NERVE CONDUCTION PATTERNS IN GUILLAIN-BARRÉ SYNDROME ASSOCIATED WITH ZIKA VIRUS INFECTION IN CUCUTA, COLOMBIA.

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NOTE OF INSTITUTIONAL RESPONSIBILITY

Universidad del Rosario, Universidad CES and Center for studies of autoimmune diseases (CREA) are not responsible for the concepts emitted by the researchers in their work, only watch over the scientific, methodological and ethical rigor of the same in the pursuit of truth and justice.

Crear es crecer.

To my beloved family.

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LIST OF TERMS

AANEM	American Association of Neuromuscular & Electrodiagnostic Medicine
AIDP	Acute inflammatory demyelinating polyradiculoneuropathy
AMAN	Acute motor axonal neuropathy
AMSAN	Acute motor and sensory axonal neuropathy
ARs	Attack rates
BMI	Body Mass Index
CIDP	Chronic inflammatory demyelinating polyradiculopathy
C. jejuni	*Campylobacter jejuni*
CMV	Cytomegalovirus
CMAP	Compound muscle action potential
CSF	Cerebro spinal fluid
CV	Conduction velocity
CHIK	Chicungunya virus
DML	Distal motor latency
DMLc:	corrected distal latency in milliseconds
DML:	Distal latency in milliseconds
dCMAP	Distal compound muscle action potential
DENV	Dengue virus
EBV	Epstein Barr virus
EDx	Electrodiagnosis
EMG	Electromyography
ELISA	Enzyme-linked immunosorbent assays
FCR	Flexor Carpi Radialis
GBS	Guillain-Barré syndrome
H. influenza	*Haemophilus influenza*

HIV	Human immunodeficiency virus
ICU	Intensive care unit
IFI	Immunofluorescence assay
LOS	Lipooligosaccharides
LLN	Lower limit of normal
m/s	Meters per second
MAC	Membrane attack complex
MAG	Myelin-associated glycoprotein
MFS	Miller Fisher syndrome
M. pneumoniae	*Mycoplasma pneumonie*
MUP	Motor unit potential
NCS	Nerve conduction studies
pCMAP/dCMAP	Proximal and distal radio of CMAP amplitude
PL	Palmar Longus
PRNT	Plaque reduction neutralization test
RCF	Reversible conduction failure
R0	Basic reproduction number
SD	Standard deviation
SNAP	Sensory nerve action potential.
SIVIGILA	Sistema Nacional de Vigilancia en Salud Pública
TLI	Terminal latency index
TLIc	Terminal latency index corrected for standard distance
ULN	Upper limit of normal
WHO	World Health Organization
ZIKV	Zika virus

ABSTRACT

Background

Zika virus (ZIKV) infection has been associated with an increased incidence of Guillain-Barré syndrome (GBS) but the relative frequency of acute inflammatory demyelinating polyradiculoneuropathy (AIDP) and axonal GBS subtypes is controversial.

Methods

Twenty-three GBS patients diagnosed according to Brighton criteria during the ZIKV outbreak in Cúcuta, Colombia, were evaluated clinically and electrophysiologically.

Electrodiagnosis of GBS subtypes was made according to a recently described criteria set that proved to have a high diagnostic accuracy on the basis of a single test. The electrophysiological features of 34 Italian AIDP patients were used as control.

Results

All patients had symptoms compatible with ZIKV infection before the onset of the GBS and the diagnosis of ZIKV infection was confirmed in 69.5 % of patients. Median time from onset of ZIKV infection symptoms to onset of GBS was 6 days (interquartile range, 6-14 days). Cranial nerve palsy was present in 82.6% of patients, facial palsy in 65.2%, autonomic dysfunction in 69.5%, and 43.4% of patients required mechanical ventilation. AIDP was diagnosed in 73.9% of patients. About 50% of nerves of the AIDP patients showed a prevalent demyelinating distal involvement but this pattern was not different from Italian AIDP patients without ZIKV infection.

Conclusions

GBS associated with ZIKV infection is clinically characterized by a high frequency of cranial nerve involvement, autonomic dysfunction and necessity of mechanical ventilation indicating an aggressive and severe course. AIDP is the most frequent electrophysiological subtype. Demyelination is prevalently distal but this pattern is not specific of ZIKV infection.

Key words

Zika virus, Colombia, Guillain-Barré syndrome, nerve conduction studies, electrophysiological criteria, acute inflammatory demyelinating polyneuropathy, axonal Guillain-Barré syndrome

RESUMEN

Contexto

La infección por el virus del Zika (ZIKV) se ha asociado con una mayor incidencia del síndrome de Guillain-Barré (GBS), pero la frecuencia relativa de la polirradiculoneuropatía desmielinizante inflamatoria aguda (AIDP) y los subtipos de GBS axonal es controversial.

Métodos

23 pacientes con GBS diagnosticados según los criterios de Brighton durante el brote de ZIKV en Cúcuta, Colombia, fueron evaluados clínica y electrofisiológicamente.

El electrodiagnóstico de subtipos de GBS se realizó con criterios recientemente descritos que demostraron tener una alta precisión diagnóstica sobre la base de una única prueba. Las características electrofisiológicas de 34 pacientes italianos AIDP se utilizaron como control.

Resultados

Todos los pacientes tenían síntomas compatibles con la infección por ZIKV antes de la aparición del GBS y el diagnóstico de infección por ZIKV se confirmó en el 69,5% de los pacientes. La mediana de tiempo desde el inicio de los síntomas de infección por ZIKV hasta el inicio del SGB fue de 6 días (rango intercuartílico, 6-14 días). La parálisis de pares craneales estuvo presente en el 82.6% de los pacientes, parálisis del nervio facial en el 65.2%, disfunción autonómica en el 69.5% y el 43.4% de los pacientes requirió ventilación mecánica. AIDP fue diagnosticado en el 73.9% de los pacientes. Alrededor del 50% de los nervios de los pacientes con AIDP mostraron una afectación distal desmielinizante prevalente, pero este patrón no fue diferente de los pacientes con AIDP italianos sin infección por ZIKV.

Conclusiones

El GBS asociado con la infección por ZIKV se caracteriza clínicamente por una alta frecuencia de compromiso de pares craneales, disfunción autonómica y necesidad de ventilación mecánica que indica un curso agresivo y grave. AIDP es el subtipo electrofisiológico más frecuente. La desmielinización es predominantemente distal, pero este patrón no es específico de la infección por ZIKV.

Palabras clave

Virus del Zika, Colombia, síndrome de Guillain-Barré, estudios de neuroconducción, criterios electrofisiológicos, polineuropatía desmielinizante inflamatoria aguda, síndrome axonal de Guillain-Barré

1. PROBLEM FORMULATION

1.1. Problem statement

ZIKV is an arbovirus of the *flaviviridae* family, declared epidemic worldwide mainly Latin America and in the South Pacific between 2015 and 2016. The increase in microcephaly and Guillain-Barré syndrome (1–3) prompted the World Health Organization to declare a “public health emergency of international concern” (4). Regarding this we decided at the Centre of study for autoimmune diseases- CREA to develop a study called RAIZ, previously reported, about the outbreak of ZIKV disease in Colombia, the neurological outcomes and the high incidence of GBS reported in geographic areas with high rates of ZIKV transmission (5).

GBS is an immune-mediated neuropathy characterized, in the classical form, by a rapidly progressive symmetrical weakness and areflexia (6). Existent scientific knowledge about molecular mimicry as a trigger for GBS is based on *Campylobacter jejuni* a common cause of human gastroenteritis (7), yellow fever vaccine (8), *Mycoplasma pneumonie* (9), *Haemophilus influenza* (10), Epstein Barr virus and Cytomegalovirus (11,12).

The lack of literature related to the molecular mimicry between immune response against ZIKV infection and antibodies against gangliosides in different nerve areas receive a great importance due to the epidemic globally reported and wide neurological compromise related to it, such as microcephaly, intracranial calcifications, transverse myelitis and Guillain-Barré Syndrome (13). Also important, to identify the neurological compromise in the population selected, including the development of GBS and determine the electrophysiological findings in people with GBS triggered by ZIKV is a priority, in order to give evidence about the neurotropism of ZIKV, support the clinical diagnosis of GBS and the classifications into clinical subtypes by electrophysiological studies. In the other hand, we want to identify specific molecules related to nerve damage in the case of GBS, due to is unknown for the case of ZIKV is the trigger for it.

1.2. Justification

From the declaration of the epidemic phase of Zika virus infection in Colombia, corresponding to the epidemiological week 40 of 2015 (11^{th} -17^{th} October) to epidemiological week 30 (24^{th} -30^{th} July) 2016, it has been 8,826 confirmed cases out of 92,319 cases reported by epidemiological suspicion (14). Since December 15^{th}, 2015 to July 30^{th}, 2016, it also has been reported to the SIVIGILA 617 neurological syndromes including Guillain-Barré syndrome, all of them with a history of febrile illness compatible with ZIKV disease. Due to the outbreak of the ZIKV in Colombia it is a necessity to study further this phenomenon, related to significant increase of expected cases of GBS.

It is unknown electrophysiological subtypes of GBS and more importantly, the relationship between the infection of Zika virus and the immune response targeting the nerves, and their subsequent damage. Reports of the electrophysiological studies in ZIKV associated GBS have provided conflicting conclusions. Studies from French-Polynesia concluded that electrophysiological findings were compatible with AMAN whereas in one series from Colombia the majority of patients had AIDP) (15–17). We also want to identify the predominance of antibodies against gangliosides and complexes in Colombian population with GBS, specifically after the outbreak of ZIKV.

The disabling after the GBS has a high impact in patient's quality of live. We notice patients with prolonged viremia for ZIKV has most severe forms of the disease therefore very slowly recovery of the primary functional grade he had. This cause a negative impact in society due to the cost and efforts he represents itself.

The actual treatment for GBS is unspecific and quite expensive. With the knowledge acquired in this research it would be possible to purpose further studies in order to

develop new, specific and easy ways to treat this neurological disease. Development of monoclonal antibodies to treat specifically the GBS could be the closest solution.

AIDP, AMAN and AMSAN are difficult to distinguish on clinical grounds and electrophysiology plays a determinant role in GBS diagnosis, classification and in establishing the prognosis (18). Nerve conduction studies are the main tool in diagnosis of GBS subtypes (19) and in the last three decades different criteria sets have been proposed (20–23). AIDP was electrophysiologically characterized by prolonged F-wave latencies, prolonged distal latencies, slowing nerve conduction velocities and temporal dispersion or conduction block (24). For axonal subtypes, in AMAN transient partial conduction block in intermediate and distal nerve segments (25), CMAP amplitudes are significantly reduced (24). In AMSAN the sensory potentials are reduced in amplitude and often absent (22). However, a transient conduction block/slowing could be highlighted in some AMAN and AMSAN patients without the development of abnormal temporal dispersion (18,23,25), called reversible conduction failure (RCF). This is a later diagnose and it is not contemplated in current electrodiagnostic criteria of GBS, missclasificating GBS patients (18,19,26).

There is a need for an innovative approach to reach an earlier and more accurate electrodiagnosis for all GBS subtypes, based on a single electrophysiologic study (19,23).

1.3. Research question

¿What is the relationship of nerve conduction patterns and anti-ganglioside antibodies present in Guillain-Barré syndrome after Zika virus infection?

Table 1. Health sciences descriptors.

	Guillain-Barré Syndrome	ZIKA virus	Gangliosides	Electrophysiological studies
DECS-Descriptores en Ciencias de la Salud	*English* descriptor: Guillain-Barre Syndrome *Spanish* descriptor: Síndrome de Guillain-Barré *Portuguese* descriptor: Síndrome de Guillain-Barré *English synonyms :* Acute Autoimmune Neuropathy Acute Inflammatory Demyelinating Polyradiculoneuropathy Acute Inflammatory Polyneuropathy Landry-Guillain-Barre Syndrome Polyradiculoneuropathy, Acute Inflammatory	*English* descriptor: Zika Virus *Spanish* descriptor: Virus Zika *Portuguese* descriptor: Zika virus *English synonyms :* Zika Fever Virus Virus, Zika Zikavirus Definition: An arbovirus in the FLAVIVIRUS genus of the family FLAVIVIRIDAE. Originally isolated in the Zika Forest of UGANDA it has been introduced to Asia and the Americas.	*English* descriptor: Gangliosides *Spanish* descriptor: Gangliósidos Descriptor Portugués: Gangliosídeos Sinónimos Español: Sialoglicoesfingolípidos *Definition:*A subclass of ACIDIC GLYCOSPHINGOLIPIDS They contain one or more sialic acid (N-ACETYLNEURAMINIC ACID) residues. Using the Svennerholm	*English* descriptor: Electromyography Spanish descriptor: Electromiografía Portuguese descriptor: Eletromiografia *Definition:* Recording of the changes in electric potential of muscle by means of surface or needle electrodes. *English* descriptor: Neural Conduction Spanish descriptor: Conducción Nerviosa Portuguese descriptor: Condução Nervosa *Definition:* The propagation of the NERVE IMPULSE along the nerve away from the site of an excitation stimulus.

	Definition: An acute inflammatory autoimmune neuritis caused by T cell- mediated cellular immune response directed towards peripheral myelin. Demyelination occurs inperipheral nerves and nerve roots. The process is often preceded by a viral bacterial infection, surgery, immunization, lymphoma, or exposure to toxins. Common clinical manifestations include progressive weakness, loss of sensation, and loss of deep tendon reflexes. Weakness of respiratory muscles and autonomic dysfunction may occur.		system of abbrevations, gangliosides are designated G for ganglioside, plus subscript M, D, or T for mono-, di-, or trisialo, respectively, the subscript letter being followed by a subscript arabic numeral to indicated sequence of migration in thin-layer chromatograms.	Annotation: along a single nerve; differentiate from NEURAL TRANSMISSION (between nerves)

MESH-Medical Subject Headings.	**MESH Heading: Guillain-Barre Syndrome** Annotation: Do not confuse X ref. *Polyradiculoneuropathy, Acute Inflammatory* with *Polyradiculoneuropathy, Chronic Inflammatory.* See Polyradiculoneuropathy, Chronic Inflammatory Demyelinating. Scope Note: An acute inflammatory autoimmune neuritis caused by T cell- mediated cellular immune response directed towards peripheral myelin. Demyelination occurs in peripheral nerves and nerve roots. The process is often preceded by a viral or bacterial infection,	**MESH Heading: Zika Virus** Annotation: Infection = Zika Virus Infection Scope Note: An arbovirus in the FLAVIVIRUS genus of the family FLAVIVIRIDAE. Originally isolated in the Zika Forest of UGANDA it has been introduced to Asia and the Americas.	**MeSH Heading: Gangliosides** Scope Note: A subclass of ACIDIC GLYCOSPHINGOLIPIDS They contain one or more sialic acid (N-ACETYLNEURAMINIC ACID) residues. Using the Svennerholm system of abbrevations, gangliosides are designated G for ganglioside, plus subscript M, D, or T for mono-, di-, or trisialo, respectively, the subscript letter being followed by a subscript arabic numeral	**MESH Heading: Electromyography** Scope Note: Recording of the changes in electric potential of muscle by means of surface or needle electrodes. **MeSH Heading: Neural Conduction** Annotation: along a single nerve; differentiate from NEURAL TRANSMISSION (between nerves) Scope Note: The propagation of the NERVE IMPULSE along the nerve away from the site of an excitation stimulus.

	surgery, immunization, lymphoma, or exposure to toxins. Common clinical manifestations include progressive weakness, loss of sensation, and loss of deep tendon reflexes. Weakness of respiratory muscles and autonomic dysfunction may occur.		to indicated sequence of migration in thin-layer chromatograms.	

2. THEORETICAL FRAMEWORK

2.1. Context of research

Demographic settings: Norte De Santander, Colombia

The country of Colombia is largely situated in the northwest of South America. Topographically, is divided into 4 regions: the central highlands, the Caribbean lowlands, the Pacific lowlands, and Eastern Colombia (east of the Andes Mountains). Norte de Santander one of 32 departments or provinces of Colombian territory.

