de Assis Aquino Gondim F, et al. Guía basada en la evidencia. Diagnóstico y manejo del síndrome de Guillain-Barré en diez pasos. Medicina (B Aires) 2021;81:817-836. [Spanish translation of Leonhard SE, *Nature Rev Neurol*, 2019]
3. Papri N*, Islam Z*, **Leonhard SE**, Mohammad QD, Endtz HP, Jacobs BC, Guillain-Barré syndrome in low- and middle-income countries: challenges and prospects, *Nature Reviews Neurology*. 2021
4. Ramos AP, **Leonhard SE**, Halstead SK, Cuba MA, Castañeda CC, Dioses JA, Tipismana MA, Abanto JT, Llanos A, Gourlay D, Grogl M, Ramos M, Rojas JD, Meza R, Puiu D, Sherman RM, Salzberg SL, Simner PJ, Willison HJ, Jacobs BC, Cornblath DR, Umeres HF*, Pardo CS*, Guillain-Barré syndrome outbreak in Peru 2019 associated with Campylobacter jejuni infection, *Neurology: Neuroimmunology & Neuroinflammation*. 2021
5. Walgaard C, Jacobs BC, Lingsma HF, Steyerberg EW, Van den Berg B, Doets AY, **Leonhard SE [...], van Doorn PA, Dutch GBS Study Group**, et al., Second intravenous immunoglobulin dose in patients with Guillain-Barré syndrome with poor prognosis (SID-GBS): a double-blind, randomised, placebo-controlled trial, *The Lancet Neurology*, 2021.
6. **Leonhard SE**, Mandarakas MR, Gondim FAA, et al. Diretrizes Baseadas em Evidências Diagnóstico e manejo da Síndrome de Guillain–Barré em dez etapas. *Revista Neurociências*, 2021. [Portuguese translation of Leonhard SE, *Nature Rev Neurol*, 2019].
7. **Leonhard SE**, Halstead S, Lant SB, et al.,Guillain-Barré syndrome during the Zika virus outbreak in Northeast Brazil: an observational cohort study, *Journal of the Neurological Sciences*. 2020
8. **Leonhard SE**, Cornblath DR, Endtz HP, et al. Guillain-Barré syndrome in times of pandemics, *Journal of Neurology, Neurosurgery and Psychiatry*. 2020
9. **Leonhard SE**, Bresani-Salvi CC, Lyra Batista JD, et al. Guillain-Barré syndrome related to Zika virus infection: a systematic review and meta-analysis of the clinical and electrophysiological phenotype, *PLOS Neglected Tropical Diseases*. 2020
10. Brito Ferreira ML, Militão de Albuquerque MFP, de Brito CAA, de Oliveira França RF, Porto Moreira ÁJ, de Morais Machado MÍ, da Paz Melo R, Medialdea-Carrera R, Dornelas Mesquita S, Lopes Santos M, Mehta R, Ramos E Silva R, **Leonhard SE** [...], Solomon T., et al.. Neurological disease in adults with Zika and chikungunya virus infection in Northeast Brazil: a prospective observational study. *Lancet Neurology*. 2020.
11. **Leonhard SE**, Mandarakas MR, Gondim FAA, et al. Diagnosis and management of Guillain-Barré syndrome in ten steps. *Nature Reviews Neurology*. 2019
12. Aspahan MC, **Leonhard SE**, Gomez RS, et al. Neuromyelitis optica spectrum disorder associated with Zika virus infection, Neurology Clinical Practice. 2019
13. **Leonhard SE**, Conde RM, Gondim FAA, et al. Diagnosis and treatment of Guillain-Barré syndrome during the Zika virus epidemic in Brazil: a national survey study. *Journal of the Peripheral Nervous System*. 2019
14. **Leonhard SE**, Lant S, Jacobs BC, et al., Zika virus infection in the returning traveller: what every neurologist should know. *Practical Neurology*. 2018
15. **Leonhard SE**, Munts AG, van der Eijk AA, et al. Acute-onset chronic inflammatory demyelinating polyneuropathy after Zika virus infection. *Journal of Neurology Neurosurgery and Psychiatry*. 2017

16. **Leonhard SE**, Fritz D, van de Beek D, et al. Cryptococcal meningitis complicating sarcoidosis. *Medicine*. 2016
17. **Leonhard SE**, Fritz D, Eftimov F, et al. Neurosarcoidosis in a Tertiary Referral Center: A Cross-Sectional Cohort Study, Medicine. 2016.



