UNIVERSITÀ DEGLI STUDI DI
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Ciclo XXXVI

The role of IgM oligoclonal bands in cerebrospinal fluid as a new biomarker of diagnosis, pathogenesis and prognosis in Guillain-Barrè syndrome

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Abstract

Introduction

Guillain-Barré Syndrome (GBS) is an acute, immune-mediated polyradiculoneuropathy characterized by high burden of disability and widely unpredictable course. Diagnostic and prognostic biomarkers are needed to identify specific subgroups of patients, reliably predict prognosis and help unravelling pathogenetic mechanisms. The presence of OligoClonal Bands (OCBs) in the CerebroSpinal Fluid (CSF) is a sensitive and specific index of IgM or IgG intrathecal production, which plays a well-known diagnostic and prognostic role in other immune-mediated neurological diseases such as multiple sclerosis. Previous studies report no significant role for IgG OCBs in CSF in GBS patients, while no data are available for IgM OCBs. This study aims to evaluate the presence and pathognetic significance of IgM OCBs in CSF and their potential role as diagnostic and prognostic biomarkers for GBS.

Patients and methods

All patients admitted at the Neurology Unit of the University Hospital of Modena for GBS between 2006 and 2020 were screened for enrollement. Inclusion criteria included high diagnostic probability (class 1 or 2 of Brighton criteria), completely available clinical history, neurophysiological study and laboratory data about CSF analysis and a regular 12-months neurological follow up after the onset of neuropathy. All the included patients underwent thorough Nerve Conduction Study (NCS) and lumbar puncture during the acute phase of the disease. Test for IgM OCBs presence was performed on stored CSF sample for each patient. Many independent demographic, clinical, neurophysiological and laboratory variabls as well as multiple outcome measures during the follow up were collected for each patients. Statistical methods were based on group comparison with non-parametric test and multivariate regression and survival analysis in order to identify significant associations between IgM OCBs, patients' characteristics and outcome measures.

Results

The study included 187 patients, 29 of which (15%) had IgM OCBs in their CSF. Clinically, their presence identified a specific subgroup of patient mainly characterized by more frequent involvement of bulbar nerves and dysfunction of the autonomic system, higher prevalence of pure motor phenotype, more severe clinical picture at hospital admission (i.e. lower scores at the Medical Research Council -MRC- scale for muscle strength and inflammatory Rasch-build Overall Disability Scale -iRODS-) and worse performance at validated prognostic scores at hospital admission (i.e. modified Erasmus GBS Outcome Score, Erasmus GBS Respiratory Insufficiency Score). NCS data revealed a significant association between IgM OCBs and prominent axonal involvement, with higher prevalence of Acute Motor Axonal Neuropathy and of F waves axonal abnormalities. Laboratory analysis of CSF showed a significant association between IgM OCBs presence and more severe Blood-Brain-Barrier (BBB) damage, with higher prevalence of albumin-cytological dissociation and higher concentration of CSF proteins. Of notice, no patients had IgG OCBs in their CSF and indexes of IgG intrathecal production were not significantly different between the two groups. Considering the outcome measures, multivariate regression and survival analysis confirmed a statistically significant association between IgM OCBs, more severe clinical course (MRC and disability at nadir) and worse outcome after 12 months in terms of higher residual muscle weakness (MRC), higher residual

disability (GBS Disability Scale, iRODS, loss of the ability of walking without support) and lower chance of complete recovery.

Discussion and conclusions

In our study, IgM OCBs were present in the CSF of a restricted but specific subgroup of GBS patients, characterized by a more severe form of the disease, with prominent axonal and motor involvement. Their association with nerve roots damage and higher BBB dysfunction suggests a role as index of intrathecal active inflammation causing widespread insult of proximal nerves. Not surprisingly, therefore, their presence is associated with worse long-term outcome and lower chance of recovery. Such results support the potential role of IgM OCBs as diagnostic, pathogenetic and prognostic biomarkers of GBS, deserving further investigations and validation in wider cohorts of patients.

Section 1: introduction

1.1 Guillain-Barrè syndrome

This section represents a comprehensive review about the state of the art of our knowledge about Guillain-Barrè Syndrome (GBS).

1.1.1 General aspects

Guillain-Barré syndrome (GBS) is a rare and potentially life-threatening autoimmune disorder that affects the peripheral nervous system. It was first described by the French neurologists Georges Guillain, Jean Alexandre Barré and André Strohl in 1916. Since then, our understanding of the syndrome has evolved significantly. Initially, it was considered a mysterious disorder with no clear cause. However, over the years, research has shed light on various aspects of GBS, including its epidemiology, pathophysiology, and clinical manifestations.

GBS is relatively rare, with an estimated incidence of 1-2 cases per 100.000 people annually. While it can affect individuals of all ages, there is a slightly higher prevalence in adults and males. Certain viral and bacterial infections have been associated with an increased risk of developing GBS, making it an intriguing area of study in the field of infectious diseases.

The exact cause of GBS remains a topic of ongoing research and debate. However, it is widely accepted that GBS is an autoimmune disorder in which the body's immune system mistakenly attacks the peripheral nerves. This immune response often follows an infection, with several microorganisms implicated as potential triggers. Among these, the bacterium Campylobacter Jejuni and two neurotropic viruses (i.e. Epstein-Barr Virus -EBV- and CitoMegalo Virus –CMV-) have been the most commonly identified culprits.

The hallmark of GBS is demyelination, a process in which the protective myelin sheath surrounding peripheral nerves is damaged or destroyed. This disrupts the normal conduction of nerve signals, leading to the characteristic symptoms of muscle weakness, tingling, and in severe cases, paralysis. The exact mechanisms by which the immune system targets peripheral nerves and triggers this demyelination process are complex and multifactorial.

GBS is a clinically diverse disorder, with a wide spectrum of symptoms and severity. The classic presentation often begins with tingling or numbness in the extremities, which may then progress to muscle weakness. This weakness typically starts in the legs and can ascend to affect the arms, eventually leading to quadriplegia in severe cases. GBS can also lead to life-threatening complications, such as respiratory failure, which requires mechanical ventilation. In addition to muscle weakness, GBS can cause a range of other symptoms, including pain, sensory disturbances, and autonomic dysfunction. Pain can be severe and is often described as aching or cramping. Sensory symptoms may include heightened sensitivity to touch, temperature, or vibration. Autonomic dysfunction can manifest as blood pressure fluctuations, heart rate abnormalities, and issues with bowel and bladder control.

Diagnosing GBS involves a combination of clinical evaluation, laboratory tests and neurophysiology. One of the key diagnostic criteria for GBS is the progressive nature of muscle weakness, usually reaching its peak within four weeks. Cerebrospinal fluid analysis can show elevated protein levels without an increase in white blood cells, a finding known as albumin-cytologic dissociation. Electrophysiological studies, such as Nerve Conduction Studies (NCS) and electromyography (EMG), are crucial in confirming the diagnosis and characterizing the extent of nerve damage.

The management of GBS is multifaceted and typically involves supportive care, immunomodulatory therapy and rehabilitation. Patients often require hospitalization and close monitoring. Intravenous immunoglobulin (IVIG) and plasma exchange (plasmapheresis) are the primary immunomodulatory treatments used to reduce the immune response and limit further nerve damage. The choice between IVIG and plasma exchange is often based on clinical factors, availability and patient preferences. Rehabilitation also plays a critical role in GBS recovery. The recovery process can be lengthy and may vary from one individual to another. Some

patients experience a near-complete recovery, while others may have residual weakness and long-term neurological deficits.
The prognosis of GBS is highly variable and depends on several factors, including the speed of disease progression, the severity of muscle weakness and the availability of prompt and appropriate treatment. While the majority of patients experience at least some degree of recovery, a significant number are left with long-term neurological impairment. Factors that have been associated with a worse prognosis include older age, severe muscle weakness, and the presence of certain complications, such as respiratory failure.
Since the incomplete knowledge about pathogenesis and the lack of prognostic markers of disease, the study of GBS continues to be a dynamic and evolving field after more than a century from its definition as a specific syndrome. Researchers are working to better understand the pathophysiology of the syndrome, identify novel treatment approaches and improve the ability to predict outcomes (**figures 1, 2**). Other ongoing exploration of the relationship between GBS and various infectious agents, as well as the genetic factors that may predispose certain individuals to develop the condition.

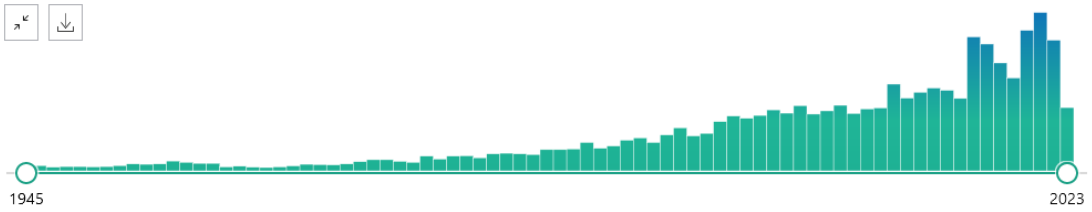


Figure 1: *timeline of publications on PubMed for "Guillain-Barrè pathogenesis" (years 1945-2023, 24 publications in year 2022).*

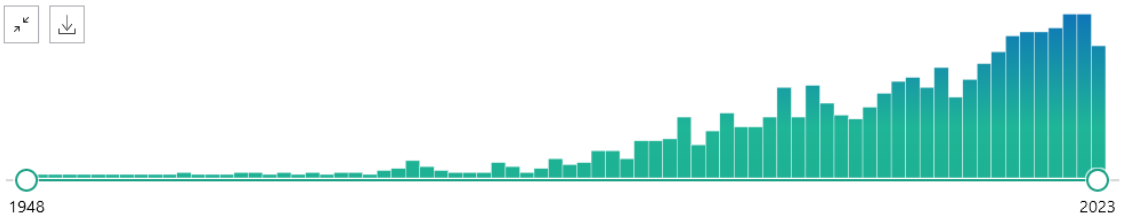


Figure 2: *timeline of publications on PubMed for "Guillain-Barrè prognostic factors" (years 1948-2023, 81 publications in year 2022).*

1.1.2 Epidemiology of Guillain-Barré Syndrome

GBS is a relatively rare, worldwide neurological disorder with an incidence that varies globally. While the exact numbers may differ across studies, it is estimated that the annual incidence of GBS ranges from 1 to 2 cases per 100.000 people worldwide (**figure 3**). Prevalence, on the other hand, is often lower, as GBS is an acute condition with a considerable number of patients experiencing significant recovery.[1]

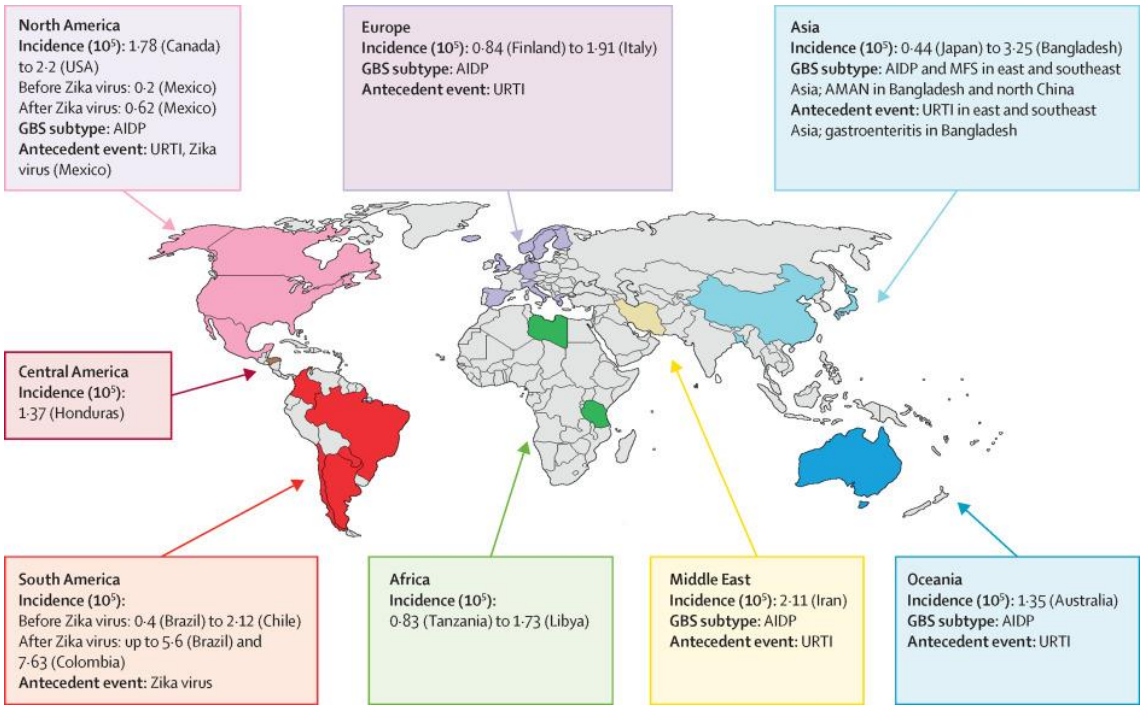


Figure 3: *worldwide incidence of GBS. Reproduced from: Shahrizaila N et al, Guillain-Barré syndrome, Lancet, 2021.*

GBS can affect individuals of all ages, but there are distinctive age patterns in its occurrence. In adults, GBS show an age-dependent distribution of incidence, peaking among older patients, typically between the ages of 50 and 74 years (**figure 4**).[2] GBS affects both genders, but there is a male preponderance in most cases. However, this gender difference may vary by age group and geographical region (**figure 4**).[2]

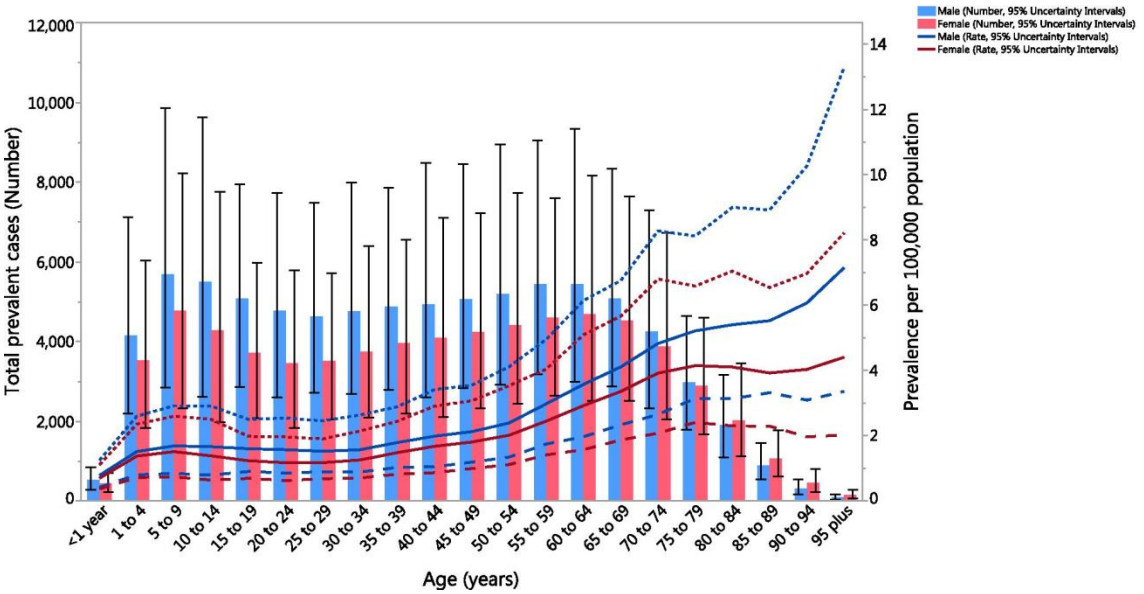


Figure 4: *global total number of cases (bars) and the prevalence of Guillain–Barre syndrome per 100,000 population (lines), by age and sex in 2019. Reproduced from: Bragazzi NL et al, Global, regional, and national burden of Guillain-Barré syndrome and its underlying causes from 1990 to 2019, J Neuroinflammation, 2021.*

The incidence of GBS displays regional variations, that may be attributed to factors such as genetics, climate, environmental exposures and healthcare infrastructure. The areas with higher incidence are some parts of Asia, particularly northern China and Japan, where GBS incidence is notably higher compared to other parts of the world (**figure 5**).[2] In particular, in Japan and Eastern Asia the purely motor, axonal variant (AMAN) of GBS shows a partularly high incidence than in the rest of the world, representing up to 30% of all GBS cases. This may be considered as an example of unknown geneticly-based predisposition or environmental exposure to some hidden facilitating agent (**figure 6**).[3]

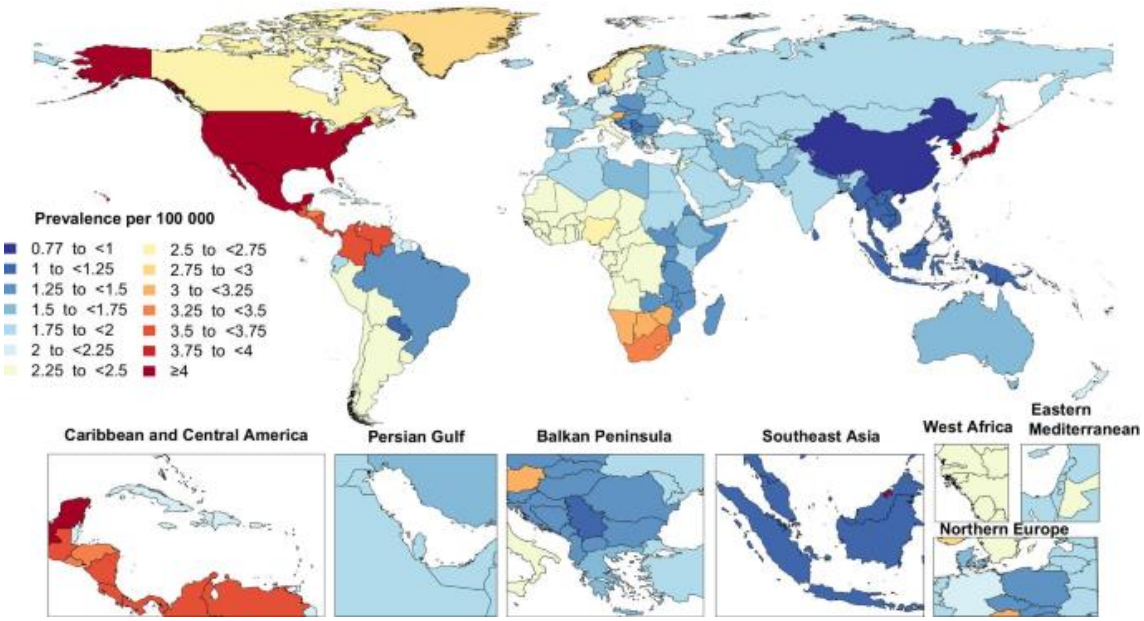


Figure 5: *annual incidence of GBS in different countries of the world for year 2019. Reproduced from: Bragazzi NL et al, Global, regional, and national burden of Guillain-Barré syndrome and its underlying causes from 1990 to 2019, J Neuroinflammation, 2021.*

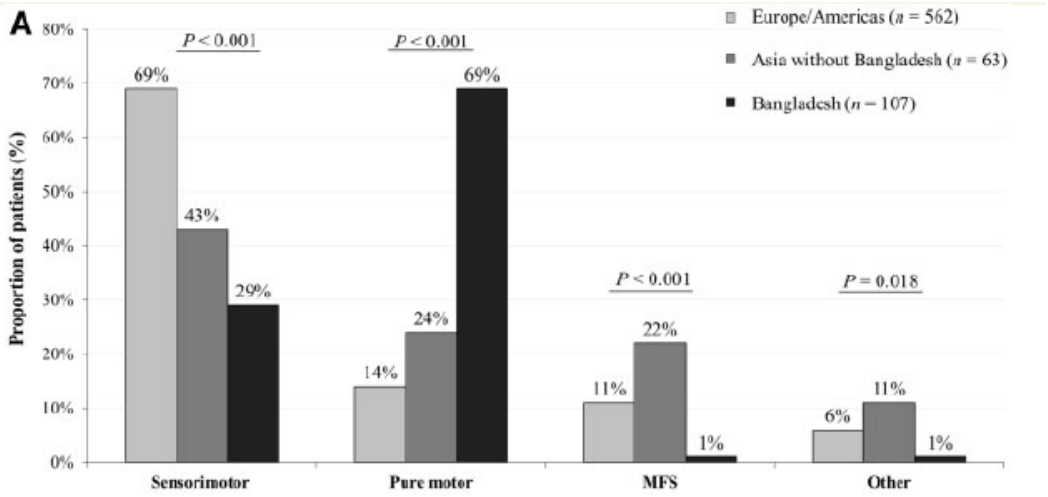


Figure 6: *Clinical variants in different geographical areas. Reproduced from: Doets A et al, Regional variation of Guillain-Barré syndrome, Brain, 2018.*

Conversely, some regions report lower GBS incidence rates. Even though intriguing, because understanding why GBS is less prevalent in these areas may provide insights into protective factors against the condition, it should be noted that GBS incidence is generally lower among low-income countries. In other words, a low rate of reports might be due to unefficient healthcare systems that prevent the access to hospital admission and treatments to GBS patients (**figure 7**).[4]

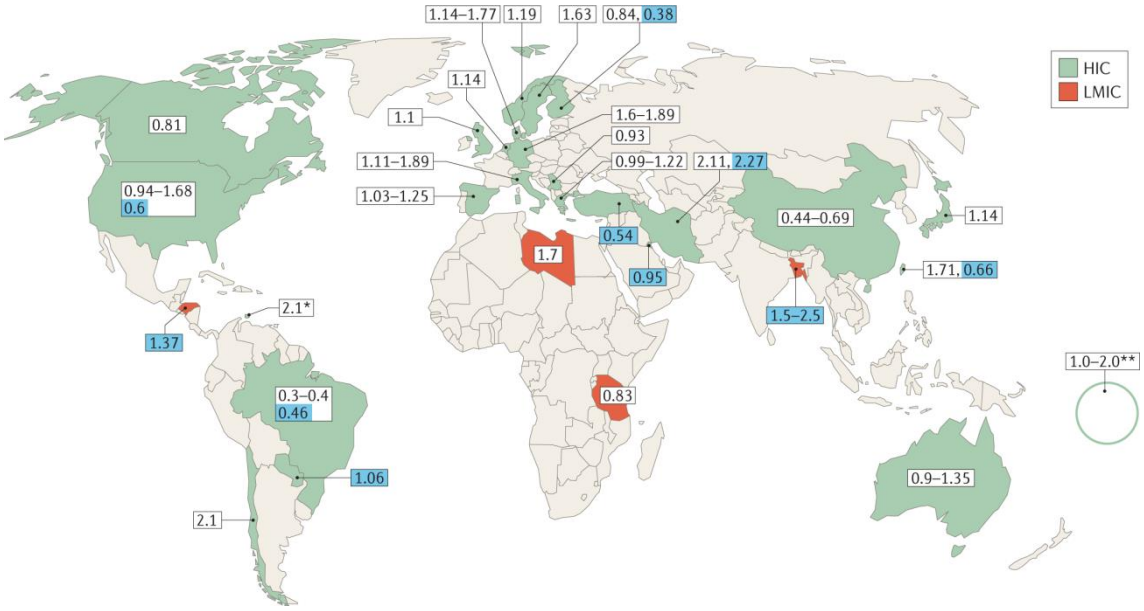


Figure 7: *annual incidence of GBS in different countries of the world, in relation to annual gross national income per capita. HIC: high income countries. LMIC: low-middle income countries. Reproduced from: Papri N et al, Guillain-Barré syndrome in low-income and middle-income countries: challenges and prospects, Nat Rev Neurol, 2021.*

Temporal trends in GBS incidence have been a subject of interest, particularly concerning outbreaks or clusters of cases. Some instances of increased GBS incidence have been linked to infectious disease outbreaks or vaccination campaigns. Such evenience happened, for example during the Zika virus outbreak in Pacific islands and South America in 2013-2016 that was epidemiologically linked to an anomalous time-locked increase of GBS incidence.[5,6] On the other hand, despite an increased incidence during pandemic, an analogue causative relation has never been demonstrated between GBS and Sars-CoV-2.[7,8] These trends provide insights into the complex relationship between GBS and infectious agents. Up to now, six infectious agents have been significantly associated with GBS on the basis of epidemiological patterns of incidence. They are mainly Campylobacter Jejuni and Epstein-Barr virus (EBV), followed by CitoMegalo virus (CMV), Mycoplasma Pneumoniae, Hepatitis E virus (HEV) and Zika virus.[1,9] However, the mechanisms underlying the such relationship between infections and GBS are still largely obscure. Vaccination has been a topic of concern in relation to GBS. While vaccines, particularly those for influenza, have been associated with a slightly increased risk of GBS, the absolute risk remains low. As well as before, even in presence of a slightly increased risk of developing GBS, no significant causal relation has ever been demonstrated between the different vaccinations against Sars-CoV-2 and GBS.[10,11]

1.1.3 Pathogenesis of Guillain-Barré Syndrome

Despite substantial progress in elucidating its pathogenesis, GBS remains a challenging disorder due to its various clinical forms and triggers. The syndrome's pathophysiology is generally attributed to autoimmune processes targeting peripheral nerves, which include many different cellular and molecular mediators was well as different possible targets, but the exact mechanisms and triggers remain the subject of ongoing investigation (**figure 8**).[9]

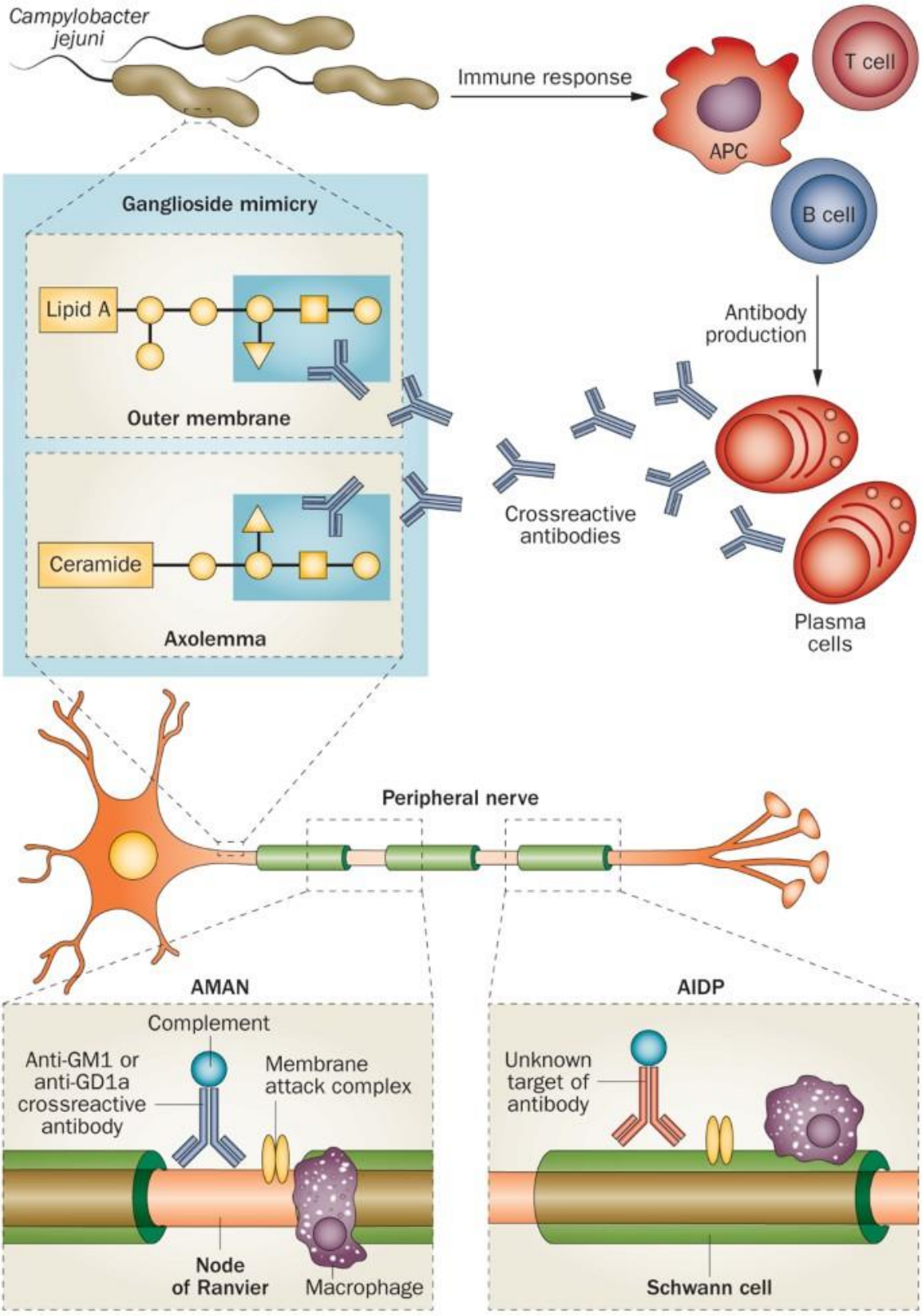


Figure 8: *overview of molecular mimicry and autoimmune response in GBS. Reproduced from: Van Der Berg B et al, Guillain-Barré syndrome: pathogenesis, diagnosis, treatment and prognosis, Nat Rev Neurol, 2014.*

One of the central themes in GBS pathogenesis is molecular mimicry. This concept proposes that the body's immune system, while attempting to combat infections, may mistake components of the peripheral nerves for pathogens, leading to an autoimmune attack. This autoimmune response is believed to be the primary driver behind the demyelination and axonal damage seen in GBS.[9,12] Molecular mimicry can occur when certain pathogens, especially viruses and bacteria, share structural similarities with components of peripheral nerves, such as gangliosides. Gangliosides are complex glycolipids found on the surface of nerve cells that play a critical role in nerve function, conditioning membrane structure and integrity, regulating molecular signaling and facilitating electrical conductance. The presence of molecular mimicry triggers an immune response, causing antibodies and immune cells to attack both the pathogen and the nerve cells, leading to inflammation and nerve damage.

Different possible trigger for molecular mimicry have been proposed. The bacterium Campylobacter Jejuni has been one of the most consistently identified infectious triggers for GBS. This microorganism is often associated with gastroenteritis, and it is believed that the immune response generated against C. jejuni cross-reacts with gangliosides found on peripheral nerves, setting off the autoimmune cascade that characterizes GBS. Research has shown that specific strains of C. jejuni carry lipopolysaccharides (LPS) that resemble gangliosides found in human nerves. This structural similarity can result in molecular mimicry, initiating the autoimmune response against peripheral nerves (**figure 9**).[9,12]

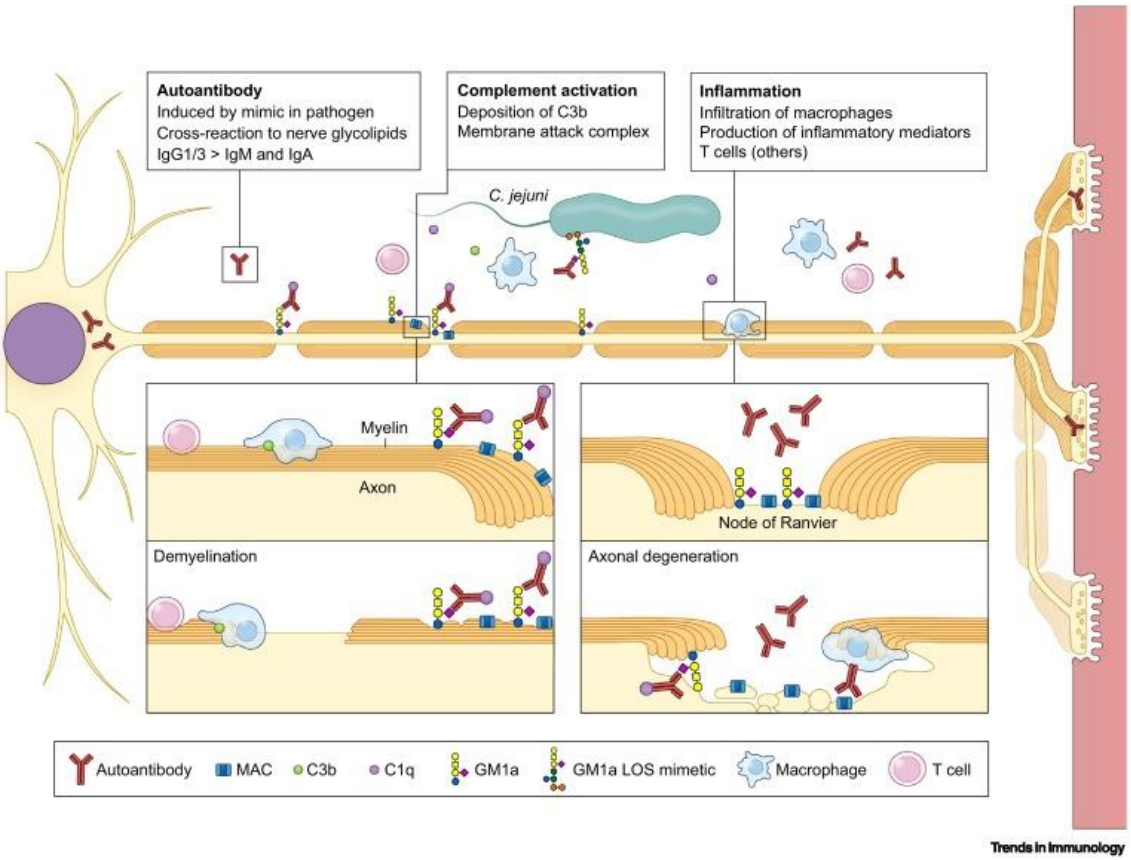


Figure 9: *overview of molecular mimicry and autoimmune response in GBS. Reproduced from: Laman JD et al, Guillain-Barré syndrome: expanding the concept of molecular mimicry, Trends Immunol, 2022.*

In addition to C. jejuni, other infections have been linked to GBS, although less consistently. As already said before, the most significant epidemiological links with GBS have been demonstrated for Epstein-Barr virus (EBV), cytomegalovirus (CMV) and Zika virus. These pathogens can trigger the immune system's response, leading to the development of GBS in susceptible individuals. The specific mechanisms of how these infections contribute to GBS

pathogenesis can vary, but molecular mimicry between pathogen components and nerve structures remains a common thread.[13-15]

However, not all patients infected by these and other organisms develop GBS and, on the contrary, for the majority of patients no infective trigger can be identified. Therefore, it is intuitive that other factors play a role determining patient predisposition to molecular mimicry. Certain genetic polymorphisms and variations may influence an individual's likelihood of developing GBS and the specific clinical manifestations they experience. In particular, human leukocyte antigen (HLA) genes, which are essential for the immune system's recognition of self and non-self antigens, have been implicated in GBS. Specific HLA alleles have been associated with an increased risk of GBS, i.e. DRB1*0401 and DRB1*1301, suggesting that variations in these genes may impact the immune response to infections and the likelihood of developing the syndrome.[16] Other genetically determined factors involve cytokine genes, whose polymorphism can influence the production and regulation of these molecules, affecting the immune system's behavior. Some studies have explored associations between certain cytokine gene variations and GBS susceptibility, highlighting the role of genetic factors in GBS pathogenesis.[17] Finally, genes involved in the recognition of pathogens and the regulation of immune response have been investigated in the context of GBS, with potential influence on the development and severity of GBS.[18]