Norte de Santander is located in northwestern zone of the Colombian Andes Mountains. The total extension area of the department is 21.648 km². It has 850,000 habitants (27) and it is found 320 meter above sea level, main temperature is 27.6 °C, high temperatures are around 38 °C. This province is divided into 40 municipalities or districts, gathered into six subregions: North, West, East, Central, South-west and South-east. At the East is found Cucuta, North lat. 7º53' 00" y West long. 72º 30' 19'', which is the capital city of the province and it is situated in relation to Venezuelan border (27).

City of Cúcuta

San Jose de Cucuta is the capital city of Norte de Santander province or department, this in turn is the core of the Metropolitan area. It is divided into 10 administrative districts and 10 communes. Cucuta is located on the west bank Pamplonita river, 10 and 5 minutes from Venezuela, the municipalities of San Antonio del Tachira and Pedro María Ureña, respectively.

The city is located 325m above sea level in the hydrographic basin, formed by Pamplonita River, the Eastern Andes range, Zulia, Tachira and Guaramito. This valley is very seismic and geographically corresponds to the continental area of Maracaibo's Lake (27). Among the valley you find wide variety of ecosystems

characterized by unestable ground, slopes, deforestation and highly eroded by wind and rain. Those geographical accidents has never been a limit for the progress, even though, large number of building are built in risk areas (27).

Cucuta is border on the North by Puerto Santander, Tibu and Venezuela, on the South side by Bochalema, Los Patios and Villa del Rosario, at the East by the Republic of Venezuela and on the West with Sardinata, El Zulia and San Cayetano.

The territorial extension is 1,176 km2By the current year 2016, the Administrative National Department of statistics (DANE), consider the total population of Cucuta in 656.414 people (28). The head municipality concentrates 96.62% of the total population and represents 46.21% of the provincial population. This data shows an unbalanced among the population density located in Cucuta with regard to the rest of the province. Cucuta- Venezuela border area facilitates the population´s settlement, transit and migration between those countries (27).

Weather in Cucuta is warm and dry, characterized by high temperatures ranging 27°C to 29°C. Crosswinds in the months of June and August reach speeds 37 to 74Km/h, making the weather more enjoyable. The average of rainfall is higher during the months of April, May, June, September, October and November, approximately 655mm of rainfall.

The annual average of relative humidity is 70 to 75%, their lands are covered in warm thermal floor. The territory, due to its large size, comprises two very different landscapes, the warm, dry valley where the city and jungle areas of abundant rainfall in the north of the municipality.

2.2. Definitions

Zika virus (ZIKV) is a little-known emerging mosquito-borne flavivirus, of the Flaviviridae family, which is closely related to the Flavivirus genus. ZIKV contains a positive, single-stranded genomic RNA encoding a polyprotein that is processed into three structural proteins, i.e., the capsid (C), the precursor of membrane (prM), and

the envelope (E), and seven nonstructural proteins (29). Many different *Aedes* species mosquitoes can account (30)t for the transmission of ZIKV, including *Aedes aegypti*, which at present is considered to be the main vector of the virus in South and Southeast Asia (31,32), as in South America including Colombia.

A patient with GBS is an immune-mediated peripheral neuropathy characterized by injury or axonal myelin and represents the most common cause of symmetrical flaccid paralysis and areflexia (33). The diagnosis is supported in clinical features and also, with paraclinical findings such as cytoalbumin dissociation in the CSF and specific damage patterns of the nerve, observed in electrophysiological studies (34).

About electrophysiological study, based on the conduction of motor and sensitive nerves, assessing velocity, onset and amplitude of the action potential. Electromyography is the second compound of the study, it consists on determine the denervation of the muscle and it is useful to assess neuromuscular diseases (30).

Gangliosides are glycosphingolipids that are mainly located in brain tissue, acting as a ligand of myelin-associated glycoprotein (MAG) which maintains stability, structure of the myelin sheath in the axon and helps control nerve regeneration (35). In autoimmune neuropathies, broadly accepted the pathogenesis as the presence of a specific humoral response directed against membrane glycolipids. As for the GBS, the first autoantibodies associated with this syndrome date from 1988 (36). It has also described molecular mimicry mechanism in the GBS, widely documented in a prior infection with *Campylobacter jejuni* (9).

2.3. ZIKV: magnitude, frequency and distribution in Colombia.

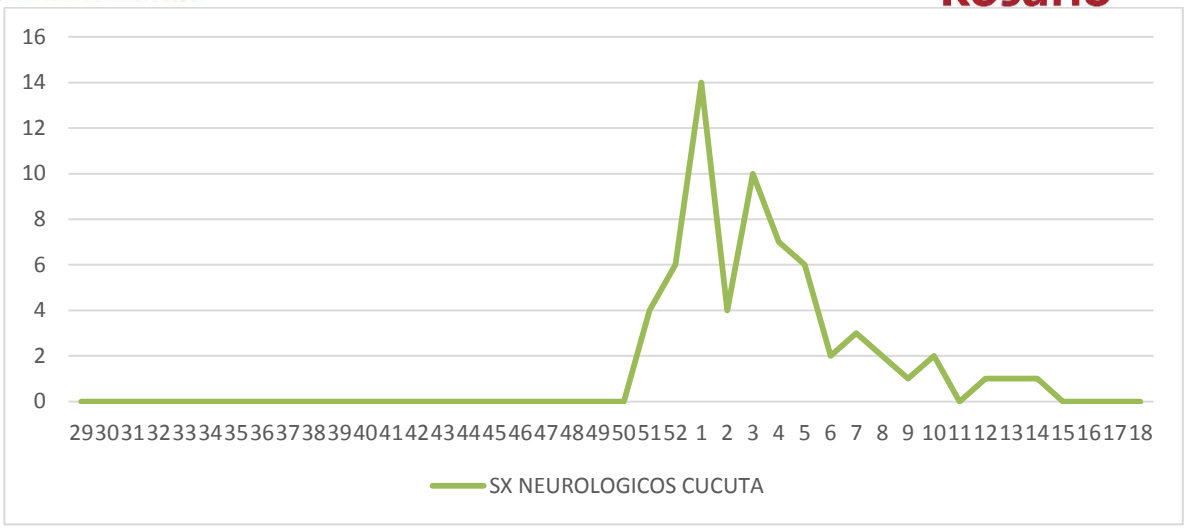
From the declaration of the epidemic phase of Zika virus infection in Colombia, corresponding to the epidemiological week 40 of 2015 (11^{th} -17^{th} October) to epidemiological week 30 (24^{th} -30^{th} July) 2016, it has been 8,826 confirmed cases out of 92,319 cases reported by epidemiological suspicion (14). Since December

15th, 2015 to July 30th, 2016, it also has been reported to the Colombian National System of Public Health Surveillance (SIVIGILA for “Sistema Nacional de Vigilancia en Salud Pública”), 617 neurological syndromes including Guillain-Barré syndrome, all of them with a history of febrile illness compatible with ZIKV disease (14).

According to the SIVIGILA, is the second department with the highest reported cases is Norte de Santander, with 8,505 epidemiological suspicion, of which 1,521 cases were laboratory tested. Likewise, North Santander tops the list of Colombia departments with greater reporting of neurological syndromes with a history of positive ZIKV infection, with 82 reported cases, corresponding to 13.29% of all reported cases in the country. Most studies worldwide estimate the incidence of GBS in Europe and North America between 0.8-1.9 cases per 100,000 populations per year. In Colombia an annual incidence of 3.0 per 100,000 populations has been reported (14).

Aedes aegypti mosquito is globally identified as the main vector of transmission ZIKV. First description date from 1950, following the successful inoculation from an infected mosquito to a human volunteer (13) Subsequent experiments showed mosquito transmission with mice and Zika virus has been isolated in several species of *Aedes* (37).

Figure 1. Neurological compromise after ZIKV infection in Cúcuta, 66 cases were notified to the SIVIGILA, from 29 epidemiological week 2015 to 18 epidemiological week 2016 (14)



Taken from: Instituto Departamental de Salud Norte de Santander. 2016.

GBS is an autoimmune neuropathy, life-threatening, often related to broad spectrum of complications. The mortality rate in Europe and North America is documented between 3- 7% (38). In Colombia, is approximately 4% (39). The clinical diagnostic criteria we used to identify Guillain-Barré Syndrome were the Asbury and Brighton criteria (21,40).

The electrodiagnosis were performed in two phases. First, the diagnosis of GBS was assessed with Hadden criteria (41). However, given the restrictions of diagnostic criteria sets, as Ho, Rajabally electrodiagnostic criteria, Uncini et al. identified the necessity of reach a more precise reference diagnosis for assessing the accuracy of criteria sets (18,42). Thus, based on the need for an innovative approach to reach an earlier and more accurate electrodiagnosis based on a single electrophysiologic study. They compared, the diagnostic accuracy at the first electrophysiologic test of a statistical method of supervised classification with two existing criteria sets (Ho, Rjabally electrodiagnostic criteria (18,42) and proposed a newly one which also defines at the second study, the cut-offs for RCF in motor and sensory fibers (19).

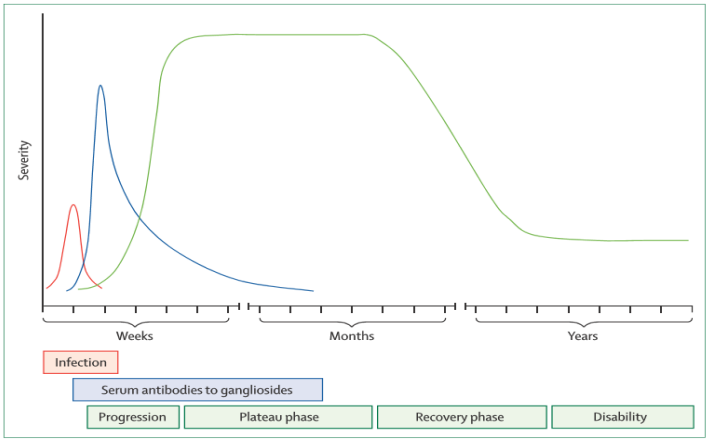
In a second-time assessment, we applied the new criteria set proposed by Uncini et al, in order to perform a more reliable diagnosis.

2.4. Guillain-Barre Syndrome: Clinical and pathological context

Guillain- Barré syndrome is an immune-mediated peripheral neuropathy characterized by injury or axonal myelin, and represents the most common cause of symmetrical flaccid paralysis and arreflexia (34). The clinical presentation was initially constituted with paresthesias in extremities (most common symptom), with occasional and slight loss of sensitivity, with low back pain and neuropathic pain at times (43). Within few days the clinic is symmetrical and distal to proximal weakness, which is described as an ascending pattern also characterized by the reduction or abolition of tendon reflexes (hypo or areflexia) is established. The facial involvement, commitment third cranial nerve, papilledema or dysphagia, suggests a presentation of clinical variants of Guillain-Barré syndrome (34).

The clinical journey through Guillain-Barré syndrome follows a typical pattern that can be readily divided into its constituent phases and components (figure 2) (44).

Figure 2. Guillain Barré Syndrome time course.



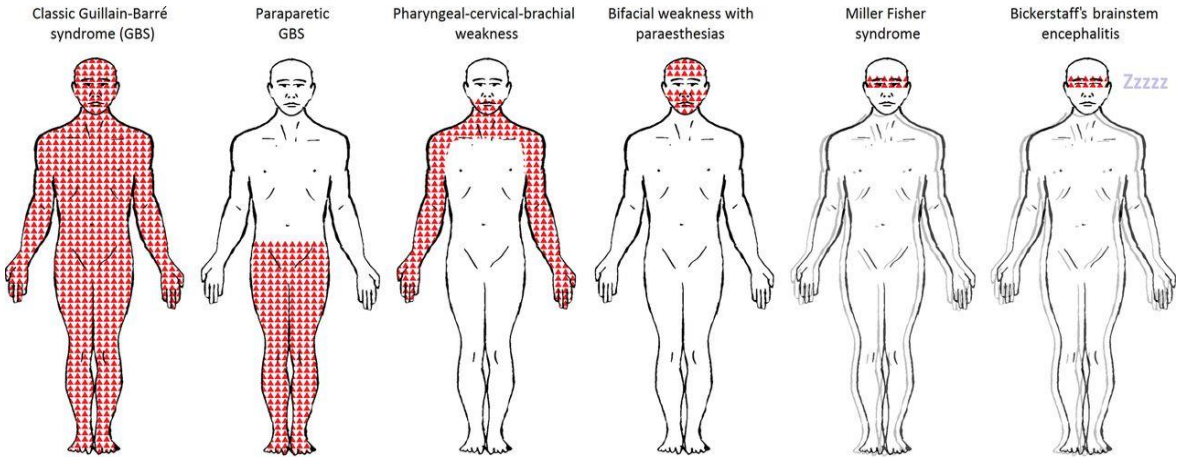
Taken from Willison HJ, Jacobs BC, van Doorn PA. Guillain-Barré syndrome. Lancet. 2016;388(10045):717-27.

GBS has one principal variant, known as Miller Fisher syndrome (MFS). Five per cent of patients with MFS develop weakness during disease course, indicating that MFS and GBS form a continuum (34). The first variant described in the clinical spectrum of SGB was MFS, distinguished by ophthalmoplegia, ataxia and areflexia without weakness in limbs (24,34,45). Usually not fully meet the three criteria; however, the diagnosis is supported by albumin- cytological dissociation and the presence of ganglioside antibodies. Stem encephalitis is a variant Bickerstaff turn MSF, with the same findings in cerebrospinal fluid and ganglioside antibodies, characterized by altered state of consciousness, hypereflexia, ataxia and ophthalmoparesis (45).

The clinical presentation of GBS-related disorders is heterogeneous and the diagnosis may not be obvious at first, because there are patients with acute flaccid paralysis and brainstem syndromes and unusual presentations of GBS-related disorders (34).

Faringocervicobraquial motor variant is defined by ptosis, weakness in the facial muscles, throat and neck flexor muscles, which then progresses to the commitment of force in upper and lower limbs, feeling of numbness and decreased or absent reflexes (24,34). Variants in the clinical spectrum of Guillain-Barré less frequent described in the literature. The frustrated forms and atypical presentations of SGB are also recognized and often correlates with the geographical area and environmental exposure or trigger the immune response generated by Guillain-Barré syndrome. Paraparetic motor variant is a typical frustrates form that selectively affects a lower limb, with areflexia and pain in lower back, simulating an acute spinal cord injury. While this could be the main differential diagnosis, the presence of ganglioside antibodies and albuminocytologic dissociation present, with or without electrophysiological changes that strengthen the diagnosis (34).

Figure 3. Clinical presentation of GBS Syndrome.



Taken from: Wakerley BR, Yuki N. Mimics and chameleons in Guillain-Barré and Miller Fisher syndromes. Pract Neurol. 2015;15(2):90-9

According to Wakerley et al, MFS is a clinical variant of GBS which may represent a major concern for many clinicians when it comes to differential diagnosis, mainly acute flaccid paraparesis or brainstem syndromes (see table 1) (34).

Table 2. Differential diagnoses for classic Guillain–Barré syndrome.

Acute flaccid paralysis
Viruses targeting anterior horn cells or motor neurons ▸ Poliomyelitis, non-polio enterovirus (enterovirus 71), West Nile virus ▸ Herpes simplex virus, cytomegalovirus, Epstein–Barr virus, varicella zoster virus ▸ Rabies virus, HIV
Transverse myelitis ▸ Mycoplasma pneumoniae ▸ Herpes simplex virus, cytomegalovirus, Epstein–Barr virus, varicella zoster virus

Spinal cord injury ▸ Acute spinal stenosis (eg, disc prolapse, epidural abscess or haematoma) ▸ Anterior spinal artery occlusion
Acute peripheral neuropathies ▸ Infections (eg, herpes simplex virus, HIV) ▸ Consumption of toxins or poisons (eg, puffer fish poisoning (tetrodotoxin), lead, thallium, arsenic) ▸ Tick paralysis, Lyme disease ▸ Porphyria
Neuromuscular junction disorders ▸ Myasthenia gravis ▸ Lambert–Eaton myasthenic syndrome ▸ Botulism
Neuromuscular weakness related to critical illness ▸ Critical illness neuropathy and myopathy
Muscle disorders ▸ Acute myositis ▸ Periodic paralysis ▸ Functional

Taken from: Wakerley BR, Yuki N. Mimics and chameleons in Guillain-Barré and Miller Fisher syndromes. Pract Neurol. 2015;15(2):90-9

2.5. Electrodiagnosis (EDx): nerve conduction and electromyography studies

Basic anatomy

A nerve is an enclosed, cable-like bundle of axons. Many types of axons are known: somatic and autonomic fibers, motor and sensory fibers, large and small fibers. Each fiber consists of an axon insulated by segments of myelin, which is thick and tightly wrapped for large myelinated fibers and thin and loosely wrapped for small unmyelinated fibers (46).