PhD Portfolio

Name:	Sonja E. Leonhard
PhD Period:	5 December 2016 – 5 March 2021
Erasmus MC Department:	Neurology
Research School:	Erasmus Postgraduate School Molecular Medicine (MolMed)
Promotors:	Prof. dr. Bart C. Jacobs Prof. dr. Hugh J. Willison

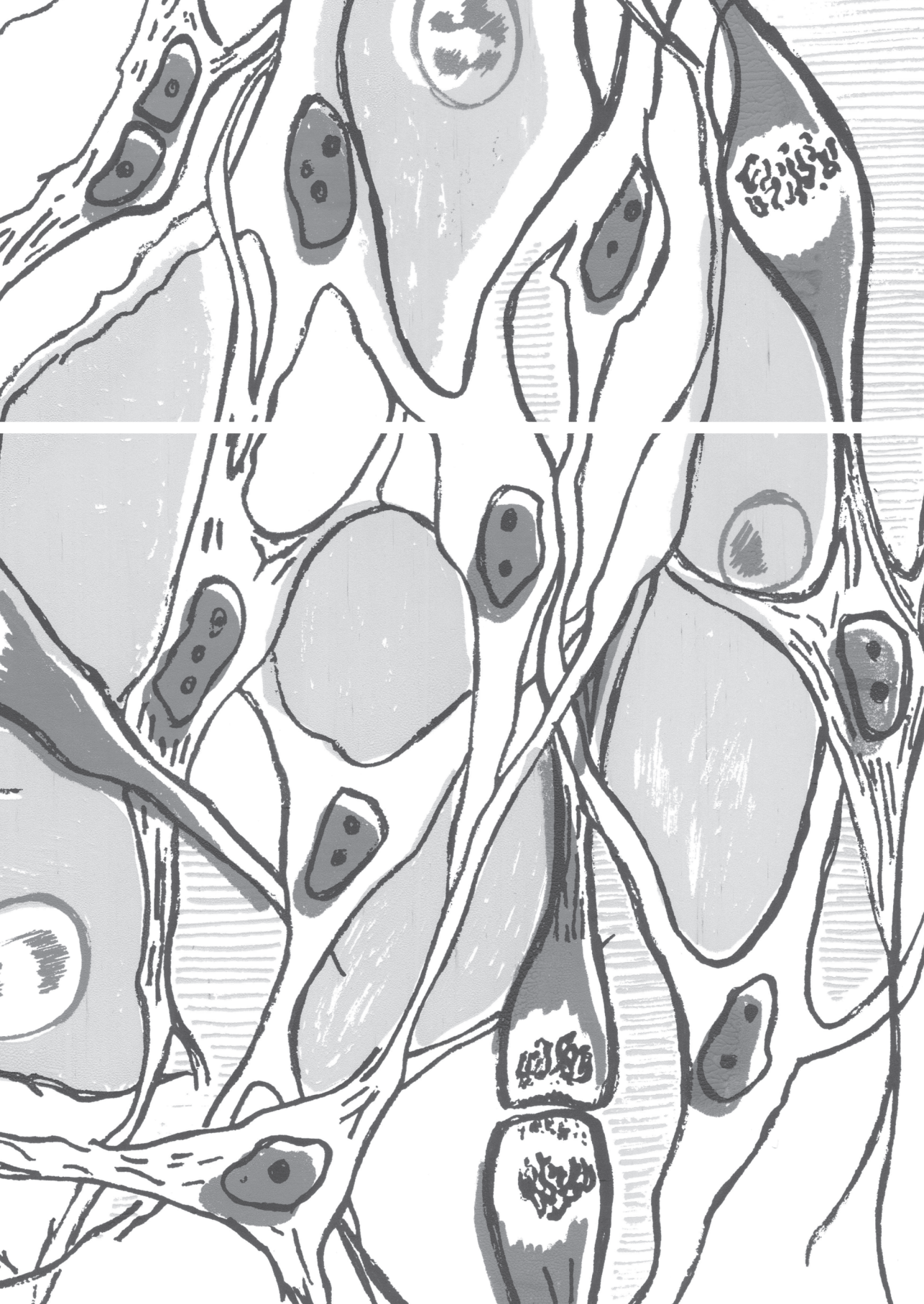
1. PhD training	**Year**	**Workload (ECTS*)**
Courses		
Patient Oriented Research: design, conduct and analisis (CPO): Erasmus MC	2017	0.3
Basiscursus Regelgeving en Organisatie voor Klinisch onderzoekers : Erasmus MC	2017	1.0
Biostatistics: Biostatistical Methods I: NIHES	2017	5.7
Portuguese language course: Instituto Latino, Amsterdam	2017	2.3
Scientific Integrity: Erasmus MC	2018	0.3
Systematic Literature search : Erasmus MC	2017, 2018	1.0
Course in Virology: MolMed	2018	1.8
Case-control Studies: Erasmus MC Summer Course	2018	0.7
Microsoft Excel 2010 Basic: MolMed	2018	0.3
Microsoft Excel 2010 Advanced: MolMed	2018	0.3
Photoshop and Illustrator CS6 Workshop : MolMed	2018	0.3
Koninklijke Nederlandse Akademie voor de Wetenschappen Honours program Arts/Science: Amsterdam	2018	1.2
Biomedical English Writing Course: MolMed	2019	2.0
Immunology: Molmed,	2019	1.0
GraphPad Prism: Molmed	2020	0.9
Neuroinfectious Diseases Course: University of Liverpool (virtual)	2021	0.6
Seminars and workshops		
Symposium neuromuscular diseases (Boerhaave) (4x): Amsterdam	2017, 2018, 2020, 2021	1.2
Belgian – Dutch neuromuscular study club (2x): Zeist/Utrecht	2017	0.3
Zika virus capacity training: Erasmus MC	2017	0.5
Muscles to meet 2019 (neuromuscular diseases symposium): Zeist	2019	0.3
Symposium Zoonotic disease emergence: Erasmus MC	2019	0.2
Scientific presentations and meetings		
Poster: Annual congress Dutch Neurology Association (NVN): Nunspeet	2017	0.6
Oral: Peripheral Nerve Society Annual Meeting: Sitges, Spain	2017	1.4
Oral: ZikaPLAN Annual Meeting: Havana, Cuba	2017	1.2
Oral & Poster: Peripheral Nerve Society Annual Meeting: Baltimore, USA	2018	1.4
Oral: ZikaPLAN Annual Meeting: London, UK	2018	0.6
Poster (x3): 28th annual Brazilian Neurology conference: São Paulo, Brazil	2018	0.9
Oral & Poster: Peripheral Nerve Society Annual Meeting: Genoa, Italy	2019	1.4