Other than bacteria or viruses, environmental factors such as vaccinations have been scrutinized for their potential association with GBS, particularly the influenza vaccine. Altough the risk of GBS following vaccination is considered extremely low, some studies have suggested a slight increase in GBS incidence in the weeks following influenza vaccination.[19,20] The exact mechanisms through which vaccines may trigger GBS remain under investigation, but molecular mimicry seems to play a key role among predisposed patients. It is essential to emphasize that the overall benefits of vaccination in preventing infectious diseases significantly outweigh the minimal risks associated with GBS. Beyond vaccinations, various environmental factors have been explored in the context of GBS pathogenesis. These include exposure to certain toxins, such as pesticides, and recent surgical procedures. However, the evidence for these associations is often limited, and more research is needed to establish causal links between these factors and GBS.[1,9]

Whatever trigger may induce molecular mimicry, the consequent autoimmunity cascade in GBS involves both cellular and antibody-mediated immune responses.[1,9,21,22] The presence of autoantibodies is a defining feature of antibody-mediated immune responses in GBS. Several autoantibodies have been identified in GBS patients, including anti-ganglioside, anti-sulphatide and anti-neurofascin antibodies. Moreover, many different gangliosides, such as GM1, GD1, GQ1 and GT1 have been associated with different clinical subtypes of GBS. The diversity of autoantibodies underscores the complexity of GBS immunopathology (**figure 10**).[23]

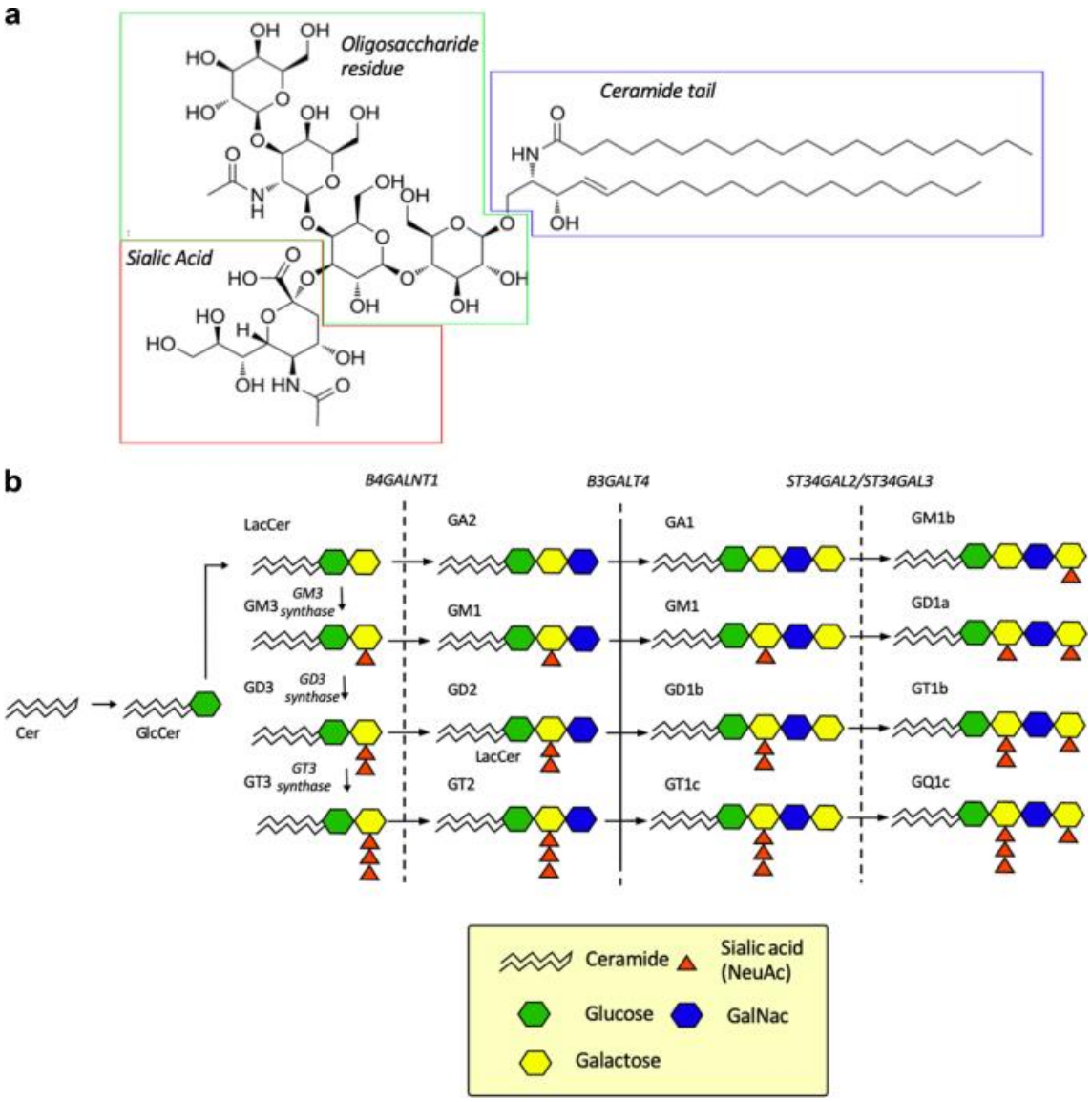


Figure 10: *overview of different gangliosides. Reproduced from: Cutillo G et al, Physiology of gangliosides and the role of antiganglioside antibodies in human diseases, Cell Mol Immunol, 2020*

Gangliosides are complex glycolipids found on the neuronal surface, where they play a key role in keeping the structure and function of the membrane. Autoantibodies against gangliosides lead to the activation of the immune response on the surface of peripheral nerves, causing damages of axonal membrane and/or depauperation of the myelin sheets around the axon. Anti-ganglioside antibodies, in fact, are able to activate the migration of leucocytes from the blood stream, leading to the production of cytokines and other inflammatory mediators and, finally, can opsonize the antigen activating the complement cascade directly on site. The thight interface between the nerve and blood (i.e. blood-nerve barrier, BNB) is damaged as well, becoming more permeable to immune cells and inflammatory mediators, that increase and perpetrate the neural damage in a vicious spiral **(figure 11)**.[1,9,21-23]

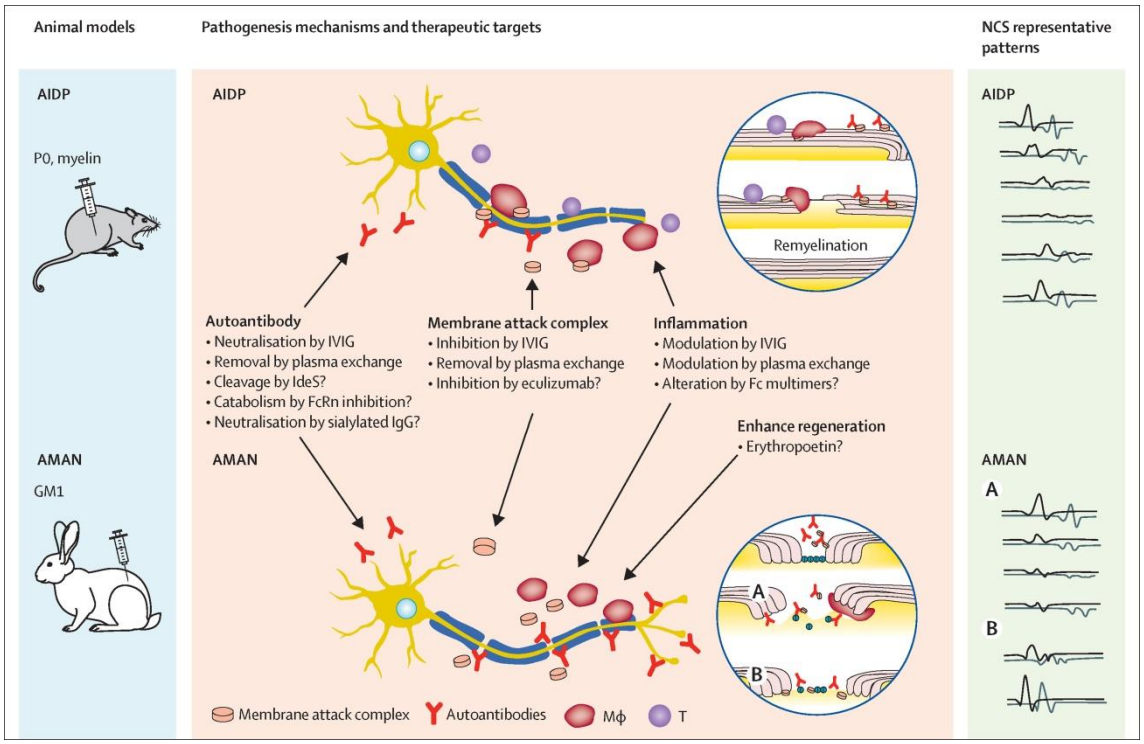


Figure 11: *overview of antibody-mediated immune riesponse in GBS and different pathway of nerve damage. Reproduced from: Willison HJ et al, Guillain-Barré syndrome, Lancet, 2016.*

Gangliosides are expressed differently along the nerve, in relation to their specific function. Some gangliosides, for example, are highly expressed at he interface between axonal surface and Schwann cell ant the node of Ranvier, were they play a role in keeping in place the tight junction between the neuron and the myelin sheet. GM and neurofascin 155 (NF155) are among these gangliosides, being highly concentrated at the node of Ranvier. When antibodies against GM1 or NF155 are present, the immune-mediated response disgregates the structure of the node of Ranvier, damaging the axonal membrane end preventing the salutatory nerve conduction, thus inducing the impairment of nerve function (**figure 12**).[24,25] In general, the combination of different types of gangliosides and their heterogeneous distribution along nerves may partially explain the relation between different anti-gangliosides antibodies, different neurophysiological pattern of damage and different clinical disease phenotypes (**figure 11**).[1,9,21-23]

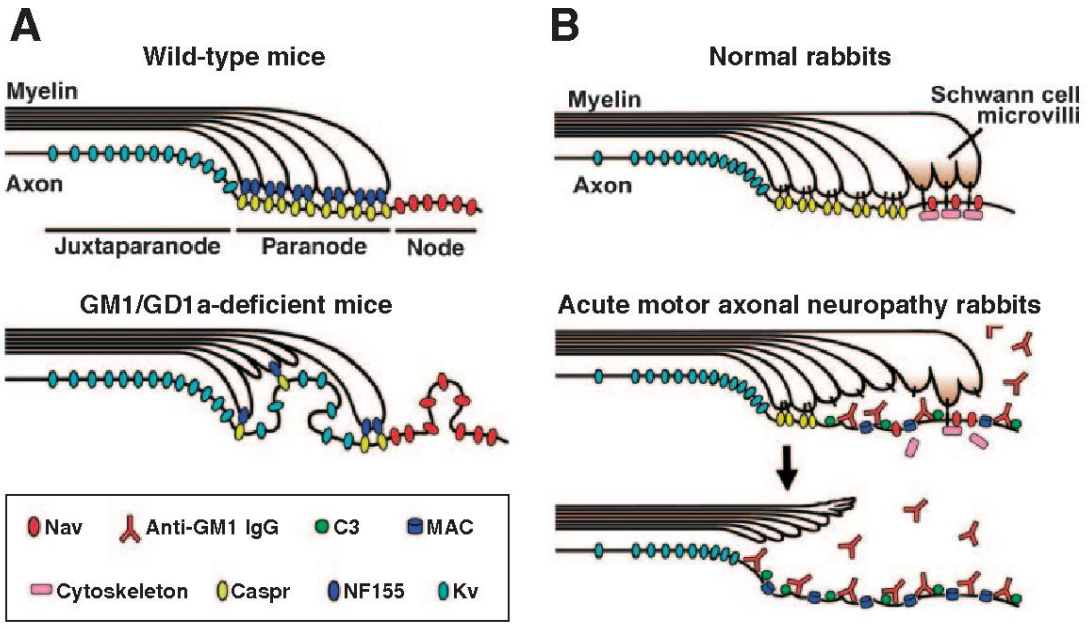


Figure 12: *the role of GM1 and NF155 at the node of Ranvier in animal models (A) and schematic representation of the attack og anti-GM1 antibodies in animal model of AMAN (B). Reproduced from: Yuki N, Guillain-Barré syndrome and anti-ganglioside antibodies: a clinician-scientist's journey, Proc Jpn Acad Ser B Phys Biol Sci, 2012.*

The best known interactions between anti-ganglioside antibodies and neurons in GBS involve the peripheral neurons, but gangliosides are present on cells of the central nervous system as well, suggesting a potential interaction at that level. On the other hand, it is well known that in GBS one of the most frequent and potentially severe site of neural damage is the nerve root. This the short segment of the nerve between the emergency from the spinal cord and the neural foramina. This is the only, brief tract of the peripheral nerve that is still wrapped by the dural layer of the meninges and, therefore, protected by the blood-brain barrier (BBB). It is well known that, other than the BNB, a crucial component of GBS pathogenesis is the immune-mediated damage of the BBB, leading to the unregulated passage of proteins and inflammatory molecules such as cytokines from the blood directly into the cerebro-spinal fluid, activating the autoimmune cascade of damage inside the subdural space.[26]
An indirect proof of the role of antibody-mediated immune response is the efficacy of the current therapeutical approaches for GBS. Both intravenous immunoglobulin (IVIG) and plasmapheresis aim to modulate the immune response, neutralizing autoantibodies. Furthermore, other emerging immunotherapies, including monoclonal antibodies and B-cell-targeted therapies, are being explored as potential treatments for GBS. These therapies specifically target components of the immune response, offering promise for more personalized and effective management of the disorder.[27,28]
Nevertheless, it is important to notice that with current laboratory methods anti-gangliosides antibodies are found in sera of GBS patients only in about 40% of cases.[1] Therefore, there might be other molecular targets we still do not know: advancements in autoantibody profiling techniques and the identification of novel autoantibodies in GBS continue to expand our understanding of the disorder and may give to the clinicians potential targets for treatment as well as biomarkers for diagnosis and prognosis.

While antibodies are central to the autoimmune response in GBS, T-cells also play a significant role in the pathogenesis. Infiltration of T-cells into peripheral nerves is a common feature of GBS and these immune cells are thought to contribute to nerve damage by releasing pro-inflammatory cytokines and recruiting other immune cells to the site of injury. T-cell-mediated damage is particularly relevant in axonal variants of GBS, where demyelination is less prominent and axonal degeneration is the primary pathological process.[29-31]

All subgroups of T-cells are involved in GBS autoimmune response: CD4+ or helper, CD8+ or cytotoxic and Tregs. The immune response in GBS primarily involves the activation of CD4+ T cells, which lead to the production of pro-inflammatory cytokines, including chemotactic factors and proteases that favour the activation and grouping of CD+, macrophages and neutrophils. The cell-mediated inflammatory response in GBS is aspecific: the most involved cytokines are IL-1, IL-5, IL-7, TNFα and IFNγ. This immune attack can result in both demyelination and axonal damage (**figure 13**).[29-31]

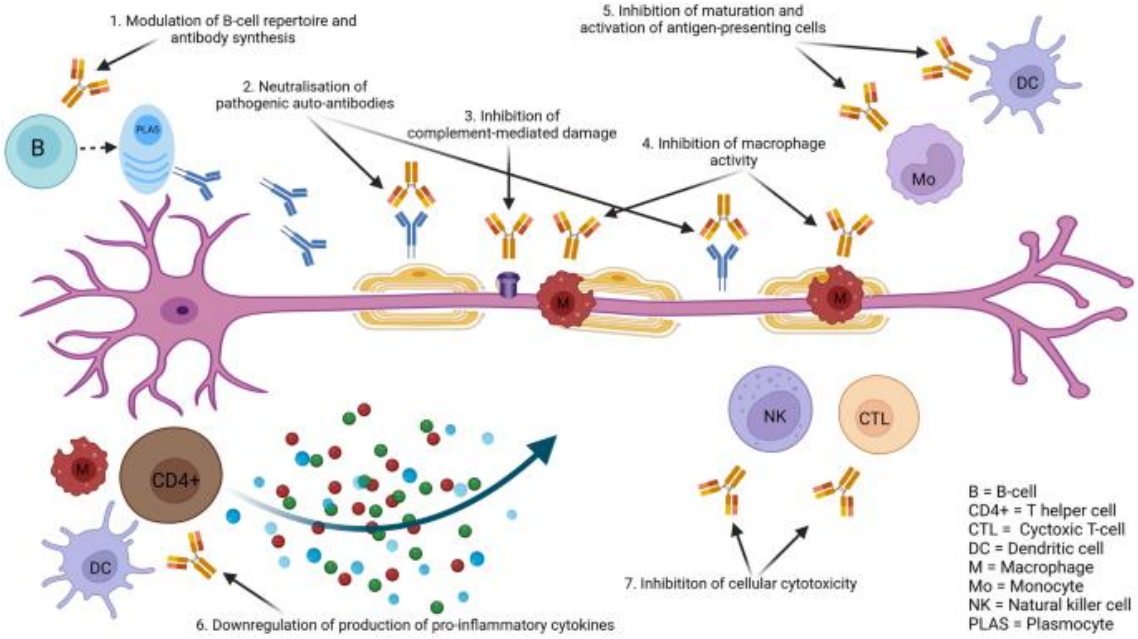


Figure 13: *schematic representation of cell-mediated immunity in GBS. Reproduced from: Rajabally YA, Immunoglobulin and Monoclonal Antibody Therapies in Guillain-Barré Syndrome, Neurotherapeutics, 2022.*

1.1.4 Clinical aspects of Guillain-Barré Syndrome

Guillain-Barré Syndrome (GBS) is defined as a immune-mediate polyneuropathy presenting with symmetrical ascending weakness and areflexia, characterized by monophasic course with subacute onset, rapid progression and slow recovery.[1] However, GBS may present with a wider range of clinical features that muste be promptly recognized in order to ensure timely diagnosis, appropriate management and better outcomes.

GBS typically begins with a prodromal phase, often characterized by non-specific symptoms such as fever, upper respiratory tract infections or gastrointestinal complaints. These symptoms, usually appearing in the four weeks before the onset of neuropathy, are frequently attributed to preceding infections, which may be involved in triggering the autoimmune response that leads to GBS (**figure 14**).[22]

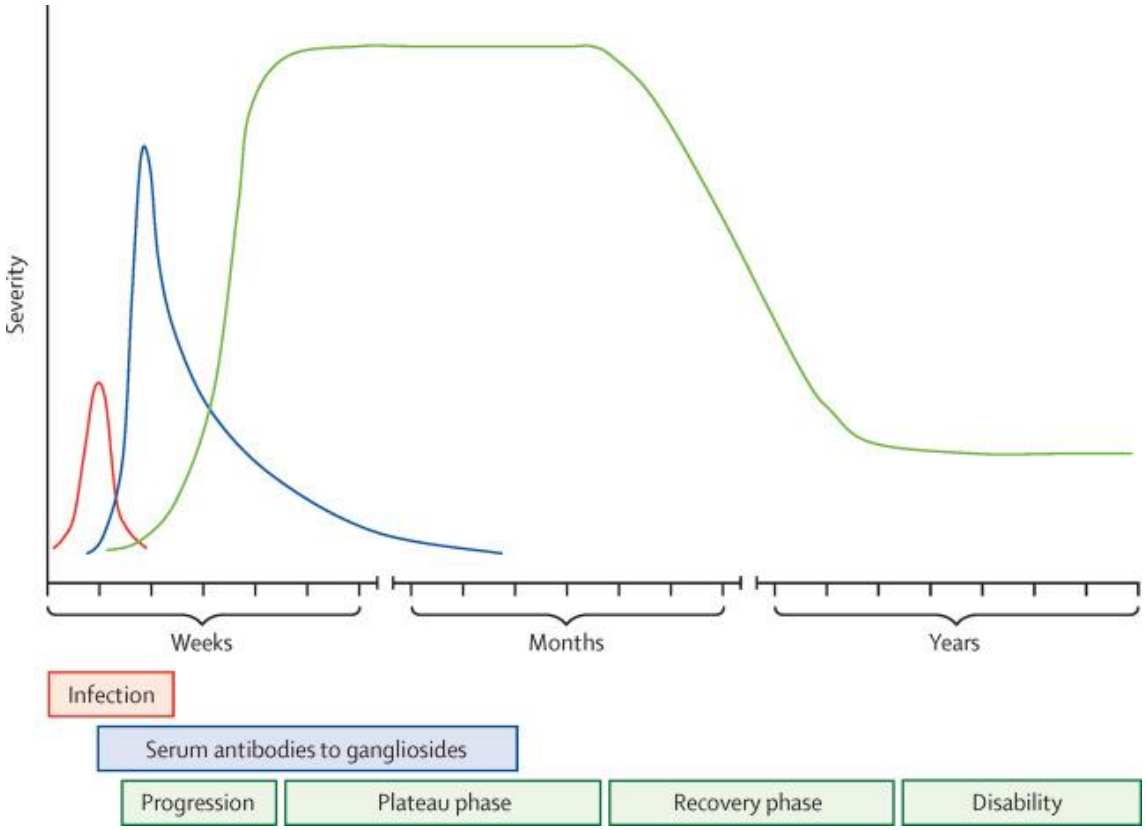


Figure 14: *typical time course of GBS. Reproduced from: Willison HJ et al, Guillain-Barré syndrome, Lancet, 2016.*

The onset of GBS may include muscle weakness, sensory disturbances, autonomic dysfunction, and life-threatening complications. However, the hallmark of GBS is the acute and symmetrical onset of muscle weakness. This muscle weakness usually begins in the lower limbs and may ascend over hours or days to involve the upper limbs and, in severe cases, the muscles used for respiration. Weakness progressively worsens, reaching its peak within several weeks (the so called "nadir"). The pace of progression can vary, but in most severe form GBS can lead to respiratory failure within hours or days. This potential for rapid deterioration makes GBS a medical emergency, requiring immediate hospitalization and supportive care.[1,9,22]

Sensory disturbances are also common in GBS and may include tingling, numbness, increased sensitivity to touch, temperature or vibration. These sensations are typically experienced in the extremities and often precede the onset of muscle weakness. Pain is another common symptom in GBS and can be severe and disabling. Patients often describe it as aching, cramping, or a deep, burning sensation. Pain is frequently localized to the back and at the four extremities. The exact mechanisms underlying pain in GBS are not fully understood, but it is believed to result from inflammation and nerve damage.[1,9,22]

Autonomic dysfunction is a notable aspect of GBS. It can manifest as fluctuations in blood pressure, heart rate abnormalities and issues with bowel and bladder control. Orthostatic hypotension, where blood pressure drops when transitioning from sitting to standing, is a frequent autonomic feature in GBS. Dysautonomia may represent a life-threatening complication, often deserving intensive care and life support.[1,9,22]

In some GBS cases, cranial nerves, which control facial and eye movements, may be affected. This can result in symptoms such as facial weakness, double vision, difficulty swallowing and speech problems. The presence of ocular nerve involvement is uncommon and usually is suggestive of specific GBS subtypes, such as Miller Fisher syndrome. Bulbar dysfunction may impair breathing and swallowing functions, thus requiring assisted ventilation through oro-tracheal intubation.[1,9,22]

The diagnosis of GBS is based upon its clinical characteristics, supported by instrumental confirmation by neurophysiology and laboratory tests. The most widely recognized criteria are the Brighton criteria (**figure 15**), which classify GBS into four levels of diagnostic certainty based on clinical and laboratory findings.[32]
The clinical criteria for diagnosing GBS include progressive and symmetrical muscle weakness and decreased or absent deep tendon reflexes. Worsening must reach the nadir between 12 hours and 28 days after the onset of symptoms. All differential diagnosis of acute polyneuropathy must be excluded.
Cerebrospinal fluid (CSF) analysis is an essential component of GBS diagnosis. Patients with GBS often have elevated protein levels in their CSF, without a corresponding increase in white blood cells. This finding, known as albumin-cytologic dissociation, is a characteristic feature of GBS.[33-35]
Electrophysiological studies, such as Nerve Conduction Studies (NCS), play a crucial role in GBS diagnosis and can help confirm the diagnosis, characterize the pattern of nerve damage (i.e. demyelinating or axonal) and define the extent and severity of nerve damage.[36]

	Levels of diagnostic certainty			
	1 (definite)	**2 (probable)**	**3 (possible)**	**4 (uncertain)**
Bilateral and flaccid limb weakness	+	+	+	+/-
Decreased or absent deep tendon reflexes in affected limbs	+	+	+	+/-
Monophasic course and time between onset and nadir included between 12 hours and 28 days	+	+	+	+/-
Absence of alernative diagnosis for weakness	+	+	+	+/-
CSF cell count <50 per ml	+	+/-*	-	+/-
CSF protein concentration >60 mg/dl	+	+/-*	-	+/-
NCS findings consistent with one of the subtypes of GBS	+	+/-*	-	+/-

Figure 15: *Brighton criteria for diagnosis of GBS.*

NCS is an indispensable tool for early detection, subtype classification and prognosis assessment in GBS. Accurate and timely diagnosis is pivotal in optimizing patient outcomes and NCS has become central to this process.[36] NCS evaluates the electrical function of peripheral nerves and is instrumental in identifying the characteristic findings of GBS, such as conduction blocks, prolonged distal latencies and reduced nerve conduction velocities. These findings aid in distinguishing GBS from other neurological conditions with similar clinical presentations. Furthermore, neurophysiological diagnosis is crucial in subtype classification and prognosis assessment in GBS.[36,37] Essentially, NCS is able to distinguish between demyelinating and axonal forms of GBS (**figure 16, 17**). The first typically presents with slowed nerve conduction velocities, conduction block, temporal dispersion of the composite motor action potential

(cMAP) and with prolonged F waves latency (**figure 18b**). On the other hand, axonal variants show reduced CMAP amplitude, reversible conduction failure and absent F waves due to axonal damage (**figure 18a**). Distinguishing these subtypes is critical for predicting outcomes and tailoring treatment strategies.[38] Finally, NCS is helpful for grading the severity of nerve damage in terms of extent of demyelination or axonal loss, predicting the course of GBS and guiding the timing of interventions. In particular, the degree of axonal damage is correlated with poorer outcomes.[36,37] In some cases, NCS may be used for monitoring patient's response to treatment, by means of serial neurophysiological studies demonstrating improvements in nerve conduction parameters.[36-38]

	Ho et al. [6] (1995)	Hadden et al. [2] (1998)	Rajabally et al. [25] (2015)
Criteria for AIDP	Must have one of the following in two nerves	Must have one of the following in two nerves	Must have one of the following in two nerves
CV	<90% LLN (<85%, if distal amp <50% LLN)	<90% LLN <85%, if distal amp <50% LLN)	<70% LLN
DML	>110% ULN (>120%, if distal amp <LLN)	>110% ULN (>120%, if distal amp <LLN)	>150% ULN
TD	Unequivocal	Not considered	
CB	Not considered	Proximal–to–distal amp ratio <0.5 and distal amp >20% LLN	Proximal–to–distal amp ratio <0.7 in two nerves (except tibial nerve), plus an additional parameter in one other nerve
F–wave latency	>120% ULN	>120% ULN	>120% ULN (>150%, if distal amp <50%) or F–wave absence in two nerves with distal amp ≥20% LLN, plus an additional parameter in one other nerve
Criteria for AMAN	No evidence of demyelination in the above nerves	None of the above except in one nerve if distal amp <10% of LLN	None of the above except in one nerve
			If distal amp <10% of LLN, one demyelinating feature allowed in one nerve, and at least one of the following:
	Distal amp <80% in two nerves	Distal amp <80% in two nerves	(1) Distal amp <80% in two nerves.
			(2) F–wave absence in two nerves with distal amp ≥20% LLN, with no demyelinating feature in any nerve.
			(3) Proximal–to–distal amp ratio <0.7 in two nerves (except tibial nerve).
			(4) F–wave absence in one nerve with distal amp ≥20% LLN or proximal–to–distal amp ratio <0.7 in one nerve (except tibial nerve), with distal amp <80% LLN in one other nerve.

Figure 16: *historical sets of neurophysiological criteria for GBS diagnosis. Reproduced from: Yoon BA et al, Electrodiagnostic findings in Guillain-Barré syndrome, Annals Clin Neurophysiol, 2020.*

Criteria for AIDP		Criteria for AMAN	
In first or second NCS, at least one of the following in at least two nerves:		In first and second NCS, none of the AIDP features in any nerve (demyelinating features allowed in one nerve if distal CMAP <20% LLN)	
√	Motor CV <70% LLN.	In first NCS, at least one of the following in each of two nerves:	
√	DML >130% ULN.	√	Distal CMAP <80% LLN.
√	Distal CMAP duration >120% ULN.	√	Proximal–to–distal CMAP amplitude ratio <0.7 (excluding tibial nerve).
√	Proximal–to–distal CMAP duration ratio >130%.	√	Isolated F–wave absence (or <20% persistence).
√	F–wave latency >120% ULN.	In second NCS, at least one of the following in two nerves is evidence of axonal degeneration:	
		√	Persistent or further reduction of distal CMAP amplitude.
Or one of the above in one nerve plus either or the following:		√	Proximal–to–distal CMAP amplitude ratio <0.7 in first test, which recovers because of a decrease in distal
√	Absent F–waves in two nerves with distal CMAP >20% LLN.		CMAP without increased TD (distal CMAP duration <130% and proximal–to–distal CMAP duration ratio <130%).
√	Abnormal ulnar SNAP amplitude and normal sural SNAP amplitude.	In second NCS, at least one of the following in two nerves is evidence of reversible conduction failure:	
		√	>150% increase in distal CMAP amplitude without increased distal CMAP duration (<130% ULN).
		√	Proximal–to–distal CMAP amplitude ratio <0.7 in first test, which improves by >0.2 because of increased proximal CMAP without TD (proximal–to–distal CMAP ratio <130%).
		√	Isolated F–wave absence (or <20% persistence) that recovers without increased minimal latency (<120% of ULN).

Figure 17: *Uncini's criteria for demyelinating and axonal GBS. Reproduced from: Uncini A et al, The electrodiagnosis of Guillain-Barré syndrome subtypes: Where do we stand? Clin Neurophysiol, 2018.*

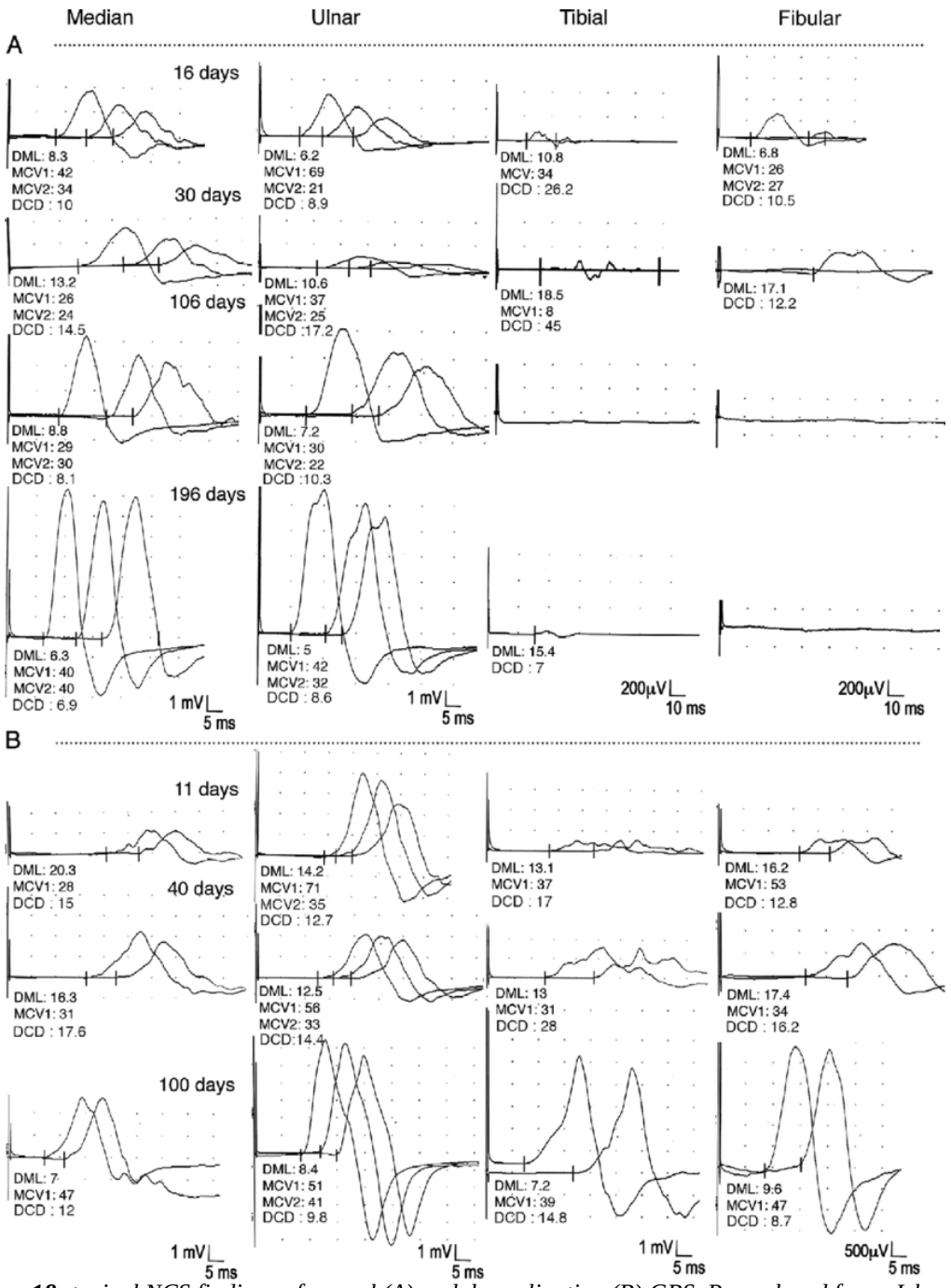


Figure 18: *typical NCS findings of axonal (A) and demyelinating (B) GBS. Reproduced from: Islam B et al. Electrophysiology of Guillain-Barré syndrome in Bangladesh: A prospective study of 312 patients, Clin Neurophysiol Pract, 2021.*

Nevertheless, while neurophysiological diagnosis has significantly enhanced our understanding and management of GBS, challenges and avenues for future research remain. Firstly, the development of standardized neurophysiological criteria for GBS diagnosis and subtype classification is needed to ensure consistency across different centers and improve the accuracy of assessments.[39] In some cases, further tests after the first examination are needed to correctly define subtype and extension of nerve damage.[38] Secondly, the identification of neurophysiological biomarkers that correlate with clinical outcomes and therapeutic responses could improve our knowledge of the disease as well as its clinical management.[36-39]

Combining clinical and neurophysiological findings, the clinical spectrum of GBS may be classified in distinct subtypes, which often correlate with differences in the underlying pathological processes (**figure 19**):[3,40]

- acute inflammatory demyelinating polyneuropathy (AIDP): it is the most common subtype of GBS. It is characterized by widespread demyelination of peripheral nerves, resulting in the loss of myelin, which impairs nerve signal conduction. The hallmark of AIDP is ascending muscle weakness, which typically starts in the lower limbs and progresses upward. AIDP is often associated with anti-ganglioside antibodies.[1,3,9,22,40]
- acute motor axonal neuropathy (AMAN) and acute motor-sensory axonal neuropathy (AMSAN): AMAN and AMSAN are less common GBS variants characterized by axonal damage rather than demyelination. These subtypes often present with more severe motor deficits and are frequently associated with specific infectious triggers, such as Campylobacter jejuni. In AMAN and AMSAN, the immune response targets axons, resulting in nerve conduction block and axonal degeneration.[1,3,9,22,40]
- Miller-Fisher syndrome (MFS): it is a rare GBS variant characterized by a triad of symptoms: ataxia (lack of muscle coordination), ophthalmoplegia (paralysis of the eye muscles) and areflexia (absence of deep tendon reflexes). This syndrome is often associated with anti-GQ1b antibodies and cranial nerve involvement, particularly in the absence of limb weakness.[1,3,9,22,40]

Other regional, more specific variants of GBS are rare: paraparethic variant, pharyngo-cervico-brachial (PCB), facial diplegia (FD), pure sensory GBS.[3,40,41]

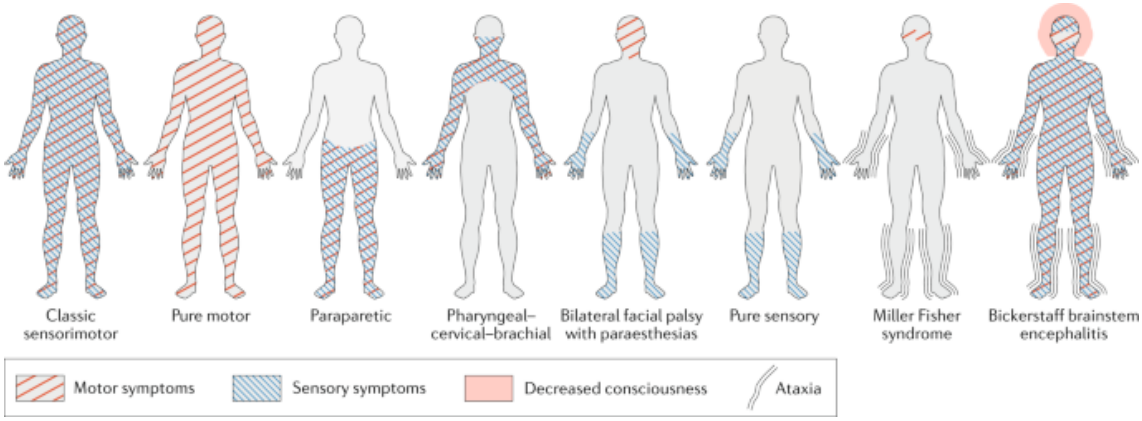


Figure 19: *variants of GBS. Reproduced from: Leonhard SEet al, Diagnosis and management of Guillain-Barré syndrome in ten steps, Nat Rev Neurol, 2019.*

GBS can lead to a range of complications, some of which can be life-threatening:[1,9,22]

- Respiratory failure is one of the most serious and potentially life-threatening complications of GBS. As muscle weakness progresses, patients may struggle to breathe effectively, leading to respiratory insufficiency or failure. Mechanical ventilation is often required to support breathing in such cases.
- Immobility due to muscle weakness in GBS can increase the risk of thromboembolic events, such as deep vein thrombosis (DVT) and pulmonary embolism (PE). Appropriate measures, including anticoagulation therapy and physical therapy, are often employed to prevent these complications.
- Autonomic dysfunction can lead to fluctuations in blood pressure, heart rate abnormalities, and issues with bowel and bladder control. These symptoms can be challenging to manage and may require specialized care.
- Immobility and sensory disturbances in GBS can increase the risk of developing pressure ulcers. Frequent repositioning, skin care, and pressure-relief devices are essential in preventing and managing these ulcers.