The functional and electrodiagnostic implications of different nerve fiber diameters and their degree of myelination is varied in nerve fiber conduction velocities. Myelinated fibers have faster velocities as a result of saltatory conduction (30–60 m/s), whereas unmyelinated fibers conduct relatively slowly (<1 m/s). Routine nerve conduction studies assess exclusively larger myelinated nerve fibers, as the contributions from smaller myelinated and unmyelinated fibers to the recorded signal are by comparison minimal. Special tests can assess these fibers but are not commonly performed and rarely help with characterization of common neuropathies (47).

Neurophysiological changes have a crucial role in the diagnosis of GBS. Its sensitivity is however heavily depended on the demyelinating nature of the peripheral nerves (41). It is standardized the technique to perform electrodiagnosis, such as electromyography and nerve conduction studies (including late potentials) (48).

Electromyography (EMG): Needle examination

EMG is the technique used for the electrical detection of signals arising from the depolarization of skeletal muscle, evaluates the integrity of the motor unit and it is useful to determine whether there is damage to nerve fibers to individual muscles

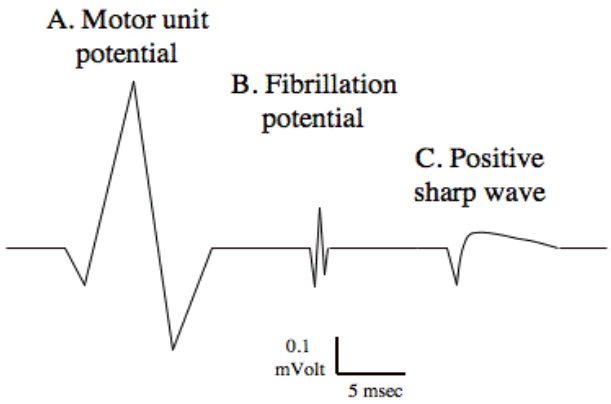
(46). Usually is performed with a needle placed directly in the muscle, but it also could be measure from skin surface electrodes. This measure the amplitude and morphology of the electrical signal within skeletal muscle. Alterations found in these patterns may suggest denervation of the muscle and muscular diseases (48).

A normal muscle is electrically silent when recording from a needle electrode (48). Some findings appear when the needle is moved in the muscle, this is called *insertional activity*. The activity ends immediately upon termination of the movement, with restoration of electrical silence. The only place within the muscle that is not electrically silent is the motor end-plate. This spikes can be misinterpreted as evidence of denervation or of increased insertional activity and membrane instability, this distinction requires some care (46).

After you stablish the insertional activity and the electrically silent, you ask the patient to voluntarily contract the muscle. Contraction takes place by activating motor neurons to the muscle, each of which is connected to many muscle fibers scattered throughout the muscle, termed a motor unit. The electrical signal that is recorded as a motor unit potential (MUP) (46). At this point, you assess the *amplitude* of MUP. As the strength of contraction is slowly increased, motor units are recruited in a very orderly sequence, called recruitment pattern. Delayed recruitment is a reflection of loss of motor units within the muscle. Muscle diseases can produce some membrane instability if the disease is very active. This can result in the appearance of "fibrillation potentials" that represent the contraction of individual muscle fibers (46).

When the disease of the muscle is based on the motor unit or in the distal motor axon, the effect is showed by fibrillation potentials that represent the contraction of individual muscle fibers. The finding of fibrillations and positive sharp waves are called acute denervation (one week at least up to 12 months after the damage), is the most reliable and objective test that there is for damage to motor axons to the muscle (48).

Figure 4. Electromyography wave forms. Guillain-Barré. A. Motor unit potential. (MUP), B. Fibrillation potential, C. Positive sharp wave.



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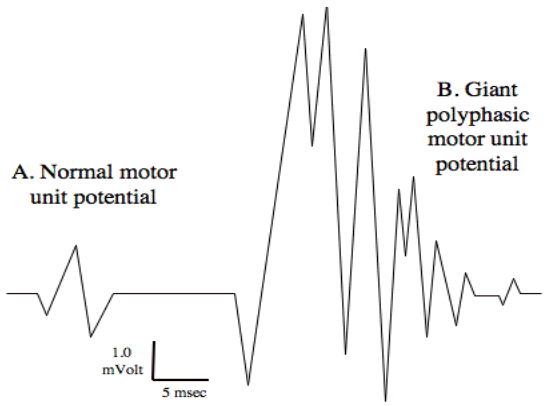
Reinnervation may occur whenever a muscle is partially denervated. This process results in the development of clumps of reinnervated muscle fibers attached to individual motor neurons, which is not the physiological pattern (one motor unit innervates one muscle fiber). According to this, the motor units become significantly larger both in amplitude and duration, the MUP often become more irregular termed polyphasic, and this late finding may suggest the presence of chronic denervation (49).

Figure 5. Electromyography wave forms. A. Normal motor unit potential, B. Giant polyphasic motor unit potential.



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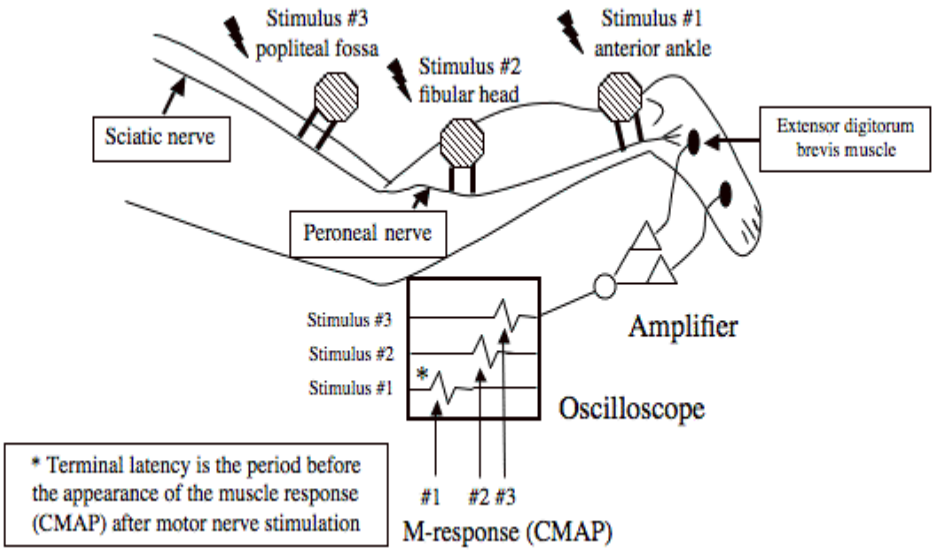
Motor conduction velocity (CV)

Motor conduction studies are performed by stimulating a motor nerve while is being recording the response from its target muscles. The compound muscle action potential, CMAP is recorded following motor nerve stimulation. When motor nerve fibers are stimulated close to the muscle, the time between the stimulus and the start of depolarizing muscle is called the terminal latency. This value includes both the amount of time that it takes the nerve to conduct from the point of stimulation to the motor end plate area and the amount of time for the neuromuscular junction transmission to activate the muscle (46,47,50).

The motor nerve conduction velocity is a mathematical relationship who involves the distance between the two simulation sites and the difference in the terminal latencies recorded from the more distal and more proximal sites. The value comes from

dividing the distance by the time gives the nerve conduction velocity over the segment in between the stimuli; expressed in m/s.

Figure 6. Motor nerve conduction (peroneal nerve). Stimulus #1 is placed in anterior ankle, near to malleolus externus (anterior ankle), where the extensor digitorium brevis muscle is found, in relation to peroneal nerve. Stimulus #2 is placed near to fibular head, where the peroneal nerve can be reached. Stimulus #3 is placed in popliteal fossa, and the stimulus will reach the sciatic nerve. Every stimulus is amplified in the machine´s screen.



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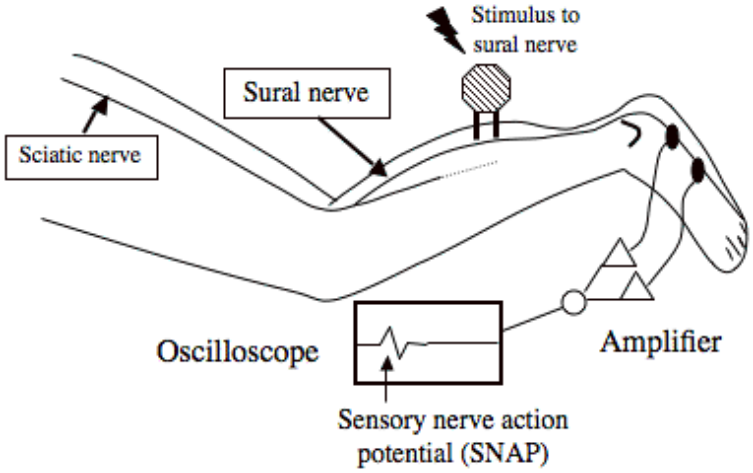
Guillian-Barré Syndrome preferentially damage the myelin of the largest, fastest conducting fibers. This causes slowing as manifest by decreased conduction

velocity. Actual blockage of conduction can occur due to damage to the myelin of 3-4 internode segments (46).

Sensory conduction velocity (CV)

This test can be performed in either an orthdromic (i.e. distal stimulation and proximal recording) or antidromic (i.e. proximal stimulation and distal recording) direction (48). The recording is made directly from the sensory nerve, called the *sensory nerve action potential, SNAP* (quite smaller than the CMAP). To determine the sensory nerve conduction velocity over the segment you divide the distance between the point of stimulation/recording, over the latency measured.

Figure 7. Sensory nerve conduction. The stimulus is performed in the sural nerve, 12cm above the malleulus externus, between gastrocnemius head.



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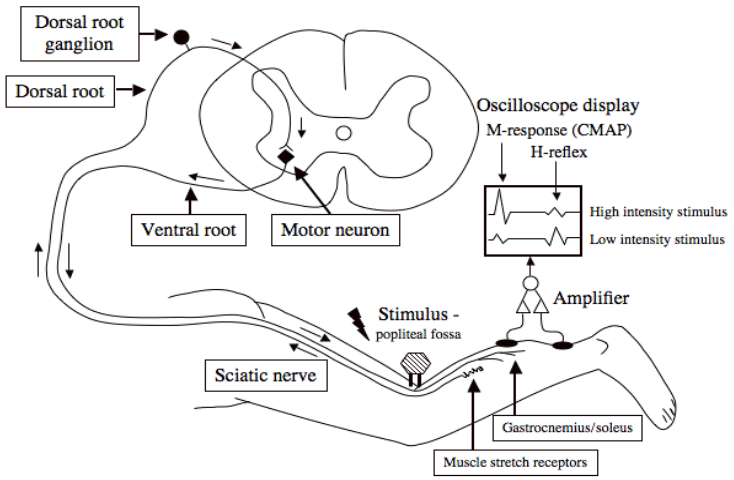
Late potentials

The late potentials are electrodiagnostically-elicited responses in muscle, called in that way due to they appear more than 10-20 m/s after stimulation of motor nerves (48).

- H-reflex. Commonly tested by electrical stimulation of the tibial nerve, with recordings from the gastrocnemius/soleus muscle complex (i.e., the triceps surae). Therefore, this response utilizes the same neural pathway as the ankle jerk reflex (46). The electrical stimuli required to depolarize the largest nerve fibers is lower, because of the heavily myelinated sheets around them. Since the largest nerve fibers in a peripheral nerve are those arising from muscle stretch receptors, there should be a stimulus intensity that activates muscle stretch afferent nerve fibers without directly activating many motor nerve axons (which are slightly smaller in diameter).

  After the electrical stimuli, a monosynaptic reflex contraction will be elicited in the muscle, leading the sensory axon all the way back to the spinal cord before synapsing on the motor neuron, and since the motor response must then traverse the length of the motor axon to reach the triceps surae muscle. Damage to any portion of the reflex arc, can result in loss or slowing of the reflex response. It is measure the amplitude of response and the time expressed in m/s, required to perform the reflex (46).

Figure 8. H-reflex. Late response commonly tested by electrical stimulation of the tibial nerve, with recordings from the gastrocnemius/soleus muscle complex.



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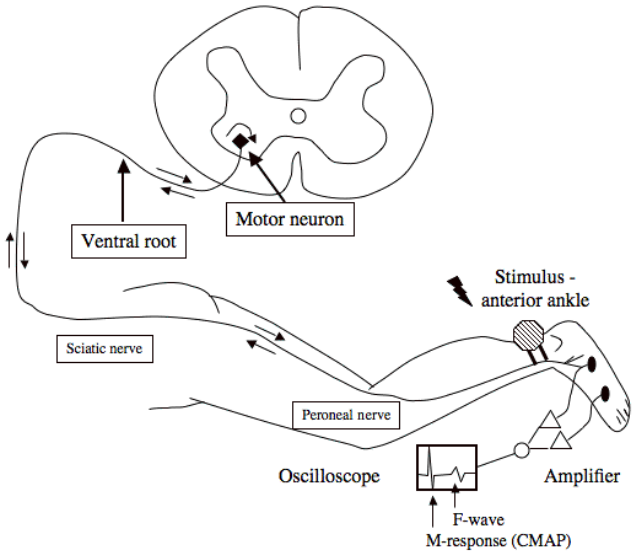
- F-wave. This response occurs in muscles during a motor nerve conduction study long after the initial contraction of the muscle (the CMAP response can be normally recorded in the muscle approximately 25 -55 milliseconds later. When this antidromic (opposite to the normal direction of conduction) depolarization reaches the motor neurons in the spinal cord, a percentage of these motor neurons are activated a second time.

  This results in an orthodromic electrical signal being conducted in the normal (orthodromic) direction from the spinal cord to the muscles innervated by the nerve. This second, later activation produces a small muscle contraction that is termed the F-response (46).

Figure 9. F-wave. Response that occurs in muscles during a motor nerve conduction study long after the initial contraction of the muscle.



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Delay in the F-response indicates some slowing of conduction of the motor axon. Since the F-response traverses more proximal portions of the motor axons (twice, in fact) it may be useful in the investigation of proximal nerve pathology such as root pathology seen in radiculopathy, Guillian Barre Syndrome, or Chronic Inflammatory Demyelinating Polyradiculopathy (CIDP) (46).

The diagnosis of SGB is performed by according to clinical and electrophysiological criteria. Several criteria sets has been proposed for the identification to subfenotypes of GBS (acute inflammatory demyelinating polyneuropathy –AIDP, acute motor axonal neuropathy –AMAN, acute motor and sensory axonal neuropathy –AMSAN). There is a lack of agreement worldwide according to the standard criteria set who best identify and classified the patient with GBS, also having among them

differences in terms of criteria listed. Criteria sets to identify AIDP subfenotypes, the parameters indicative of demyelination, the cut-off limits and the number of required abnormalities shows different sensitivities. Criteria sets for AMAN and AMSAN were proposed on the initial assumption that these subtypes were pathologically characterized by simple axonal degeneration (26).

Some AMAN patients show transient conduction block or slowing in the intermediate and distal nerve segments, mimicking demyelination but without the development of abnormal temporal dispersion, named reverse conduction failure (RCF). This lack of distinction leads to classify AMAN patients with RCF as AIDP or AMAN with axonal degeneration (26). Thus, taken into consideration RCF, it was proposed a new criteria set by Uncini, (table 2) in an attempt to consolidate all parameters existing and to update the existing ones.