Oral: ZikaPLAN Annual Meeting: Cali, Colombia	2019	0.9
Oral: Peripheral Nerve Society Annual Meeting: virtual	2020	1.2
Oral: ZikaPLAN Annual Meeting: virtual	2020	0.3
Oral: ZikaPLAN Annual Meeting: virtual	2021	0.3
Poster: Peripheral Nerve Society Annual Meeting: virtual	2021	0.6
Conference attendance		
2nd International Meeting on Arboviruses and their Vectors: Glasgow, United Kingdom	2017	0.6
10th European Congress on Tropical Medicine & International Health: Antwerp, Belgium	2017	0.3
Annual congress Dutch Neurology Association (NVN): Nunspeet	2018	0.6

2. Teaching activities		
Lectures		
Poster: Muscle Disease Congres (Spierziektencongres): Veldhoven	2017	0.3
Invited speaker: 1st International Symposium on GBS and other neurological diseases: Mexico City, Mexico	2018	0.6
Invited speaker: Neuronon, annual training for neurologists: Zwolle	2018	0.3
Oral & Poster: Muscle Disease Congres (Spierziektencongres): Veldhoven	2019	0.3
Invited speaker: GBS-CIDP Symposium: Rotterdam	2019	0.3
Invited speaker: Mexican Academy of Neurology 2020 Annual Meeting: virtual	2020	0.3
Invited speaker: Global Health Network webinar “Lessons from a Zika Network to Covid-19 response and beyond”	2020	0.3
Invited speaker: European Academy of Neurology 2021 Educational course :virtual	2021	0.3
Invited speaker: Peripheral Nerve Society 2021 Educational course :virtual	2021	0.3

Other		
Reviewing papers for international peer reviewed journals (x23)**	2018-2021	2.0
Website design and maintenance: IGOS-Zika (for researchers)	2018	2.0
Website design and maintenance: The Global Health Network (for clinicians/researchers)	2020-2021	2.0
Website design and maintenance: GBS Expertise Centre (for clinicians/patients)	2020	1.0
Member of PNS Outreach Committee	2019-2021	2.0
total		**46.5**

*1 ECTS (European credit transfer system)= 28 study hours
**17 different journals, including *Neurology*, *The BMJ*, *Vaccine* and *New England Journal of Medicine*



Appendix

Diagnóstico e manejo da síndrome de Guillain–Barré em dez etapas

Portuguese translation of the publication *Diagnosis and management of Guillain–Barré syndrome in ten steps, Nature Reviews Neurology 15, 671–683 (2019).*

Diagnóstico y manejo del síndrome de Guillain-Barré en diez pasos

Spanish translation of the publication *Diagnosis and management of Guillain–Barré syndrome in ten steps, Nature Reviews Neurology 15, 671–683 (2019).*