1.1.5 Treatment of Guillain-Barré Syndrome

GBS treatment involves a multidisciplinary approach, including neurologists, intensive care specialists, physical therapists and other healthcare professionals.[41]

Supportive care is a critical component of GBS management, particularly in the acute phase of the syndrome. Patients with GBS often require hospitalization to address complications, such as respiratory failure, autonomic dysfunction, and muscle weakness. The key aspects of supportive care in GBS include:[42,43]

- Respiratory support: respiratory failure is one of the most serious and potentially life-threatening complications of GBS. Mechanical ventilation is often required to support breathing in these cases. Intensive care units (ICUs) are equipped to provide mechanical ventilation, monitor oxygen levels, and manage the respiratory status of GBS patients. In some instances, non-invasive ventilation techniques, such as bilevel positive airway pressure (BiPAP) or continuous positive airway pressure (CPAP), may be employed. These techniques provide ventilatory support without intubation and are suitable for GBS patients with impending or mild respiratory failure.
- Hemodynamic support: GBS can result in autonomic dysfunction, leading to blood pressure fluctuations and heart rate abnormalities. Patients may experience orthostatic hypotension, which can result in a drop in blood pressure upon standing. Intravenous fluids and medications, such as pressors or vasopressors, may be administered to maintain stable blood pressure. Close monitoring in the ICU is essential to manage these autonomic disturbances effectively.
- Pain management: pain is a common symptom in GBS and can be severe, often described as aching, cramping, or a deep, burning sensation. Managing pain is an integral part of supportive care. Analgesic medications, including non-steroidal anti-inflammatory drugs (NSAIDs), opioids, and adjuvant therapies, may be prescribed to alleviate pain and discomfort.
- Prevention of complications: immobilization due to muscle weakness in GBS increases the risk of complications, such as deep vein thrombosis (DVT) and pulmonary embolism (PE). Prophylactic measures, including anticoagulation therapy and physical therapy, are crucial to prevent these complications. Frequent repositioning, the use of compression stockings, and pneumatic compression devices are employed to maintain blood flow and prevent venous stasis.
- Nutritional support: dysphagia, or difficulty swallowing, can occur in GBS, especially when cranial nerves are affected. Ensuring adequate nutrition is essential. In cases of severe dysphagia or when there is a risk of aspiration, enteral nutrition via nasogastric or gastrostomy tubes may be necessary to maintain proper caloric intake.
- Skin care: immobility, sensory disturbances, and the use of medical devices (such as ventilators) can increase the risk of developing pressure ulcers. Skin care, including frequent assessment, repositioning, and the use of pressure-relief devices, is essential to prevent and manage these ulcers.

Other than supportive care, the use of immunomodulatory therapies is a cornerstone of GBS treatment. These therapies aim to reduce the immune response and limit further nerve damage. The two primary options for immunomodulatory treatment in GBS are intravenous immunoglobulin (IVIG) and plasma exchange (plasmapheresis).

- Intravenous Immunoglobulin (IVIG):[44] intravenous administration of high-dose immunoglobulin, which contains pooled immunoglobulins from healthy donors. IVIG has been shown to be effective in reducing the severity and duration of GBS symptoms. The precise mechanism of action of IVIG in GBS is not fully understood, but it is believed to involve the modulation of the immune response. IVIG is thought to have immunomodulatory effects, including the suppression of pro-inflammatory responses and the inhibition of the complement system. Additionally, IVIG may have a role in promoting remyelination and axonal repair. The recommended dosage of IVIG for GBS

is typically 0.4 grams per kilogram of body weight per day for five consecutive days. This dosage may vary depending on clinical factors, such as disease severity and the patient's response to treatment. IVIG is preferred over plasmapheresis in many cases due to its convenience, lower risk of complications, and greater accessibility. Side effects of IVIG are generally mild and may include headache, fever, and allergic reactions. IVIG is a well-tolerated treatment, but it should be administered under the supervision of healthcare professionals, and patients should be monitored for adverse effects.

- Plasma Exchange (PE):[45] also known as plasmapheresis, it is an alternative immunomodulatory treatment for GBS. It involves the removal of the patient's plasma, which contains pathogenic antibodies and immune components, and its replacement with fresh or albumin-containing plasma. Plasmapheresis is believed to be effective in GBS by removing the antibodies that contribute to the immune attack on peripheral nerves. This process helps reduce inflammation and limit further nerve damage. Plasmapheresis is typically administered in a series of sessions over several days. The exact number of sessions and the volume of plasma exchanged may vary depending on clinical factors. Patients often require central venous access for plasmapheresis sessions, which are performed in a clinical setting. Plasmapheresis may be considered when IVIG is contraindicated or when there is an inadequate response to IVIG. Potential side effects of plasmapheresis include hypotension, bleeding, infection, and electrolyte imbalances. The choice between IVIG and plasmapheresis is based on individual patient factors, availability of treatment modalities, and clinical judgment.

In some cases, a combination of IVIG and plasmapheresis may be considered, especially for patients with severe or refractory GBS. The rationale for combination therapy is to target different aspects of the immune response and potentially enhance treatment efficacy. However, the optimal use of combination therapy and its long-term outcomes require further investigation.[46]

Rehabilitation is a crucial component of GBS management, focusing on helping patients regain function, improve mobility, and enhance their quality of life. The goals of rehabilitation in GBS include:[47,48]

- Regaining Muscle Strength: physical therapy plays a vital role in helping patients rebuild muscle strength and function. Exercises are tailored to the individual's capabilities and progress over time.
- Restoring Mobility**:** occupational therapy is essential in restoring mobility and daily living skills. This includes activities like dressing, bathing, and using assistive devices. Occupational therapists work with patients to improve their independence and quality of life.
- Speech and swallowing therapy: when cranial nerves are involved in GBS, patients may experience difficulties with speech and swallowing. Speech and language therapists assist in regaining these functions. They provide exercises and strategies to improve speech clarity and ensure safe swallowing.
- Preventing complications, such as contractures and pressure ulcers.

Rehabilitation is a long-term process that continues well beyond the acute phase of GBS. It is tailored to the individual patient's needs and may extend for several months to years, depending on the extent of neurological deficits and the pace of recovery. The ultimate goal is to help GBS patients achieve the best possible functional outcomes and quality of life.[47,48]

Several promising areas of investigation are shedding light on potential new treatment strategies. Complement inhibitors, such as eculizumab, are being studied to determine their potential in reducing nerve injury and improving GBS outcomes. Research is ongoing to identify targeted immunomodulatory agents that may offer more specific and effective treatments for GBS, aiming to suppress the immune response without the broad immunosuppression associated with treatments like corticosteroids.[49]

1.1.6 Prognostic factors in Guillain-Barré Syndrome

The prognosis of GBS varies widely and depends on several factors. While the majority of patients experience at least some degree of recovery, a significant number are left with long-term neurological deficits (**figure 20**).[1,3] In some cases, GBS can result in persistent disability, including weakness, pain, and sensory disturbances. For others, the recovery may be near-complete, although it can take several months to years. Predicting individual outcomes remains challenging due to the variability in GBS presentation and the influence of genetic, immunological, and clinical factors. Different factors have been associated with a worse prognosis, including older age at onset, severe muscle weakness, disease subtype, the presence of specific antibodies, treatment response and the development of certain complications, such as respiratory failure.[50-58]

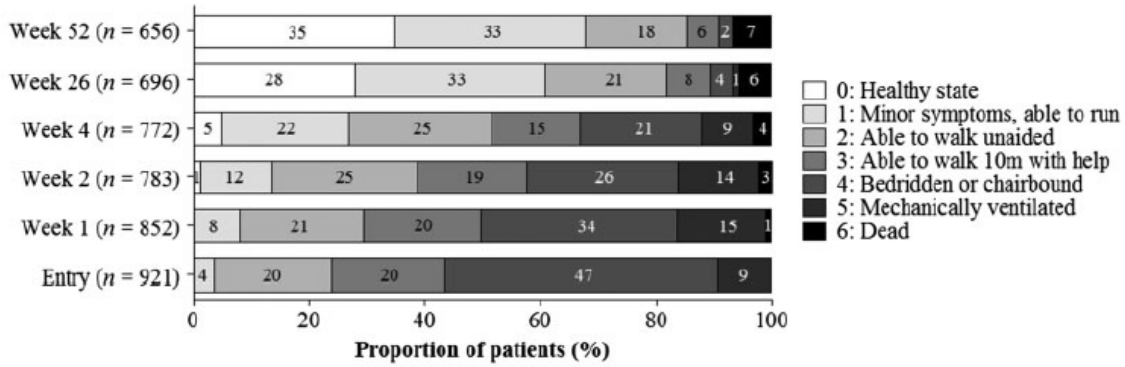


Figure 20: *Outcome of GBS in the IGOS cohort. Reproduced from: Doets AY et al, Regional variation of Guillain-Barré syndrome, Brain, 2018.*

Age is a significant prognostic factor in GBS. Younger patients, especially children and adolescents, tend to have more favorable outcomes and higher chances of complete recovery. Older adults, particularly those over the age of 50, often face a more prolonged and less complete recovery. Advanced age is associated with a higher risk of complications and a poorer prognosis in GBS. The reasons for age-related differences in GBS prognosis are not entirely understood but may be related to factors such as reduced regenerative capacity in older individuals, increased comorbidities, and age-related changes in the immune system.[1,9,22,50-58]

The presence of specific antibodies in GBS patients can provide valuable prognostic information. Anti-GM1 antibodies are often detected in patients with AMAN and are associated with axonal variants of GBS. The presence of anti-GM1 antibodies may indicate a more severe clinical course and slower recovery. These patients are more likely to experience persistent motor deficits. On the contrary, anti-GD1a and anti-GQ1b antibodies are often found in GBS variants that involve cranial nerve abnormalities, such as MFS. These patients typically have a better prognosis, with a higher likelihood of near-complete recovery.[1,9,22,50-58]

Genetic factors have gained attention as potential prognostic markers in GBS. Certain HLA alleles have been associated with increased susceptibility to GBS and may influence the clinical course. HLA-DR2, HLA-DR3, and HLA-DQB1*03 alleles have been linked to a higher risk of GBS, more severe forms of the disease and slower recovery.
Variations in cytokine gene polymorphisms, such as those affecting tumor necrosis factor (TNF) and interleukin-1 (IL-1), have also been investigated for their potential impact on GBS prognosis. These polymorphisms may influence the intensity of the immune response and contribute to differences in clinical outcomes.
While genetic markers hold promise as prognostic factors, further research is needed to establish their clinical utility. Genetic testing in GBS is not yet a routine practice for prognosis determination but may become more relevant as our understanding of genetic contributions to GBS advances.[1,9,22,50-58]

The initial clinical presentation of GBS varies widely among patients and plays a crucial role in determining prognosis. The pattern and severity of muscle weakness at onset is a critical prognostic factor in GBS.
Cranial nerve involvement, such as facial weakness, double vision, or swallowing difficulties, is another prognostic factor in GBS. The presence of cranial nerve abnormalities, particularly in isolation or in combination with limb weakness, is often associated with a less severe clinical course and a better prognosis. These patients tend to recover more quickly and with fewer long-term deficits.
The presence and extent of sensory disturbances can impact GBS prognosis. Patients with predominantly motor deficits and minimal sensory involvement tend to have better outcomes. On the other hand, those with significant sensory disturbances, such as numbness, tingling, or heightened sensitivity, may experience more prolonged recovery and persistent sensory deficits.
Autonomic dysfunction, characterized by blood pressure fluctuations, heart rate abnormalities, and bowel and bladder problems, can be a challenging aspect of GBS. Its presence does not necessarily predict a poorer prognosis, but it may complicate the clinical course and require specialized care.[1,9,22,50-58]

The specific GBS subtype can significantly influence prognosis.
AIDP is the most common and typically less severe subtype of GBS. It is characterized by demyelination of peripheral nerves, resulting in the loss of myelin and impairment of nerve signal conduction. Many patients with AIDP experience good recovery, although the pace and extent of recovery can vary.
AMAN and AMSAN subtypes are associated with axonal damage rather than demyelination. These variants often present with more severe motor deficits, and recovery may be slower and less complete. Patients with AMAN and AMSAN may face more prolonged disability and persistent motor deficits.
MFS is a distinct variant of GBS characterized by a triad of symptoms: ataxia (lack of muscle coordination), ophthalmoplegia (paralysis of the eye muscles), and areflexia (absence of deep tendon reflexes). While the symptoms can be debilitating, patients with MFS tend to have a favorable prognosis and often experience near-complete recovery.
The specific GBS subtype can provide valuable insights into the clinical course and potential outcomes. While these subtypes are not a strict predictor of prognosis, they offer guidance for clinicians and may influence treatment decisions.[1,9,22,50-58]

The timing of immunomodulatory treatment in GBS can impact prognosis. Early initiation of treatment, such as intravenous immunoglobulin (IVIG) or plasma exchange (plasmapheresis), is associated with better outcomes. These treatments aim to suppress the immune response and limit further nerve damage. Patients who receive treatment within the first few weeks of symptom onset tend to experience more rapid recovery and may achieve near-complete remission. In contrast, delayed treatment initiation can result in a more prolonged recovery and increased risk of complications.
The response to immunomodulatory treatment, such as IVIG or plasmapheresis, can serve as a significant prognostic factor. Patients who demonstrate a robust and rapid response to treatment tend to have more favorable outcomes. Conversely, those who do not respond well to initial treatment may face a more prolonged recovery and persistent deficits. Monitoring treatment response is essential for adapting the treatment approach. Patients who do not improve with one form of immunomodulatory therapy may be considered for alternative treatments or a combination of therapies to achieve a better response.[1,9,22,50-58]

The occurrence of complications, such as respiratory failure, thromboembolic events and autonomic dysfunction can complicate the clinical course and impact recovery. Patients who develop respiratory failure, often requiring mechanical ventilation, may experience a more prolonged recovery. The extent of muscle weakness and the duration of ventilator support are

key factors in determining outcomes. Autonomic dysfunction, such as fluctuations in blood pressure and heart rate abnormalities, can complicate the clinical course and may require specialized care. Managing these issues is crucial for prognosis.[1,9,22,50-58]

Rehabilitation plays a critical role in GBS recovery and, by extension, prognosis. The extent of rehabilitation services and the patient's engagement in therapy can significantly impact outcomes. Patients who actively participate in physical therapy, occupational therapy, and speech therapy tend to experience better recovery and improved quality of life. Physical therapy helps patients regain muscle strength and mobility, occupational therapy focuses on daily living skills, and speech therapy assists in restoring speech and swallowing functions.[1,9,22,50-58]

Recently, two commonly used scores have been validated for clinical prediction of prognosis of GBS: the modified Erasmus GBS Outcome Score (mEGOS) and the Erasmus GBS Respiratory Insufficiency Score (EGRIS). These scores serve as valuable tools for clinicians in the evaluation and management of GBS patients, helping to guide treatment decisions and improve patient care. They provide a structured and objective framework for evaluating GBS patients and can be particularly useful in the acute phase when swift and accurate assessment is critical.
The modified Erasmus GBS Outcome Score (mEGOS) is a clinical scoring system developed to assess the overall disability and recovery in GBS patients. It evaluates motor function, presence of gastrointestinal antecedent and age at onset. Patients with high initial mEGOS scores are more likely to experience a more prolonged and severe course of the disease, while those with low scores tend to have a more favorable prognosis (**figure 21**).[59]
The Erasmus GBS Respiratory Insufficiency Score (EGRIS) is a specific scoring system developed to assess the risk of respiratory insufficiency in GBS patients. Respiratory insufficiency is a life-threatening complication of GBS, and early detection and intervention are crucial to prevent its progression. EGRIS takes into consideration motor function, bulbar weakness and time between onset and hospital admission. Higher EGRIS scores suggest a higher risk of respiratory insufficiency and need of mechanical ventilation. EGRIS is a valuable tool for identifying patients who may require close monitoring, respiratory support, or early interventions, such as mechanical ventilation (**figure 22**).[60]

mEGOS at hospital admission

		Score
Age at onset		
	≤40	0
	41-60	1
	>60	2
Preceding diarrhea		
	Absent	0
	Present	1
MRC sum score		
	51-60	0
	41-50	2
	31-40	4
	≤30	6
Total score		**0 - 9**

Figure 21: *modified Erasmus GBS Outcome Score (mEGOS).*

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

Figure 22: *Erasmus GBS Respiratory Insufficiency Score (EGRIS).*

Further research is needed to identify specific and reliable prognostic factors of GBS. Developing subtype-specific scoring systems may help better capture the unique features and prognostic factors associated with different GBS subtypes. In addition, the integration of clinical scores with biomarkers, such as neurofilament levels and specific anti-ganglioside antibodies, can offer a more comprehensive prognostic assessment. While no single factor can definitively forecast outcomes, a comprehensive assessment of these factors can provide valuable insights into the likely course of the disease.[61]

1.1.7 Biomarkers in Guillain-Barré Syndrome

There is great interest in literature about the research of reliable, affordable and specific for GBS. Such molecules could help the understanding of GBS pathogenesis, improving diagnosis, clinical stratification of prognosis and monitoring the response to treatment.

Nevertheless, still nowadays the only validated biomarker for GBS diagnosis is albumin-cytological dissociation, as defined in the Brighton diagnostic criteria. This findings however, identified in the sixties, has low specificity and sensitivity and should be replaced by more reliable and modern biomarkers.[33]

Many potential new molecules have been proposed as biomarkers for GBS, both in serum and in CSF. Anti-GM1 antibodies are commonly associated with axonal forms of GBS, which have the worst clinical picture with poorer recovery after treatment.[62-65] The heterogeneous world of cytokine expression has been explored through proteomic approaches: IL-8 overexpression and Th17 pathway deregulation have been identified in GBS, but they lack specificity.[66-70] The neutrophile to lymphocyte ratio has been related to prognosis, but it has no diagnostic value.[71,72] Other identified molecules were S100, Tau, phosphorylated neurofilament heavy chain, all related to worse long term prognosis.[73] More recently, two promising small molecules has been identified in CSF. Sphingomyelin is a component of the myelin sheat that surrounds the axon: it is present at a higher concentration in patients with demyelinating GBS.[74] On the other hand,

peripherin is contained specifically inside the axons and is released in CSF case of axonal damage.[75] These two potential biomarkers are promising helps for diagnosis and classification of GBS subtypes, but their prognostic value has not been established yet. Moreover, they are expensive and difficult to test, so they are mostly used for research purposes to demonstrate and evaluate the entity of demyelinating or axonal damage.

Certainly, the most promising among the new biomarkers for GBS prognosis are neurofilament light chain levels in CSF and blood.
Neurofilaments are a family of structural proteins found in neurons, where they contribute to the maintenance of axonal structure and support essential neuronal functions.[76] These proteins are predominantly located in the axons of neurons, providing stability and assisting in the transport of materials along axonal processes.[76] The three major subunits of neurofilament proteins are designated as light (NF-L), medium (NF-M) and heavy chain (NF-H). These subunits combine to form the intermediate filament structure within neurons (**figure 23**).[77]

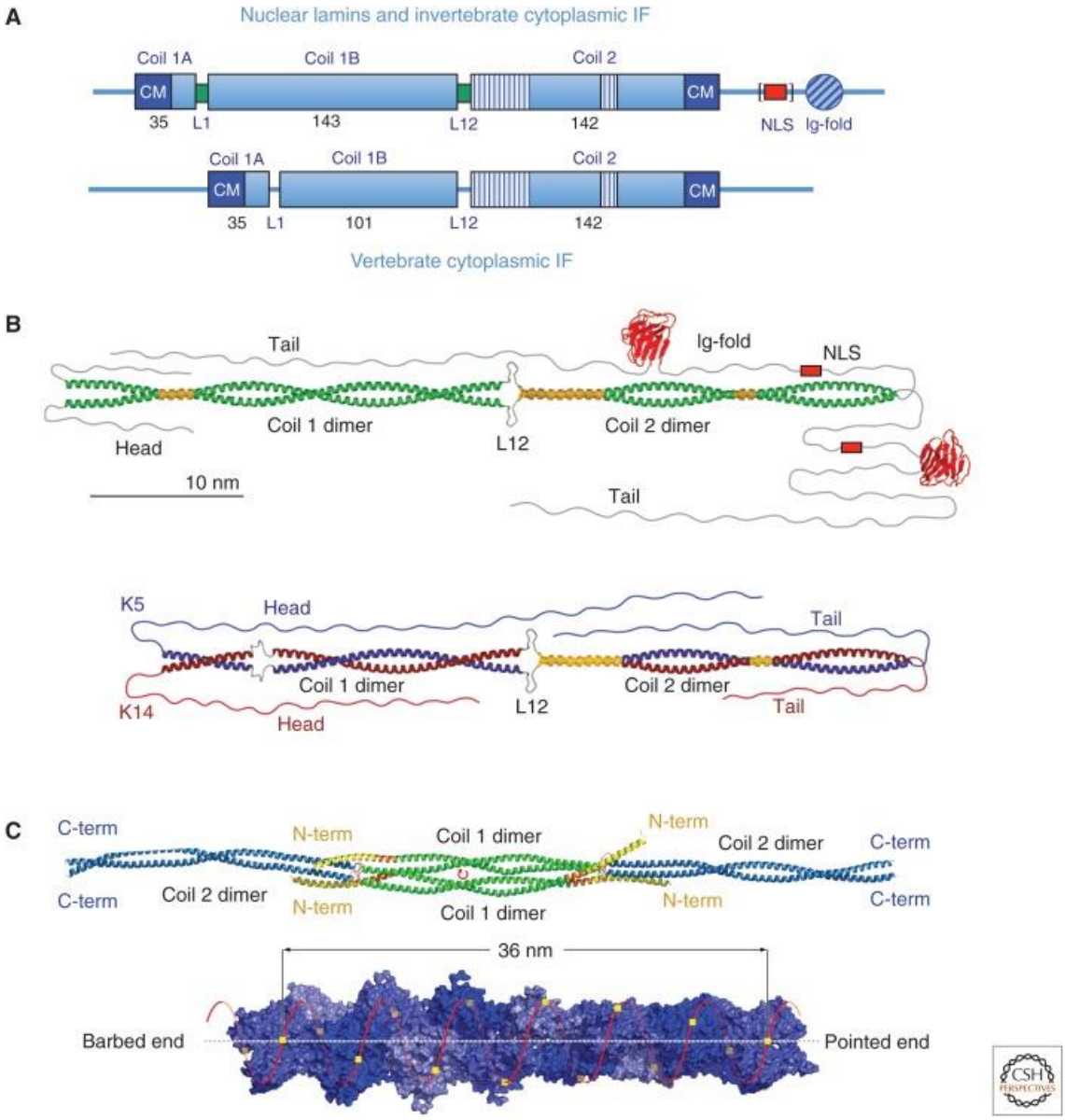


Figure 23: *intermediate filament structure in neurons. Reproduced from: Herrmann H et al, Intermediate Filaments: Structure and Assembly, Cold Spring Harb Perspect Biol, 2016.*

Abnormalities in neurofilament proteins can disrupt the structural integrity of axons, leading to axonal damage and impairing neuronal function. The release of neurofilament proteins into the cerebrospinal fluid (CSF) and blood can occur following axonal damage or degeneration. In neurological conditions, including GBS, the concentration of neurofilament proteins reflect the severity and extent of axonal injury.[78-80] Thus, elevated levels of neurofilament proteins in cerebrospinal fluid (CSF) and blood have been associated with axonal damage, a more severe clinical course and poorer recovery in GBS (**figure 24**).[81-85]

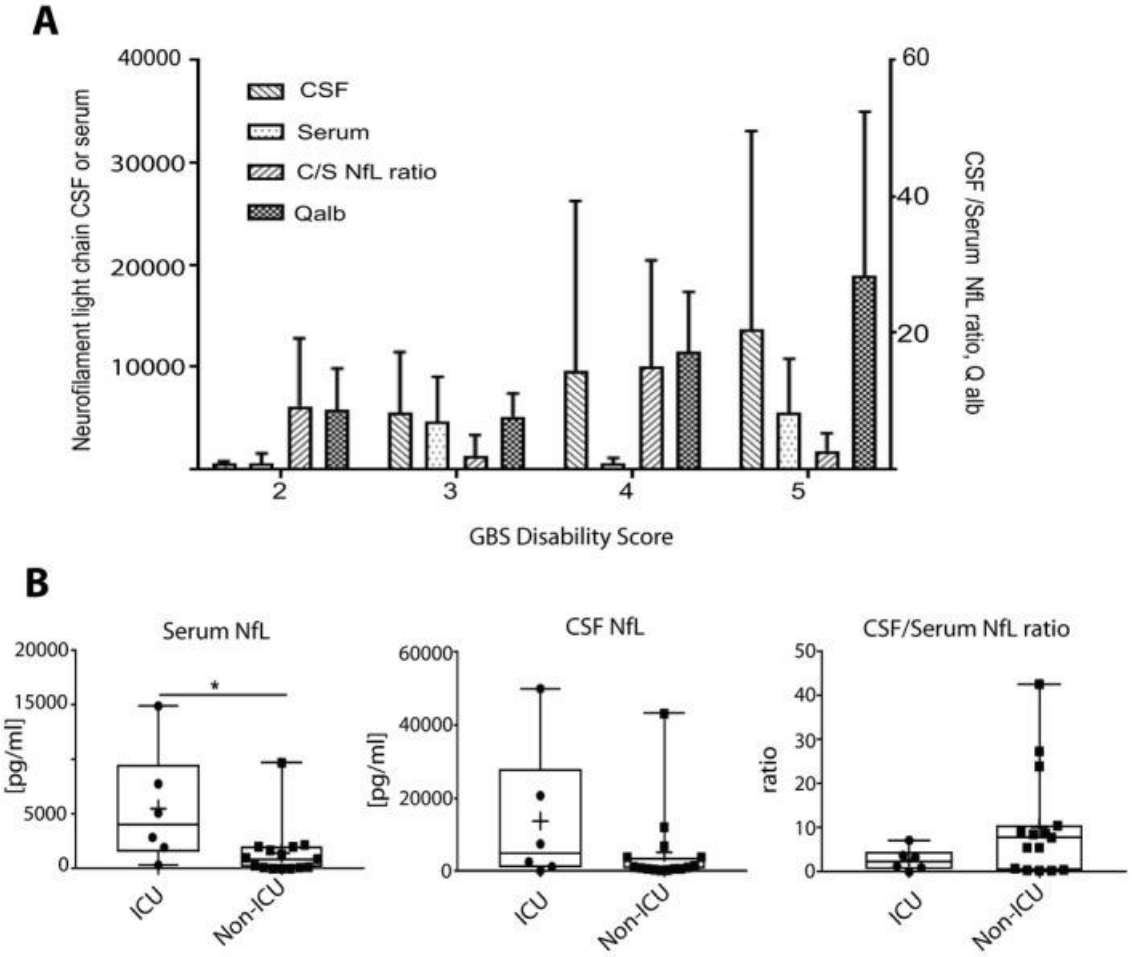


Figure 24: *prognostic relation beteen neurofilaments and GBS severity. Reproduced from: Körtvelyessy P et al, Ratio and index of Neurofilament light chain indicate its origin in Guillain-Barré Syndrome, Ann Clin Transl Neurol, 2020.*

The assessment of neurofilament levels in the cerebrospinal fluid (CSF) has provided valuable insights into axonal injury in GBS. CSF is in direct contact with the central nervous system and, as such, can contain markers of axonal damage. Actually, elevated CSF neurofilament levels are often observed in GBS subtypes associated with axonal damage, such as Acute Motor Axonal Neuropathy (AMAN) and Acute Motor-Sensory Axonal Neuropathy (AMSAN).[78-80] Several studies have demonstrated that elevated neurofilament levels in CSF are associated with a more severe clinical course, increased disability and poorer recovery in GBS (**figure 25**).[82,83]

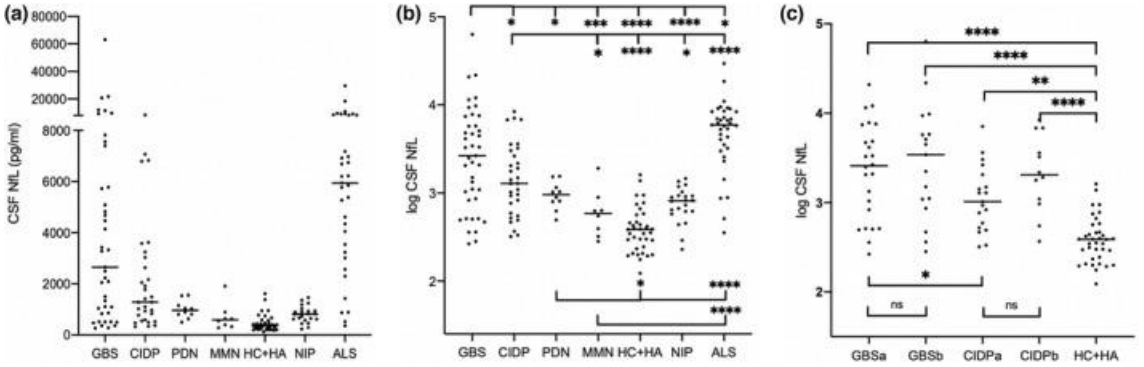


Figure 25: *neurofilament ligh chain level in CSF of patients with GBS and other neurological conditions. Reproduced from: Kmezic I et al, Neurofilament light chain and total tau in the differential diagnosis and prognostic evaluation of acute and chronic inflammatory polyneuropathies, Eur J Neurol, 2022.*

In addition to CSF, researchers have explored the role of neurofilament levels in the blood (serum) as prognostic markers in GBS. Blood samples are more accessible and less invasive to obtain than CSF samples, making serum neurofilament measurements more practical for routine clinical use. Studies revealed that even elevated serum neurofilament levels were associated with more severe disease, prolonged recovery and increased disability (**figure 26**).[84-85] Another potential application of NF-L chain levels might be the monitoring of disease progression and treatment response through repeated measurements during the follow up.[78-85]

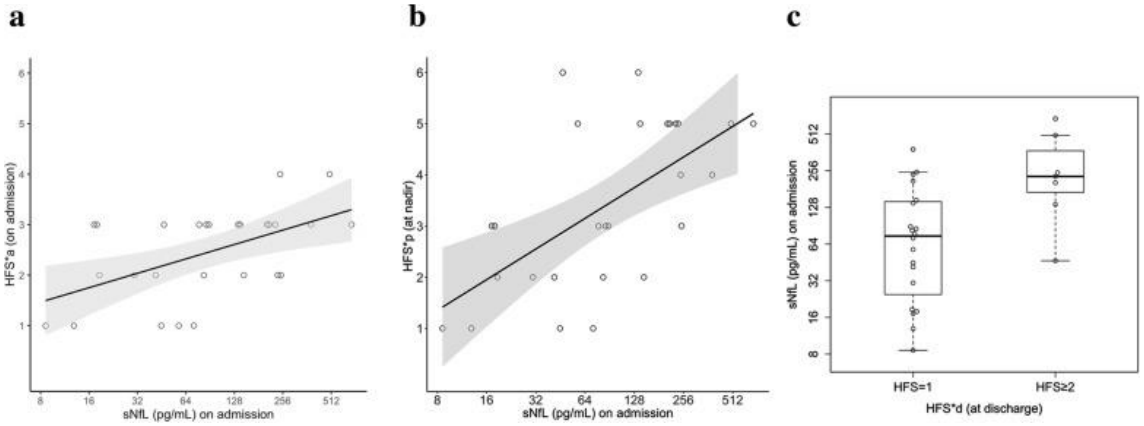


Figure 26: *relation between serum neurofilament ligh chain level and disability in GBS. Reproduced from: Altmann P et al, Increased serum neurofilament light chain concentration indicates poor outcome in Guillain-Barré syndrome, J Neuroinflammation, 2020.*

Altough their potential clinical use as diagnostic and prognostic biomarkers, NF-L chain levels have some limitations. First of all, neurofilament levels can vary widely among individuals and absolute cutoff values to define severity are yet to be established. The interpretation of neurofilament levels must therefore consider individual variations and should be integrated with clinical assessments. This intrinsic characteristic is particularly evident in GBS, due to its great diversity in clinical presentation, underlying pathogenic mechanisms and disease severity. As a consequence, up to date the use of neurofilaments as a biomarker finds a clinical application only when connected with other clinical and neurophysiological assessments and in combination with other potential prognostic biomarkers. Practically, the actual value of neurofilament level measurement is mostly related to repeated determinations in each single patient, while inter-individual variability greatly limits its application as an absolute and transversal marker.[78-85] Secondly, NF-L chain measurement is still quite expensive and available only in selected laboratories, making it difficult to apply extensively.[78-85]

1.2 IgG and IgM oligoclonal bands

This section resumes the foundamentals about IgG and IgM OligoClonal Bands (OCBs) and their role as a diagnostic and prognostic factors in some neurological diseases, inculiding GBS.