Table 3. Criteria set employed for electrodiagnosis of GBS subtypes.

AIDP	AMAN	AMSAN	Unexcitable	Equivocal
At least one of the following in at least two nerves: ‣ MCV <70% LLN ‣ DML>130 % ULN ‣ dCMAP duration >120% ULN	None of the AIDP features in any nerve (demyelinating features allowed in one nerve if dCMAP <20% LLN) And at least one of the following in each of two nerves:	‣ Same criteria of AMAN in motor nerves, plus: ‣ SNAP amplitudes < 50% LLN in at least two nerves	‣ Distal CMAP absent in all nerves (or present in only one with distal CMAP <10% LLN)	‣ Abnormal findings however not fitting criteria specific for other subtypes

▸ pCMAP/dCMAP duration ratio >130% ▸ F-response latency>120% ULN Or one of the above in one nerve, plus: ▸ Absent F waves in two nerves with dCMAP > 20% LLN ▸ Abnormal ulnar SNAP amplitude and normal sural SNAP amplitude	▸ dCMAP<80% LLN ▸ pCMAP/dCMAP amplitude ratio <0.7 (excluding tibial nerve) ▸ Isolated F wave absence (or <20% persistence)			

AIDP, acute inflammatory demyelinating polyradiculoneuropathy; AMAN, acute motor axonal neuropathy; AMSAN, acute motor and sensory axonal neuropathy; ULN, upper limit of normal; LLN, lower limit of normal; DML, distal motor latency; MVC, motor conduction velocity; CMAP, compound muscle action potential; dCMAP, distal compound muscle action potential; pCMAP/dCMAP ratio between proximal and distal amplitude compound muscle action potential; SNAP, sensory nerve action potential.

Also, as part of our interest, it may be important to demonstrate that there is a prevalent distal involvement in your AIDP patients. The terminal latency index (TLI)

is a calculated electrophysiological parameter and it was used to compare the distal segment (distal of nerve stimulation) with the intermediate segment (i.e. wrist to elbow) (23). As distal motor latency (DML) and TLI values depend on the distance between the recording electrode and the site of distal nerve stimulation and in order to apply normal values settled by Kaku et al (Capasso, Clin Neurophysiology, 2002). If TLIc <0.25 it indicates a prevalent involvement of distal nerve segments compared to intermediate ones (23).

DML and TLI was corrected for a standard distance of 70mm, using the following formula:

$$DMLc: DML – [(d-70)/MCV]$$

DMLc: Milliseconds (corrected for standard distance)

DML: Distal latency in milliseconds

MCV: Motor conduction velocity in meters per second (m/s)

TLI was calculated using the formula:

$$TLIc= 70/MCV/DMLc$$

DMLc: Milliseconds (corrected for standard distance)

TLIc: Terminal latency index corrected for standard distance

If TLI is <0.25, indicates a prevalent involvement of distal nerve segments compared to intermediate ones (23).

Moreover, because distal distance was reported in not all the patients, so it was impossible to establish relationship among them if the measure of interest was not trusty done. Interestingly, we wanted to demonstrate if the AIDP subtype observed in GBS associated to ZIKV has a different segment nerve involvement so we applied distinction patterns in AIDP patients. Moreover it was interesting to verify in how

many nerves and patients prolonged DML and increased dCMAP duration were combined or dissociated (23).

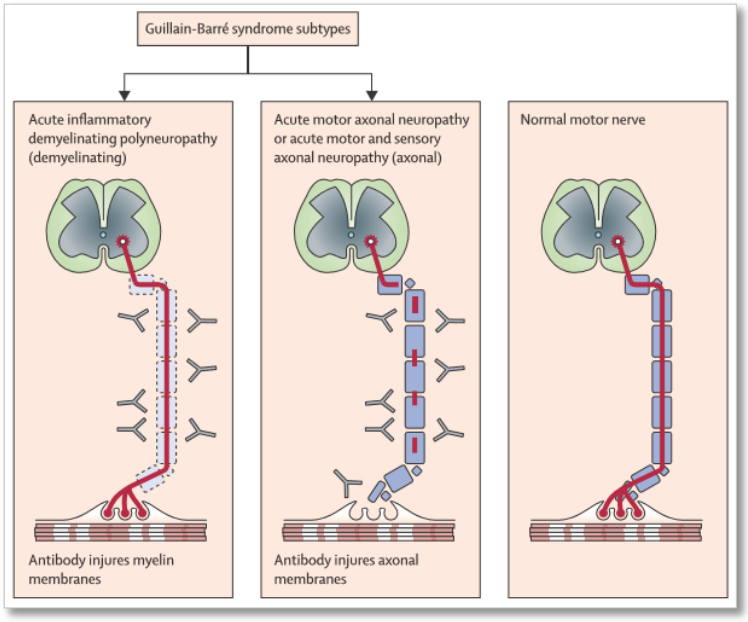
Table 3. Criteria to define the pattern of prevalent demyelinating involvement in nerve segments.

Distal	Intermediate	Diffuse	Unclassifiable
DML>130 % ULN and/or dCMAP duration >120% ULN **and** MCV >70% LLN and pCMAP/dCMAP duration ratio <130%	MCV <70% LLN and/or pCMAP/dCMAP duration ratio >130% **and** DML<130 % ULN and dCMAP duration <120% ULN	DML>130 % ULN and/or dCMAP duration >120% ULN MCV <70% LLN **and** MCV <70% LLN and/or pCMAP/dCMAP duration ratio >130%	Normal nerve, unexcitable or that does not reach the cut-offs for demyelination in distal and/or intermediate nerve segments

ULN, upper limit of normal; LLN, lower limit of normal; DML, distal motor latency; MVC, motor conduction velocity; CMAP, compound muscle action potential; dCMAP, distal compound muscle action potential; pCMAP/dCMAP ratio between proximal and distal amplitude compound muscle action potential.

According to the electrophysiological criteria, GSB it is categorized into two major subtypes or classic, acute inflammatory demyelinating polyneuropathy (AIDP) and Acute Motor Axonal Neuropathy (AMAN) (38).

Figure 10. Major Guillain-Barré Syndrome subtypes in which antibody-mediated effector pathways, including complement activation, cause glial or axonal membrane injury with consequent conduction failure).



Taken from Willison HJ, Jacobs BC, van Doorn PA. Guillain-Barré syndrome. Lancet. 2016;388(10045):717-27.

This classification was based initially on electrophysiological and pathological studies subsequently supported with antibodies identifying biomarkers in axonal motor neuropathy, directed against the neuronal membrane gangliosides GM1 and GD1a mainly (38,43). In relation to biomarkers, electrophysiological findings and clinical subtype of the SGB, must take into account race and ancestry, since the incidence of AIDP and AMAN varies across the world depending on the breed that mainly affects so as the microorganism that triggers the immunogenic response.

In the last 20 years they have devoted efforts to understand the mechanism of pathogenesis of GBS and molecular mimicry is involved. Few studies have determined the relationship and immunogenicity of the protein myelin specific T cell-mediated (43,51).

AMAN is strongly related to ganglioside GM1 antibodies and circulating GD1a.In more than 50% of the studies reported in Colombia about SGB, they have not described the electrophysiological findings (39), suggesting the need to report our findings, in order to identify and recognize the electrophysiological patterns in our population. On the other hand, it seeks to provide information on the epidemiological SGB in our country.

Likewise, it seeks to determine the most frequent clinical subtype and the correlation with the severity and prognosis specifically in our Colombian population. We intend to publish our results in the scientific community, so our findings are recorded and serve as a basis to depart for further research. In this way, we can have an estimate of epidemiological and clinical behavior of SGB, predicting severity and determining probability of recovery versus time.

2.6. Gangliosides & Antibodies

Gangliosides are part of a large family of compounds glycosphingolipid ceramide portion attached to an oligosaccharide having sialic acid. They are about 0.6% of the total lipids of the brain and are mainly located in brain tissue forming part of the membranes of nerve cells, acting as a ligand of the myelin-associated glycoprotein (MAG) which maintains stability and structure of the sheath myelin on axon and helps control nerve regeneration (52). There are five gangliosides (GM1, GD 1a, GD 1b, GT1a and GQ1b) which together account for the vast majority (97%) of adult nerve tissue gangliosides. In autoimmune neuropathies, it is generally accepted as an initial factor in its pathogenesis the presence of a specific humoral response directed against membrane glycolipids. The first autoantibodies associated with this syndrome were found in 1988 (53).

It has been proposed that these autoantibodies with, complement deposition on Schwann cells, axons or myelin being the first visible element in this syndrome is

demonstrated. Once bound autoantibodies to the presynaptic membrane ganglioside muscle weakness occurs by: complement fixation, pore formation complement membrane attack complex (MAC) and influx of calcium into the nerve terminal. The MAC leads to conduction block engine, axonal cytoskeleton degradation and damage of mitochondria molecular mimicry theory between the antigens of the infectious agent and peripheral nerve in the development of this autoimmune disease nerve (51).

2.7. Molecular mimicry between gangliosides and microbial glycan

Among the pathogenic characteristics of GBS are the presence of inflammatory infiltrates composed mainly of macrophages and CD4 + T cells. However, the main feature already mentioned above is the presence of anti-ganglioside antibodies. The presence of an infectious agent or vaccine that precedes the development of this neuropathy recently suggests a causal relationship between the infectious antecedent and the autoimmune pathogenesis. The infectious agent most closely related and best characterized with the development of GBS is *Campylobacter jejuni*, although other infectious agents such as *Mycoplasma pneumonie*, *Haemophilus influenzae*, Epstein Barr virus and Cytomegalovirus are known (12). Also, the relationship of some vaccines as precipitants of this syndrome has been studied.

Due to the direct relationship that has been found between the previous infection by one of these agents and the development of this syndrome, it has been proposed the theory of molecular mimicry between antigens of the infectious agent and the peripheral nerve in the development of this autoimmune disease (54,55). The theory of molecular mimicry observed when a susceptible host acquires an infection with an agent that has antigens that are immunologically similar to theirs but that differ enough to induce an immune response when they are presented to T cells.

As a result, the tolerance to the autoantigens is broken and the specific immune response that is generated against the pathogen cross-reacts against host structures to cause damage to the tissue and finally the disease (54). This is the case of infection with *Campylobacter jejuni*. After infection with this agent, antibodies against lipooligosaccharides (LOS) appear in the bacterial wall, suggesting that Campylobacter contains polysaccharides that resemble sialic acids found in the gangliosides of The human nerve tissues (56) Specifically, Campylobacter LOS are structurally identical to some ganglioside residues GM1 and GD1a present in nerve and neuromuscular junction. *Mycoplasma pneumoniae* infection may be another candidate for the pathogenesis of chronic polyneuropathy in GBS, serum of patients with this polyneuropathy after an infection with this bacterium reacts to gangliosides GM1 and GD1b, suggesting that galactosyl may be the target antigen (57).

On the other hand, cytomegalovirus (CMV) is the most common viral agent that precedes GBS among the possible explanations for the association between infection with this virus and GBS, including the stimulation of immune responses to glycoconjugates or viral peptides similar to Those found in myelin or Schwann cells. Antibodies to GM2 ganglioside have been described in many patients with GBS following infection with CMV (58).

Although anti-ganglioside reactivity may be directed against a single ganglioside, in some cases this reactivity is polyspecific due to the presence of identical epitopes in different gangliosides such as gangliosides GD1b, GQ1b, GT1b, GD2 and GM3 that share a disialosyl epitope found. In patients with sensitive-ataxic variants of GBS (58).

3. OBJECTIVES

3.1. General objective

To describe the nerve conduction patterns in a people infected with Zika virus and subsecuently, developed neurological syndromes. Moreover, correlate the clinical subtypes of GBS with anti-ganglioside antibodies, in a sample of population in Cucuta Norte de Santander, Colombia.

3.2. Specific objectives

- To identify social and demographic characteristics of the population based on the study.
- To determine the most common clinical subtype of the population in Cucuta with Guillain-Barré syndrome and assess the relationship, with the triggering infectious agent.
- To measure the prevalence of the variants included in the clinical spectrum of SGB.
- To evaluate the major nerve involvement in the GBS subtypes. Also, to identify if there is a common pattern observed in nerve damage in this patients, e.g. sural sparing.
- To describe the patterns for SNAP and CMAP mainly affected in each nerve, in order to correlate the distal or proximal compromise (conduction velocity, amplitude abnormal).

4. METHODOLOGY

4.1. Focus of the research

Quantitative Research was used to quantify and identify the ZIKV-GBS status, codifying variables into numerical and dummies variables. In the other hand, qualitative focus was applied in nerve conduction studies analysis and laboratory testing for arboviruses (DENV, ZIKV, CHIKV), Eipstein-Barr virus, cytomegalovirus, *Campilobacter jejuni*, *Mycoplasma pneumoniae* and antiganglioside antibodies, in order to gain an understanding of underlying reasons, opinions, and motivations.

4.2. Type and study design

This is a cross sectional study, derivated from our previous study (6). I selected all population infected with ZIKV who developed neurological compromise including GBS, with electrodiagnostic studies (electromyography and nerve conduction studies) and blood sample, in order to detect antibodies against gangliosides related to GBS and clinical subtypes of it. The period of time was between January to July 2016. No follow up were performed.

We already have the information available and collected by myself and the CREA work team. No further ethical considerations need to be made, electrophysiological studies are part of diagnostic procedures and does not need additional informed consent. Patients who had studies conducted during their hospitalizations due to GBS did not need a new electrophysiological study. Failing which, and expertise neurophysiology Dr. Ernesto Ojeda (EO) and a research assistant Diana González (DG) performed 13 studies. Blood withdraw was performed by trained personnel, prior signature of informed consent from the subject of study. This research is considering as minor risk for the patient.

4.3. Population

Our work team accessed most of the patient included in this analysis. All of those are included in our base study called RAIZ. We cite them to perform a complete neurological evaluation, extraction of blood for measurement of auto-antibodies and performance of electromyography and nerve conductions in those who did not have this study within their medical records. Some patients did not attend the appointment, so we do not have more data than those stipulated in the medical records, lacking important data such as the age and date of onset of neurological symptoms. For those patients highlighted in purple (16), we only had access to their electrodiagnostic study, without having a medical history that could complement the information.

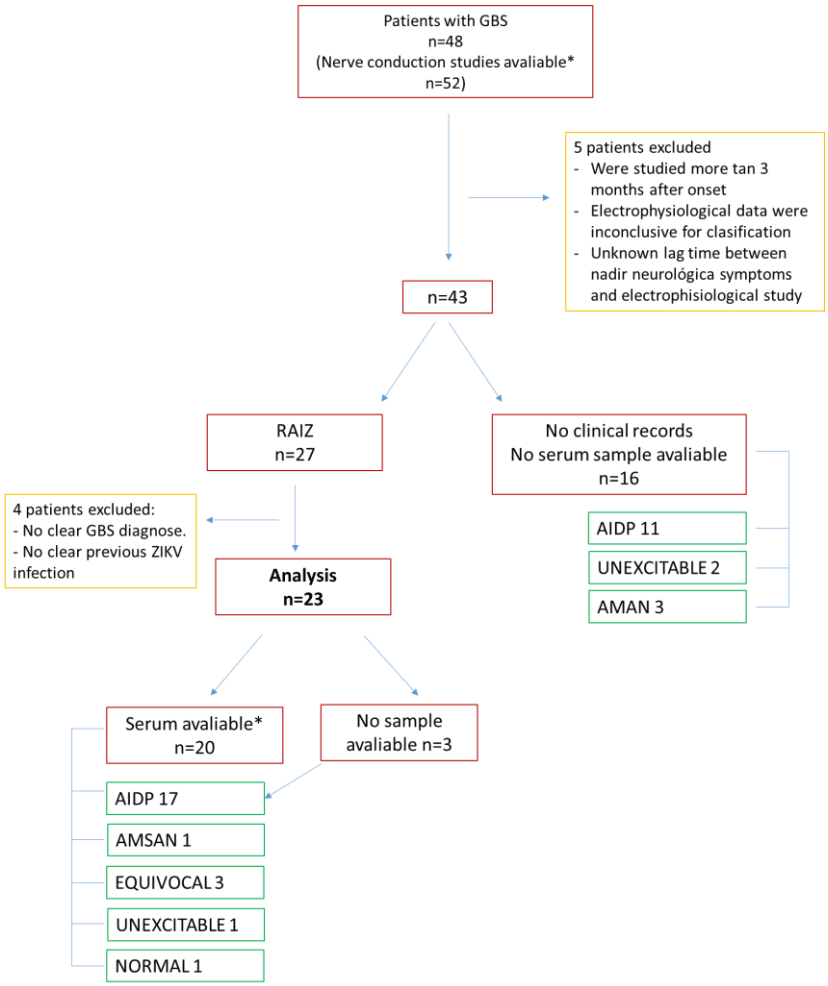
4.4. Sampling

Our study RAIZ (5) inicially identified 66 patients with neurological compromise, during the period of time previously described. We excluded from the study 3 patients who were not born in Cúcuta with 63 patients who fulfilled the eligibility criteria. All individuals (cases and controls) were contacted by telephone and will be referred to clinical evaluation days, which will be done jointly with the Health Secretariat of Cúcuta in the IPS Comuneros. Those who attend will be invited to participate through informed consent. In the following flow diagram I explain how the patients from our first research were obtained. The 39 patients included in RAIZ project have electrophysiological tests. We have blood sample of all patients. Additional 9 records were obtained but we do not have blood of them, due to the impossibility of contact them for the withdraw and the inclusion in the study. We performed electromyography and nerve conduction studies in those patients who did not have this study within their medical records. Some patients did not attend the appointment, so we do not have more data than those stipulated

in the medical records, lacking important data such as the age and date of onset of neurological symptoms. We have 16 patients that we only have access to their electrodiagnostic study, without having a medical history that could complement the information. This is a limitation of the study.

Within this group, we have a great majority with nerve electrophysiological studies. The current research (cross sectional study) is based on 48 electrophysiological studies, 33 of them had the GBS as a diagnosis and 18 had other neurological commitment. Not all of them were able to withdraw blood, so we have 39 blood samples in order to identify antibodies against antigangliosides.

Figure 11. Flow chart of patient's selection, according inclusion/exclusion criteria.



4.5. Inclusion and exclusion criteria

Inclusion and exclusion criteria applied to the population are listed below.

Table 4. Elegibility criteria for patients under study.

Inclusion	Exclusion
Patient with GBS (diagnose according Brighton criteria)	Patients without GBS or not fulfillment Brighton criteria
Probable or confirmed disease by ZIKV, before the onset of neurological symptoms	Patients without clinical records or blood sample available
People reported to the SIVIGILA for ZIKV (confirmed or probable) and GBS related	Normal parameters in electrophysiological records
Neurological complete examination made by our neurologist team	
At least 1 electrophysiological study is needed	
The electrophysiological record must be clearly identifiable with one of the patterns in the new criteria set proposed by Uncini.	
Blood sample available	
Clinical record available in order to confirm diagnose and follow the natural history of the disease in each patient	
Alive	
Patients from Cúcuta, Norte de Santander, Colombia	

4.6. Variable description

Dummy variables were used as follows:

1: Has the condition/abnormal value found.

0: Nerve was assessed but the response was absent.

-1: when the measure of the nerves were not performed and 0 when the nerve was assessed but the response was absent.

Normalization of values

Nerve Conduction Studies (NCS) are performed to evaluate the physiological function and to diagnose disorders of peripheral nerves. Despite the importance, there is no universal standard for NCS (48,59). However, many published studies for normal and references values do not meet contemporary statistical and methodological standards (59). The American Association of Neuromuscular & Electrodiagnostic Medicine (AANEM) formed the Normative Data Task Force (NDTF) to establish a set of evidence based criteria to screen the peer-reviewed published literature, regarding 11 routinely studied nerves (59). Full articles were obtained and reviewed in detail to determine whether they were focused on deriving normative data and if they appeared to meet NDTF criteria. Articles that appeared to meet most of the NDTF criteria were circulated to all members for review.

We based our knowledge to perform the nerve conduction studies in this parameters for the evaluation of normal and abnormal values, in order to have a control group. As follows, we bring together the standardized techniques for major motor and sensory nerve conduction studies in adults, the Reference values for 6 major sensory nerve, measured antidromically and for 4 major motor nerve conduction studies in adults.

Table 5. Standardized techniques for major motor and sensory nerve conduction studies in adults.

	Techniques for electrode placement (recommend) *Ground electrode always placed between the stimulating (G1) and recording electrodes (G2)				**Machine**	**Settings**
Nerves	**Stimulating Electrode (G1)**	**Recording Electrode (G2)**	**Stimulating Site (SS)**	**Distance (G1 to SS) (cm)**	**Display sensitivity (uV/div)sensory, (mV/div) motor**	**Sweep (ms/div)**
Superficial radial Sensory	Extensor pollicis longus tendon	Base of thumb	Along the radius	10	5–10	1
Median sensory	Index finger Slightly distal to the second MCP	4 cm distal to G1	Wrist: between the flexor carpi radialis and the palmaris longus tendons Palm: midway between the 14-cm stimulation point and G1	14 7	20	1
Ulnar sensory	Fifth digit	4 cm distal to G1	Slightly to the radial side of the flexor carpi	14	20	1

	Slightly distal to the fifth MCP		ulnaris tendon			
Medial antebrachial cutaneous sensory	Medial forearm	Distal: 3 cm bar	Midway between the medial epicondyle and the distal biceps tendon	10	10	1
Lateral antebrachial cutaneous sensory	On a line to the radial pulse	Distal: 3 cm bar	Just lateral to the distal biceps tendon	10	10	1
Median motor	Abductor pollicis brevis motor point Midpoint of wrist crease and the first MCP	Distal to first MCP	Wrist: between the flexor carpi radialis and the palmaris longus tendons Elbow: medial to the brachial pulse	8	5	2
Sural sensory	Posteroinferior to the lateral malleolus	Distal: 3 cm bar	At or slightly lateral to the calf midline	14	2–5	1
Ulnar motor	Hypothenar eminence	Slightly distal to the	Wrist: slightly radial to the flexor carpi	8	5	2

	Halfway between the pisiform and the MCP	fifth MCP joint Elbow flexion to 90°	ulnaris tendon Below elbow: 4 cm distal to the medial epicondyle Above elbow: 10 cm proximal to the below-elbow site, measured in a curve behind the medial epicondyle to a point slightly volar to the triceps muscle Axillary: 10 cm proximal to above elbow site			
Peroneal (fibular) motor	Midpoint of extensor digitorum brevis	Just distal to fifth MTP	Ankle: lateral to the tibialis anterior tendon	8	5	5

			Below fibular head: posteroinferi or to the fibular head Above fibular head: 10 cm proximal to the below fibular head site and slightly medial to the tendon of the biceps femoris			
Tibial motor	Medial foot (slightly anterior/inferi or to the navicular tubercle)	Slightly distal to first MTP (medial aspect of joint)	Ankle: posterior to the medial malleolus Knee: midpopliteal fossa	8	5	5
F-wave Median motor	Abductor Pollicis Brevis (medial between MCP joint of thumb and	Distal phalanx of the thumb	Wrist: 2cm proximal to the distal crease between the Flexor Carpi Radialis	14	200 or 500 uV/div	5 msec/div (upper limbs)

	midpointof distal wrist crease)		(FCR) and Palmar Longus (PL) tendons			10 msec/div (lower limbs)
H-reflex Tibial motor Median motor	Medial foot (slightly anterior/inferior to the navicular tubercle) Abductor pollicis brevis motor point	Slightly distal to first MTP (medial aspect of joint) Distal to first MCP	Knee: midpopliteal fossa Cubital fossa			

Adapted from: Chen S., Andary M.,Buschbacher R., Del Toro D., Smith B., So Y., Zimmermann Z., Dillingham T. AANEM Practice Topic: Electrodiagnostic Reference Values For Upper And Lower Limb Nerve Conduction Studies In Adult Populations. Muscle Nerve 54: 371–377, 2016. DOI 10.1002/mus.25203. Lewis J. Natus Neurology- Neurology Training Academy. Clinical Training: Nerve Conduction Studies. February 28- March 3, 2017. Middleton, WI, USA.

Normal values

Table 6. Reference values for 6 major sensory nerve conduction studies in adults.

		Amplitude: lower limit (3rd percentile) (uV)		Latency: upper limit (97th percentile) (ms)	
Nerve	**Size (N)**	**Onset-to-peak**	**Peak-to-peak**	**Onset**	**Peak**
Superficial radial sensory (antidromic, 10 cm)	212	7	11	2.2	2.8
Median sensory* (antidromic to second digit, wrist 14 cm, palm 7 cm)		11 (wrist), 6 (palm) Amplitude (wrist) by age and BMI+: Age 19–49; BMI <24 ------ 17 Age 19–49; BMI ≥24 ------ 11 Age 50–79; BMI <24 ------ 9 Age 50–79; BMI ≥24 ------ 7	13 (wrist), 8 (palm) Amplitude (wrist) by age and BMI+: Age 19–49; BMI <24 ------ 19 Age 19–49; BMI ≥24 ------ 11 Age 50–79; BMI <24 ------ 15 Age 50–79; BMI ≥24 ------ 8	3.3 (wrist), 1.6 (palm)	4 (wrist), 2.3 (palm)
Ulnar sensory (antidromic to fifth digit, 14 cm)	258	10 Amplitude (wrist) by age and BMI+:	9 Amplitude (wrist) by age and BMI+:	3.1	4

		Age 19–49; BMI <24 ------ 14 Age 19–49; BMI ≥24 ------ 11 Age 50–79; BMI <24 ------ 10 Age 50–79; BMI ≥24 ------ 5	Age 19–49; BMI <24 ------ 13 Age 19–49; BMI ≥24 ------ 8 Age 50–79; BMI <24 ------ 13 Age 50–79; BMI ≥24 ------ 4		
Medial antebrachial cutaneous sensory (antidromic, 10 cm)	207	4	3		2.6
Lateral antebrachial cutaneous sensory (antidromic, 10 cm)	213	5	6		2.5
Sural sensory (antidromic, 14 cm)	230	4	4	3.6	4.5

BMIs calculated as follows: BMI 5 (W/H^2), where W is the patient's weight (in kilograms) and H is the patient's height (in meters).

*Median sensory NCS at II digit.

⁺The lower limits of onset-to-peak and peak-to-peak amplitudes are shown as mean – 2SD, showing the statistically significant effects of age and BMI on the amplitudes of the median and ulnar sensory nerves at the wrist ($P < 0.01$). Data sets normalized by square-root transformation.

Adapted from: Chen S., Andary M.,Buschbacher R., Del Toro D., Smith B., So Y., Zimmermann Z., Dillingham T. AANEM Practice Topic: Electrodiagnostic Reference Values For Upper And Lower Limb Nerve Conduction Studies In Adult Populations. Muscle Nerve 54: 371–377, 2016. DOI 10.1002/mus.25203. Lewis J. Natus Neurology- Neurology Training Academy. Clinical Training: Nerve Conduction Studies. February 28- March 3, 2017. Middleton, WI, USA.

Table 7. Reference values for 4 major motor nerve conduction studies in adults.

		Distal Amplitude (mV)		**Conduction Velocity (m/s)**		**Distal Motor Latency (ms)**	
Nerves	**Size (n)**	**Subgroups (years)**	**Low limit 3rd %**	**Subgroups**	**Low limit 3rd %**	**Subgroups**	**Upper limit 97th %**
Median motor	249	All ages DA by age: 10-39 40-59 60-79	4.1* 5.9 4.2 3.8	All ages CV age-sex: 10-39y women 10-39y men 40-79y women 40-79y men	49* 49 53 47 51	All ages DL years-sex: 10-39y women 10-39y men 40-79y women 40-79y men	4.5* 4.6 4.4 4.7 4.4
Ulnar motor	248	All ages	7.9*	CV Below elbow CV Across elbow CV Above elbow CV drop across the elbow CV drop across the elbow (%)	52* 43* 50* 15* 23%*	All ages	3.7*
Fibular (peroneal) motor	242	All ages	1.3*	CV ankle to below fibular head CV age-height:	38* 43	All ages	6.5*

				10-39y <170cm 10-39y >170cm 40-79y <170cm 40-79y >170cm	37 39 36		
		DA by age: 10-39y 40-79y % drop in amplitude from ankle to below fibula % drop in amplitude across fibular head	 2.6 1.1 32%* 25%*	CV across fibular head CV drop across the fibular head % drop in CV across fibular head	42* 6* 12%*		
Tibial motor	250	All ages DA by age: 19-29 30-59 60-79 Amplitude drop from ankle to knee % drop in amplitude from ankle to knee	4.4* 5.8 5.3 1.1 10.3%* 71%*	All ages CV age-height: 19-49y <160cm 19-49y 160-170cm 19-49y ≥170cm 50-79y <160cm 50-79y 160-170cm 50-79y ≥170cm	39* 44 42 37 40 37 34	All ages	6.1*

*Values for the entire sample for each nerve encompassing all ages.

Adapted from: Chen S., Andary M.,Buschbacher R., Del Toro D., Smith B., So Y., Zimmermann Z., Dillingham T. AANEM Practice Topic: Electrodiagnostic Reference Values For Upper And Lower Limb Nerve Conduction Studies In Adult Populations. Muscle Nerve 54: 371–377, 2016. DOI 10.1002/mus.25203. Lewis J. Natus Neurology- Neurology Training Academy. Clinical Training: Nerve Conduction Studies. February 28- March 3, 2017. Middleton, WI, USA.

Table 8. Distal CMAP duration in normal subjects.

	Low frequency filter: 2Hz	**Low frequency filter: 5Hz**	**High frequency filter: 10Hz**	**High frequency filter: 20Hz**
Nerve*	**Duration (ms) (SD)**	**Duration (ms) (SD)**	**Duration (ms) (SD)**	**Duration (ms) (SD)**
Median	6.1 (0.9) +2SD 7.9	5.8 (0.9)	5.6 (0.8)	5.1 (0.7)
Ulnar	6.5 (1.0) +2SD 8.5	6.1 (1.0)	6.0 (0.9)	5.4 (0.7)
Peroneal	6.1 (0.9) +2SD 7.9	6.0 (0.9)	5.8 (0.8)	5.5 (0.8)
Tibial	5.6 (0.9) +2SD 7.4	5.5 (0.9)	5.5 (0.9)	5.3 (0.9)

Normal values found in a population n=147, for Distal CMAP in normal subjects.

Adapted from: Mitsuma S, Van den Bergh P, Rajabally YA, et al. Effects of low frequency filtering on distal compound muscle action potential duration for diagnosis of CIDP: A Japanese-European multicenter prospective study. Clin Neurophysiol. (9):1805-10. 2015. doi: 10.1016/j.clinph.2014.11.027. PMID: 25591830.

Diagram of variables

Figure 12. Diagram of variables included in the study.