1.2.1 Generalities about IgG and IgM OCBs and neurological disorders

OligoClonal Bands (OCBs) are different clonal immunoglobulins, mainly IgM or IgG, visible as distinct pattern ("bands" or lines) in electrophoretic analysis of serum or CerebroSpinal Fluid (CSF). These bands represent a limited number of immunoglobulin-producing clones, as opposed to the broad polyclonal pattern, visible as a homogeneous and continuous pattern, normally found in serum.[86,87] Techniques like isoelectric focusing (IEF) and immunoblotting are commonly used to detect and characterize OCBs. These methods enable the differentiation between OCBs present in the CSF and those found in the blood. In fact, comparison between electrophoretic analysis of CSF and serum may result essentially in three types of patterns (**figure 27**):[86,88]

- Presence of OCBs in CSF that are not visible in serum
- Presence of the same OCBs in CSF and in serum ("mirror pattern")
- Presence of OCBs in serum that are not visible in CSF

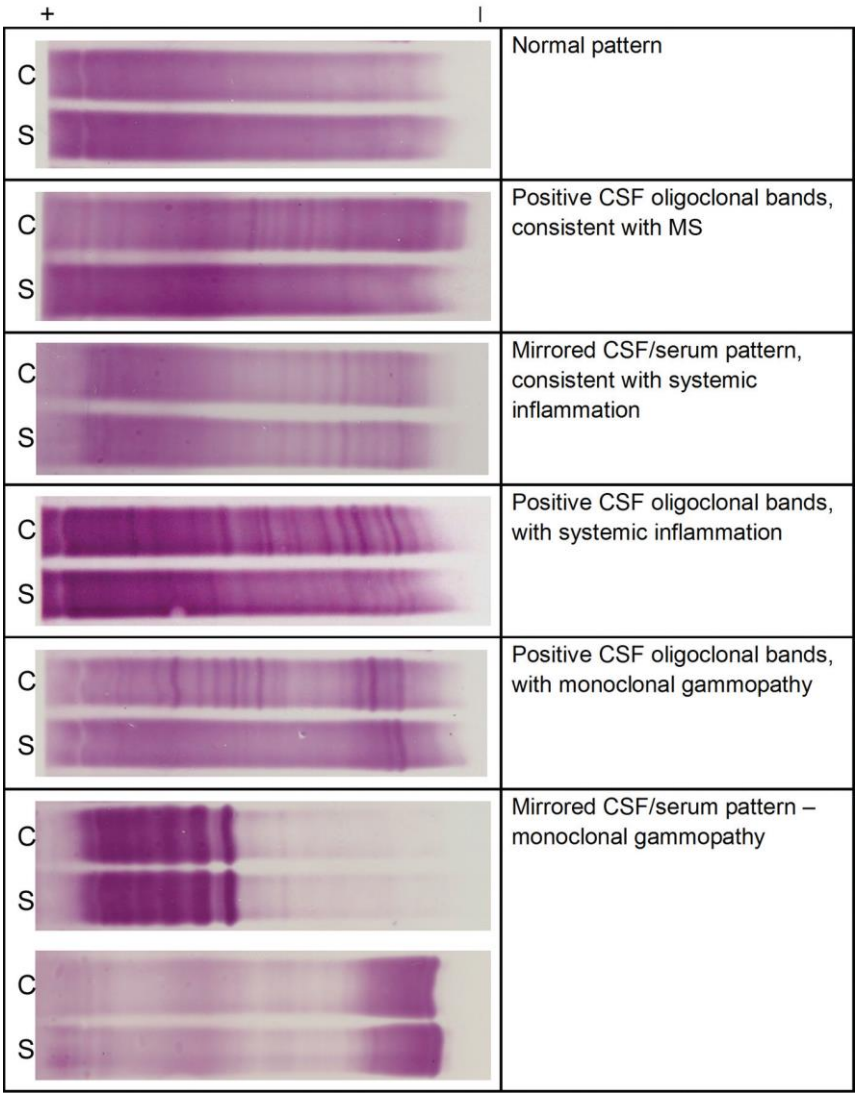


Figure 27: *examples of CSF and serum IEF: second, fourth and fifth examinations shows OCBs in CSF that are not visible in serum. Reproduced from: Chen Y, Laboratory Performance on Reporting Monoclonal Gammopathy During Cerebrospinal Fluid Oligoclonal Banding Analysis from External Quality Assessment Surveys, J Appl Lab Med, 2018.*

The first of these pattern (private OCBs present only in CSF, not visible in serum) represents the expression of intrathecal production of clones of immunoglobulins. The exact mechanisms behind their formation remain under investigation, but it is known that the presence of OCBs is the most sensitive marker of the production of immunoglobulins specifically and primarily inside the central nervous system (CNS).[86,87,89,90] This characteristic might give information about diagnosis and pathogenesis in different neurological conditions related to immune-mediated damage of the central nervous system. In the following sections, talking about OCBs we will focus on this specific case of OCBs found in CSF and absent in serum.
Investigating the pathophysiological significance of IgG and IgM OCBs provides insights into the mechanisms underlying neurological disorders. First of all, the presence of OCBs in CSF, whether IgG or IgM, suggests ongoing immune activation within the CNS. This underscores the autoimmune nature of many neurological disorders, as these antibodies are likely directed against specific neural antigens.[86,87,89,90] Secondly, OCBs have been associated with blood-brain barrier dysfunction, allowing immune cells and antibodies to enter the CNS. The interaction between OCBs and blood-brain barrier disruption plays a crucial role in the pathophysiology of different neurological conditions.[86,87,89,90] Finally, OCBs may directly contribute to nerve damage and inflammation within the CNS.[86,87,89,90]
The clinical relevance of IgG and IgM OCBs in CSF extends across various neurological disorders. They serve mainly as diagnostic biomarkers, particularly IgG OCBs in multiple sclerosis (MS), which are part of the recently revised McDonald's diagnostic criteria.[91] Studies are needed to clarify the prognostic value of OCBs and their potential role as target of personalized therapeutic approaches.

1.2.2 IgG and IgM OCBs in multiple sclerosis

Among all neurological diseases, the role of IgG and IgM OCBs in CSF was better elucidated for multiple sclerosis (MS). MS and its experimental models are the archetype for the study of the immune-mediated damage of the CNS, characterized by inflammation, demyelination and neurodegeneration with secondary axonal loss.

The presence of IgG OCBs in CSF is the typical liquoral hallmark of MS, beingfound in over 80% of MS patients.[92,93] This finding is so specific and relevant for diagnosis that it has been included among the internationally validated latest diagnostic criteria of 2017 (McDonald's revised).[91]
In MS, the presence of IgG OCBs in CSF represents the most sensitive and specific marker of local, intrathecal synthesis of antibodies, which is the most important phenomenon at the basis of autoimmune response.[92,93] The exact origin of these antibodies is stil debated, but as for other immune-mediated diseases, they souhld represent the activation and clonal expansion of mature B cells, differentiating in IgG-producing plasma cells, directly inside the CNS (**figure 28**).[92,93]
The antigens driving this response are still unknown, even though clinical observation and experimental models point at some major constituent of myelin sheat, such as myelin basic protein.[94,95] However, not all the antibodies produced in MS are directed against myelin. Furthermore, it is not clear why there is a oligo-clonal response instead of a single clonal antibody production. It is evident that the immune response is not entirely specific against one single antigen and the OCBs detectable in the CSF are the expression of this more widespread activation of the humoral immune response in the CNS, possibly with different pathogenetic mechanisms involved.[94-96] In any case, as for all IgGs, even those represented in OCBs are able to directly damage CNS structures by means of opsonization, complement activation and stimulation of cell-based immune response.[92,93]

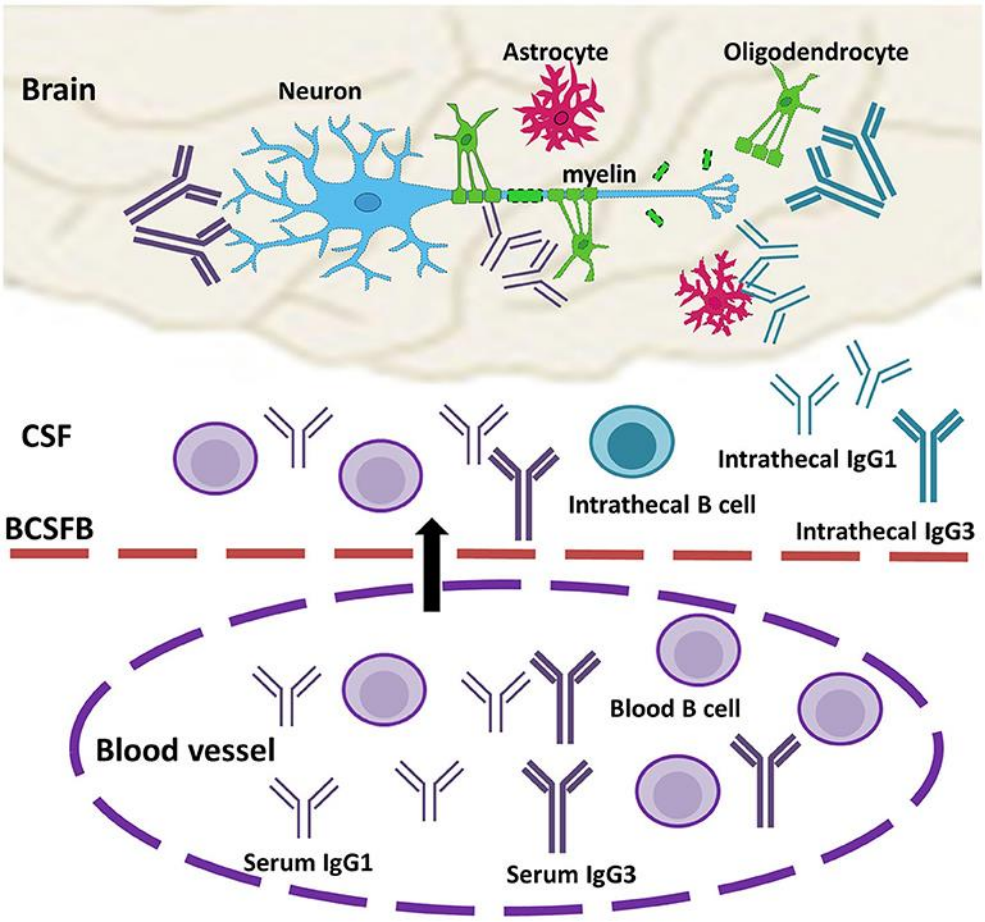


Figure 28: *schematic representation of production and targeting of IgG OCBs in CSF in MS. Reproduced from: Yu X et al, The Role of Antibodies in the Pathogenesis of Multiple Sclerosis, Front Neurol, 2020.*

IgG OCBs have been associated with Blood-Brain Barrier (BBB) dysfunction, leading to a higher and less specific permeability to molecules from blood stream, normally excluded from the CNS environment (i.e. pro-inflammatory cytokines, chemotactic agents and matrix metallo-proteases). In turn, this mechanism can lead to a much widespread and self-sustained activation of immune response, with more severe damage of nervous structures and of the BBB.[97] Togheter with a potential direct role of oligo-clonal IgGs in myelin or neural damage, this is one of the reasons why IgG OCBs are suspected to be directly involved as a powerful mediator of CNS damage in MS.

Although they are part of diagnostic criteria for MS, IgG OCBs are not specific for this disease. They must be always evaluated togheter with clinical and neuroradiological findings in order to interpretate correctly their diagnostic relevance. For instance, OCBs may be the marker of infections or other chronic neurodegenerative diseases of the CNS, even though a monoclonal response (i.e. a single clonal IgG band in CSF) is more characteristic of these conditions.[98,99]

Many studies have found an association between the number and persistence of OCBs in CSF and disease activity and progression in MS.[100-106] Patients with a high OCB count may experience a more severe clinical course with an increased risk of clinical relapses and a greater likelihood of disability progression (**figure 29, 30**).[100-106] In other words, the presence of OCBs can be interpreted as an indicator of ongoing intrathecal inflammation and immune activity.[100-106]

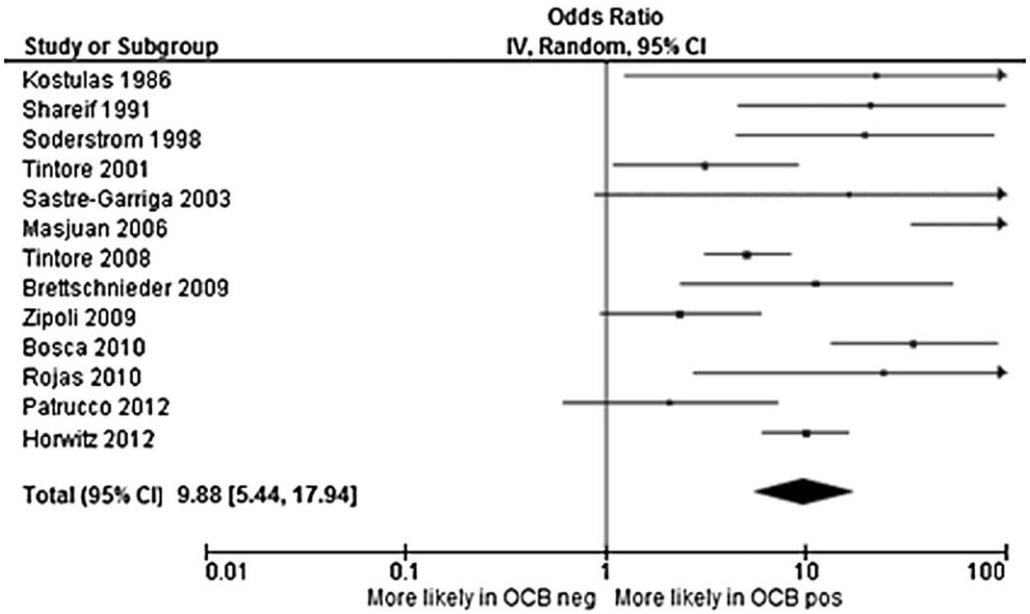


Figure 29: *metanalysis of the risk of progression from a clinically isolated syndrome to MS on the basis of IgG OCBs presence in CSF. Dobson R et al, Cerebrospinal fluid oligoclonal bands in multiple sclerosis and clinically isolated syndromes: a meta-analysis of prevalence, prognosis and effect of latitude, J Neurol Neurosurg Psychiatry, 2013.*

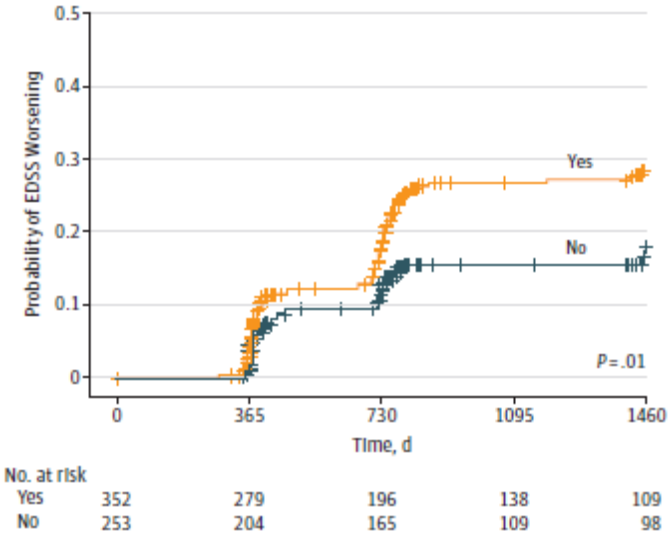


Figure 30: *Kaplan-Meier survival estimates of the risk of disability progression plotted against the presence or absence of IgG OCBs in CSF of MS patients. Reproduced from: Gasperi C et al, Association of Intrathecal Immunoglobulin G Synthesis With Disability Worsening in Multiple Sclerosis, JAMA Neurol, 2019.*

These profiles may help in the classification of MS patients into distinct disease subgroups, identifying those with a refractory or more rapid course. As a consequence, the presence, number or pattern change of OCBs in CSF could be a useful biomarker for monitoring disease activity and predict prognosis in MS.[100-106]

As a consequence, the presence of OCBs could influence therapeutic decisions, being a marker of actie inflammatory response and more aggressive disease course, prompting the clinician to precociously adopt advanced lines of treatment. Conversely, the disappearance of OCBs may suggest an effective treatment response. Finally, the identification of specific antigens recognized by IgG OCBs could be offer a target for tailored therapeutic approaches.[107]

While IgG OCBs role in MS has been widely elucidated by hundreds of studies during the last decades, much less is known about the presence and significance of IgM OCBs.
As for IgGs, even IgM OCBs are considered the most sensitive and specific hallmark of intrathecal IgM production, as the result of B cells and plasma cells activation directly inside the CNS.[108,109] Nevertheless, IgM and IgG OCBs are distinct and not always co-existing, representing therefore different pathways of immune activation.[108,109] IgM differs from IgG in many ways. From a structural point of view, in the extra-cellular space IgMs aggregate in big pentameric complexes. As a consequence, they are less prone to pass through membranes, such as the BBB, and they may represent a more sensitive marker of primarily intrathecal antibody production. Secondly, pentameric IgM tend to precipitate on surfaces, leading to a stronger complement activation and a more severe damages of structures as myelin, neurons or the BBB itself, depending on the specific antigen reaction. Finally, IgM production represents an earlier stage of antibody release from partially mature B cells, which will successively refine their production towards IgG class antibodies. Actually, IgMs may represent a less specific antibody response than that mediated by IgGs.[108]
For all these reasons, IgM OCBs have been recently evaluated as a possible marker of more widespread, severe and precoucious immune response inside the CNS, with a heavier burden of neural damage and worse BBB dysfunction.[110-116] Actually, some studies have foud a correlation between the presence of IgM OCBs in CSF and severity of disease in MS, with patients presenting with higher grades of disability and a more aggressive clinical course. Moreover, representing an initial phase of antobidy production during immune response, the presence of IgM OCBs resulted predictive of an increased risk of relapses. Finally, on the basis of their possibily higher damage potential against CNS structures, IgM OCBs were also evaluated as markers of secondary or primary progressive MS. Actually, a positive correlation between IgM OCBs in CSF and progressive forms of disease was confirmed, suggesting a role of these antibodies in determining the characteristic axonal loss seen in these patients.[110-116]

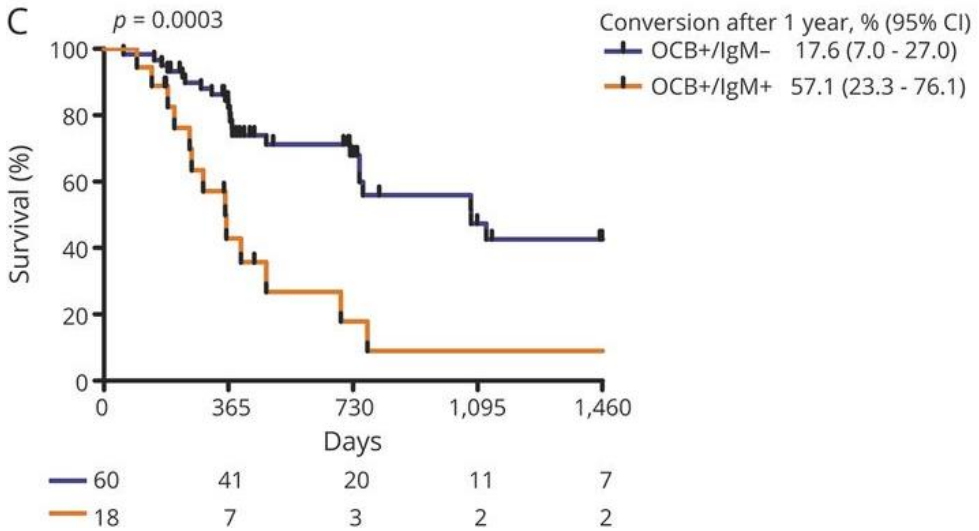


Figure 31: *Kaplan-Meier failure estimates of the risk of conversion from clinically isolated syndrome to MS plotted against the presence or absence of IgG OCBs in CSF. Reproduced from: Pfuhl C et al, Intrathecal IgM production is a strong risk factor for early conversion to multiple sclerosis, Neurology, 2019*

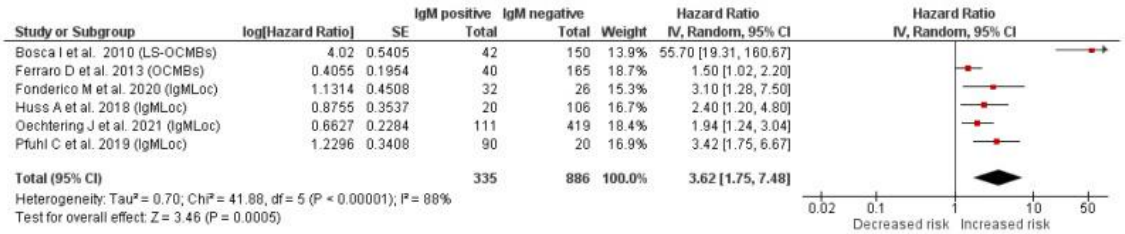


Figure 32: *metanalysis of the risk of a second clinical relapse and presence of IgM OCBs in CSF of MS patients. Reproduced from: Fonderico M et al, Cerebrospinal Fluid IgM and Oligoclonal IgG Bands in Multiple Sclerosis: A Meta-Analysis of Prevalence and Prognosis, Brain Sci, 2021.*

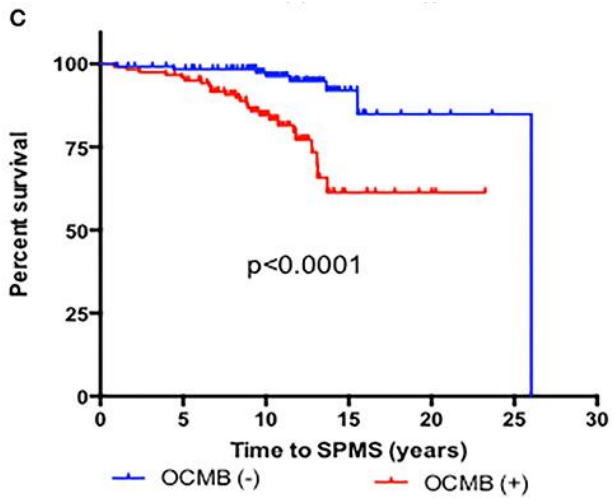


Figure 33: *Kaplan-Meier failure estimates of the risk of evolution to secondary progressive MS plotted against the presence or absence of IgG OCBs in CSF. Reproduced from: Alcalá Vicente Cet al, Oligoclonal M bands and cervical spinal cord lesions predict early secondary progressive multiple sclerosis, Front Neurol, 2022.*

The number of studies regarding IgM OCBs in MS is still quite low, mainly because of the relative technical difficulty in laboratory testing, that is more complex and time consuming in comparison with IgG OCBs.[117,118] A further limit of IgM OCBs search in standard clinical practice is the relatively little proportion of patients that actually have them in their CSF.[117,118] Therefore, all these promising findings about the potential prognostic role of IgM OCBs need confirmation in surveys on wider populations.[117,118] Moreover, little is known about the specific antigens against which IgM reacts and, as a consequence, about the potential role of such antigens as targets of tailored therapeutic approaches.

1.2.3 IgG and IgM OCBs in immune-mediated neuropathies, with particular reference to Guillain-Barré syndrome

Given the encouraging results found for multiple sclerosis, some researchers turned their attention to the potential presence and significance of OCBs in chronic and acute immune-mediated polyneuropathy, including GBS. Unfortunately, however, literature reports only a very restricted number of studies exploring this item, often conducted on small samples of patients. Overall, the presence of IgG or IgM OCBs in immune mediated neuropathies has never been reported in a convincing way. Therefore, up to date, all the described cases may represent the result of random association and no casual link between OCBs and the immune mediated pathogenesis of inflammatory polyneuropathies has been demonstrated yet. Here it follows a brief review of all the published studies evaluating the association between OCBs and neuropathies.

In their 2020 paper, Pannewitz-Makaj et al screened the CSF of 3622 patients with every kind of neurological diseases searching for clues of intrathecal production of IgG (i.e. Reiber index and IgG OCBs).[119] Among 470 patients affected by generic neuropathy, about 5% had CSF-restricted IgG OCBs, which is a way less frequent finding if compared with the expected proportion among MS patients (i.e. 80% or more).[119] Moreover, similar low frequencies were found for many of the other screened neurological conditions (**figure 34**).[119] Authors concluded that a low rate of IgG OCBs presence may be reported in many different neurological conditions, representing a non specific, casual finding among neurological patients.[119] However, it is likely that frequency for "neuropathies" could have been higher if observation were restricted to defined immune-mediated neuropathies, such as GBS or Chronic Immune Demyalinating Polyradiculoneuropathy (CIDP).

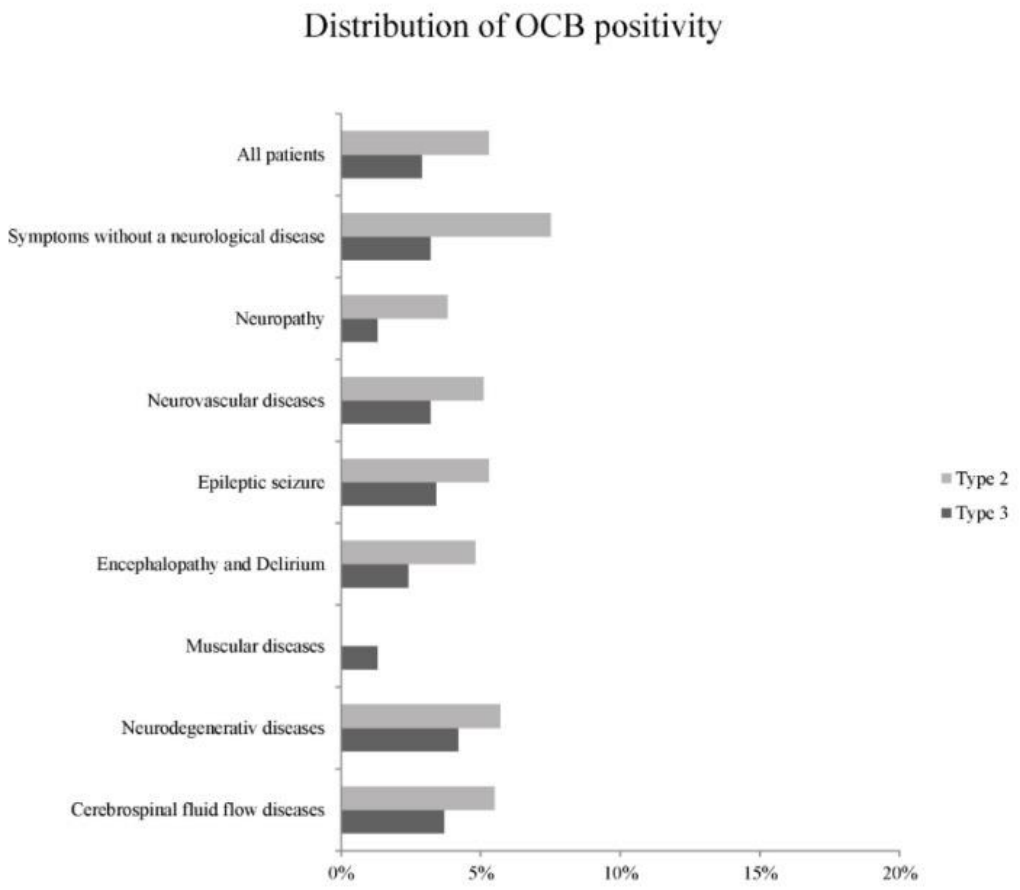


Figure 34: *proportion of patients with IgG OCBs in CSF in different neurological conditions. Reproduced from: Pannewitz-Makaj K et al,Evidence of Oligoclonal Bands Does Not Exclude Non-Inflammatory Neurological Diseases, Diagnostics (Basel), 2020.*

Similar findings, even though based on a much smaller sample of 17 patients, had already been observed by Grimaldi et al, in their 1986 study.[120] Among the 10 patients with MS, all presented IgG OCBs in CSF. On the contrary, only 3 of the other 7 patients without MS had IgG OCBs in CSF (43%), and one of these was diagnosed with GBS.[120]

In 2021, Ruiz et al evaluated the possibile intrathecal production of IgG in Chronic Immune Demyelinating Polyradiculoneuropathy (CIDP).[121] They recruited 48 patients with CIDP and screened them for presence of IgG OCBs in CSF and serum, other than calculating albumin and IgG crude concentrations and CSF/serum albumin ratio (Qalb). Obtained data were then compared with 32 patients with GBS, 18 with anti-myelin associated glycoprotein (MAG) antibody neuropathy, 4 with multifocal motor neuropathy (MMN) and 32 with non inflammatory neuropathies. Only one CIDP patient (about 2%) and none of the GBS patients

had IgG OCBs in CSF. Conversely, mirror pattern OCBs was present in 9 CIDP patients (19%) and 13 GBS patients (40%). Qalb was significantly higher among CIDP patients than those affected by non inflammatory neuropathies (p=0.0003). The Authors concluded that inflammatory neuropathies show a higher degree of BBB damage but no significant signs of intrathecal IgG production.[121] Similar results were found by Tu et al in their 2021 case-control study on CIDP and GBS (**figure 35**).[122]

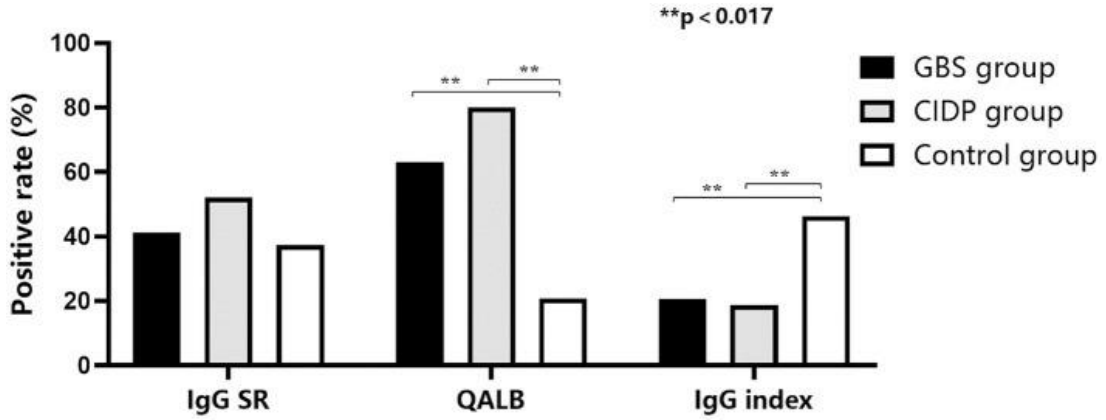


Figure 35: *proportion of patients with altered Qalb and IgG index in GBS group, CIDP group and control group. ** represents statistical significance. Reproduced from: Tu Yet al, The Correlation Among the Immunoglobulin G Synthesis Rate, IgG Index and Albumin Quotient in Guillain-Barré Syndrome and Chronic Inflammatory Demyelinating Polyradiculoneuropathy: A Retrospective Case-Control Study, Front Neurol, 2021.*

Another study from Segurado et al in 1986 showed a high prevalence of IgG OCBs in CSF (68% of GBS patients and 79% of CIDP patients), but these bands corresponded perfectly to those present in serum.[123] Therefore, the Authors interpreted this finding as a consequence of BBB damage with extra-intrathecal passage of serum-produced IgGs.[123] No mention was made in the paper about the presence of CSF-restricted IgG OCBs in their patients.[123]
In 1975 Link described the case of a woman with GBS and demonstrated the presence and long-term persistence of IgG OCBs in CSF, without correspondence in serum.[124]
However, sequent larger studies could not confirm such findings. In 1979, Siden et al found specular IgG OCBs in CSF and in serum of 17% of 27 screened patients with GBS.[125] Some years later, in 1981, Kruger et al obtained similar results testing with IEF the CSF and sera of 16 patients with GBS. Thirteen of them had IgG OCBs: 10 only in serum, 3 both in CSF and serum(19%), but none showed private intrathecal synthesis of IgG OCBs.[126]
In 1985, Vedeler et al found no CSF or serum IgG OCBs among a cohort of 80 GBS patients.[127]
Similarly, in their 1993 review of 146 patients, Zeman et al found 16 patients diagnosed with GBS: none of them had IgG OCBs in CSF.[128] In their 1987 review, Harrington et al concluded that in GBS the presence of IgG OCBs in CSF is transitory and represent the passage of antibodies from blood into CSF through a damaged BBB.[129] More recently (2006), the same conclusions were drawn by Mata et al in their study of CSF and serum of 73 GBS patients and 43 CIDP patients.[130] None of them had IgG OCBs in CSF.[130]

Only one study in literature explored the potential presence of IgM OCBs in the CSF of GBS patients. In 2016, Ferraro et al assessed the frequency of EBV-specific IgG and IgM OCBs in CSF of 50 patients with clinically isolated syndrome (CIS) and 27 controls affected by GBS.[131] Quite surprisingly, six GBS patients (22%) showed EBV-specific IgG OCBs in both CSF and serum ("mirror pattern") while 3 patients (16%) had EBV-specific IgM OCBs both in CSF and serum.[131] Analogue proportions were found in the CIS group of patients. No significant association with analyzed variables nor prognostic value was found at statistical analysis.[131]

1.3 Premises and rationale of the study

Guillain-Barrè syndrome (GBS) is a rare but potentially severe polyneuropathy with partially unknown pathogenesis and highly variable and unpredictable clinical course. Reliable and innovative biomarkers are needed to shed a light on pathological mechanisms, to help predicting the evolution of the disease in each patient and to guide treatment approach.
IgM oligoclonal bands (OCBs) in the cerebrospinal fluid (CSF) are a sensitive and specific marker of intrathecal antibody production. They may have a direct role in damaging the proximal structures of nerves (i.e. nerve roots) inside the subdural space and causing the malfunctioning of the blood-brain barrier (BBB), thus playing a potential role in maintaining the inflammatory response. While IgM OCBs have been well studied in diseases of the central nervous system (CNS), such as multiple sclerosis, there is no report in literature about their potential role as a biomarker in acute immune-mediated neuropathy such as GBS.

Given these premises, the present study aims to answer the following questions:

1. Are IgM OCBs present in the CSF of GBS patients?

2. If yes, do IgM OCBs identify a specific subgroup of GBS patients, identified by peculiar clinical and neurophysiological characteristics?

3. Do IgM OCBs in CSF associate with other markers of specific neural damage, thus supporting their potential role as direct players in GBS pathogenesis?

4. Are IgM OCBs in CSF a prognostic marker of outcome for GBS patients?

To find these answers, a cohort of GBS patients will be screened for the presence of IgM OCBs in CSF using a specific immunoelectroforetic test on CSF and serum.
Then, the potential presence of IgM OCBs will be related with different clinical, neurophysiological and laboratory variable in order to identify significant differences between the two groups of patients (i.e. with or without IgM OCBs) in terms of clinical characteristics and specific pathogenetic pathway.
Finally, the potential prognostic role of IgM OCBs will be evaluated by means of statistical measures of association (i.e. regression and survival analysis) with different outcomes specifically designed for GBS.

The following sections present in detail the criteria for patient selection, the adopted methods and the results of the study.