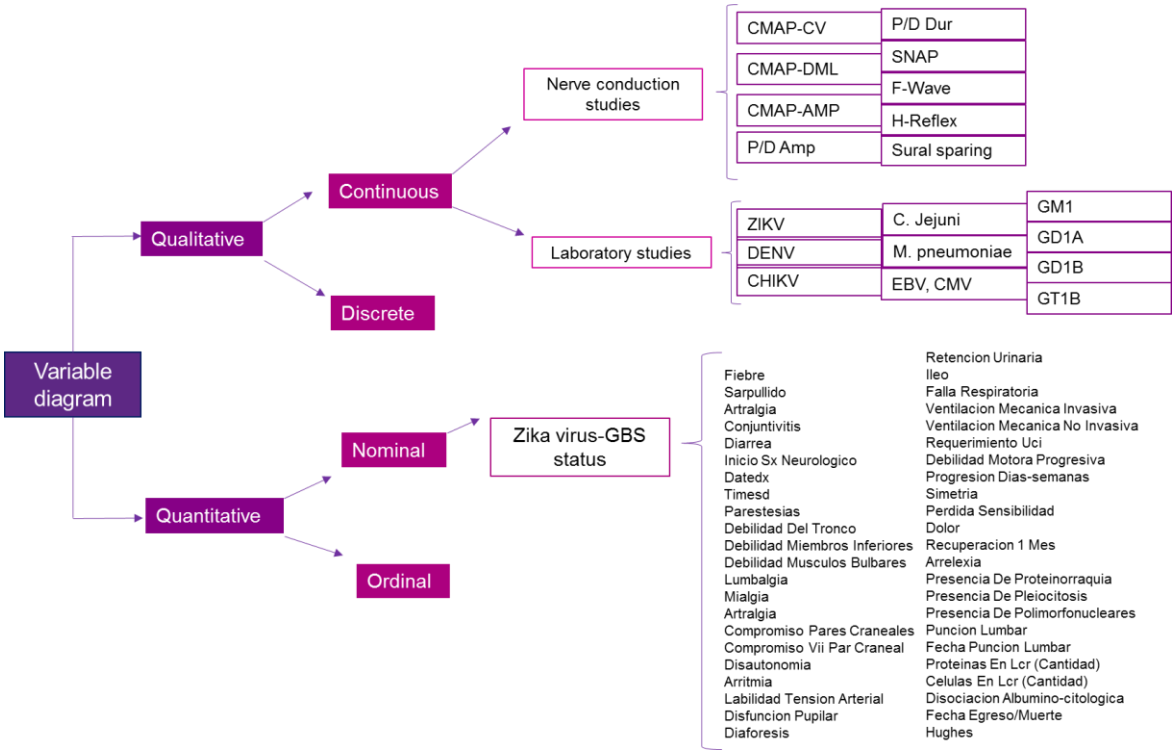


Table of variables

Further information is available in annexed section. See Supplementary material 1.

4.7. Information gathering techniques

Source of information

Our base study called RAIZ (RAIZ, article submitted) have focused on the epidemiology and immunobiology of Zika virus (ZIKV) infection and factors associated with the development of Guillain-Barre syndrome (GBS) and other

neurological syndromes in Cúcuta, the capital of North Santander department, Colombia. Data of patients with ZIKV disease reported to the national population-based surveillance system were used to calculate the basic reproduction number (R0) and the attack rates (ARs) as well as to develop epidemiological maps. Patients with neurological syndromes were contacted and their diagnoses were confirmed. A case-control study in which 29 patients with GBS associated with ZIKV compared with 74-matched control patients with ZIKV infection alone was undertaken.

In this first assessment, we identify high rates of cranial nerves involvement and dysautonomia were present in 82% and 75.9%, respectively. Intensive care unit (ICU) admission was necessary in 69% of the GBS patients. Most of the patients disclosed a high disability condition (Hughes grade 4). Also, we dysautonomia was the main risk factor of poor GBS prognosis (i.e., ICU admission and disability).

Given this observation, we decided to design a second study in order to identify the severity of ZIKV infection in relation to GBS syndrome presentation.

Information gathering instrument

Quantitative data collection methods include various forms of surveys, in this specific case we employed self-reported questionaries, forms to be filled with physician assistance and all clinical information adquired was supported by clinical records (see annexed material). Qualitative data collection methods vary using unstructured or semi-structured techniques, such as ELISA, IFI, PRNT and nerve conduction studies (for further information, see processing techniques).

Process of obtaining information

- Neurologist and research assistant evaluated patients in Cúcuta in four occasions.
- Review of each clinical record available

- CREA registration form- Developed to include patients in RAIZ project (RAIZ, article submitted)
- Informed consent, assent were obtained, as needed.

4.8. Bias and bias control

- Inclusion bias
- Sample selection
- Operator-dependent: two different experts assessing the electrophysiological studies.

4.9. Processing techniques and data analysis

Laboratory studies

Sera from the 20 convalescent patients were tested within a median of 96.5 days (IQR: 69-132) after the onset of the GBS, with a positive IgG detected by ELISA and neutralizing antibodies with $PRNT_{90}$. These patients have been already reported (5). Three additional patients were included, but serum was not available. IgG and IgM antibodies against ZIKV were assayed using a standardized enzyme-linked immunosorbent assays (ELISA). Detection of IgG against ZIKV was also performed using an indirect immunofluorescence (IFI) assay on serum samples (Euroimmun, Germany). In addition, negative and positive controls provided by the manufacturer were analyzed in parallel. For ELISA, serum samples were diluted 1:101, following manufacturer instructions and IgG and IgM was considered positive ≥ 1.1 relative units (RU/ml). Regarding IFI, all samples were processed for IgG at 1/10 dilution, with microscope parameters: 600ms, high, bar to 900. Results were determined according to the positive and negative controls and according to the IFI patterns for the virus in

agreement with the manufacturer's instructions. CSF, saliva, urine or tissue samples were not collected.

Serum samples were screened for ZIKV neutralizing antibody utilizing a PRNT on Vero cells (ATCC #CCL-81). End point titrations of reactive sera, utilizing a 90% cutoff ($PRNT_{90}$), were performed against ZIKV strain H/PF/2013 as described (for details, see supplementary material) (60). Plaques generated by test sera at varying dilutions and the control preparation were counted. The percentage of plaques counted in test sera were compared with the number of plaques from the control preparation. Log dilutions of test sera preparations were as follows: 1:128, 1: 256, 1: 512, 1: 1024, 1:2048, 1: 4096. The amount of formed foci were counted using an ELISPOT plate reader (ImmunoSPOT-Cellular Technology). Data of corresponding transformed dilutions (Log(1/Dilution)) against neutralization percentages per sample was plotted and a best-fit line drawn to interpolate $PRNT_{90}$ values.

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Table 9. Methodology of Plaque reduction neutralization test (PRNT).

Plaque reduction neutralization test (PRNT)
Cells: African Green Monkey kidney cells (Vero) were obtained from ATCC (ATCC; Manassas, VA, USA) and grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1.5 g/l sodium bicarbonate, 100 U/ml of penicillin, 100 µg/ml of streptomycin, and incubated at 37°C in 5% CO2.
Virus: ZIKV strain H/PF/2013 (GenBank:KJ776791), provided by Dr. Jorge Osorio (University of Wisconsin). Virus stocks were performed by inoculation onto a confluent monolayer of Vero cells as described. (Dudley DM, et al. Nature Communications. 2016)
Plaque Reduction Neutralization Test: Serum samples were serially diluted mixed with 150 PFU of the ZIKV H/PF/2013 strain and incubated for 1 hour at 37°C. This serum/virus mixture was added to confluent layers of Vero cells in 96 well plates and incubated for 1 hour at 37°C, after which the serum/virus mixture was removed and overlay solution (carboxyl-methyl cellulose) was added. After 48 hours of infection, the monolayers were fixed, washed, and then incubated with Rabbit anti-Zika antibody (GeneTex) overnight at 4°C. Plates were washed and peroxidase-labeled detection antibody was incubated for 2 hours at 37°C. Following incubation, cells were washed and developed using enzyme substrate. The amount of formed foci were counted using an ELISPOT plate reader (ImmunoSPOT-Cellular Technology). Neutralization percentages (Nx) were calculated per sample/replicate/dilution as follows: Nx={100-[100(A/Control)] where A corresponds to the amount of foci counted in the sample and Control

is the geometric mean of foci counted from wells treated with cells and virus only.

Since Cúcuta is one of the most affected regions of Colombia for arboviruses (61), IgG and IgM against Dengue virus and Chikungunya virus were also quantified using an ELISA from Vircell (Granada, Spain) and Abcam (Cambridge, United Kingdom), respectively. Additionally, IgG against CHIKV and each of the 4 serotypes of DENV were assayed on serum samples using an indirect immunofluorescence assay (Euroimmun, Luebeck, Germany). Moreover, as GBS can also be triggered by some common infections, antibodies against *Mycoplasma pneumoniae, C. jejuni*, Epstein-Barr virus (EBV) and Cytomegalovirus (CMV) were also tested (5).

Electrophysiological studies

Nerve conduction studies were performed by a Cadwell Sierra Ascent (Cadwell, USA) with a median interval from GBS onset of 68.4 days (IQR: 28-129) according to standardized techniques. In median, ulnar, peroneal and tibial motor nerves distal motor latency (DML), amplitude and duration of negative peak of compound muscle action potential (CMAP) from different stimulation sites, motor conduction velocity (MCV) and minimal F-wave latency were measured. Proximal/distal (p/d) CMAP amplitude and duration ratios were assessed in each nerve segment. Sensory studies were performed antidromically in median, ulnar and sural nerves and amplitude of sensory nerve action potential (SNAP) was measured baseline to negative peak. Electrophysiological findings were normalized as percentages of upper (ULN) and lower limits of normal (LLN) according to the reference values proposed by the Normative DataTask Force of the Association of Neuromuscular & Electrodiagnostic medicine (59). The cut-off values for the distal CMAP duration were determined according to normal values for 2Hz low frequency filter + 2 SD

(23). In three patients nerve conduction studies were repeated with an interval of 111-114 days from the first study.

Electrodiagnostic criteria

We employed for the electrodiagnosis of GBS subtypes a recently described electrodiagnostic criteria set that at the first study and in a cohort with a balanced number of AIDP and axonal GBS showed the highest diagnostic accuracy compared with two other criteria sets (table 2) (19,23,62). In this criteria set the increased duration of dCMAP and p/d CMAP duration ratio were introduced as parameters of demyelination, and a p/d CMAP amplitude ratio <0.7 was considered only for axonal GBS subtypes. SNAP amplitudes and sural sparing, defined as abnormal ulnar and normal sural SNAP amplitude, and were also taken into account. To investigate whether in AIDP nerves there was a prevalent involvement of distal or intermediate nerve segments we employed the criteria reported in table 3. The control group was made by 34 Italian AIDP patients diagnosed, according to the criteria reported in table 2, at the University Hospital of Chieti and with a median interval from disease onset and electrophysiological test of 28 days (IQR: 13,5-34,2). These 34 patients had no IgG antibodies to gangliosides GM1, GM1b, GD1a, GalNAcGD1a, GD1b, GT1a and GQ1b.

Data analysis

Univariate analysis was applied to determine distribution of clinical and electrodiagnostic findings. Descriptive univariate analysis was performed in IBM SPSS Statistics 24. The Chi square and Kruskal-Wallis tests and spearman correlation coefficient accordingly. Fisher's exact tests were performed to established differences between categorical variables and outcomes of interest (i.e. diagnosis according criteria, previous neurological symptoms). Kruskal–

Wallis test was performed for assessing possible differences in continuous variables on outcomes of interest. Cohen's kappa coefficient was applied in Hadden criteria and the new criteria set proposed by Uncini. Bivariate statistical analysis was performed in R 3.3.2. Generalized additive models were used to estimates PRNT90. Statistical analysis was performed in the R statistical software version 3.3.2 (R Core Team, 2016) Chi-square test was employed to determine statistical significant differences between Colombian and Italian AIDP patients. A p-value < of 0.05 was considered statistically significant. Figures were performed on version 7 of GraphPad Prism software.

Software employed in data analysis:

- SPSS Statistics Desktop 22.0 (IBM SPSS Statistics for Windows, NY: IBM Corp, 2016)
- R statistical software version 3.3.2. (R Core Team, 2016)
- GraphPad Prism software (GraphPad Software, Inc., 2016)

5. ETHICS

This study will be conducted within the ethical standards that have their beginning in the latest official version of the Declaration of Helsinki.

1. Value: The value of an investigation is measured by the scientific, social and clinical importance that it has; Our research complies with the above insofar as the Guillain Barré association has not previously been studied in Colombia as a consequence of previous infection with the Zika virus with anti-ganglioside antibodies. Finding a strong association between these antibodies and the severity and type of Guillain Barré could eventually initiate the development of some medicine that works as an immunoglobulin against these antiganglioside antibodies with a social, clinical and scientific impact very beneficial and even generalizable to all populations Not just Colombian.

When measuring the value of responsible use of resources, we realize that our research uses money and time in the most optimal way possible, since we start from a database already known (SIVIGILA), selecting the patients With Zika and Guillain Barré to whom the antibodies are taken in blood, without unnecessarily exploiting the resources since the previous analysis gives us the approach towards the patients that we need.

2. Scientific validity: This second point goes hand in hand with the previous point of value because it is useless to have a research question with scientific impact if there is no adequate design and methodology of the study. In this case, we intend to study the association of anti-ganglioside antibodies with the severity and type of Guillain Barré that occurs after infection with the Zika virus. This is what we intend to carry out by knowing through the SIVIGILA database the patients confirmed with the virus presented by Guillain Barré in Cúcuta, to whom

the samples would be taken to establish the presence of anti-ganglioside antibodies, knowing Beforehand the result of nerve conduction studies.

3. Equitable selection of the subject: The selection of the subjects is equitable because the patients will be selected for reasons related to the research questions, that is, they will not be chosen based on the risks or benefits that the research can bring. The city of Cúcuta was chosen because it is the most infected place with the Zika virus in Colombia, where all the patients who have had Zika and Guillain Barré who accept to participate will be studied.

4. Favorable risk-benefit ratio: Based on the fundamental ethical principles of non-maleficence and beneficence, all research should seek to maximize benefits for both the subject and society, while minimizing the risks to the subject of study. In the case of our research, the patient's risk of participating is minimal compared to the benefit that would bring society to find the relationship between anti-ganglioside antibodies and the severity and type of Guillain Barré developed by patients who were infected with Zika virus for the reason already discussed.

5. Independent evaluation: The investigation was reviewed by appropriate experts, not affiliated with the study, with the authority to suggest changes, approve or cancel the investigation. On the other hand, independent evaluation of compliance with ethical requirements, of a study or research, guarantees to the society that the people registered for the tests will be treated ethically and not only as mere means.

6. Informed Consent: All individuals participated in the proposed clinical investigation, were given information about the current health situation, complications and outcome of the Zika virus and Guillaín Barré's Syndrome, in order to preserve respect for people and respect for Autonomous decisions.

7. Respect for enrolled subjects: Respect is to allow the subject to change their opinion, to decide that the research does not match their interests or preferences, and to withdraw without penalty. Second, since substantial information will be collected on. De is very emphatic in saying that during the course of clinical research, new data can be obtained, information about the risks and benefits of the interventions used.

This study will be conducted in accordance with the requirements for the development of research activity in health posed in resolution 8430 of the Ministry of Health in 1993, prevailing in this research the criterion of respect for the dignity and protection of the rights and welfare of subjects included in the study.

Informed consent will be given, as part as the participation in RAIZ project, in which all the information necessary for the patient to take the decision to participate or not in the studio is located.Informed consent will include a special section for the individual to decide what to do with your sample (blood, saliva and/or minor salivary gland tissue) after this study is complete.

This research is classified as "Research with minimal risk", according to the provisions of resolution 8430 of the Ministry of Health in 1993, including registration data through consistent common procedures: physical or psychological routine examinations, obtaining saliva or salivary tissue and blood collection by venipuncture with minimum volume (less than 450 ml in two months). A report of adverse events classified as serious or not serious, related to the taking of the biological sample is performed.

6. RESULTS

6.1. Demographic characteristics

Demographic characteristics, clinical features and electrodiagnosis of GBS subtypes are summarized in table 10 and 11. The median age of the patients with GBS was 42.1 years and 56.1% were females.

Table 10. Demographic and clinical characteristics of 23 patients with Guillain-Barré Syndrome and previous ZIKV infection.

Characteristics	***n=23*** *no.(%), median [IQR]*
Female sex	13 (56.5)
Age (years)	42 [27.0-50.7]
ZIKV infection symptoms	
Fever	15 (65.2)
Rash	18 (78.2)
Arthralgia	16 (69.5)
Conjunctivitis	12 (52.1)
Zika Infection Diagnostic Category	
Suspected	3 (10.0)
Confirmed	20 (86.9)

Time from onset of ZIKV infection symptoms and onset of GBS (days)	**6 [6.0-14.0]**
GBS diagnostic certainty according to Brighton criteria	
Level 1	6 (26.1)
Level 2	12 (52.1)
Level 3	3 (13.0)
Level 4	2 (8.6)

In order to determine the most common clinical subtype of the population in Cucuta with Guillain-Barré syndrome and assess the relationship, with the triggering infectious agent, we describe clinical information regarding ZIKV infection and the subsequent development and presentation of GBS. We identify the frequency of clinical variants of SGB, in case to be different from the typical presentation described for *C. jejuni.*

Table 11. Clinical and electrodiagnostic findings of 23 patients with Guillain-Barré Syndrome and previous ZIKV infection.

Neurological features, n= 23 ***no. (%)***	
Symmetrical weakness	19 (82.6)
Lower limbs weakness	23 (100)

Upper limbs weakness	22 (95.6)
Symmetrical arreflexia	19 (82.6)
Arreflexia lower limbs	21 (91.3)
Arreflexia upper limbs	19 (82.6)
Paresthesias	20 (86.9)
Sensory deficit	16 (69.5)
Pain	15 (65.2)
Cranial neuropathies	
Any (III, VII, IX, X)	18 (78.2)
Oculomotor nerve (III)	1 (4.3)
Facial nerve (VII)	15 (65.2)
Bulbar nerves	11 (47.8)
Dysautonomia	
Any	20 (86.9)
Unstable blood pressure	15/20 (75.0)
Arrhythmia	7/20 (35.0)
Pupillary dysfunction	1/20 (5.0)
Diaphoresis	4/20 (20.0)
Bladder dysfunction	9/20 (45.0)
Ileus	7/20 (35.0)

Severity	
ICU admission	18 (78.2)
Respiratory failure	14 (60.8)
Mechanical invasive ventilation	10 (43.4)
Non-invasive mechanical ventilation	4 (17.3)
Progression of neurological symptoms from days to weeks	21 (91.3)
Hughes disability scale at hospital leave	
1	2 (8.6)
2	2 (8.6)
3	3 (13.04)
4	13 (56.5)
5	1 (4.3)
6	0 (0)
Data not available	2 (8.6)
GBS subtypes	
Acute inflammatory demyelinating polyradiculoneuropathy (AIDP)	17 (73.9)
Acute motor axonal neuropathy (AMAN)	0 (0)

Acute motor and sensory axonal neuropathy (AMSAN)	1 (4.3)
Equivocal	3 (13.0)
Unexcitable	1 (4.3)
Normal	1 (4.3)
Treatment	
None	5 (21.7)
Intravenous immunoglobulins	16 (69.5)
Plasmapheresis	0 (0)
Intravenous immunoglobulin and plasmapheresis	1 (4.3)
Data not available	1 (4.3)

6.2. Zika virus and other arbovirus infection

All patients had a history ZIKV infection preceding the onset of GBS. The median time interval between the onset of the ZIKV infection and the onset of GBS was 6 days [IQR 6.0-14.0]. The most frequent symptoms were rash (78.2%) (table 8). No CSF, saliva, urine or tissue, were not collected at the moment of infection. Three patients belongs to probable category given the impossibility to get blood samples from them.

Positive IgM against ZIKV by ELISA was found in one patient (4.3%). Serum samples, even after 3 months from onset ZIKV infection, showing that it could be

reaching the end of the acute phase supporting by the finding of low IgM titters closer to negative threshold. IgG titters were elevated, demonstrating indeed, the ZIKV previous infection. All patients had IgG antibodies positive for ZIKV, using ELISA and IFI assay. Concerning other arbovirus, all patients were positive for anti-DENV IgG, while only 17.3% were positive for IgM antibodies. For CHIKV, 65.2% of the patients were anti-CHIKV IgG antibodies positive and none had IgM antibodies.

6.3. Neurological features

Clinical presentation was characterized by rapidly progressive bilateral mainly symmetrical weakness (table 9). A high percentage of patients (73.9%) had cranial nerve involvement, 73.9 % had swallowing difficulties and 65.2% had facial palsy. Autonomic dysfunction was present during the disease course in 86.9% of patients, 78.2% of patients were admitted to intensive care unit and 43.4% required invasive mechanical ventilation. Hospital stayed time had a median of 27 days, IQR 12-42. Disability at the hospital leave was measured through Hughes disability scale, in which more than half of the patients were confined to a chair or bed during the acute phase of the disease. Further details in supplementary table 1.

6.4. Treatment

Out of 12 patients (52.1%) received treatment within 7 days from the onset of neurological symptoms, and 5 patients (21.7%) received treatment after more than 7 days. Treatment consisted on intravenous immunoglobulins in patients 69.5% of patients. One patient (4.3%) received a combination of immunotherapy and plasmapheresis. Five patients (21.7%) did not received specific therapy for GBS because of a benign course of the disease.

6.5. Laboratory studies

CSF analysis was performed in 8 patients (30.8%). All patients (100%) had albuminocytologic dissociation, indicated by increased protein levels (>52 mg per deciliter) in the absence of pleocytosis (<50 cells per cubic millimeter).

Considering CMV and EBV, none of the patients had IgM antibodies against these viruses. Whereas all patients were positive for CMV and EBV IgG antibodies, indicating a previous infection, at any time life. None of the patients had detectable levels of IgM antibodies against M. pneumoniae, but 73.9% of the patients showed IgG antibodies. Perception of pneumonia did not correlate with a previous M. pneumoniae infection. For C. jejuni only IgG antibodies were measured, and were found to be present in 21.7%.

6.6. Electrodiagnosis

Electrophysiological studies were performed in all patients, with a median interval from GBS onset of 68.4 days [IQR 28-129]. AIDP was diagnosed in 17 (73.9%) patients (table 9). AMSAN was diagnosed in only one patient by a study performed 67 days after the onset. One patient had 7 days after the onset a normal study, another had inexcitable nerves and three patients had an equivocal pattern. In the AIDP patients 103 nerves were classified according to the prevalent electrophysiologic pattern of demyelination in nerve segments (table 10). The most frequent pattern was distal (45.6%) followed by the diffuse (14.5%) and intermediate (4.8%). About 35% of nerves were normal, unexcitable or did not reach the cut-offs required for demyelination and were unclassifiable. However, these frequencies did not differ from the results obtained in 142 nerves of 34 Italian AIDP. In three AIDP

patients electrophysiology was repeated with an interval of 111-114 days and the distal demyelinating involvement was, albeit improved still evident, in a least two motor nerves. Further details in supplementary table 1.

Table 12. Pattern of prevalent segmental nerve involvement in AIDP patients associated to ZIKV disease and in an Italian AIDP cohort.

	ZIKV associated AIDP n= 17, (%)	**Italian AIDP* n=34, (%)**
Nerves	103	142
Distal	47 (45.6)	68 (47.8)
Intermediate	5 (4.8)	5 (3.5)
Diffuse	15 (14.5)	19 (13.4)
Unclassifiable	36 (34.9)	50 (35.2)

* Significant differences were not found (Chi-square: 0.386, p-value: 0.943)

7. DISCUSSION

The incidence of GBS in Cúcuta increased 4.41 times during the ZIKV outbreak (5). In the patients we report herein the median interval between ZIKV infection and the median onset of neurological symptoms was only 6 days suggesting a parainfectious pattern similarly to a subgroup of 20 patients recently reported from Colombia that showed neurological symptoms during or immediately after the viral syndrome associated to ZIKV infection (17). This interval seems to be too rapid to represent an autoimmune reaction to a first exposure to a virus and is different from the classical postinfectious profile described in classical GBS usually developing up to 4 weeks after an infection (62,63). The reason of this parainfectious profile is uncertain but a hyperacute immune response, possibly favored by previous infections (e.g., flavivirus, M. pneumoniae) or a direct viral neuropathogenic mechanism could be hypothesized (5).

The frequency of cranial nerve (65.2%) and the need for ventilator support (43.4%) is higher than in a large European GBS cohort (36% and 28%, respectively) (64). Facial palsy (often bilateral) is a characteristic feature being present in 65% of patients of our cohort and described in up to 79% of French-Polynesian patient. Autonomic dysfunction, especially the life threating unstable blood pressure and arrhythmia, was also very frequent in the population we report. All together, these features indicate that GBS associated with ZIKV infection has an aggressive and severe course that should be carefully monitored.

In our first analysis (5) dysautonomia was identified as the main risk factor of poor GBS prognosis (i.e., intensive care unit admission and disability). Dysautonomia was observed in a much higher percentage (75.9%) than in previous reports in which such condition has been reported in a range between 22% and 47.1%. Although there are not conclusive data about the burden of GBS in Colombia, it is estimated to be elevated with a high rate of patients requiring hospitalization and one-third of

them needing admission to the intensive care unit because of respiratory failure, dysautonomia or medical complications (65).

The electrophysiological results were consistent with the AIDP subtype in 73.9 % of patients similarly to the percentage (78%) reported in a larger Colombian cohort and in contrast with the AMAN diagnosis in all patients from French-Polynesia (15–17). This differences may reflect a changed pathological and electrophysiological subtype of ZIKV-associated GBS due to mutations of the virus spreading from South Pacific to America or different host-dependent factors in the two geographic areas. Anyway, a simpler explanation can be found by examining the electrophysiological data. In the two reports from the French Polynesia, 37 patients were studied at the first week of disease onset and at 4 months (15,16). Only the mean of the mean electrophysiological values were reported and no classification according to electrodiagnostic criteria was attempted. In the first week of disease mean DMLs were greatly increased: median nerve was 335%, ulnar 202% and peroneal 203% of ULN. The mean distal CMAP duration was also increased (peroneal nerve 128%, median 155% of ULN), with individual values up to 320% of ULN. No conduction block or substantial mean conduction slowing in the intermediate nerve segments was reported (although the lowest reported conduction velocities ranged from 24.7 m/s in the peroneal nerve to 29.9 m/s in the median). Studies repeated at 4 months in 19 patients showed improvement of CMAP amplitudes and DMLs; although the mean DMLs remained substantially prolonged.

The authors concluded that the electrophysiology was consistent with AMAN with reversible conduction failure prevalently of distal nerve segments as indicated by a reduction of the mean terminal latency index (15,16). The diagnosis of AMAN was reinforced, according to the authors, by the not significantly decreased mean SNAP amplitudes of radial and sural nerve although the lower limits of the range reported were decreased and 83% of patients had paresthesia. In another report from Cúcuta, 10 out of 14 (71%) patients were classified as AMAN, although the authors described

prolonged DMLs (without reporting the actual values) and a sural-sparing pattern typically found in AIDP (66). (Arias et al. 2017)

In our opinion the greatly increased values of DML and distal CMAP duration reported in the French Polynesian cohort are indeed more in line with a de-remyelinating process such as AIDP. AMAN with RCF is characterized by recovery within a few weeks of slightly prolonged DMLs, conduction slowing, reduced distal CMAP amplitudes and conduction block in intermediate nerve segments without the development of excessive temporal dispersion of CMAPs (18,19,26). Moreover, the "normal" radial and sural SNAP amplitudes in most of patients can be explained by the fact that these nerves, tested in an intermediate segment, are usually less affected in AIDP than the distal segments of median and ulnar sensory nerves. Overall, although the presentation of the French Polynesian does not allow an individual classification in subtypes we deem that most of patients were actually AIDP. Regarding the preferential distal involvement, we found in our cohort that about half of nerves had evidence of prevalent distal demyelination compared with about only 5% showing a prevalent intermediate involvement. However a similar preferential distal pattern of nerve involvement was found in an Italian AIDP cohort not associated to ZIKV infection and reemphasize the well-known notion that in AIDP, the distal nerve terminals, where the blood–nerve barrier is deficient, are preferentially affected. To corroborate our opinion that ZIKV infection is mainly associated to AIDP is the only one histopathologic evaluation of peripheral nerve reported up to now that showed demyelination and mononuclear cell inflammation with some axonal degeneration consistent with the classical AIDP picture (67,68).

Although the retrospective analysis could be considered as a shortcoming, the characteristic electrophysiological features were detectable even at prolonged time intervals.

8. CONCLUSIONS

In conclusion GBS associated to ZIKV infection has mainly a parainfectious onset, an aggressive and severe course and is mostly demyelinating in nature as GBS cases associated to other flavivirus infections (19). To gain a greater understanding of the pathogenesis through electrophysiology, extensive data acquisition and serial studies are recommended. This should be followed by the electrodiagnostic characterization of the individual patient rather than cohort analysis, which is likely to introduce significant inaccuracies in the conclusions.

Primary health strategies should be enforced, beyond just educating people, and also provide essential health care, through means accessible to all individuals and families in the community. Further studies and continued efforts from the government aimed at eradicating the vector are warranted. We suggest that ZIKV infection is an ideal system in which a systemsbiology type of analysis would be appropriate to identify host factors, concurrent health conditions that influence susceptibility and outcome, and finally, of course, effector pathways.

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10. SUPPLEMENTARY MATERIAL

Supplementary material 1. Operational definitions of variables.

	VARIABLE			NAME	DEFINITION	TYPE	SCALE OF MEASUREMENT
ID	ID number			ID	ID number	Numerical	-
Age	Age			Age	-	Ratio	Years
Sex	Sex			Sex	**1=** Women **2=** Men	Categorical	
Definition of Compromise	ZIKV and neurological commitment, including GBS			ZIKN-	**1=** ZIKV infection without neurological commitment	Nominal	
				ZIKN+	**2=** ZIKV infection with neurological commitment ***except*** GBS		
				ZIKV/GBS	**3=** ZIKV infection and GBS		
				NDA	**4=** No data available		
CMAP-Motor conduction velocity	Median	LEFT	WRIST	LMEDIAN-	> 50 m/s = Prolongated	Ratio	**m/s**
			ELBOW	LMEDIAN-			
			AXILA	LMEDIAN-			
		RIGHT	WRIST	RMEDIAN-			
			ELBOW	RMEDIAN-			
			AXILA	RMEDIAN-			
	Peroneal	LEFT	ANKLE	LPERON-	> 38m/s =		
			KNEE	LPERON-			
		RIGHT	ANKLE	RPERON-			
			KNEE	RPERON-			
	Tibial	LEFT	ANKLE	LTIBIAL -	> 35m/s =		
			KNEE	LTIBIAL -			
		RIGHT	ANKLE	RTIBIAL -			
			KNEE	RTIBIAL -			
	Ulnar	LEFT	WRIST	LULNAR-	>53 m/s		
			B ELBOW	LULNAR-			
			A ELBOW	LULNAR-			
		RIGHT	WRIST	RULNAR-			
			B ELBOW	RULNAR-			
			A ELBOW	RULNAR-			
CMAP-Onset	Median	LEFT	WRIST	LMEDIAN-	< 4.2 ms = Decreased		
			ELBOW	LMEDIAN-			
			AXILA	LMEDIAN-			
		RIGHT	WRIST	RMEDIAN-			
			ELBOW	RMEDIAN-			
			AXILA	RMEDIAN-			
			ANKLE	LPERON-			