Section 2: patients and methods

2.1 Study design

The study has been designed as a mixed retrospective-prospective, partially blinded, multiphase cohort study. It started in November 2020 and ended in October 2023 and its development can be resumed by the following 4 steps:

1. Patient selection
2. Testing for the presence of IgM or IgG in CSF samples of included patients
3. Collection of clinical, neurophysiological, serological data as well as measures of outcome
4. Statistical analysis

For the first phase, we retrospectively evaluated the clinical charts and conclusive relations of all the neuromuscular patients admitted in the Neurology Service of the University Hospital of Modena from July 1st, 2006 to October 31st, 2020. In addition, the diagnostic database of patients admitted in the same Unit for the same time interval was queried in a cross search for the key words "Guillain", "Barrè", "Guillain Barrè", "Guillain-Barrè", "neuropathy", "polyneuropathy", "radiculopathy", "polyradiculopathy", "radiculoneuropathy", "polyradiculoneuropathy", "acute neuropathy", "acute polyneuropathy", "acute radiculopathy", "acute polyradiculopathy", "acute radiculoneuropathy", "acute polyradiculoneuropathy", "neuritis", "polyneuritis", "radiculitis", "polyradiculitis", "radiculoneuritis", "polyradiculoneuritis", "acute neuritis", "acute polyneuritis", "acute radiculitis", "acute polyradiculitis", "acute radiculoneuritis", "acute polyradiculoneuritis", "paralysis", "plegia", "acute paralysis", "acute plegia", "hypo/areflexia". We then evaluates each one of the more than 2000 clinical files identified by this research.
As a result, 186 patients with possible GBS diagnosis were retrospectively found. Thiry-three of them were excluded fromt the study because unfitting the selection criteria (see the next section for details about patient selection), while the remaining 153 were included, as a result of a retrospective search covering the time period between July 2006 and October 2020.
Since November 1st, 2020 until June 30th, 2022, all patients with a confirmed diagnosis of GBS admitted in the Neurology Service of the University Hospital of Modena were included prospectively in the study. Patients fitting inclusion criteria were 24. After enrollement, all of them were followed for a time of at least 12 months since the onset of symptoms.
At the end of the retrospective and prospective enrollement phase, the cohort of GBS patients considered for the study consisted of 187 patients. All these patients had a confirmed GBS diagnosis, fitted all inclusion criteria (see next section about patient selection) and were regularly followed for at least 12 months after the onset of neurological symptoms.

Between the beginning of 2021 and June 2023, the CSF of all the 187 patients included in the study were tested for the presence of IgM and IgG oligoclonal bands. All examinations were performed by the same, specialized Biologist in our Laboratory of Neuroimmunology (see further the section about laboratory testin for further details about the methodic). The Biologist was blinded towards the clinical and neurophysiological data of the patients as well as the performance of outcome measures during the follow up. The only known variables to the Biologist were name, sex, date of birth of the patients and time of collection of the CSF sample.
In parallel, the retrospective search and the prospective enrollement of GBS patients, the collection of clinical data as well as the clinical follow up of the prospectively enrolled patients, were all conducted by the same specialized neurologist. Even the neurologist was blinded towards the presence or absence of IgM OCBs in CSF in the included patients, in order to minimize the risk of biases in the clinical evaluation of outcome measures. Only after all clinical data were collected and all follow up time were concluded, the name of the IgM OCBs positive patients were unveiled to the evaluating Neurologist.

The second and third phases (i.e. CSF testing for IgM and IgG OCBs and data collection with clinical follow up) proceeded in parallel between the end of 2020 and June 2023. After all clnical data were collected and the one year follow time was concluded for all patients, the statistical analysis (fourth phase) could be performed, In the next sections, further details about clinical variables, outcome measures and statistical methods adopted are provided.

The study was approved by the locally competent Ethical Committee, with protocol number 22107/43/2022, SIRER ID 3853, last amendment and review September 6th, 2023.

Figure 36 shows a flow chart that schematically resumes the main phases of the study discussed in this section.

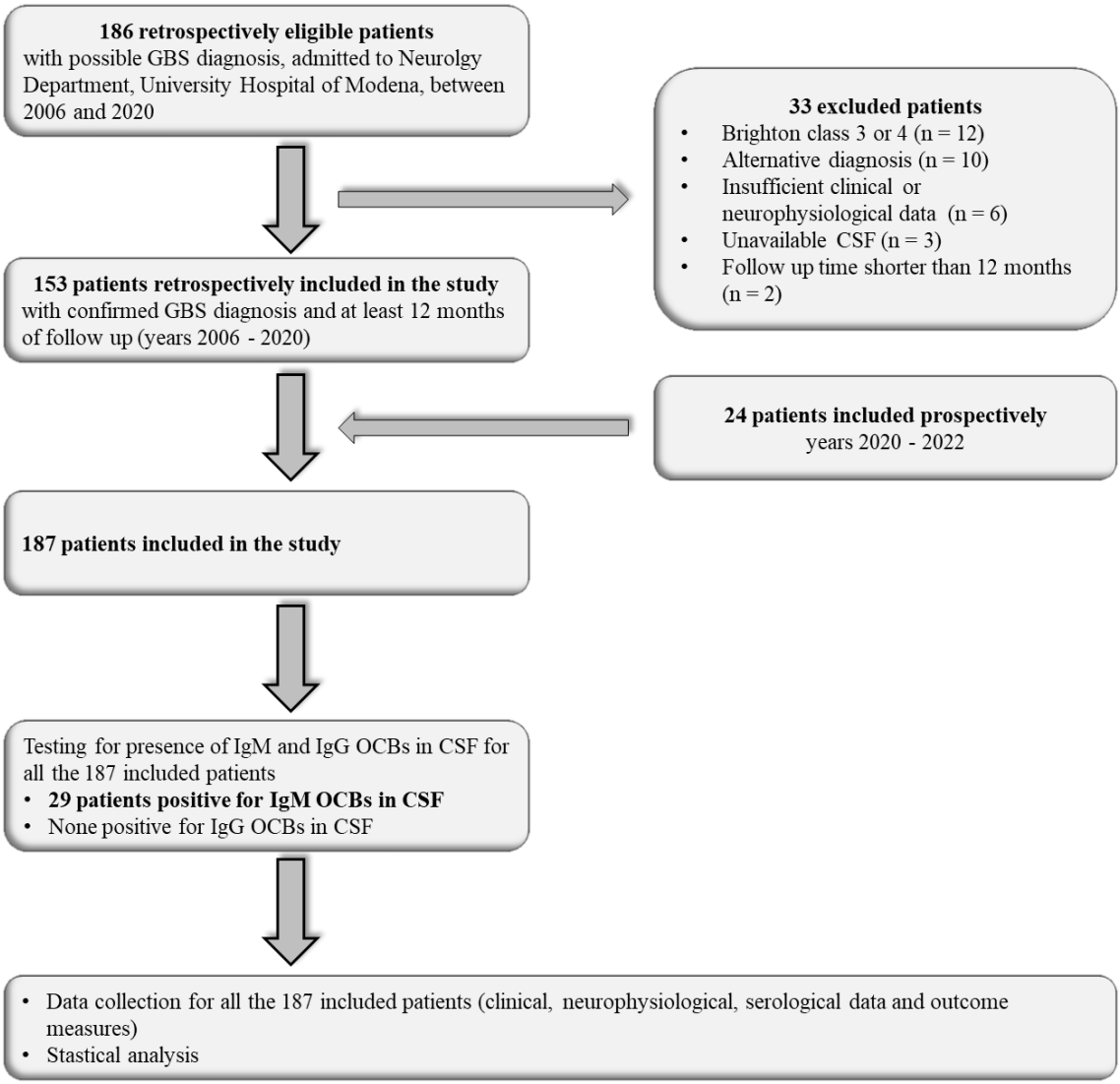


Figure 36: *design of the study.*

2.2 Patient selection

Table 1 resumes the inclusion criteria adopted in the study.

Inclusion criteria
Confirmed GBS diagnosis (class 1 or 2 of Brighton criteria)
Nerve Conduction Study during the acute phase of the disease
Lumbar puncture during the acute phase of the disease
Complete and available clinical documentation
Complete and available NCS
Available CSF sample for IgM OCBs testing
Regular neurological follow up of at least 12 months

Table 1: *inclusion criteria.*

In order to increase diagnostic specificity avoiding false positive results (i.e. presence of IgM OCBs in patients with different diseases than GBS), all patients must have met a high grade of diagnostic certainty to be included in our cohort. Therefore, all patients not fitting class 1 or 2 of Brighton criteria for GBS were excluded from the study (i.e. definite or probable diagnosis) (**table 2**).[32] Thus, it means that all of them must have:

- Bilateral and flaccid limb weakness
- Hypo/areflexia in the affected limbs
- Monophasic course and nadir time included between 12 hours and 28 days from the onset of symptoms
- Absence of alternative diagnosis for weakness

In addition, all of the included patients had at least one of the following instrumental support criteria:[32]

- Consistent NCS findings, or
- Albumin-cytological dissociation in CSF (i.e. high protein concentration -over 60 mg/dl- with normal cell count –lower than 50/ml-)

Of the 153 patients identified retrospectively, 12 were excluded because of low diagnostic certainty. Most of them (n = 9) had normal NCS study and no sing of albumin-cytological dissociation, other 2 had a pure sensory variant and one patient showed non treatment-related relapses during the first year of follow up.

	Levels of diagnostic certainty			
	1 (definite)	**2 (probable)**	**3 (possible)**	**4 (uncertain)**
Bilateral and flaccid limb weakness	+	+	+	+/-
Decreased or absent deep tendon reflexes in affected limbs	+	+	+	+/-
Monophasic course and time between onset and nadir included between 12 hours and 28 days	+	+	+	+/-
Absence of alernative diagnosis for weakness	+	+	+	+/-
CSF cell count <50 per ml	+	+/-*	-	+/-
CSF protein concentration >60 mg/dl	+	+/-*	-	+/-
NCS findings consistent with one of the subtypes of GBS	+	+/-*	-	+/-

Table 2: *Brighton criteria for diagnosis of GBS.*

A second criteria of exclusion from the study was the presence of an alternative diagnosis than GBS. Ten patients were excluded for this reason: in particular, 4 of them had neuroborreliosis confirmed with serological analysis on blood and CSF, other 4 showed a chronic remitting-relapsing disease course consistent with CIDP (i.e. Chronic Inflammatory Demyelinating Polyradiculoneuropathy), one was affected by a vasculitis of the peripheral nervous system and another patient had a subacute multineuropathy associated with systemic vasculitis (eosinophilic granulomatosis with angiitis er Churg-Strauss syndrome).

All patients included in the study must have sufficient clinical documentation to collect all the required clinical data, i.e. a complete medical chart, available and complete serological analysis on blood and CSF and a thoroughly evaluable NCS study. Again, to reduce bias selection, a complete screening for other possibile causes of acute neuropathy was necessary for study inclusion. At this regard, all patients must have undergone: clinical, serological and radiological screening for neoplastic and paraneoplastic diseases; serological analysis for borreliosis, HIV, siphilis, HBV and HCV-related hepatitis and other neurotrophic viruses; a complete serological and clinical evaluation for autoimmune diseases; clinical and serological testing for toxic, iatrogenic or drug-induced neuropathies. In particular, all included patients must have been serologically screened for Campilobacter Jejuni, Epstein Barr Virus (EBV), Mycoplasma Pneumoniae and CitoMegaloVirus (CMV). Four patients did not satisfy such premises, having only partial or equivocal available clinical data. Other 2 patients were excluded beacause of unavailable NCS examination. Three patients did not undergo lumbar puncture, so we had no informations about CSF nor sample left for search of IgM OCBs: as a consequence, they were not included in the cohort, as well.

Finally, but most importantly, all included patients must have been regularly evaluated for a follow up time not inferior than 12 months after the onset of symptoms. This means that all included patients have been evaluated with a neurological examination at least every 3 months,

including MRC sum score calculation and specific inquiring about disability and timing of recovery. Two patients were lost during the follow up and, therefore, excluded from the study.

In conclusion, we obtained a cohort of 187 patients representing a homogeneous and selected group with the following characteristics:

- High certainty of GBS diagnosis, with a typical clinical picture supported by at least one instrumental specific confirmation test (class 1 or 2 of Brighton criteria)
- Thorough screening for exclusion of alternative diagnosis
- Complete report of clinical, neurophysiological and laboratory data, available for consultation
- Regular neurological follow up for a minimum period of 12 months after the onset of symptoms

2.3 Evaluated variables

For every patient included in the study, a number of clinical, neurophysiological and laboratory independent variable was collected. Everyone of them was referred primarily to the whole cohort and then weighted between patient with or wihout IgM OCBs in CSF, in order to perform group comparisons. The main considered independent variables are resumed in **table 3.**

Clinical characteristics	Neurophysiology
Sex	
Female	Time from onset to first NCS study (days)
Male	Second NCS study
Age at onset of neuropathy	AIDP
Age ≥ 60 years	AMAN
Infective prodromes (overall)	AMSAN
Gastrointestinal	Axonal NCS
Upper airways	Abnormal F waves
Other	Prolonged F waves latency
Antecedent vaccinations	F waves absence
Gangliosides (overall)	Sensory involvement
GM1	
GD-GQ-GT	
Clinical involvement	**Laboratory**
Motor	Time from onset to lumbar puncture (days)
Hypo/areflexia	Albumin-cytological dissociation
Sensory	Protein level in CSF (mg/dl)
Bulbar	Cells in CSF (n/µl)
Ataxia	BBB damage index
Autonomic	Abnormal
Clinical phenotype	CSF IgG level (mg/dl)
Classic	Serum IgG level (mg/dl)
Pure motor	CSF albumin level (mg/dl)
Miller-Fisher syndrome	Serum albumin level (mg/dl)
Regional variants	Link index
Brighton diagnostic criteria	Abnormal
Class 1	Reiber index
Class 2	Abnormal
mEGOS at hospital admission	**Presence of IgM OCBs in CSF**
EGRIS at hospital admission	Presence of IgG OCBs in CSF
Time from onset to admission (days)	Presence of mirror pattern IgM
Treatment (overall)	Presence of mirror pattern IgG
IVIG	
PE	
Both therapies	
IgG monoclonal component in serum	
IgM monoclonal component in serum	

Table 3: *independent variables considered in the study.*

Clinical characteristics:

- Age of patient at the onset of neuropathy: treated both as a continuous and a dichotomic variable (i.e. <60 or ≥60 years of age)
- Sex: male or female
- Time from the onset of symptoms to hospital admission, in days
- Presence of prodromes in the 4 weeks before the onset of neuropathy, with further distinction between gastrointestinal (i.e. diarrhea), upper airways involvement or other infective prodromes
- Antecedent vaccinations in the 4 weeks before the onset of neuropathy
- Presence of anti-ganglioside antibodies, with further distinction between anti-GM1 antibodies or anti-GD/GQ/GT antibodies
- Copathologies, with particular reference to diabetes, autoimmune diseases, ematological or solid tumors, chronic respiratory insufficiency, chronic heart and vascular diseases, chronic kidney or liver diseases
- Presence of monoclonal IgM or IgG component in serum
- Type of clinical involvement at hospital admission: motor deficits, sensory deficits, absent or reduced deep tendon reflexes, cranial/bulbar involvement, ataxia, dysautonomia
- Clinical phenotype at hospital admission: classic, pure motor neuropathy, Miller-Fisher syndrome, regional variants (i.e. paraparetic variant, bilateral facial palsy, pharyngo-cervico-brachial variant)
- Class of Brighton diagnostic criteria at hospital admission (1 or 2)
- Modified Erasmus GBS Outcome Score (mEGOS) at hospital admission (**table 4**)[59]
- Erasmus GBS Respiratory Insufficiency Score (EGRIS) at hospital admission (**table 5**)[60]
- Underwent treatment, furtherly distinguished in intra-venous immune globulins (IVIG), plasmapheresis (PE) or both
- Follow up time, in months

mEGOS at hospital admission		
		Score
Age at onset		
	≤40	0
	41-60	1
	>60	2
Preceding diarrhea		
	Absent	0
	Present	1
MRC sum score		
	51-60	0
	41-50	2
	31-40	4
	≤30	6
Total score		**0 - 9**

Table 4: *modified Erasmus GBS Outcome Score (mEGOS).*

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

Table 5: *Erasmus GBS Respiratory Insufficiency Score (EGRIS).*

Neurophysiological study (all included patients underwent at least one Nerve Conduction Study –NCS- during the acute phase of the disease):

- Time from onset to performing of first NCS, in days
- Possible second NCS
- Neurophysiological classification of GBS: Acute Immune Demyelinating Polyradiculoneuropathy (AIDP), Acute Motor Axonal Neuropathy (AMAN) or Acute Motor-Sensory Axonal Neuropathy (AMSAN)
- Alteration of late muscular responses, or F-waves, considered as follows:
    - Overall alteration of F-waves latency or persistence in at least 2 nerves
    - Increase of F-waves minimal latency in at least 2 nerves
    - F-waves absence in at least 2 nerves

Further detail about the technical performance of NCS, the considered criteria and the used normal values are provided in the specific section below.

Cerebro-Spinal Fluid characteristics (all included patients underwent lumbar puncture in the acute phase of the disease with the analysis of the following parameters):

- Time from onset of neuropathy to performing of lumbar puncture, in days
- Number of cells per ml
- Protein concentration, in mg/dl
- Presence of the so-called "albumin-cytological dissociation", which consist of an elevation of protein concentration with normal number of cells per volume unit
- Concentration of albumin in serum and in CSF, in mg/dl
- Concentration of IgG in serum and in CSF, in mg/dl
- Blood-Brain-Barrier (BBB) damage index, i.e. the 100-fold ratio between the concentrations of albumin in CSF and in IgG:

BBB damage index = QAlb x 100 = [Alb(CSF)/Alb(serum)]x100

The value has been considered both as a continuous variable and a discrete variable, dichotomized as normal (<0.7) or abnormal (≥0.7)

- Indexes of intrathecal synthesis of IgG:

  Link index: QIgG/QAlb = [IgG(CSF)/IgG(serum)] / [Alb(CSF)/Alb(serum)]

  Reiber index or Antibody Index: QIgG(spec)/QIgG(total)

  Both parameters were evaluated as a continuous variable and a dichotomic variable (normal vs abnormal)
- Presence of IgG oligoclonal bands in CSF (with further specification about the presence of a mirror pattern between serum and CSF)
- Presence of IgG oligoclonal bands in CSF (with further specification about the presence of a mirror pattern between serum and CSF)

Further details about the laboratory test for IgM OCBs detection in CSF are provided in the specific section below.

2.4 Outcome measures

At every neurological evaluation during the follow up, every patient was screened for all the following outcome measures. The different outcomes were therefore considered in different time-point during the follow up and, for most of them, the time to the event was registered in order to perform survival analysis. **Table 6** resumes the considered outcome measures.

Outcome measures
Hospital admission
MRC scale
GB-DS
i-RODS
Unable to walk unaided
Nadir
MRC scale
GB-DS
i-RODS
Time to nadir (days)
Unable to walk unaided
Time to aided walking (days)
Bedridden patients
Time to bedridden (days)
12 months follow up
MRC scale
GB-DS
i-RODS
Unable to walk unaided
Recovery of unaided walking
Time to recovery of unaided walking (weeks)
Complete recovery
Time to complete recovery (weeks)
Death
Time (weeks)
Mechanical ventilation
Time (days)

Table 6: *outcome measures considered in the study.*

Hospital admission:

- Medical Research Council scale for muscle power (MRC scale), as shown in **table 7**: three district for the upper limb (arm abduction, elbow flexion, wrist extension) and for the lower limb (hip flexion, knee extension, ankle dorsiflexion) for each side of the body, with a 0-to-5 points score for each tested item. Total score ranges from 60 (normal muscle power in all tested districts) to 0 (complete tetraplegia).[132] MRC score has been treated as a continuous variable
- Guillain-Barrè Disability Scale (GB-DS), as shown in **table 8**: total score ranges from 0 (no disability) to 6 (death).[133] GB-DS has been treated as an ordinal variable
- Inflammatory Rasch-build Overall Disability Scale (i-RODS), as shown in **table 9**: it is a list of 24 daily activities scoring 0 to 2 points each (0 = impossible to perform; 1 = performed with difficulties; 2 = easy to perform). Total score ranges from 0 (maximal disability) to 48 (no disability)[134,135]
- Inability to walk unaided at hospital admission (i.e. neeed of any kind of support for deambulation or patient unable of walking at all). It is a dichotomic variable

At nadir (i.e. the timepoint with the most severe clinical involvement):

- Time from onset of neuropathy to nadir, in days
- MRC scale (as defined in the previous section), continuous variable
- GB-DS (as defined in the previous section), ordinal variable
- Inability to walk unaided (as defined in the previous section), dichotomic variable
- Time from onset of neuropathy to inability of walking unaided, in days
- Bedridden patient, i.e. the inability of the patient of walking even with assistance, so he/she is confined to bedTime from onset of neuropathy to inability of walking unaided
- Time from onset of neuropathy to bedridden patient, in days

At 12 months (i.e. the end of the minimum follow up time):

- MRC scale (as defined in the previous section), continuous variable
- GB-DS (as defined in the previous section), ordinal variable
- Inability to walk unaided (as defined in the previous section), dichotomic variable
- Complete recovery, defined as the complete absence of all neuropathic symptoms with recovery of walking, running and climbing stairs without assistance
- Time from the onset of neuropathy to complete recovery, in weeks

For all patients, two more outcome were considered:

- Need of invasive mechanical ventilation during the acute phase of the disease, with time from the onset of neuropathy to intubation, in days
- Death in consequence of GBS during the first 6 months of disease, with time from the onset of neuropathy to intubation, in weeks

Finally, we condered separately the group of patients who lost the ability to walk unaided during the acute phase of the disease (146 patients). In this smaller subcohort, another more specific outcome measure was considered for the analysis:

- Recovery of the ability of walking without any assistance, with time from the onset of neuropathy to the event, in weeks

MRC sum score

Movement tested on each side	**Score for each movement**
Arm abduction	0 = no movement
Elbow flexion	1 = flicker of movement
Wrist extension	2 = movement with gravity eliminated
	3 = movement against gravity
Hip flexion	4 = movement against resistance
Knee extension	5 = normal power
Ankle dorsiflexion	
Total score	**0 - 60**

Table 7: *Medical Research Council score for muscle strenght.*

Guillain-Barrè disability score (GB-DS)

Patient condition	Score
Healthy	0
Minor symptoms or signs of neuropathy, but able of manual working and running	1
Able to walk without support of a stick (5 meters across an open space) but unable of manual working or running	2
Able to walk with a support (5 meters across an open space)	3
Chairbound or bedridden	4
Requiring assisted ventilation	5
Death	6

Table 8: *GBS Disability Score (GB-DS).*

	Activity	Impossible to perform = 0	Performed with difficulty = 1	Easy to perform = 2
1	Reading	0 points	1 point	2 points
2	Eating			
3	Brushing teeth			
4	Washing upper body			
5	Sitting on the toilet			
6	Making a sandwich			
7	Dressing upper body			
8	Washing lower body			
9	Moving a chair			
10	Turning a key in the lock			
11	Going to the general pratictioner			
12	Having a shower			
13	Doing dishes			
14	Doing shopping			
15	Catching an object (i.e. a ball)			
16	Bending and picking up an object			
17	Walking one flight of stairs			
18	Travelling by public transport			
19	Avoiding obstacles while walking			
20	Walking up to 1 Km outside			
21	Carrying a heavy object (about 10 Kg)			
22	Dancing			
23	Standing for hours			
24	Running			
	Total score		**0 - 48**	

Table 9: *inflammatory Rasch-build Overall Disability Scale (i-RODS).*

2.5 NCS study

All included patients underwent complete Nerve Conduction Study (NCS) in the acute phase of the disease. The complete report of each examination, including raw data and traces, was reviewed by a specialized and trained neurophysiologist. In case that findings at first examination resulted equivocal or inconclusive, a second study was performed after 2-4 weeks. Overall, all patients had abnormal findings at NCS, consistent with GBS diagnosis. All NCS examinations were performed at Neurohpysiology Lab of the Neurology Unit, University Hospital of Modena, using a Dantec Keypoint© G4 electromyograph. Single-used, pre-gelled surface electrodes 15x20 mm by SEI inc. were used for surface recording. If sensitive potentials were absent at surface recording, a second assessment with monopolar needle electrodes was tried in all patients.

As internal protocol, the following nerves were examined for all patients: bilateral sural, bilateral tibial, bilateral peroneal, unilateral radial, at least one among unilateral median or ulnar (**table 10**). More specifically, the following data were collected for al patients:

- Sensory Action Potentials (SAPs): bilateral sural nerves, at least one radial nerve and at least one among median or ulnar nerve
- Compound Motor Action Potentials (cMAPs): bilateral tibial nerves, bilateral peroneal nerves, at least one among median or ulnar nerve
- Late Muscle Response or F-waves: bilateral tibial nerves, bilateral peroneal nerves, at least one among median or ulnar nerve

Overall, for all patients it was possible to collect at least 4 SAPs, 5 cMAPS and 5 F-waves from different bilateral nerves of both lower and upper limbs.

Stimulation parameters and recording techniques followed strictly the published guidelines and recommendations from the most important International Scientific Societies of Neurophysyology (International Federation of Clinical Neurophysiology, IFCN; American Association of Neuromuscular and Electrodiagnostic Medicine, AANEM; European Academy of Neurology/Peripheral Nerve Society, EAN/PNS).[136-139] In particular:

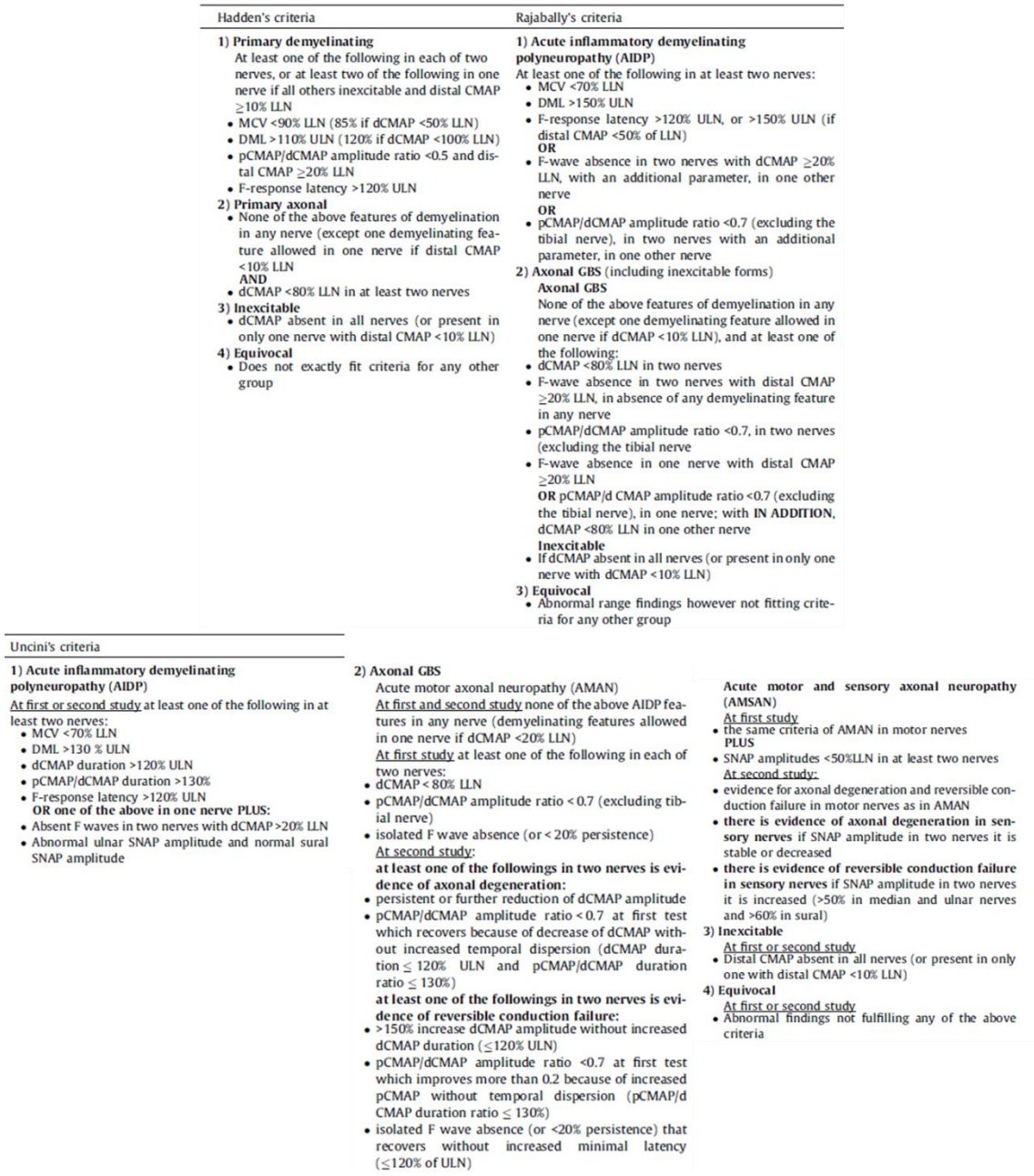
- Each nerve was stimulated progressively up to supramaximal current intensity and at least two superimposable responses were obtained and recorded
- The following parameters were measured for SAPs: distal latency at first deviation from the baseline or at first positive peak, amplitude from baseline to peak, duration from onset to return to baseline, sensory conduction velocity (SCV) in m/s
- Each cMAP was recorded at the most distal stimulating site and at least at one proximal stimulating site, in order to find possible motor conduction blocks
- The following parameters were measured for cMAPs: distal latency at negative onset, negative peak amplitude, duration from onset to return to baseline of the last negative peak, motor conduction velocity (MCV) in m/s, reduction or amplitude or area of cMAP between proximal and distal stimulation in percentage, increase in cMAP duration between proximal and distal stimulation in percentage
- The following parameters were measured for F-waves: persistence (i.e. the ratio between the number of obtained responses over the number of given stimuli, in percentage), minimal latency (at least two reproducible onset points)
- All tests were conducted in a noise-screened and safe laboratory, with controlled skin temperature and after accurately cleaning body surface at the recording sites

The obtained parameters for SAPs, cMAPs and F-waves were then compared with internally validated normal values, normalized for age, sex and height of patients.

Nerve Conduction Study (NCS)		
Motor conduction study (at least two stimulation points: distal and proximal)	Bilateral tibial nerves, bilateral peroneal nerves, at least one of median/ulnar nerve	Distal latency Negative amplitude Negative duration Negative area Motor conduction velocity Proximal/distal area reduction Proximal/distal duration increase
F waves	The same as for motor conduction studies	Minimal latency Persistence
Sensory conduction study	Bilateral sural nerves, radial unilateral nerve, at least one of median/ulnar nerve	Distal latency Peak-peak amplitude Negative duration Sensory conduction velocity

Table 10: *Nerve Conduction Study (NCS) protocol.*

All recorded examination were reviewed and evaluated applying the validated neurophysiological criteria for GBS, as proposed and publiseh by Uncini and Kuwabara in 2018 (**Figure 37**).[38] Such criteria were chosen because, although more complex, they showed higher sensitivity and specificity than previously reported critieria from Hadden and Rajabally, with greater reliability in classifying GBS patients on the basis of the neurophysiological nature of neural damage (i.e. demyelinating vs axonal).[38] In fact, on the basis of NCS study, it was possible to classify all patients included in our cohort among one of the proposed neurophysiological cathegories: Acute Inflammatory Demielinating Polyradiculoneuropathy (AIDP), Acute Motor Axonal Neuropathy (AMAN) or Acute Motor-Sensory Axonal Neuropathy (AMSAN).[38]



Hadden's criteria

1) Primary demyelinating
At least one of the following in each of two nerves, or at least two of the following in one nerve if all others inexcitable and distal CMAP ≥10% LLN
- MCV <90% LLN (85% if dCMAP <50% LLN)
- DML >110% ULN (120% if dCMAP <100% LLN)
- pCMAP/dCMAP amplitude ratio <0.5 and distal CMAP ≥20% LLN
- F-response latency >120% ULN

2) Primary axonal
- None of the above features of demyelination in any nerve (except one demyelinating feature allowed in one nerve if distal CMAP <10% LLN
  AND
- dCMAP <80% LLN in at least two nerves

3) Inexcitable
- dCMAP absent in all nerves (or present in only one nerve with distal CMAP <10% LLN)

4) Equivocal
- Does not exactly fit criteria for any other group

Rajabally's criteria

1) Acute inflammatory demyelinating polyneuropathy (AIDP)
At least one of the following in at least two nerves:
- MCV <70% LLN
- DML >150% ULN
- F-response latency >120% ULN, or >150% ULN (if distal CMAP <50% of LLN)
  OR
- F-wave absence in two nerves with dCMAP ≥20% LLN, with an additional parameter, in one other nerve
  OR
- pCMAP/dCMAP amplitude ratio <0.7 (excluding the tibial nerve), in two nerves with an additional parameter, in one other nerve

2) Axonal GBS (including inexcitable forms)
Axonal GBS
None of the above features of demyelination in any nerve (except one demyelinating feature allowed in one nerve if dCMAP <10% LLN), and at least one of the following:
- dCMAP <80% LLN in two nerves
- F-wave absence in two nerves with distal CMAP ≥20% LLN, in absence of any demyelinating feature in any nerve
- pCMAP/dCMAP amplitude ratio <0.7, in two nerves (excluding the tibial nerve
- F-wave absence in one nerve with distal CMAP ≥20% LLN
  OR pCMAP/d CMAP amplitude ratio <0.7 (excluding the tibial nerve), in one nerve; with **IN ADDITION**, dCMAP <80% LLN in one other nerve

Inexcitable
- If dCMAP absent in all nerves (or present in only one nerve with dCMAP <10% LLN)

3) Equivocal
- Abnormal range findings however not fitting criteria for any other group

Uncini's criteria

1) Acute inflammatory demyelinating polyneuropathy (AIDP)
At first or second study at least one of the following in at least two nerves:
- MCV <70% LLN
- DML >130 % ULN
- dCMAP duration >120% ULN
- pCMAP/dCMAP duration >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

2) Axonal GBS
Acute motor axonal neuropathy (AMAN)
At first and second study none of the above AIDP features in any nerve (demyelinating features allowed in one nerve if dCMAP <20% LLN)
At first study at least one of the following in each of two nerves:
- dCMAP < 80% LLN
- pCMAP/dCMAP amplitude ratio < 0.7 (excluding tibial nerve)
- isolated F wave absence (or < 20% persistence)

At second study:
at least one of the followings in two nerves is evidence of axonal degeneration:
- persistent or further reduction of dCMAP amplitude
- pCMAP/dCMAP amplitude ratio < 0.7 at first test which recovers because of decrease of dCMAP without increased temporal dispersion (dCMAP duration ≤ 120% ULN and pCMAP/dCMAP duration ratio ≤ 130%)

at least one of the followings in two nerves is evidence of reversible conduction failure:
- >150% increase dCMAP amplitude without increased dCMAP duration (≤120% ULN)
- pCMAP/dCMAP amplitude ratio <0.7 at first test which improves more than 0.2 because of increased pCMAP without temporal dispersion (pCMAP/d CMAP duration ratio ≤ 130%)
- isolated F wave absence (or <20% persistence) that recovers without increased minimal latency (≤120% of ULN)

Acute motor and sensory axonal neuropathy (AMSAN)
At first study
- the same criteria of AMAN in motor nerves

PLUS
- SNAP amplitudes <50%LLN in at least two nerves

At second study:
- evidence for axonal degeneration and reversible conduction failure in motor nerves as in AMAN
- **there is evidence of axonal degeneration in sensory nerves** if SNAP amplitude in two nerves it is stable or decreased
- **there is evidence of reversible conduction failure in sensory nerves** if SNAP amplitude in two nerves it is increased (>50% in median and ulnar nerves and >60% in sural)

3) Inexcitable
At first or second study
- Distal CMAP absent in all nerves (or present in only one with distal CMAP <10% LLN)

4) Equivocal
At first or second study
- Abnormal findings not fulfilling any of the above criteria

Figure 37: *the different neurophysyiological subsets of criteria proposed for GBS diagnosis: Hadden's criteria (2006), Rajabally's criteria (2014), Uncini's criteria (2018). Reproduced from: Uncini A et al, The electrodiagnosis of Guillain-Barré syndrome subtypes: Where do we stand? Clin Neurophysiol, 2018.*

In addition, for the purpose of our study, we specifically considered the following parameters about F-waves, in order to distinguish a demyelinating or an axonal damage of nerve roots:

- Increase >120% of the Upper Limit of Normal (ULN) of minimal latency in at least two different nerves with distal cMAP amplitude >20% of the Lower Limit of Normal (LLN), suggesting primary demyelinating damage of nerve root
- F-waves absence or persistence <20% in at least two different nerves with distal cMAP amplitude >20% of LLN, suggesting primary axonal damage of nerve root

2.6 IgM OCBs detection

For the detection of IgM OCBs in CSF we used the method described and validated by Villar et al in 2001 and replied by Ferraro et al in 2013.[140,141]

All CSF and serum samples were stored at -80 Celsius degrees before the analysis. Serum samples were diluted 1:800 with saline solution, in order to make IgM concentration comparable between serum and normal CSF. Both diluted serum and CSF samples were then incubated in a final 50 mM concentration of 1,4-DiThioTheritol (DTT) (Merck) and 0.1 M of tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 9.5) at room temperature for 30 minutes.