	Peroneal	LEFT	KNEE	LPERON-	< 6.1 ms =	Ratio	**ms**
		RIGHT	ANKLE	RPERON-			
			KNEE	RPERON-			
	Tibial	LEFT	ANKLE	LTIBIAL -	< 6.1 ms = Decreased		
			KNEE	LTIBIAL -			
		RIGHT	ANKLE	RTIBIAL -			
			KNEE	RTIBIAL -			
	Ulnar	LEFT	WRIST	LULNAR-	< 4.2 ms = Decreased		
			B ELBOW	LULNAR-			
			A ELBOW	LULNAR-			
		RIGHT	WRIST	RULNAR-			
			B ELBOW	RULNAR-			
			A ELBOW	RULNAR-			
CMAP- Motor amplitude	Median	LEFT	WRIST	LMEDIAN-	> 5 mV = Increased	Ratio	**mV**
			ELBOW	LMEDIAN-			
			AXILA	LMEDIAN-			
		RIGHT	WRIST	RMEDIAN-			
			ELBOW	RMEDIAN-			
			AXILA	RMEDIAN-			
	Peroneal	LEFT	ANKLE	LPERON-	> 2.5 mV =		
			KNEE	LPERON-			
		RIGHT	ANKLE	RPERON-			
			KNEE	RPERON-			
	Tibial	LEFT	ANKLE	LTIBIAL -	> 3 mV = Increased		
			KNEE	LTIBIAL -			
		RIGHT	ANKLE	RTIBIAL -			
			KNEE	RTIBIAL -			
	Ulnar	LEFT	WRIST	LULNAR-	> 3 mV = Increased		
			B ELBOW	LULNAR-			
			A ELBOW	LULNAR-			
		RIGHT	WRIST	RULNAR-			
			B ELBOW	RULNAR-			
			A ELBOW	RULNAR-			
SNAP - Sensory conduction	Median	LEFT	WRIST	LMEDIAN-	> 39 m/s =	Ratio	**m/s**
		RIGHT	WRIST	RMEDIAN-			
	Sural	LEFT	CALF	LSURAL -	> 35 m/s =		
		RIGHT	CALF	RSURAL -			
	Ulnar	LEFT	WRIST	LULNAR-	> 38 m/s =		
		RIGHT	WRIST	RULNAR-			
SNAP – Peak_ Distal	Median	LEFT	WRIST	LMEDIAN-	< 3.6 ms =	Ratio	**ms**
		RIGHT	WRIST	RMEDIAN-			
	Sural	LEFT	CALF	LSURAL -	< 4.0 ms =		
		RIGHT	CALF	RSURAL -			
	Ulnar	LEFT	WRIST	LULNAR-	< 3.7 ms =		
		RIGHT	WRIST	RULNAR-			
SNAP – Sensory Amplitude	Median	LEFT	WRIST	LMEDIAN-	> 10 mV =	Ratio	**mV**
		RIGHT	WRIST	RMEDIAN-			
	Sural	LEFT	CALF	LSURAL -	> 5mV = Increased		
		RIGHT	CALF	RSURAL -			
	Ulnar	LEFT	WRIST	LULNAR-	> 15 mV =		
		RIGHT	WRIST	RULNAR-			
F wave- Max Mean	Median	LEFT		LMEDIAN-	< 33 ms = Decreased	Ratio	**ms**
		RIGHT		RMEDIAN-			
F wave- L-R Mean	Median	LEFT		LMEDIAN-FwL-	< 33 ms = Decreased		
		RIGHT		RMEDIAN-FwL-			
H reflex- Latency	Tibial	LEFT	Gastroc	LTIBIAL-HrLat	-	Ratio	**ms**
		RIGHT	Gastroc	RTIBIAL-HrLat			
	Tibial	LEFT	Gastroc	LTIBIAL-HrL-RMea	<2 ms = Decreased		

H reflex- L-R Latency Mean		RIGHT	Gastroc	RTIBIAL-HrL-RMea			
			Recruitme	RRECTFEMOR			
ZIKV	Detección virus-sangre (Semicuantitativovo)		IgG	ZIKVIGG	Negative <0,8 OD Uncertainly: ≥ 0,8 y <11	Ratio	Optical Density
			IgM	ZIKVIGM	Negative <0,8 OD Uncertainly: ≥ 0,8 y <11		
DENGV	Detección virus-sangre		IgG	DENGVIGG	Negative <0,8 OD Uncertainly: ≥ 0,8 y <11	Ratio	Optical Density
			IgM	DENGIGM	Negative <0,8 OD Uncertainly: ≥ 0,8 y <11		
CHIKV	Detección virus-sangre		IgG	CHIKVIGG	Negative <0,8 OD Uncertainly: ≥ 0,8 y <11	Ratio	Optical Density
			IgM	CHIKVIGM	Negative <0,8 OD Uncertainly: ≥ 0,8 y <11		
C. jejunii	Detección bacteria-sangre		IgG	CJEJIGG	Negative <0,8 OD Uncertainly: ≥ 0,8 y <11	Ratio	Optical Density
			IgM	CJEJIGM	Negative <0,8 OD Uncertainly: ≥ 0,8 y <11		
M. pneumoniae	Detección bacteria-sangre		IgG	MPNEIGG	Negative <0,8 OD Uncertainly: ≥ 0,8 y <11	Ratio	Optical Density
			IgM	MPNEIGM	Negative <0,8 OD Uncertainly: ≥ 0,8 y <11		
EBV			IgG	EBVIGG	Negative <0,8 OD Uncertainly: ≥ 0,8 y <11	Ratio	Optical Density

	Detección virus-sangre	IgM	EBVIGM	Negative <0,8 OD Uncertainly: ≥ 0,8 y <11		
CMV	Detección virus-sangre	IgG	CMVIGG	Negative <0,8 OD Uncertainly: ≥ 0,8 y <11	Ratio	Optical Density
		IgM	CMVIGM	Negative <0,8 OD Uncertainly: ≥ 0,8 y <11		
GM1			GM1	*	Ratio	Optical Density
GD1A			GD1A	*	Ratio	Optical Density
GD1B			GD1B	*	Ratio	Optical Density
GT1B			GT1B	*	Ratio	Optical Density

Supplementary material 2. Main clinical features and electrophysiological findings on patients with GBS- ZIKV associated.

No.	*Age, sex*	*Zika virus disease*	*Main clinical features*	*Brigthon criteria- GBS diagnose*	*Electrophysiological findings*	*Subtype*
NA1	78, Man	Suspected	• Bilateral and flaccid weakness of limbs (Lower) • Paresthesia • Laryngeal muscle weakness • Monophasic course and time between onset-nadir 12 h to 28 days • No CSF available • NCS pattern conclusive for GBS • Absence of alternative diagnosis for weakness	Level 4	F wave absent Extremely prolonged DMLs in 4 nerves	AIDP
Z061	27, Woman	Confirmed	• Bilateral and flaccid weakness of limbs (Lower and upper) • Paresthesia • Decreased or absent deep tendon reflexes in weak limbs (Lower and upper) • Monophasic course and time between onset-nadir 12 h to 28 days • No CSF available but NCS pattern conclusive for GBS	Level 2	Median P/D Ampl is <0.7 dCMAP are reduced in 5 nerves and dCMAP duration is prolonged in 4 nerves. Normal sensory conductions.	AIDP

			• Absence of alternative diagnosis for weakness			
ZN001A	44, Woman	Confirmed	• Bilateral and flaccid weakness of limbs (Lower and upper) • Paresthesia • Decreased or absent deep tendon reflexes in weak limbs (Lower and upper) • Monophasic course and time between onset-nadir 12 h to 28 days • No CSF available but NCS pattern conclusive for GBS • Absence of alternative diagnosis for weakness	Level 2	DML prolonged in 3 nerves Sural sparing: Normal ulnar and median SNAP and Abnormal sural SNAP	AIDP
NA5	27, Woman	Suspected	• Bilateral and flaccid weakness of limbs (Lower) • Monophasic course and time between onset-nadir 12 h to 28 days • No CSF available • NCS pattern conclusive for GBS • Absence of alternative diagnosis for weakness	Level 4	dCMAP prolonged duration in 6 nerves	AIDP
NA7	22, Man	Suspected	• Bilateral and flaccid weakness of limbs (Lower and upper) • Laryngeal muscle weakness • Decreased or absent deep tendon reflexes in weak limbs (Lower and upper)	Level 2	DML prolonged in 6 nerves Sural sparing: Abnormal Ulnar SNAP and Normal Sural SNAP	AIDP

			• Monophasic course and time between onset-nadir 12 h to 28 days • No CSF available but NCS pattern conclusive for GBS • Absence of alternative diagnosis for weakness			
ZN022A	70, Woman	Confirmed	• Bilateral and flaccid weakness of limbs (Lower and upper) • Decreased or absent deep tendon reflexes in weak limbs (Lower and upper) • Monophasic course and time between onset-nadir 12 h to 28 days • Paresthesia • Dysauthonomia • No CSF available and NCS pattern not conclusive for GBS • Absence of alternative diagnosis for weakness	Level 3	NCS abnormalities do not fit criteria for any other group	Equivocal
ZN043A	42, Woman	Confirmed	• Bilateral and flaccid weakness of limbs (Lower and upper) • Decreased or absent deep tendon reflexes in weak limbs (Lower and upper) • Monophasic course and time between onset-nadir 12 h to 28 days • Paresthesia • Dysauthonomia	Level 1	DML prolonged in 3 nerves F-wave prolonged Sural sparing: Abnormal Ulnar SNAP and Abnormal Sural SNAP	AIDP

			• Laryngeal muscle weakness • CSF cell count <50/µl • CSF protein concentration > normal value • NCS pattern conclusive for GBS • Absence of alternative diagnosis for weakness			
ZN011A	42, Woman	Confirmed	• Bilateral and flaccid weakness of limbs (Lower and upper) • Decreased or absent deep tendon reflexes in weak limbs (Lower and upper) • Paresthesia • Dysauthonomia • Monophasic course and time between onset-nadir 12 h to 28 days • No CSF available but NCS pattern conclusive for GBS • Absence of alternative diagnosis for weakness	Level 2	DML extremely prolonged in 6 nerves Duration prolonged in 4 nerves p/d Amplitude <0.7% in 2 nerves *2nd study, 128 days after onset neurological symptoms: DML prolonged in 4 nerves Sural sparing: absent measure of ulnar and sural	AIDP *AIDP
ZN046A	49, Woman	Confirmed	• Bilateral and flaccid weakness of limbs • Decreased or absent deep tendon reflexes in weak limbs • Monophasic course and time between onset-nadir 12 h to 28 days	Level 3	only one the left ulnar dCMAP amplitude reduced, NCS abnormalities do not fit criteria for any other group	Equivocal

			• No CSF available and NCS pattern not conclusive for GBS • Absence of alternative diagnosis for weakness			
ZN005A	50, Woman	Confirmed	• Bilateral and flaccid weakness of limbs • Decreased or absent deep tendon reflexes in weak limbs Monophasic course and time between onset-nadir 12 h to 28 days • No CSF available and NCS pattern not conclusive for GBS • Absence of alternative diagnosis for weakness	Level 3	Studies were performed more than 4 months after the onset of neurological symptoms. It only has abnormal peroneal p/d amplitude ratio and there is no evidence of demyelination	Equivocal
ZN052A	27, Man	Confirmed	• Bilateral and flaccid weakness of limbs • Decreased or absent deep tendon reflexes in weak limbs Monophasic course and time between onset-nadir 12 h to 28 days • No CSF available but NCS pattern conclusive for GBS • NCS pattern conclusive for GBS • Absence of alternative diagnosis for weakness	Level 2	DMLs and dCMAP duration prolonged *2nd study: Median nerve where no inexcitable CMAP recovers but still DML prolonged Unfortunately different nerves were tested in the two studies. Study performed 138 days after onset neurological symptoms	AIDP *RCF pattern of AIDP
ZN008A	48, Man	Confirmed	• Bilateral and flaccid weakness of limbs • Decreased or absent deep tendon reflexes in weak	Level 1	DML prolonged in the left median and CV reduced in the left ulnar	AIDP

			limbs Monophasic course and time between onset-nadir 12 h to 28 days • CSF cell count <50/µl • CSF protein concentration > normal value • NCS pattern conclusive for GBS • Absence of alternative diagnosis for weakness			
ZN017A	58, Woman	Confirmed	• Bilateral and flaccid weakness of limbs • Decreased or absent deep tendon reflexes in weak limbs Monophasic course and time between onset-nadir 12 h to 28 days • No CSF available but NCS pattern conclusive for GBS • Absence of alternative diagnosis for weakness	Level 2	DML extremely prolonged in 4 nerves bilaterally *2nd study: DML still prolonged in same nerves Study performed 160 days after onset neurological symptoms	AIDP *AIDP
ZN007A	42, Man	Confirmed	• Bilateral and flaccid weakness of limbs • Decreased or absent deep tendon reflexes in weak limbs Monophasic course and time between onset-nadir 12 h to 28 days • CSF cell count <50/µl • CSF protein concentration > normal value • NCS pattern conclusive for GBS	Level 1	DML prolonged 3 nerves Sural sparing: Abnormal Ulnar SNAP and Abnormal Sural SNAP	AIDP

			• Absence of alternative diagnosis for weakness			
ZN010A	27, Man	Confirmed	• Bilateral and flaccid weakness of limbs • Decreased or absent deep tendon reflexes in weak limbs Monophasic course and time between onset-nadir 12 h to 28 days • No CSF available but NCS pattern conclusive for GBS • Absence of alternative diagnosis for weakness	Level 2	Tibial nerves p/d CMAP duration ratio increased suggesting an increased temporal dispersion	AIDP
ZN014A	70, Man	Confirmed	• Bilateral and flaccid weakness of limbs • Decreased or absent deep tendon reflexes in weak limbs Monophasic course and time between onset-nadir 12 h to 28 days • No CSF available but NCS pattern conclusive for GBS • Absence of alternative diagnosis for weakness	Level 2	DML prolonged in 3 nerves, bilaterally p/d amplitude ratio less than 0.7 sural sparing: Absent response for ulnar and sural	AIDP
ZN055A	53, Woman	Confirmed	• Bilateral and flaccid weakness of limbs • Decreased or absent deep tendon reflexes in weak limbs Monophasic course and time between onset-nadir 12 h to 28 days • CSF cell count <50/µl • CSF protein concentration > normal value	Level 1	Duration prolonged and DML prolonged in 4 nerves Sural sparing: Absent response for ulnar and sural	AIDP

			• NCS pattern conclusive for GBS • Absence of alternative diagnosis for weakness			
ZN039A	17, Man	Confirmed	• Bilateral and flaccid weakness of limbs • Decreased or absent deep tendon reflexes in weak limbs Monophasic course and time between onset-nadir 12 h to 28 days • No CSF available but NCS pattern conclusive for GBS • Absence of alternative diagnosis for weakness	Level 2	DML prolonged on 2 nerves Sural sparing: Absent response for ulnar and sural	AIDP
ZN041A	45, Woman	Confirmed	• Bilateral and flaccid weakness of limbs • Decreased or absent deep tendon reflexes in weak limbs Monophasic course and time between onset-nadir 12 h to 28 days • No CSF available but NCS pattern conclusive for GBS • Absence of alternative diagnosis for weakness	Level 2	There are no electrophysiological evidence of demyelination 7 days after the onset	Normal
ZN049A	25, Man	Confirmed	• Bilateral and flaccid weakness of limbs • Decreased or absent deep tendon reflexes in weak limbs Monophasic course and time between onset-nadir 12 h to 28 days	Level 2	DML prolonged and amplitude reduce in one nerve	AIDP

			• No CSF available but NCS pattern conclusive for GBS • Absence of alternative diagnosis for weakness			
ZN018A	37, Woman	Confirmed	• Bilateral and flaccid weakness of limbs • Decreased or absent deep tendon reflexes in weak limbs Monophasic course and time between onset-nadir 12 h to 28 days • CSF cell count <50/µl • CSF protein concentration > normal value • NCS pattern conclusive for GBS • Absence of alternative diagnosis for weakness	Level 1	There is evidence of complete demyelination patterns out of two excitable nerves	AIDP
ZN060A	31, Woman	Confirmed	• Bilateral and flaccid weakness of limbs • Decreased or absent deep tendon reflexes in weak limbs Monophasic course and time between onset-nadir 12 h to 28 days • No CSF available but NCS pattern conclusive for GBS • Absence of alternative diagnosis for weakness	Level 2	The test was performed 121 days after onset and still all nerves were unexcitable. In this case there is no guarantee you can classify if the primary pathological process was axonal whether the pattern observed correspond to severe demyelination with secondary axonal degeneration.	Unexcitable

ZN061A	35, Man	Confirmed	• Bilateral and flaccid weakness of limbs • Decreased or absent deep tendon reflexes in weak limbs Monophasic course and time between onset-nadir 12 h to 28 days • CSF cell count <50/µl • CSF protein concentration > normal value • NCS pattern conclusive for GBS • Absence of alternative diagnosis for weakness	Level 1	SNAP amplitudes are <50% LLN in all sensory nerves measured Amplitude reduced in 2 nerves	AMSAN