Agarose-based gel for isoelectrofocusing (IEF) was home-prepared mixing 3.6 g of sorbitol (Merck), 0.3 of agarose IEF (Amersham-Pharmacia) and 2.5 ml of Pharmalyte pH 5-8 (Amersham-Pharmacia) in 25 ml of distilled water. The resulting gel was cast in a small plate of 225 x 110 x 1.5 mm, which was stored in a damp chamber at 4 Celsius degrees for at least 2 hours before running the IEF. The gel production process was formerly described and validated by Keir et al in 1990.[142]

IEF was performed using a Multiphor II apparatus by Amersham-Pharmacia, previously cooled down to 10 Celius degrees. Electrode strips, dimensioned 10x200 mm, were soaked with 1 M solution of sodium hydroxide (NaOH) for the catholyte and 0.05 M of sulfuric acid (H_2SO_4) for the anolyte. For each run, five samples of 1 µl each were aligned at the anodic side of the IEF gel, using a sample application foil (Amersham-Pharmacia). IEF was run at 5 Watts for 30 minutes and then at 10 Watts until focusing was completed after 1500 Volts hours. Voltage was limited at 1275 Volts. The whole run took about 90 minutes.

After IEF, proteins were transferred to a PolyVinyldene DiFluoride (PVDF) membrane, or Millipore. Before transferring, Millipore membrane was previously wetted in methanol, than repeatedly washed in three baths of distilled water for a total time of 30 minutes. Water in excess was removed by gently blotting, and finally the membrane was placed on the gel surface. A sheet of damp filter paper was then layed on the Millipore membrane, followed by 25 layers of vry filter paper. After that, a 2 Kg weight was applied for 20 minutes to allow protein transfer from IEF gel to the PVDF membrane.

Sequently, Millipore membrane was removed and blocked in a solution of 2% dried milk in saline serum for 30 minutes. Finally, the membrane was incubated with polyclonal specific anti-human IgM antibodies (Dako), previously diluted 1:5000 in 0.2% dry milk in saline solution for 2 hours at room temperature on a platform shaker. The obtained membrane was washed 25 times with tap water and once with 0.85% saline solution for 10 minutes. At last, it was stained with Nitro Blue Tetrazolium (NBT) and Bromo-ChloroIndoleyl Phosphate (BCIP). The possible results of the analysis are shown in **figure 38**. The presence of IgM OCBs (at least two) was visually assessed by two blinded, expert neurologists.

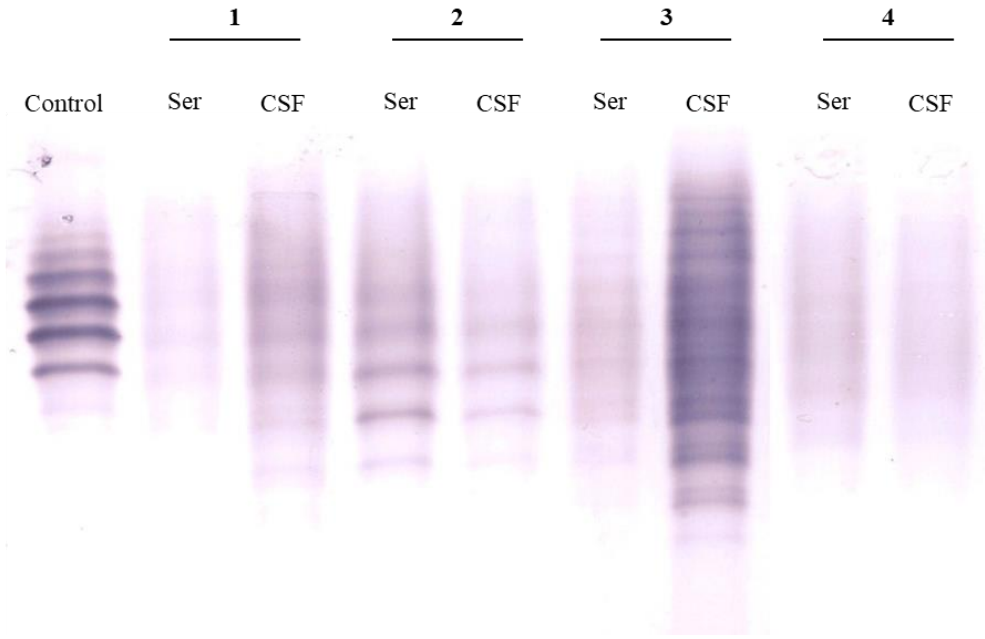


Figure 38: *IEF samples of paired serum and CSF of four patients with GBS. 1: presence of IgM OCBs in CSF. 2: IgM mirror pattern (i.e. coincident polyclonal IgM bands in serum and in CSF). 3: presence of IgM OCBs in CSF plus mirror pattern IgM. 4: absence of IgM bands. First column on the left: positive control.*

Compared to other described methods for IgM OCBs detection, the one proposed by Villar et al. is way more sensitive and reproducible thanks manily to three factors: the alkalin reduction buffer with DTT instead of distilled water, the narrower pH (5-8) and the elimination of the secondary antibody from the immunodotection stage. This way, this test avoids the cross-reaction with IgGs and improves sensitivity up to 20 ng of IgM per ml of sample. The banding pattern was clear and reproducible up to an IgM concentration of 0.4 µg/ml.

2.7 Statistical analysis

All the variables listed before were collected to fill in a Microsoft Excel© database with over 18.000 entries. No imputation was needed because no missing value was detected. Further analysis and graphs were performed using the statistical software Stata© Special Edition, version 14.2, from StataCorp LP, 4905 Lakeway Drive, College Station, Texas 77845 USA.

First of all, the characteristics of the whole cohort of patients and of the two main subgroups (IgM OCBs present vs IgM OCBs absent) were analysed using descriptive statistics:

- median and range for continuous non-parametric variables, mean ± standard deviation for continuous parametric variables
- Frequencies and percentages in two-ways tables for dichotomic variables
- Distribution, frequencies and percentages for ordinal variables

Secondly, a group comparison between IgM OCBs positive or negative patients was performed by means of:

- Mann-Whitney U-test for continuous and ordinal variables for independent samples
- Wilcoxon signed-rank test for continuous and ordinal variables for dependent samples
- Mantel-Haenszel chi-squared exact test for dichotomic variables

The third level of analysis consisted of regression analysis between dependent and independent (presence of IgM OCBs) variables:

- Linear univariate and multivariate regressions for continuous variables (reporting Coefficient, 95% Confidence Interval and P-value)
- Logistic univariate and multivariate regressions for dichotomic variables (reporting Odds Ratio, 95% Confidence Interval and P-value)
- Ordered logisitic univariate and multivariate regressions for ordinal variables (reporting Coefficient, 95% Confidence Interval and P-value)

Univariate regression analysis were conducted for all the independent variables for all the considered outcome measures. Multivariate regression analysis were performed for all the considered outcome measures including all the variables significantly related to the outcome plus the presence of IgM OCBs, excluding collinear variables.

The fourth step was survival analysis with univariate and multivariate Cox regression was performed for the five time-locked outcomes: inability to walk unaided, bedridden patient, complete recovery, death, mechanical ventilation and recovery of unaided walking (subgroup analysis of 146 patients, as explained before). All survival analysis reported Hazard Ratios, 95% Confidence Interval and P-value). Univariate Cox regression analysis were conducted for all the independent variables for all the considered outcome measures. Multivariate Cox regression analysis were performed for all the considered outcome measures including all the variables significantly related to the outcome plus the presence of IgM OCBs, excluding collinear variables. Results were reported graphically by means of Kaplan-Meyer survival or failure estimates.

For all the listed analysis, a two-sided P-value <0.05 was considered for significance, except for multivariate regression analysis. In this case, after Bonferroni's correction for different degrees of freedom, P-value <0.01 was considered significant.

Finally, the performance of IgM OCBs as predictor of the different outcomes was tested by means of the Receiver Operating Characteristic (ROC) curve, reporting Area Under the Curve (AUC) in comparison with other independent variables.

Section 3: results

3.1 Descriptive analysis of the whole sample

The following section provides an overview of clinical characteristic and outcome measures about the whole sample of patients.

3.1.1 Demographic, clinical, neurophysiological and CSF characteristics – whole sample

One hundred and eighty seven patients were included in the study. Demographic, clinical, neurophysiology and laboratory characteristics of the whole sample are reported in **table 11**.

Clinical characteristics	
Sex	
Female	69 (37%)
Male	118 (63%)
Age at onset of neuropathy	62 (45-74)
Age ≥ 60 years	99 (53%)
Infective prodromes (overall)	112 (60%)
Gastrointestinal	48 (26%)
Upper airways	49 (26%)
Other	15 (8%)
Antecedent vaccinations	9 (5%)
Gangliosides (overall)	52 (28%)
GM1	27 (14%)
GD-GQ-GT	32 (17%)
Clinical involvement	
Motor	187 (100%)
Hypo/areflexia	187 (100%)
Sensory	146 (78%)
Bulbar	73 (39%)
Ataxia	35 (19%)
Autonomic	36 (19%)
Clinical phenotype	
Classic	115 (62%)
Pure motor	27 (14%)
Miller-Fisher syndrome	21 (11%)
Regional variants	24 (13%)
Brighton diagnostic criteria	
Class 1	151 (81%)
Class 2	36 (19%)
mEGOS at hospital admission	2 (1-4)
EGRIS at hospital admission	2 (2-3)
Time from onset to admission (days)	2 (1-3)
Treatment (overall)	183 (98%)
IVIG	182 (97%)
PE	33 (18%)
Both therapies	32 (17%)
IgG monoclonal component in serum	9 (5%)
IgM monoclonal component in serum	0 (0%)

Neurophysiology	
Time from onset to first NCS study (days)	7 (5-10)
Second NCS study	121 (65%)
AIDP	152 (81%)
AMAN	26 (14%)
AMSAN	9 (5%)
Axonal NCS	35 (19%)
Abnormal F waves	106 (57%)
Prolonged F waves latency	68 (36%)
F waves absence	38 (20%)
Sensory involvement	74 (40%)

Laboratory	
Time from onset to lumbar puncture (days)	8 (4-15)
Albumin-cytological dissociation	151 (81%)
Protein level in CSF (mg/dl)	79 (55-130)
Cells in CSF (n/μl)	2 (1-3)
BBB damage index	1.1 (0.6-1.8)
Abnormal	132 (71%)
CSF IgG level (mg/dl)	6.6 (3.8-11.8)
Serum IgG level (mg/dl)	1060 (885-1340)
CSF albumin level (mg/dl)	40.7 (23.4-61.6)
Serum albumin level (mg/dl)	3976 (3521-4344)
Link index	0.6 (0.5-0.7)
Abnormal	49 (26%)
Reiber index	-1.2 (-3.6--0.5)
Abnormal	19 (10%)
Presence of IgM OCBs in CSF	**29 (16%)**
Presence of IgG OCBs in CSF	0 (0%)
Presence of mirror pattern IgM	90 (48%)
Presence of mirror pattern IgG	113 (60%)

Table 11: *characteristics of the whole sample. Results are expressed as absolute frequency (%) for dichotomic variables and as median value (IQR) for continuous variables.*

Of the 187 considered patients, 183 (98%) were of White Caucasian ethnicity, while only 3 were Black Africans and 1 was Chinese Asiatic. Sixty-three percent of patients were male, with M:F ratio of 1.7:1. The median age at onset of neuropathy was 62 years, ranging from 14 to 92 years. The median time from the onset of symptoms and hospital admission was 2 days (ranging from 1 to 7 days). For all patients, hospital admission was coincident with the first presentation of the patient to a neurology consultant evaluation in Emergency Room.

Infective prodromes were present in 60% of patients, mostly represented by gastrointestinal symptoms (i.e. gastroenteritis with diarrhea and/or vomiting) or upper airways infections (i.e. cold, flu, cough with expectorate, bronchitis). Serological analysis was positive for IgM anti-Campilobacter Jejuni in 19 patients (10%), Epstein Barr Virus (EBV) in 8 patients (4%), CitoMegaloVirus (CMV) in 5 patients (3%) and Mycoplasma Pneumoniae in 3 patients (2%).
In 9 patients (5%) the onset of GBS was preceeded by a vaccination in the previous 4 weeks. In these cases, the target of vaccinations were seasonal flu viruses (5 patients), streptococcus pneumonia (3 patients) and varicella-zoster virus (1 patient). None of the patients included in the study manifested GBS symptoms in close proximity (i.e. 4 weeks before) to anti-Sars-CoV-2 vaccination, while 3 of them resulted positive to PCR testing for Sars-CoV-2 during the acute phase of GBS. Subgroup nalysis did not show any significant difference between these patients and Sars-CoV-2 negative patients in terms of anagraphical, clinical, neurophysiological and laboratory characteristics nor outcomes.

Fifty-two patients (28%) were positive for some anti-gangliosides antibody. About a half of them (14% of the whole sample) were found positive for anti-GM1 antibodies. No patient had a monoclonal IgM component in serum.

Sixty patients were affected by one or more copathologies (32%). Diabetes was present in 18 patients (10%), 16 patients had chronic autoimmune diseases (9%), while 12 patients had antecedent or active ematological or solid tumors (6%). Other reported chronic diseases affected heart and cardiovascular system (9 patients, 5%), respiratory system (13 patients, 7%), kidney (5 patients, 3%) and liver (1 patient, 0.5%).

As for definition, all patients had some muscular weakness associated with reduction of absence of deep tendon reflexes at presentation. In addition, 78% of patients had some objective sensory involvement at presentation, 39% had cranial or bulbar involvement, 19% ataxia and 19% dysautonomia. One hundred and fifty one patients (81%) fitted the required parameters for class 1 of Brighton diagnostic criteria at presentation, while the other 19% were in class 2 beacuase of absence of albumin-cytological dissociation in CSF.

From a clinical perspective, 62% of patients were classified as a classic GBS syndrome at presentation (i.e. symmetrical, ascending, bilateral weakness with associated sensory symptoms). Among the remaining patients: 27 (14%) showed a pure motor involvement without sensory findings; 21 (11%) were characterized as Miller-Fisher syndrome (MFS) showing prominent ataxia and/or ocular involvement; 24 (13%) were affected by regional variants of GBS (mainly paraparhetic variant, followed by rare patients with facial diplegia, cervico-pharyngo-brachial variant, lateralized or predominantly upper limb forms).

The median modified Erasmus GBS Outcome Score (mEGOS) at presentation was 2 (range: 0-9), while mean Erasmus GBS Respiratory Insufficiency Score (EGRIS) was 2 (range: 1-6).

Some kind of treatment was administered to almost all patients (98%). In most cases (83% of all treated patients), the first line therapy were IntraVenous ImmunoGlobulins (IVIG), in the remaining 17% was Plasma Exchange (PE). Overall, 97% of patients were treated with IVIG, 18% with PE and 17% with both treatment sequentially.

All patients underwent a Complete Nerve Conduction Study (NCS) in the acute phase of the disease, with a median time from onset of symptoms to examination of 7 days (ranging from 2 to 28 days). In 65% of patients, a second study was performed to confirm diagnosis and condolidate neurophysiological findings. Combining the first and second tests, it was possibile to classify all patients in one the following cathegories, as proposed by Uncini and Kuwabara in their criteria:

- Acute Inflammatory Demyelinating Polyradiculopathy (AIDP): 81% of patients
- Acute Motor Axonal Neuropathy (AMAN): 14% of patients
- Acute Motor-Sensory Axonal Neuropathy (AMSAN): 5% of patients

As a whole, 35 patients (19% of the whole sample) showed features suggestive of predominant axonal damage in NCS. In 40% of patients sensory abnormalities were confirmed at NCS.
Regarding F waves, 106 patients (57%) showed some kind of alteration as defined in the already mentioned Uncini and Kuwabara criteria at first NCS test. Sixty-eight of them (36% of all patients) had a predominant demyelinating involvement of nerve root (i.e. increased F waves minimal latency), while the other 38 (20% of the whole sample) presented with major axonal damage of nerve roots at first study (i.e. F waves absence or severe reduction of persistence).

Lumbar puncture was performed in all patients during the acute phase of the disease, with a median time from the onset of symptoms of 8 days (rangin from 1 to 28 days). In the 81% of them albumin-cytological dissociation was present (i.e. an increase of protein concentration with normal cell count in CSF). All patients had a cell count of 5 or less cells per ml of CSF.
Blood-Brain-Barrier (BBB) damage index was altered in 71% of patients, while only a minority of them had an alteration of intrathecal IgG production indexes (i.e. Link index -26%- and Reiber index -10%-).
All CSF samples were tested for the presence of IgM and IgG OligoClonal Bands (OCBs). IgM OCBs were found in 29 patients (15.5% of the whole sample), while no patient had IgG OCBs in CSF (**figure 39**). On the contrary, a mirror pattern was quite common, being present in 48% of patients (IgM mirror pattern) or 60% of patients (IgG mirror pattern).

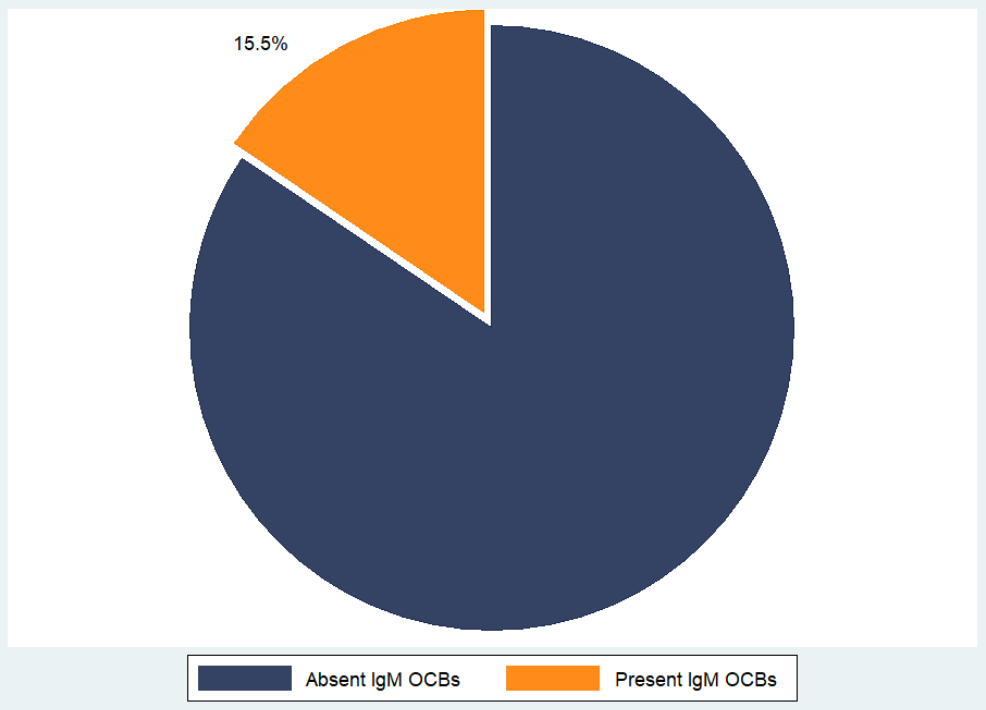


Figure 39: *frequency of IgM OCBs in CSF in our sample of patients.*

3.1.2 Outcome measures – whole sample

Table 12 shows an overview of outcome measures referred to the whole cohort of patients.

Outcome measures	
Hospital admission	
MRC scale	52 (46-56)
GB-DS	2 (2-3)
i-RODS	26 (10-35)
Unable to walk unaided	80 (43%)
Nadir	
MRC scale	46 (32-52)
GB-DS	3 (3-4)
i-RODS	15 (3-23)
Time to nadir (days)	9 (7-12)
Unable to walk unaided	146 (78%)
Time to aided walking (days)	5 (4-6)
Bedridden patients	86 (46%)
Time to bedridden (days)	8 (6-10)
12 months follow up	
MRC scale	60 (53-60)
GB-DS	1 (0-2)
i-RODS	48 (32-48)
Unable to walk unaided	41 (22%)
Recovery of unaided walking	105/146 (72%)
Time to recovery of unaided walking (weeks)	10 (8-13)
Complete recovery	127 (68%)
Time to complete recovery (weeks)	32 (20-42)
Death	11 (6%)
Time (weeks)	4 (3-12)
Mechanical ventilation	29 (16%)
Time (days)	6 (3-8)

Table 12: *outcome measures of the whole sample. Results are expressed as absolute frequency (%) for dichotomic variables and as median value (IQR) for continuous variables.*

The median score at Medical Research Council (MRC) scale was 52 at presentation, 46 at nadir and 60 at the end of the follow up time (i.e. 12 months). In analogy, the median GBS Disability Scale (GB-DS) score was 2 at presentation, 3 at nadir and 1 after 12 months of follow up. Finally, the median inflammatory Rasch-Build Overall Disability Scale (i-RODS) was 26 at hospital admission, 15 at nadir and 48 after 12 months of follow up.

At the same time points, the proportion of patients who lost the ability to walk without assistance was 43%, 78% and 22% respectively. Forty-six percent of patients were confined to bed during the acute phase of the disease, while 29 patients (16%) needed assisted ventilation and 11 patients (6%) died. Complete recovery was reached in 68% of patients during fhe 12 months follow up. Of the 146 patients who were unable to walk unaided, 105 (72%) managed to recover this ability during the follow up.

3.2 Descriptive statistics and group comparison between IgM OCBs positive and negative patients

This section describes the distribution of clinical, neurophysiological and CSF characteristics between patients with or without IgM OCBs. Then, details about outcome measures and their differente performance between groups are provided.

For every variable, the result of the statistical analysis for group comparison is reported. In the following tables, a significant difference between groups are highlighted in bold ($p<0.05$).

3.2.1 Demographic and clinical characteristics – group comparison

Table 13 reports the comparison between demographical and clinical characteristics of the two main groups of patients, i.e. with and without IgM OCBs in CSF.

Variables	Whole sample (n=187)	IgM OCB absent (n= 158)	IgM OCB present (n=29)	p
Sex				
Female	69 (37%)	57 (36%)	12 (41%)	0.586
Male	118 (63%)	101 (64%)	17 (59%)	
Age at onset of neuropathy	62 (45-74)	61 (44-74)	65 (54-71)	0.504
Age ≥ 60 years	99 (53%)	82 (52%)	17 (59%)	0.505
Infective prodromes (overall)	112 (60%)	91 (58%)	21 (72%)	0.134
Gastrointestinal	48 (26%)	40 (25%)	8 (28%)	0.797
Upper airways	49 (26%)	39 (25%)	10 (34%)	0.270
Other	15 (8%)	12 (8%)	3 (10%)	0.616
Antecedent vaccinations	**9 (5%)**	**4 (3%)**	**5 (17%)**	**0.001**
Gangliosides (overall)	**52 (28%)**	**49 (31%)**	**3 (10%)**	**0.022**
GM1	27 (14%)	22 (14%)	5 (17%)	0.640
GD-GQ-GT	**32 (17%)**	**31 (20%)**	**1 (4%)**	**0.034**
Clinical involvement				
Motor	187 (100%)	158 (100%)	29 (100%)	
Hypo/areflexia	187 (100%)	158 (100%)	29 (100%)	
Sensory	146 (78%)	126 (80%)	20 (69%)	0.197
Bulbar	**73 (39%)**	**55 (35%)**	**18 (62%)**	**0.006**
Ataxia	35 (19%)	31 (20%)	4 (14%)	0.460
Autonomic	**36 (19%)**	**25 (16%)**	**11 (40%)**	**0.006**
Clinical phenotype				
Classic	115 (62%)	99 (63%)	16 (55%)	0.446
Pure motor	**27 (14%)**	**19 (12%)**	**8 (28%)**	**0.028**
Miller-Fisher syndrome	21 (11%)	19 (12%)	2 (7%)	0.421
Regional variants	24 (13%)	21 (13%)	3 (10%)	0.663
Brighton diagnostic criteria				
Class 1	**151 (81%)**	**123 (78%)**	**28 (97%)**	**0.019**
Class 2	**36 (19%)**	**35 (22%)**	**1 (4%)**	
mEGOS at hospital admission	**2 (1-4)**	**2 (1-4)**	**4 (2-5)**	**0.010**
EGRIS at hospital admission	**2 (2-3)**	**2 (2-3)**	**3 (2-3)**	**0.007**
Time from onset to admission (days)	2 (1-3)	2 (1-3)	2 (1-3)	0.217
Treatment (overall)	183 (98%)	155 (98%)	29 (100%)	0.596
IVIG	182 (97%)	154 (98%)	28 (97%)	0.779
PE	33 (18%)	26 (17%)	7 (24%)	0.319
Both therapies	32 (17%)	25 (16%)	7 (24%)	0.274
IgG monoclonal component in serum	9 (5%)	8 (5%)	1 (4%)	0.709
IgM monoclonal component in serum	0 (0%)	0 (0%)	0 (0%)	

Table 13: *clinical characteristics and group comparison (IgM OCBs present and absent). Results are expressed as absolute frequency (%) for dichotomic variables and as median value (IQR) for continuous variables. Statistical significance ($p<0.05$) is highlighted in bold.*

Sex and age at onset of neuropathy were similar between IgM OCBs positive and negative patients, with a P-value of 0.586 and 0.504 respectively (**figures 40,41**).

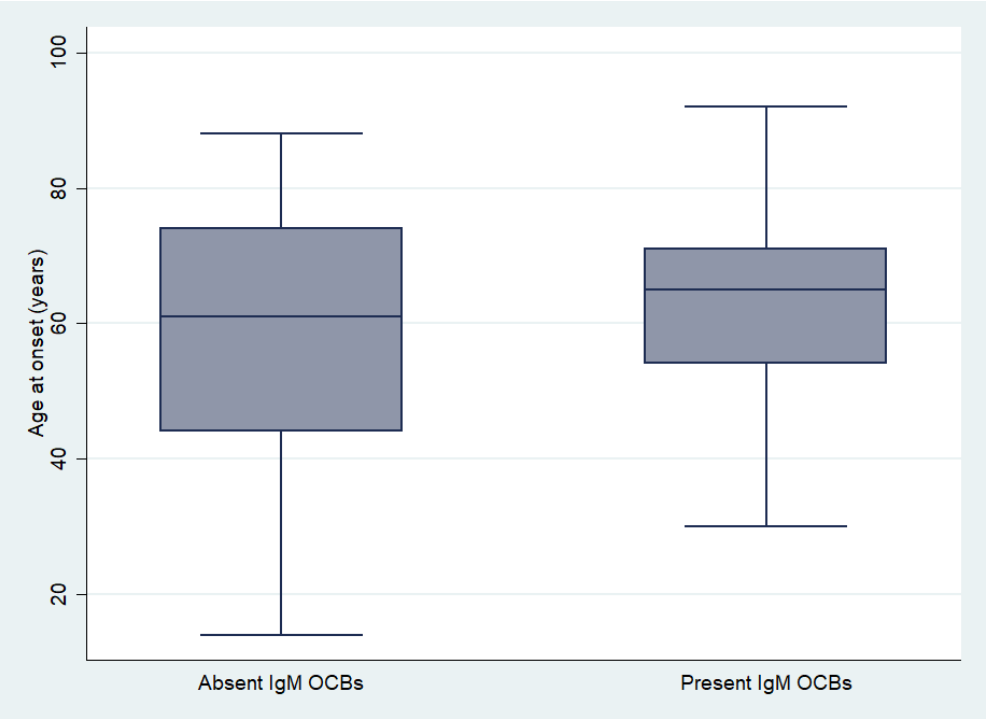


Figure 40: *box plot, median age at onset of neuropathy (years).*

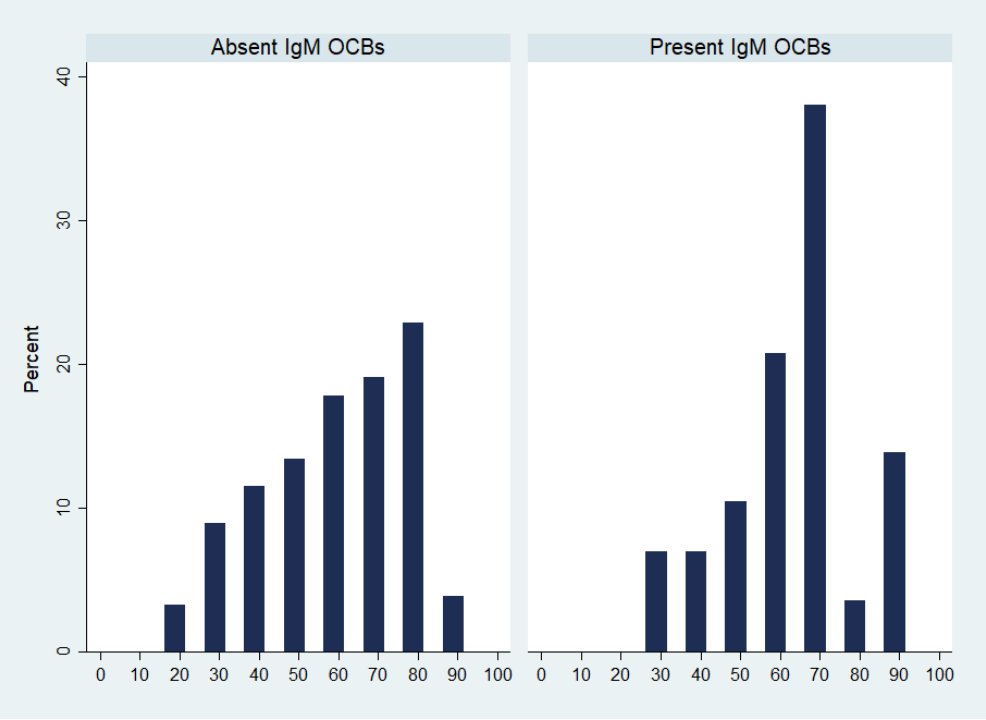


Figure 41: *distribution graph (%), age at onset of neuropathy.*

The median time interval in days from the onset of symptoms and hospital admission was not significantly different between the two groups (**figure 42**).

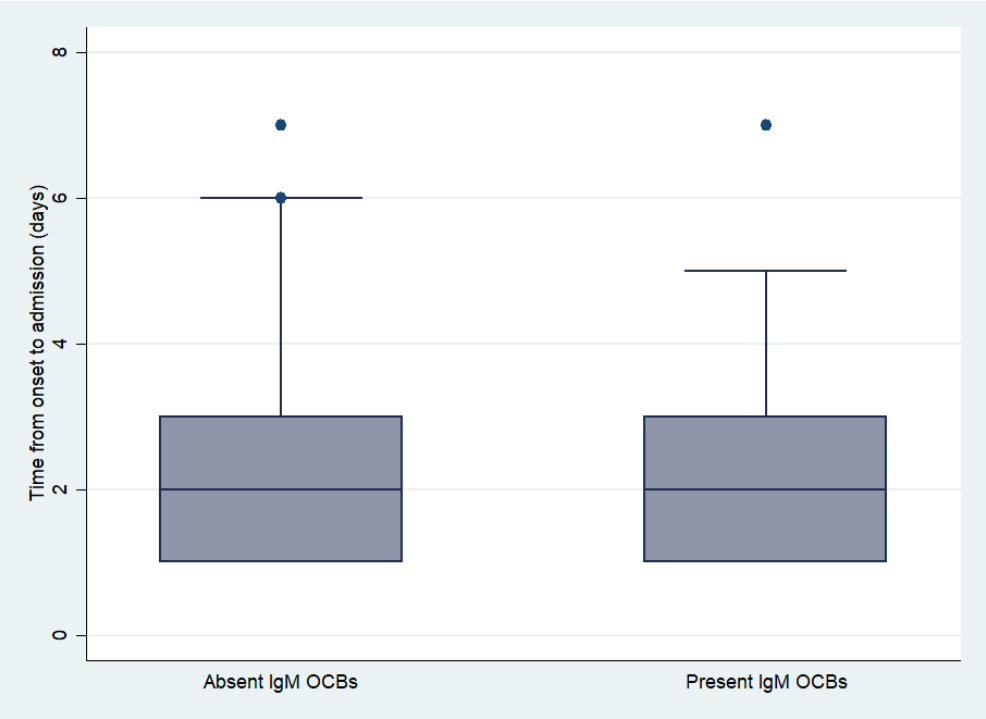


Figure 42: *box plot, median time from onset of neuropathy to hospital admission (days).*

Reported copathologies did not differed significantly among the two groups, with 9 out of 29 patients among the IgM OCBs positive group (31%) against 51 out of the 158 patients without IgM OCBs (32%) (p=0.788). In detail:

- Diabetes: 3/29 patients (10%) vs 15/158 patients (10%), p=0.896
- Chronic autoimmune diseases: 3/29 patients (10%) vs 13/158 patients (8%), p= 0.573
- Tumors: 2/29 patients (7%) vs 10/158 patients (6%), p=0.652
- Cardiovascular diseases: 1/29 patients (3%) vs 8/158 patients (5%), p=0.463
- Respiratory diseases: 2/29 patients (7%) vs 11/158 patients (7%), p=0.977
- Chronic kidney disease: 1/29 patients (3%) vs 4/158 patients (3%), p=0.651
- Chronic liver disease: 0/29 patients (0%) vs 1/158 patients (0.5%), p=0.254

Regarding the prevalence of serological IgM positivity for associated infections, no statistically significant difference was reported for any of the considered pathogen. In details:

- Campilobacter Jejuni: 15 patients among IgM OCBs positive (9%) and 4 among IgM OCBs negative (14%), with p=0.104
- EBV: 6 patients among IgM OCBs positive (4%) and 2 among IgM OCBs negative (7%), with p=0.211
- CMV: 4 patients among IgM OCBs positive (3%) and 1 among IgM OCBs negative (3%), with p=0.887
- Mycoplasma Pneumoniae: 3 patients among IgM OCBs positive (2%) and none among IgM OCBs negative (0%), with p=0.963

Serological analysis was positive for IgM anti-Campilobacter Jejuni in 19 patients (10%), Epstein Barr Virus (EBV) in 8 patients (4%), CitoMegaloVirus (CMV) in 5 patients (3%) and Mycoplasma Pneumoniae in 3 patients (2%).
The distribution of prodromes was similar in the two groups, as well. Differently, IgM OCBs positive patients showed a higher prevalence of antecedent vaccination (17% vs 3%, p=0.001) (**figure 43**).

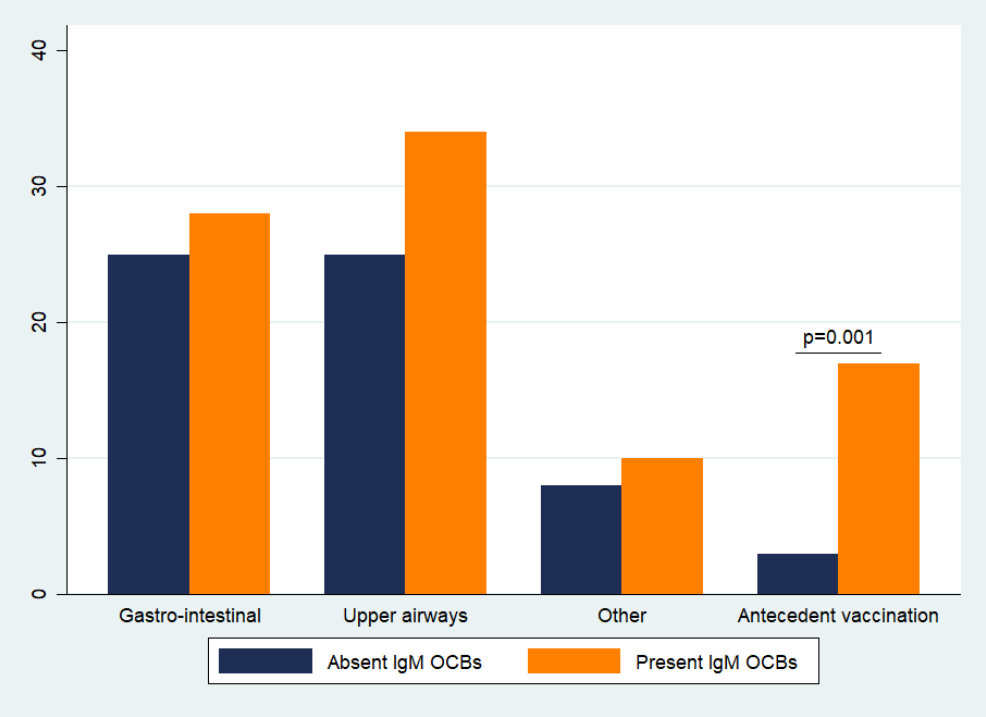


Figure 43: *bar graph with proportion (%), prodromes.*

Anti-gangliosides antibodies were significantly less present in IgM OCBs positive patients (10% vs 31%, p=0.022): more specifically, anti-GD-GQ-GT antibodies had a significantly different prevalence (4% vs 20%, p=0.034), while anti-GM1 were distributed similarly in the two groups (**figure 44**).

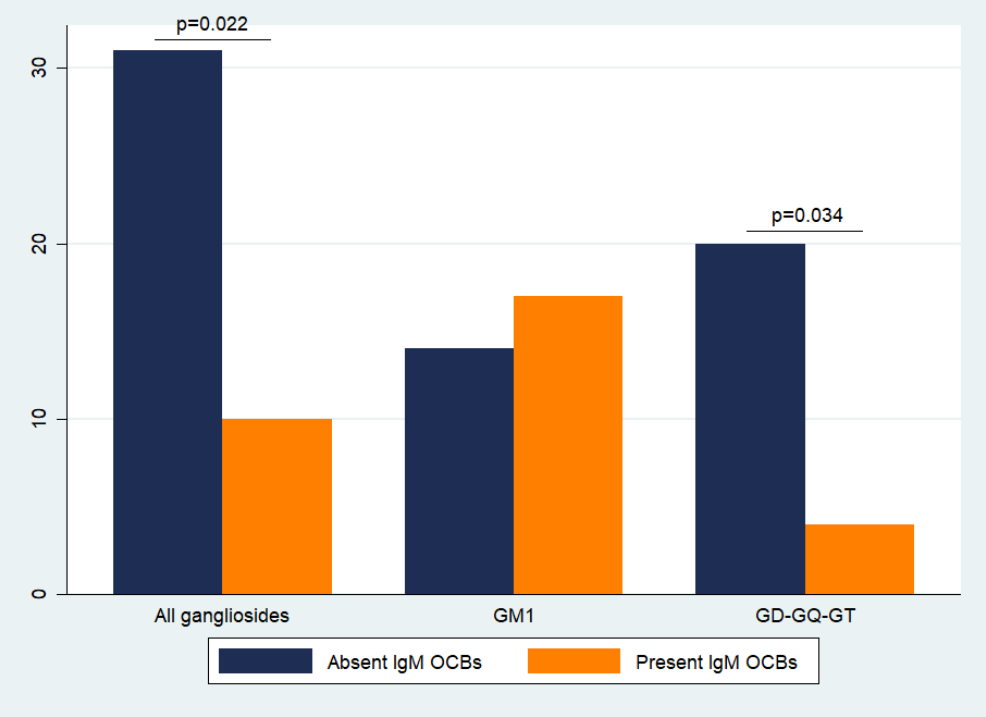


Figure 44: *bar graph with proportion (%), gangliosides.*

Regarding the clinical picture of patients, IgM OCBs positive ones showed a prominent bulbar and autonomic involvement (62% vs 35% and 40% vs 16%, respectively), with a P-value of 0.006 for both (**figure 45**).

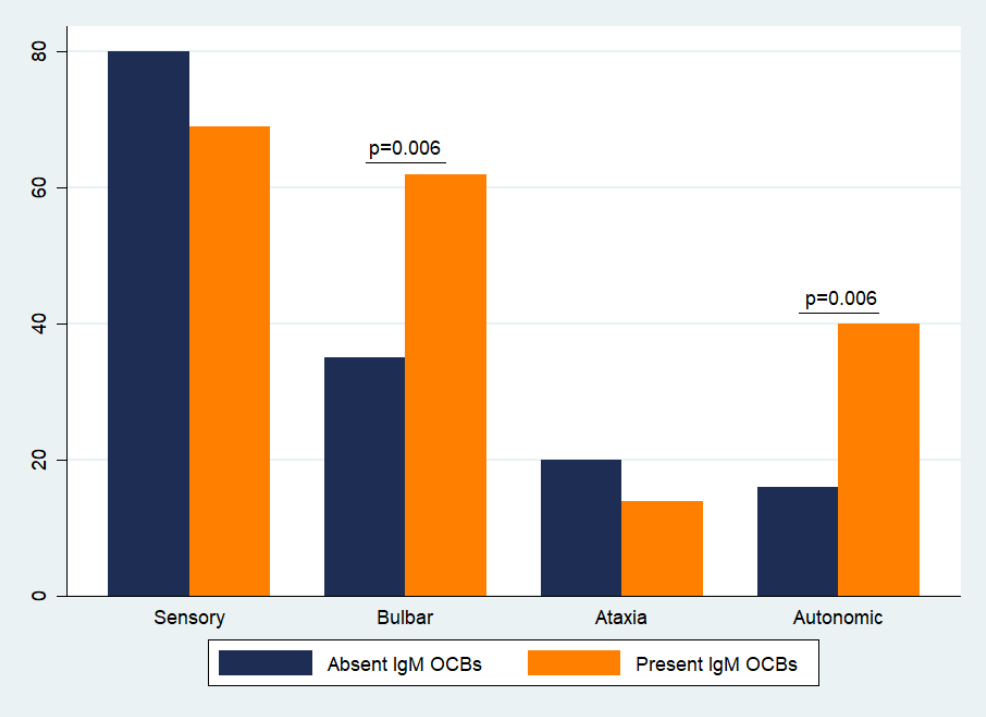


Figure 45: *bar graph with proportion (%), clinical involvement.*

In terms of clinical phenotype, a pure motor involvement was significantly more prevalent among IgM OCBs positive patients (28% vs 12%, p=0.028), while classic variant and Miller-Fisher syndrome were equally distributed between the two groups (**figure 46**).

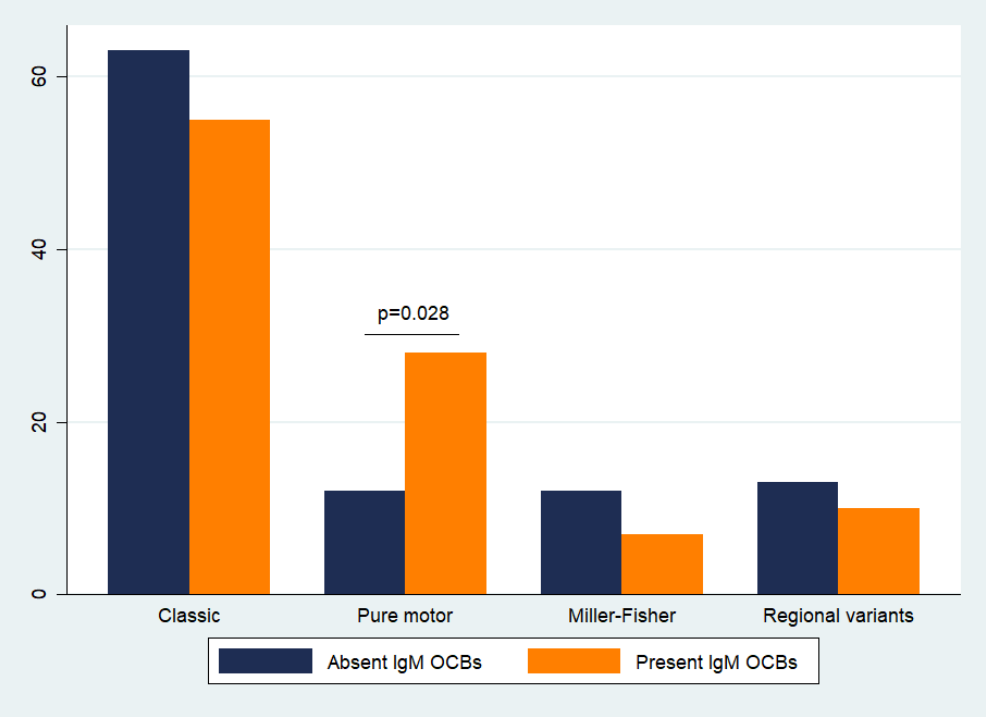


Figure 46: *bar graph with proportion (%), clinical phenotype.*

A higher proportion of IgM OCBs positive patients belonged to class 1 of Brighton criteria (97% vs 78%, p=0.019): this is related to the higher prevalence of alumin-cytological dissociation in the CSF of this group of patients (see below).

Median mEGOS and EGRIS scores at presentation were significantly higher among IgM OCBs positive patients, with 2 and 1 points of difference between groups and a P-value of 0.010 and 0.007, respectively (**figures 47, 48**).

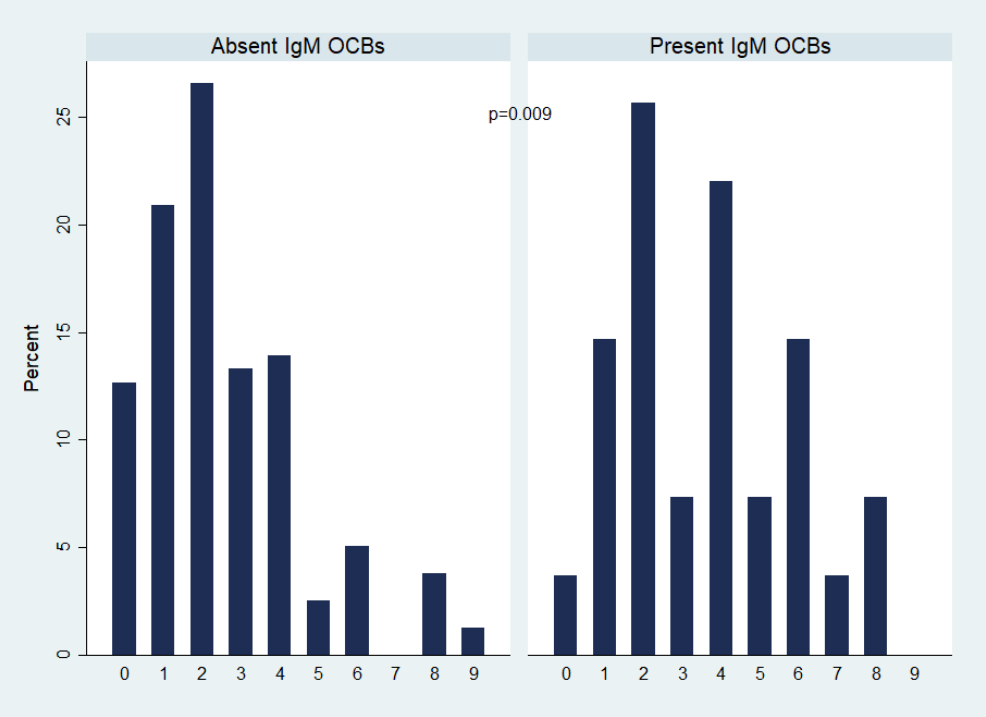


Figure 47: *distribution graph (%), modified Erasmus GBS Outcome Score (mEGOS).*

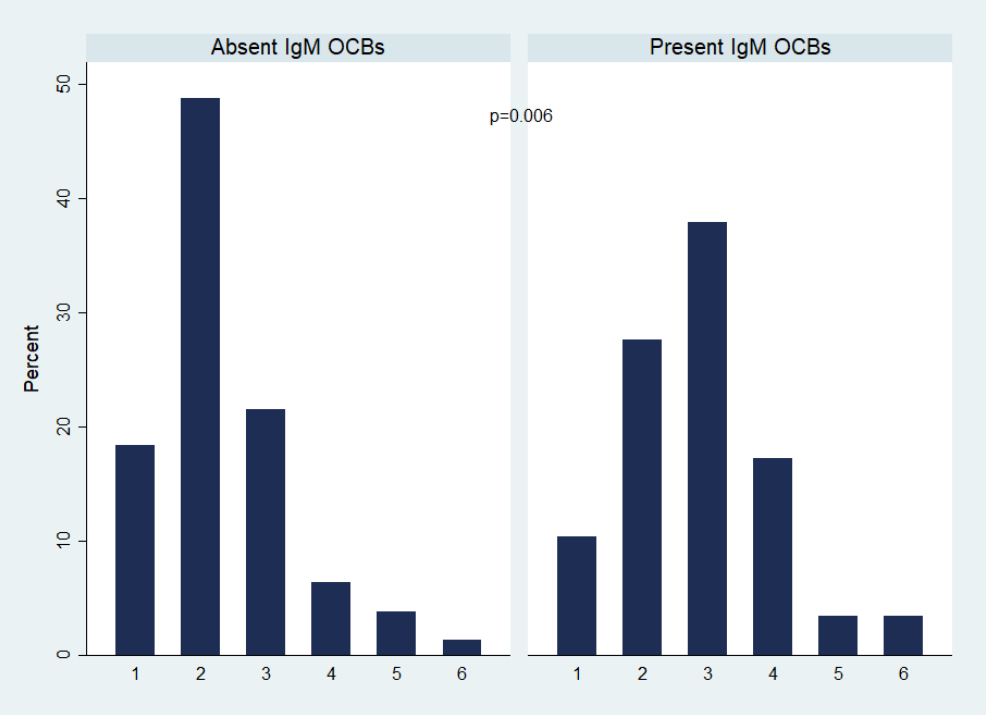


Figure 48: *distribution graph (%), Erasmus GBS Respiratory Insufficiency Score (EGRIS).*

Treatment strategies were similar in borh groups, with no significant differences between them (**figure 49**).

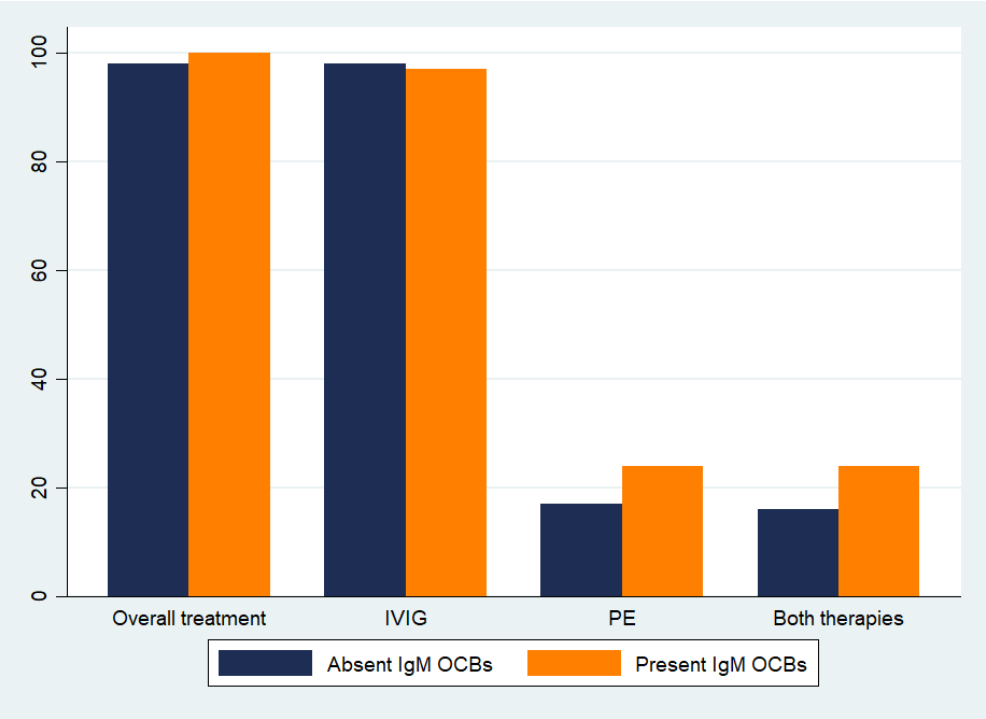


Figure 49: *bar graph (%), treatments.*

3.2.2 Neurophysiological characteristics – group comparison

Table 14 reports the comparison between neurophysiological characteristics between the two groups of patients.

	Whole sample (n=187)	IgM OCB absent (n= 158)	IgM OCB present (n=29)	p
Time from onset to first NCS study (days	7 (5-10)	7 (5-10)	8 (6-11)	0.468
Second NCS study	121 (65%)	101 (64%)	20 (69%)	0.602
AIDP	**152 (81%)**	**136 (86%)**	**16 (55%)**	**<0.001**
AMAN	**26 (14%)**	**16 (10%)**	**10 (34%)**	**<0.001**
AMSAN	9 (5%)	6 (4%)	3 (10%)	0.130
Axonal NCS	**35 (19%)**	**22 (14%)**	**13 (45%)**	**<0.001**
Abnormal F waves	**106 (57%)**	**80 (51%)**	**26 (90%)**	**<0.001**
Prolonged F waves latency	68 (36%)	54 (34%)	14 (48%)	0.147
F waves absence	**38 (20%)**	**26 (16%)**	**12 (41%)**	**0.002**
Sensory involvement	74 (40%)	62 (39%)	12 (41%)	0.829

Table 14: *neurophysiology characteristics and group comparison (IgM OCBs present and absent). Results are expressed as absolute frequency (%) for dichotomic variables and as median value (IQR) for continuous variables. Statistical significance ($p<0.05$) is highlighted in bold.*

The time from onset of neuropathy ad the first NCS examination was similar between IgM OCBs positive and negative patients (p=0.468) (**figure 50**), as well as the proportion of patients who underwent a second neurophysiological study in the two groups (p=0.602).

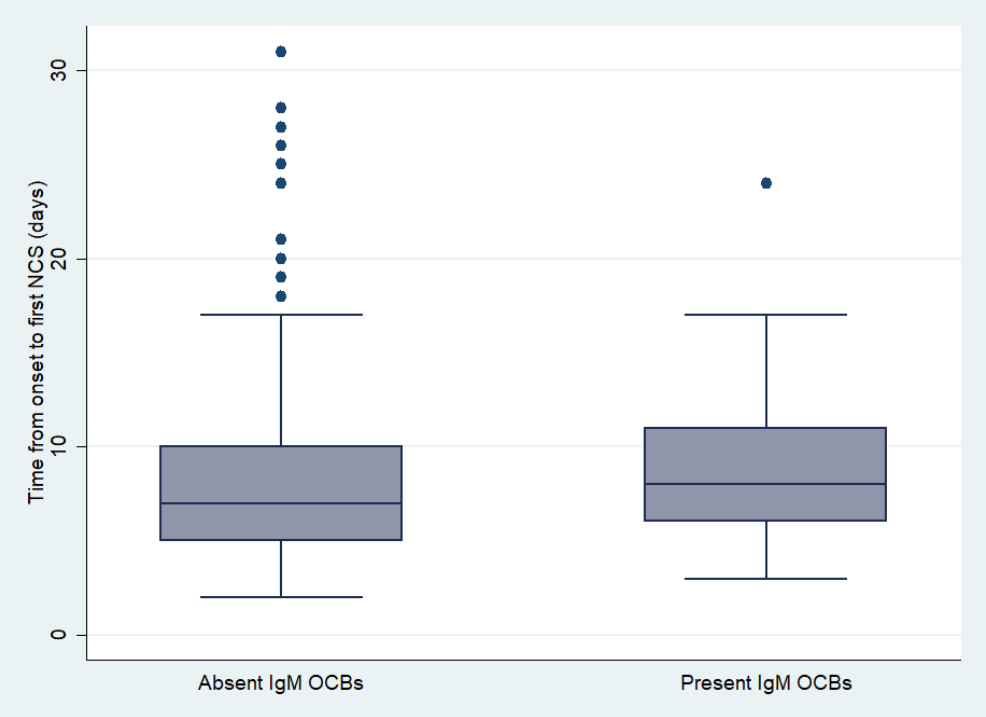


Figure 50: *box plot, median time from onset of neuropathy ant first NCS study (days).*

Nevertheless, NCS findings and, as a consequence, the electrodiagnostic distinction between demyelinating or axonal phenotype, differed significantly on the basis of the presence or absence of IgM OCBs (**figure 51**).
Plainly, AIDP prevalence was significantly higher among IgM OCBs negative patients (55% vs 86%, p<0.001) while AMAN prevalence was significantly higher among IgM OCBs positive patients (34% vs 10%, p<0.001).
As a whole, features of axonal damage at NCS examination were way more frequent in the group of IgM OCBs positive patients (45% vs 14%, p<0.001).
Finally, the proportion of sensory conductance abnormalities at NCS was superimposable between IgM OCBs positive and negative patients.

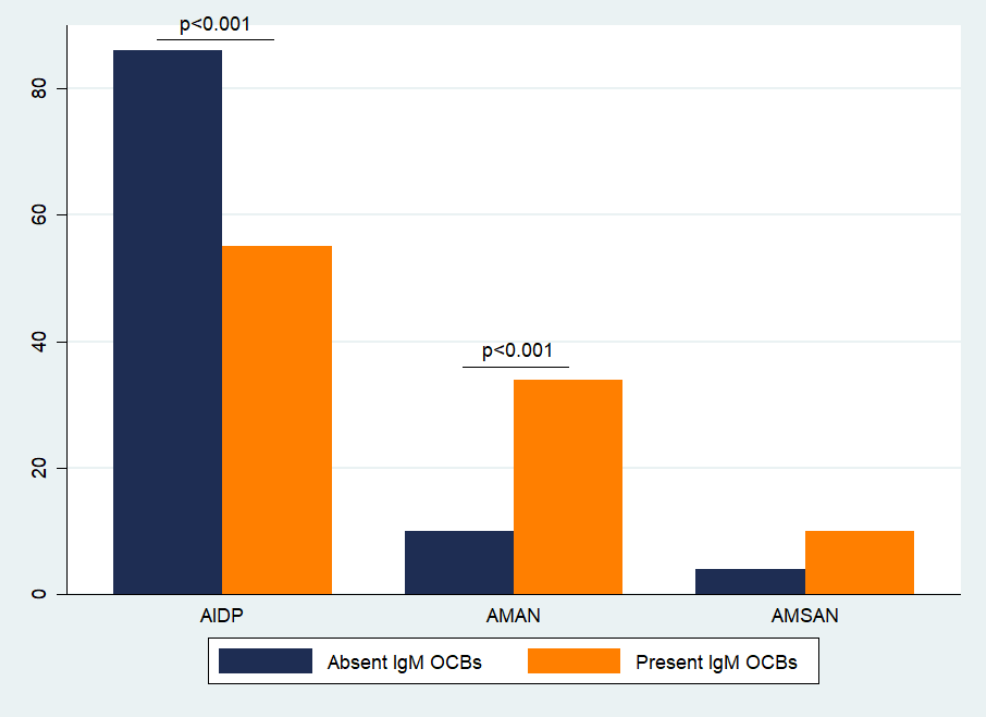


Figure 51: *bar graph (%), neurophysiological classification.*

Similarly, overall F waves abnormalities were significantly more frequent in IgM OCBs positive patients (90% vs 51%, p<0.001). Such difference can be almost completely attributed to axonal damage of nerve roots, which is highly prevalent in IgM OCBs positive population (41% vs 16%, p=0.002). On the contrary, the distribution of demyelinating features in nerve root evaluation was similar between the two groups (p=0.147) (**figure 52**).

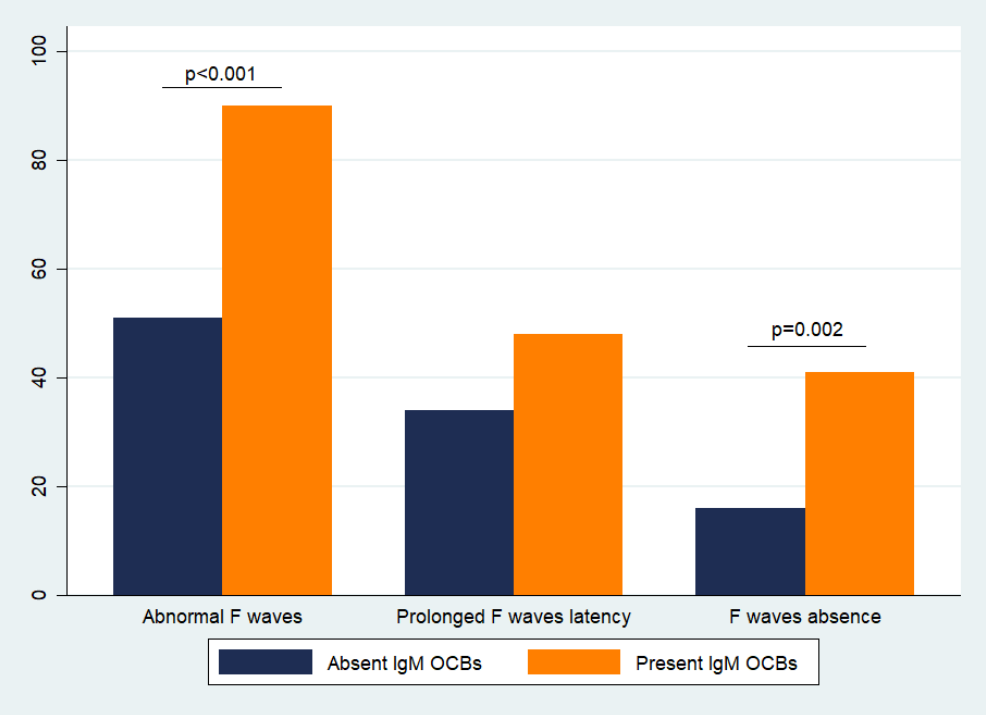


Figure 52: *bar graph (%), F waves abnormalities.*

3.2.3 CSF characteristics – group comparison

Table 15 reports the CSF characteristics in the two groups of patients.

		Whole sample (n=187)	IgM OCB absent (n= 158)	IgM OCB present (n=29)	p
Time from onset to lumbar puncture (days)		8 (4-15)	7 (4-15)	9 (5-14)	0.511
Albumin-cytological dissociation		**151 (81%)**	**123 (78%)**	**28 (97%)**	**0.022**
Protein level in CSF (mg/dl)		**79 (55-130)**	**71 (53-110)**	**164 (120-222)**	**<0.001**
Cells in CSF (n/µl)		2 (1-3)	1 (1-3)	2 (1-3)	0.127
BBB damage index		**1.1 (0.6-1.8)**	**0.9 (0.6-1.4)**	**2.8 (2.0-6.2)**	**<0.001**
	Abnormal	**132 (71%)**	**105 (66%)**	**27 (93%)**	**0.004**
CSF IgG level (mg/dl)		**6.6 (3.8-11.8)**	**5.8 (3.6-9.8)**	**16.0 (10.1 - 25.0)**	**<0.001**
Serum IgG level (mg/dl)		1060 (885-1340)	1055 (872-1331)	1060 (917-1440)	0.360
CSF albumin level (mg/dl)		**40.7 (23.4-61.6)**	**36.8 (23.0-55.2)**	**87.5 (59.4-108.0)**	**<0.001**
Serum albumin level (mg/dl)		3976 (3521-4344)	3965 (3514-4344)	4170 (3770-4847)	0.131
Link index		0.6 (0.5-0.7)	0.6 (0.5-0.7)	0.6 (0.5-0.8)	0.322
	Abnormal	49 (26%)	37 (23%)	12 (41%)	0.064
Reiber index		-1.2 (-3.6--0.5)	-1.2 (-3.2--0.5)	-1.7 (-6.1--0.5)	0.412
	Abnormal	19 (10%)	15 (10%)	4 (14%)	0.481
Presence of mirror pattern IgM		90 (48%)	72 (46%)	18 (62%)	0.102
Presence of mirror pattern IgG		113 (60%)	92 (58%)	21 (72%)	0.151
Presence of IgG OCB in CSF		0 (0%)	0 (0%)	0 (0%)	

Table 15: *laboratory characteristics and group comparison (IgM OCBs present and absent). Results are expressed as absolute frequency (%) for dichotomic variables and as median value (IQR) for continuous variables. Statistical significance (p<0.05) is highlighted in bold.*

CSF sampling was performed at a similar time point, with a median latency of 7 vs 9 days between the onset of neuropathy and lumbar puncture and a P-value of 0.511 (**figure 53**).

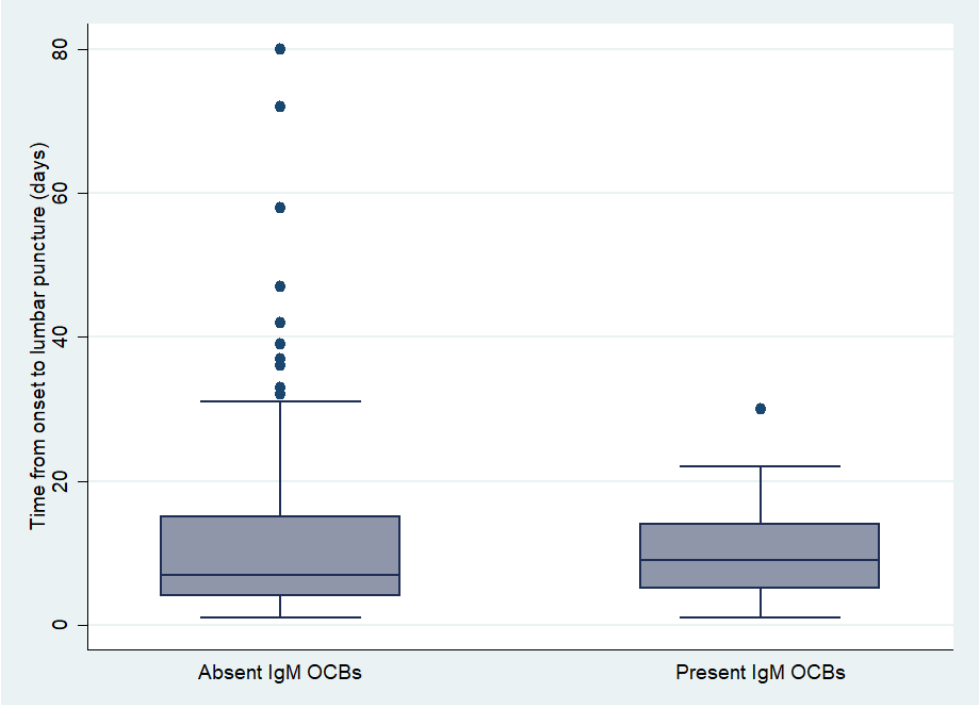


Figure 53: *box plot, median time from onset of neuropathy to lumbar puncture (days).*

The median cells number per ml of CSF was similar between the two groups (p=0.127). Conversely, the median protein concentration in CSF showed a great difference between IgM OCBs positive (164 mg/ml) and negative (71 mg/dl) patients, with p<0.001 (**figure 54**).

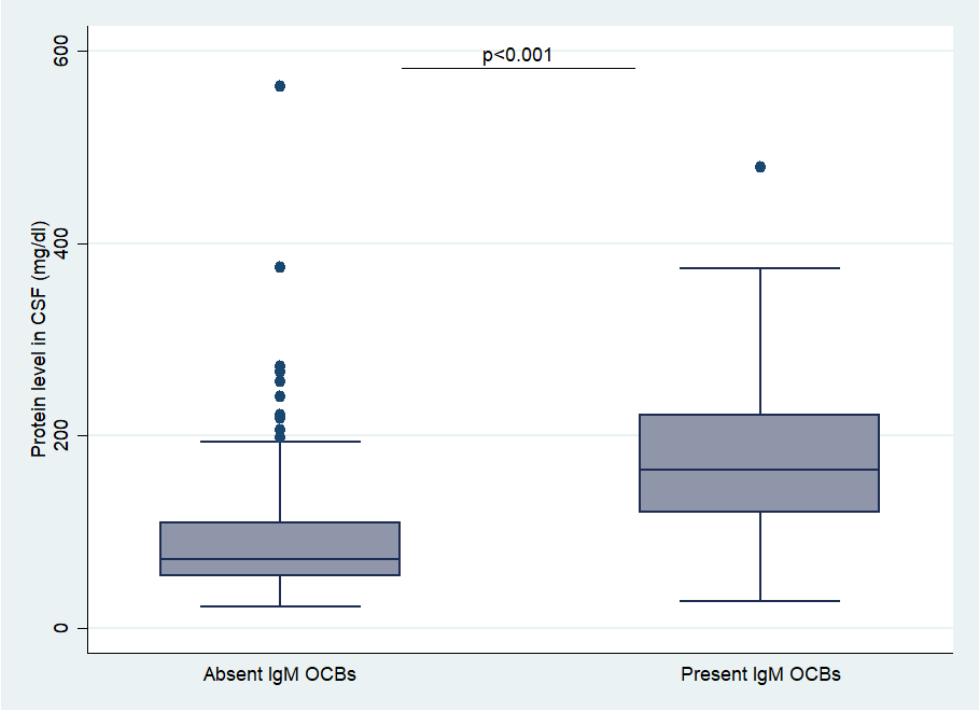


Figure 54: *box plot, median protein concentration in CSF (mg/dl).*

An analogue trend can be observed for the median albumin and IgG raw concentrations in CSF, which were significantly higher among patients with IgM OCBs (p<0.001 for both items). Meanwhile, the same can not be said for albumin and IgG concentrations in serum, which did not differ significantly between groups (p=0.131 and p=360, respectively) (**figure 55, 56**).

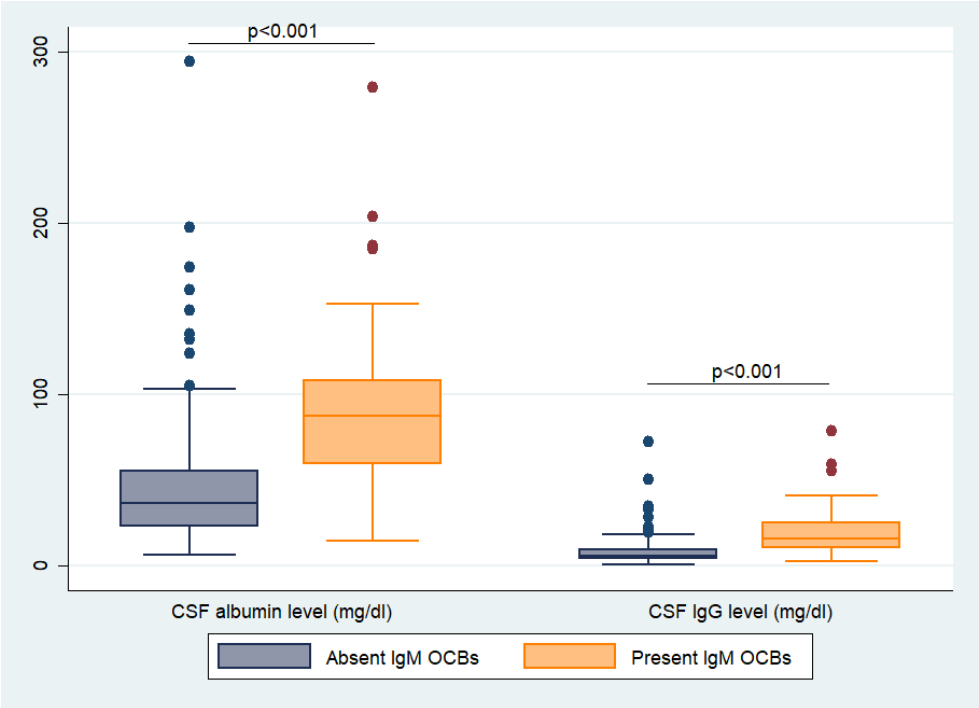


Figure 55: *box plot, median albumin and IgG concentration in CSF (mg/dl).*

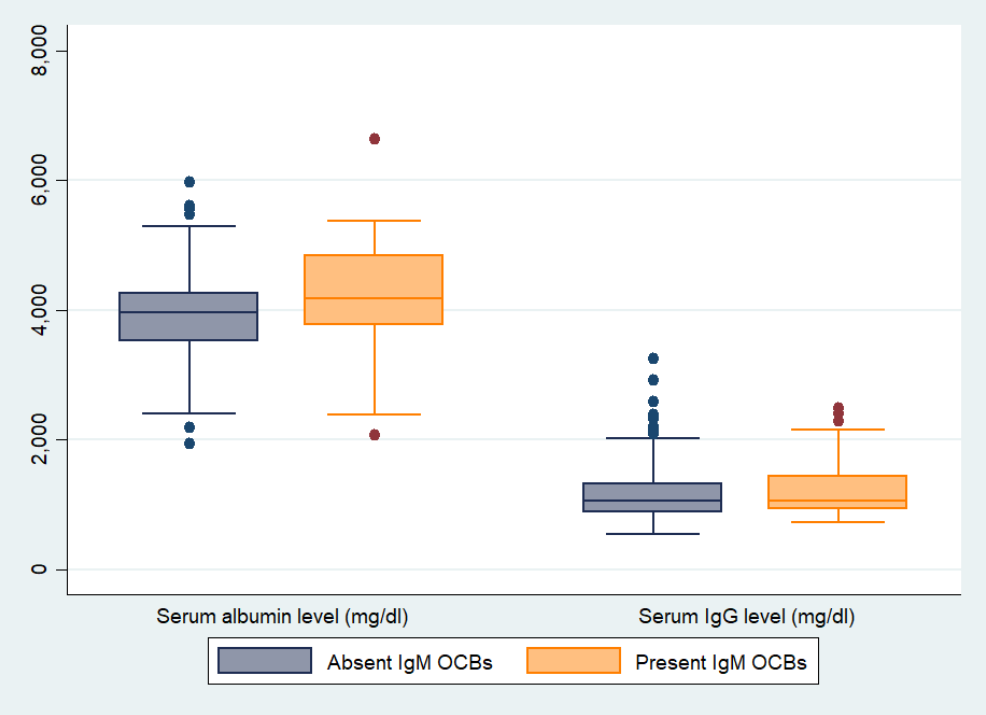


Figure 56: *box plot, median albumin and IgG concentration in serum (mg/dl).*

Given these premises, it is not surprising that the median value of BBB damage index, which is based on the albumin CSF/serum ratio, is more than 3-folds higher among IgM OCBs positive patients (p<0.001), with a prevalence of abnormal values of 93% against 66% of the IgM OCBs negative group (p=0.004) (**figure 57**).

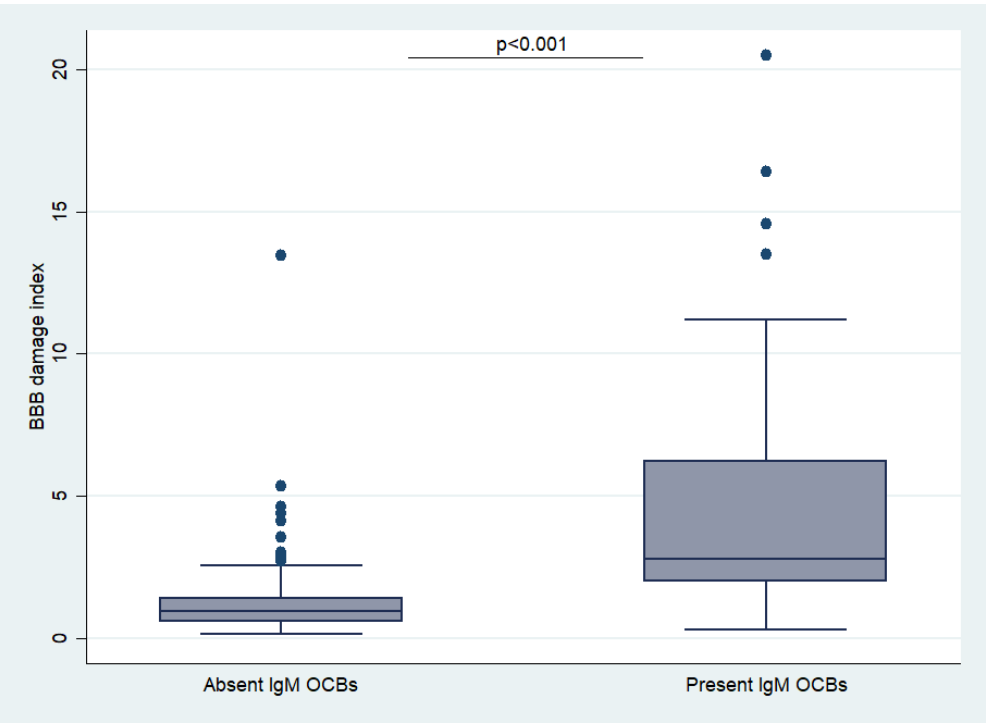


Figure 57: *box plot, median Blood Brain Barrier (BBB) damage index.*

The prevalence of albumin-cytological dissociation was significantly higher among IgM OCBs patients (97% vs 78%, p=0.022).
Even though the indexes of BBB damage are significantly more altered among IgM OCBs patients, the same did not happened for indexes of IgG intathecal production.
Actually, such indexes performed similarly between the two groups (p=0.322 and p=0.412 for Link and Reiber indexes, respectively) (**figure 58**).
As a further confirmation about the absence of IgG intrathecal production in our GBS patients, none of the CSF samples showed the presence of IgG OCBs.
Even the prevalence of mirror pattern IgG and IgM in CSF and serum was similar between IgM OCBs positive and negative patients (p=0.151 and p=0.102, respectively).

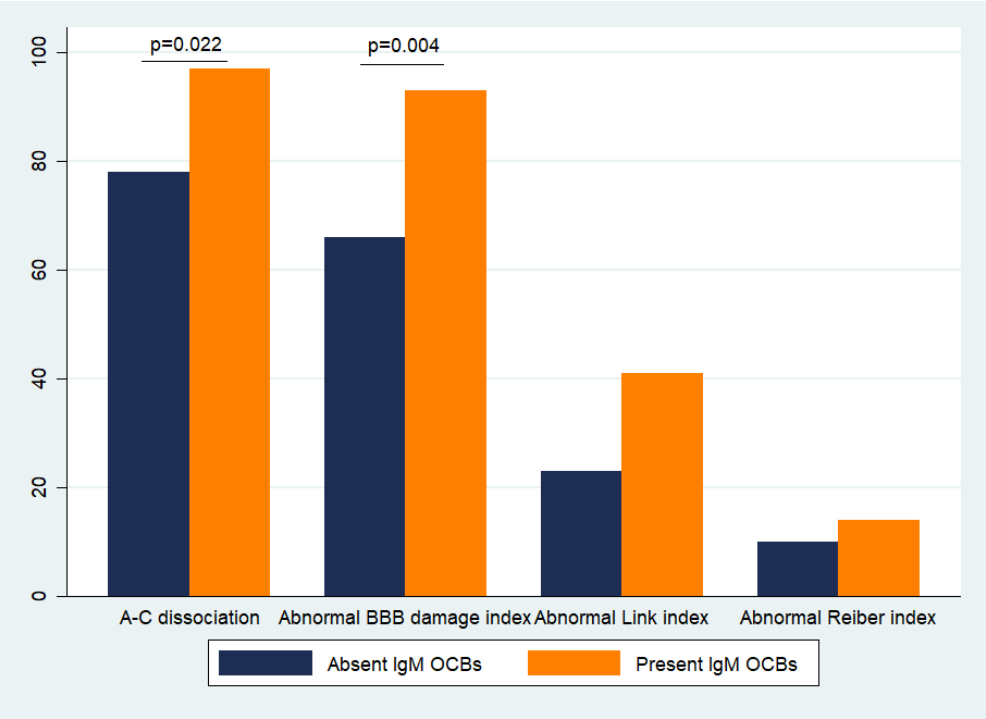


Figure 58: *bar graph (%), proportion of abnormal indexes in CSF.*

3.2.4 Outcome measures – group comparison

Table 16 shows the result of group comparison between IgM OCBs positive and negative patients referred to the different outcome measures.

	Whole sample (n=187)	IgM OCB absent (n= 158)	IgM OCB present (n=29)	p
Hospital admission				
MRC scale	**52 (46-56)**	**53 (48-56)**	**47 (42-52)**	**0.002**
GB-DS	2 (2-3)	2 (2-3)	3 (2-3)	0.083
i-RODS	**26 (10-35)**	**28 (11-36)**	**10 (6-30)**	**0.006**
Unable to walk unaided	80 (43%)	63 (40%)	17 (59%)	0.061
Nadir				
MRC scale	**46 (32-52)**	**47 (36-54)**	**32 (19-48)**	**0.002**
GB-DS	**3 (3-4)**	**3 (3-4)**	**4 (3-5)**	**0.006**
i-RODS	**15 (3-23)**	**17 (5-24)**	**3 (1-18)**	**<0.001**
Time to nadir (days)	**9 (7-12)**	**9 (7-13)**	**7 (5-9)**	**0.002**
Unable to walk unaided	**146 (78%)**	**119 (75%)**	**27 (93%)**	**0.033**
Time to aided walking (days)	**5 (4-6)**	**5 (5-6)**	**4 (3-5)**	**<0.001**
Bedridden patients	**86 (46%)**	**67 (42%)**	**19 (66%)**	**0.022**
Time to bedridden (days)	**8 (6-10)**	**9 (7-11)**	**5 (3-7)**	**<0.001**
12 months follow up				
MRC scale	**60 (53-60)**	**60 (56-60)**	**54 (32-60)**	**0.001**
GB-DS	**1 (0-2)**	**1 (0-2)**	**2 (1-4)**	**0.001**
i-RODS	**48 (32-48)**	**48 (40-48)**	**35 (16-48)**	**<0.001**
Unable to walk unaided	**41 (22%)**	**27 (17%)**	**14 (48%)**	**<0.001**
Recovery of unaided walking	**105/146 (72%)**	**92/119 (77%)**	**13/27 (48%)**	**0.002**
Time to recovery of unaided walking (weeks)	**10 (8-13)**	**10 (7-13)**	**12 (11-14)**	**<0.001**
Complete recovery	**127 (68%)**	**114 (72%)**	**13 (45%)**	**0.004**
Time to complete recovery (weeks)	**32 (20-42)**	**30 (18-40)**	**45 (40-49)**	**<0.001**
Death	11 (6%)	8 (5%)	3 (10%)	0.267
Time (weeks)	4 (3-12)	7 (3-12)	4 (2-19)	0.264
Mechanical ventilation	29 (16%)	21 (13%)	8 (28%)	0.051
Time (days)	**6 (3-8)**	**7 (4-9)**	**3 (1-6)**	**0.027**

Table 16: *outcome measures and group comparison (IgM OCBs present and absent). Results are expressed as absolute frequency (%) for dichotomic variables and as median value (IQR) for continuous variables. Statistical significance ($p<0.05$) is highlighted in bold.*

At the time of hospital admission, patients with IgM OCBs in CSF had a higher MRC score, with a median value of 47 against 53 of negative patients (p=0.002). Median MRC score at nadir was significantly lower among IgM OCBs positive patients, with a 15-points difference and a P-value of 0.002. At the end of the follow up time (12 months), the median MRC score was 8 points lower among IgM OCBs positive patients (p=0.001) (**figures 59-62**).

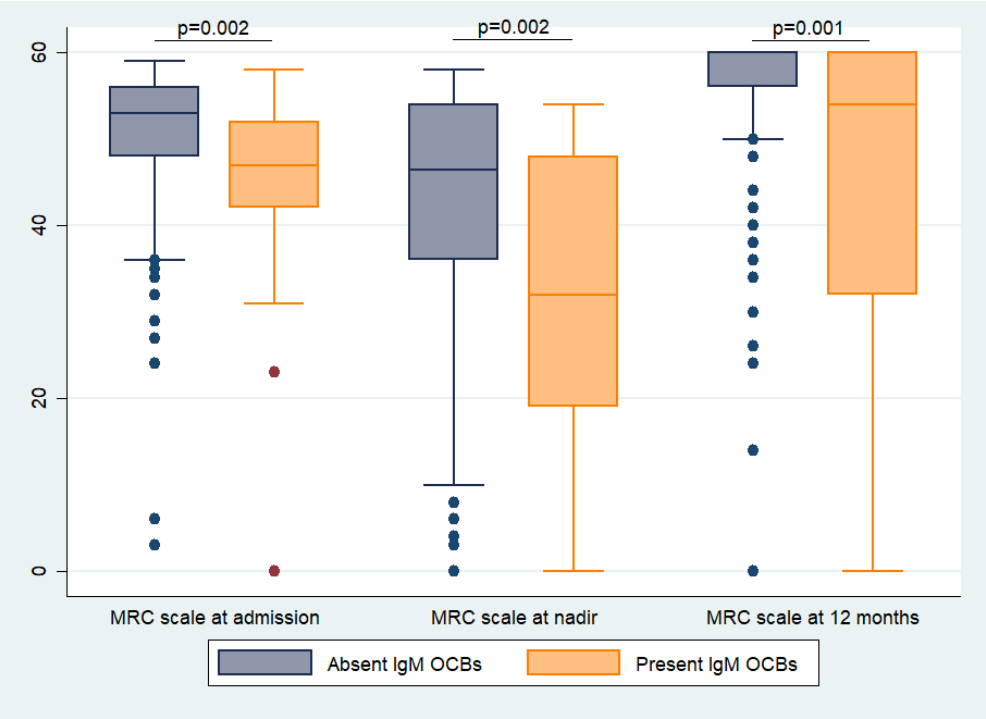


Figure 59: *box plot, median Medical Research Council (MRC) scale at admission, at nadir and after 12 months of follow up.*

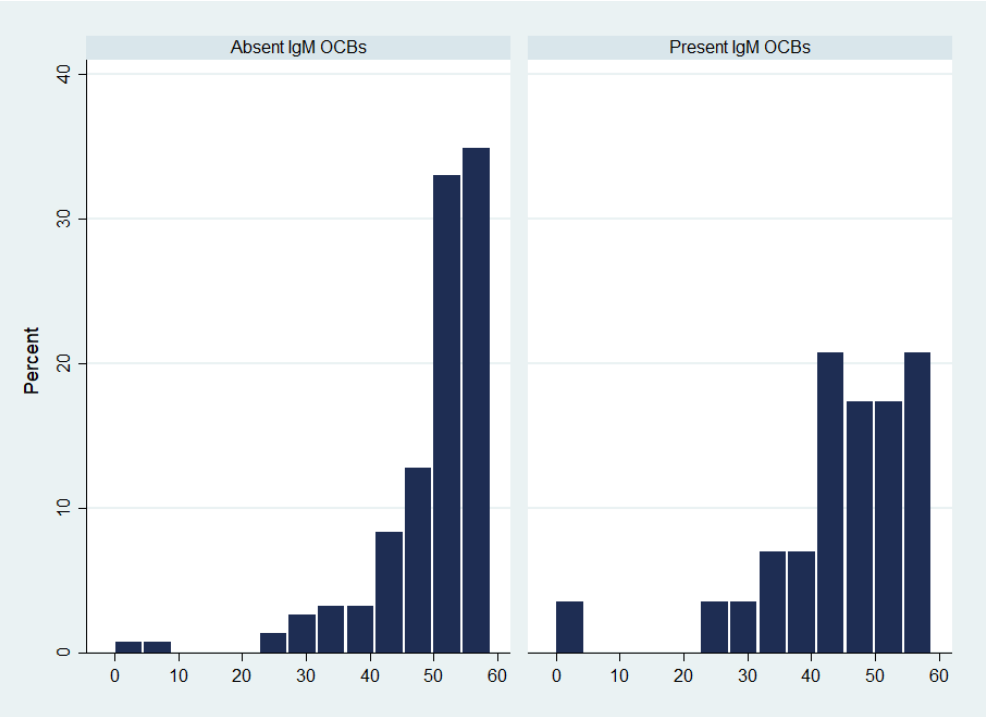


Figure 60: *distribution graph (%), Medical Research Council (MRC) scale at admission.*

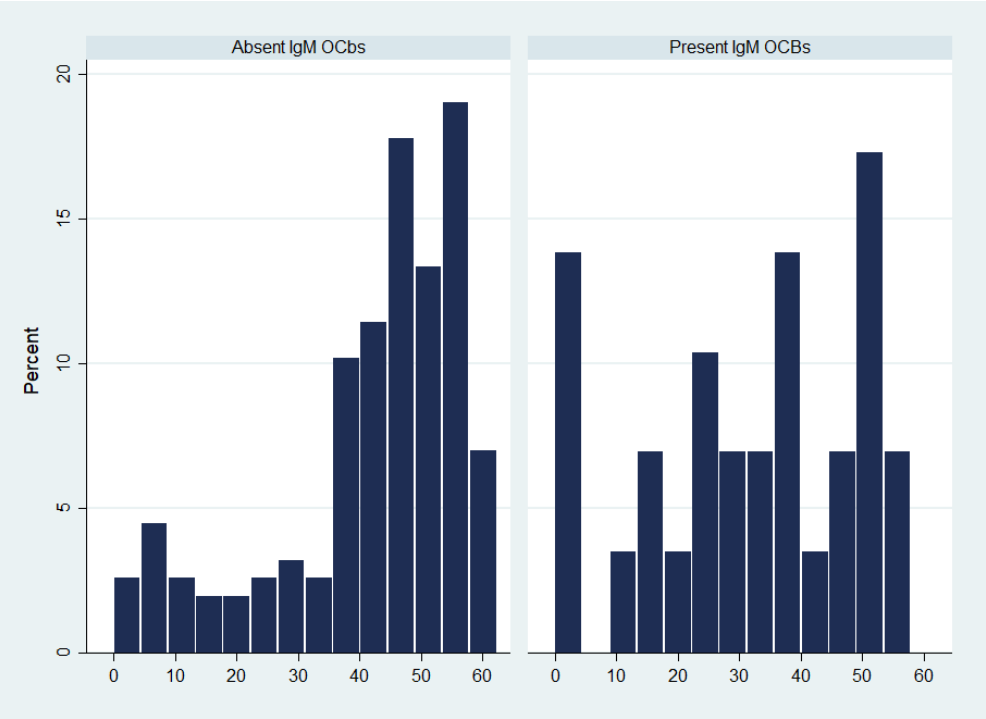


Figure 61: *distribution graph (%), Medical Research Council (MRC) scale at nadir.*

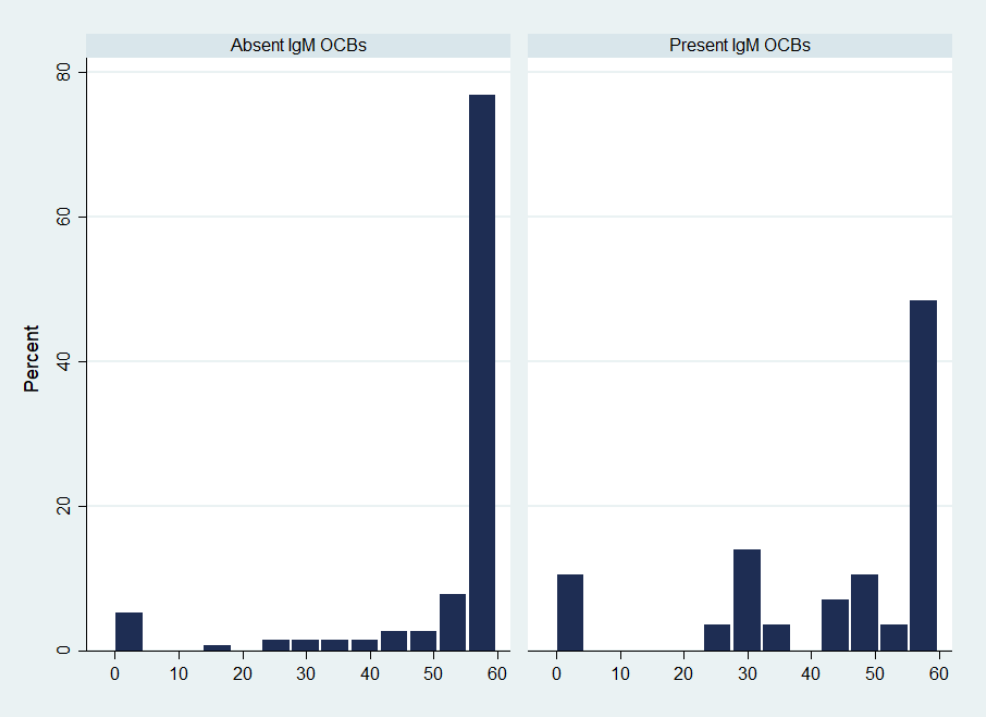


Figure 62: *distribution graph (%), Medical Research Council (MRC) scale aftert 12 months of follow up.*

Altough IgM OCBs positive patients demonstrated an evident tendency towards a higher GB-DS score, this outcome did not reach statistical significance at group comparison analysis (p=0.083). At nadir, however, median GB-DS score differed significantly, with a higher value for IgM OCBs positive patients and a P-value of 0.006. In parallel, GB-DS median score after 12 months was two-times higher among positive patients, with a P-value of 0.001 (**figures 63-66**).

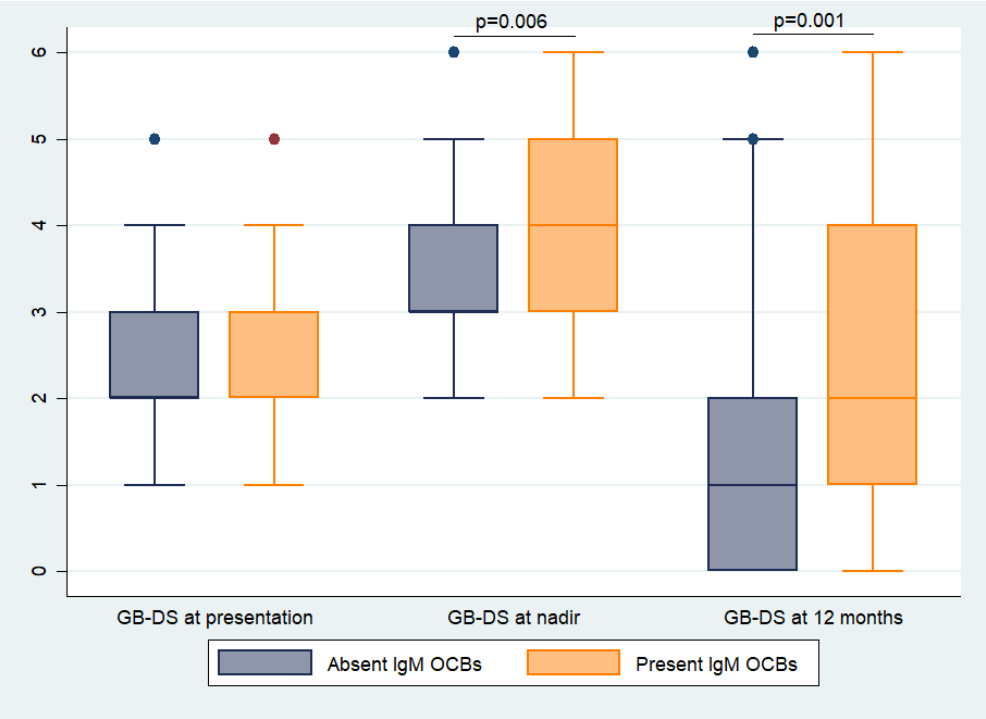


Figure 63: *box plot, median Guillain-Barrè Disability Score (GB-DS) at admission, at nadir and after 12 months of follow up.*

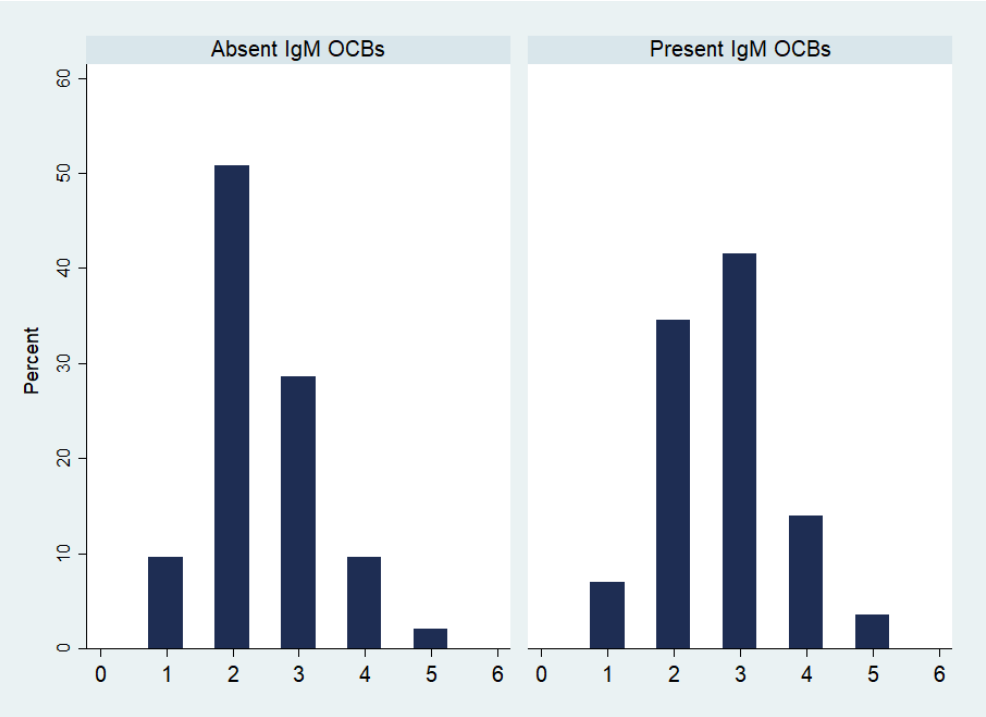


Figure 64: *distribution graph (%), Guillain-Barrè Disability Score (GB-DS) at admission.*

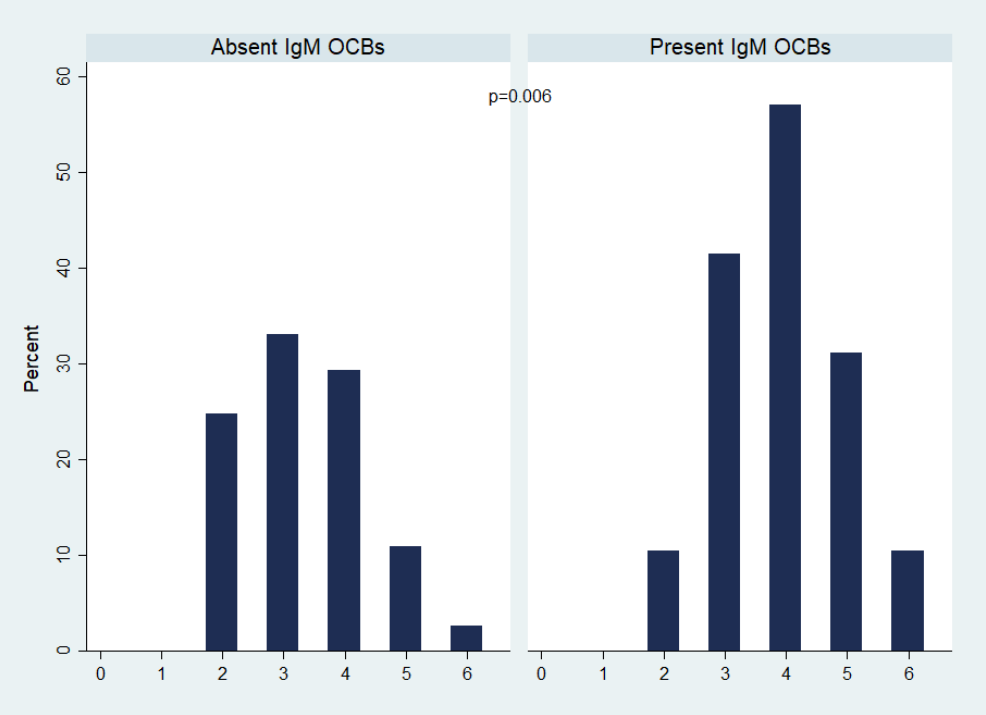


Figure 65: *distribution graph (%), Guillain-Barrè Disability Score (GB-DS) at nadir.*

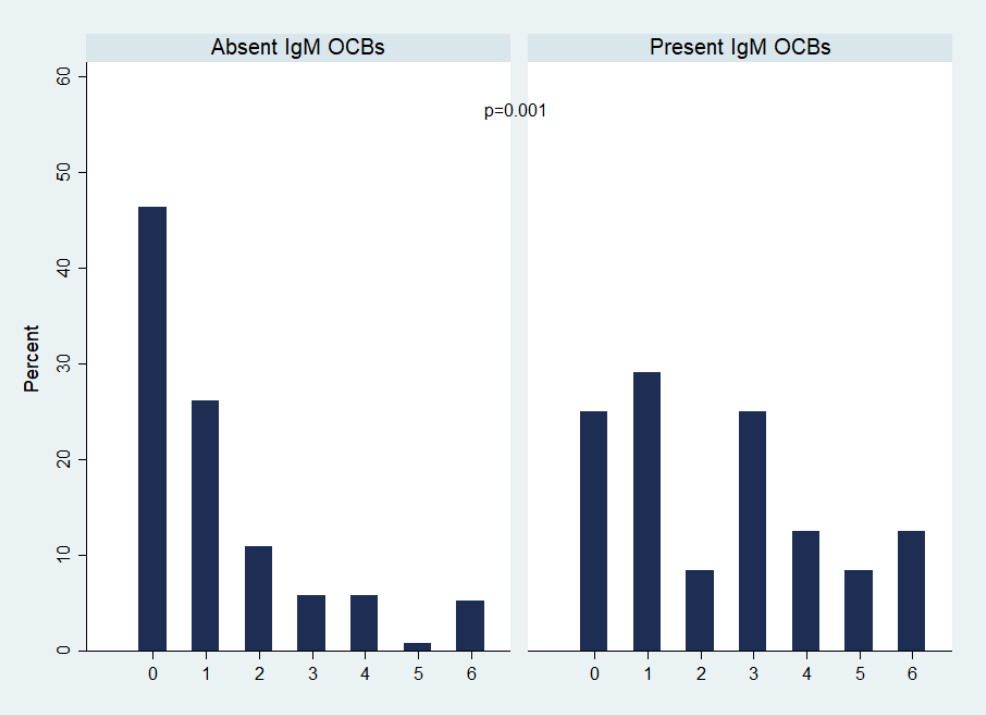


Figure 66: *distribution graph (%), Guillain-Barrè Disability Score (GB-DS) after 12 months of follow up.*

Differently from GB-DS, the median i-RODS score was significantly different at hospital admission between IgM OCBs positive and negative patients (10 vs 28 points respectively, p=0.006). An analogue significant different was also mantained at nadir and after 12 months of follow up, with 14 and 13-points lower scores for patients with IgM OCBs in CSF and highly significant P-values (p<0.001 in both cases) (**figures 67-70**).

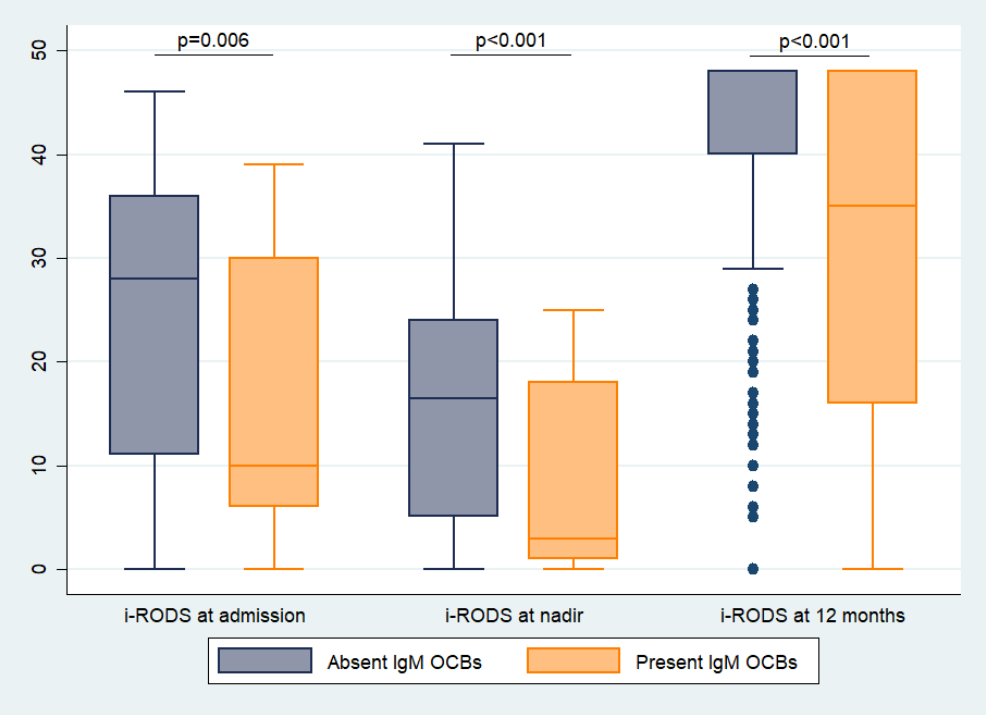


Figure 67: *box plot, median inflammatory Rasch-build Overall Disability Scale (i-RODS) at admission, at nadir and after 12 months of follow up.*

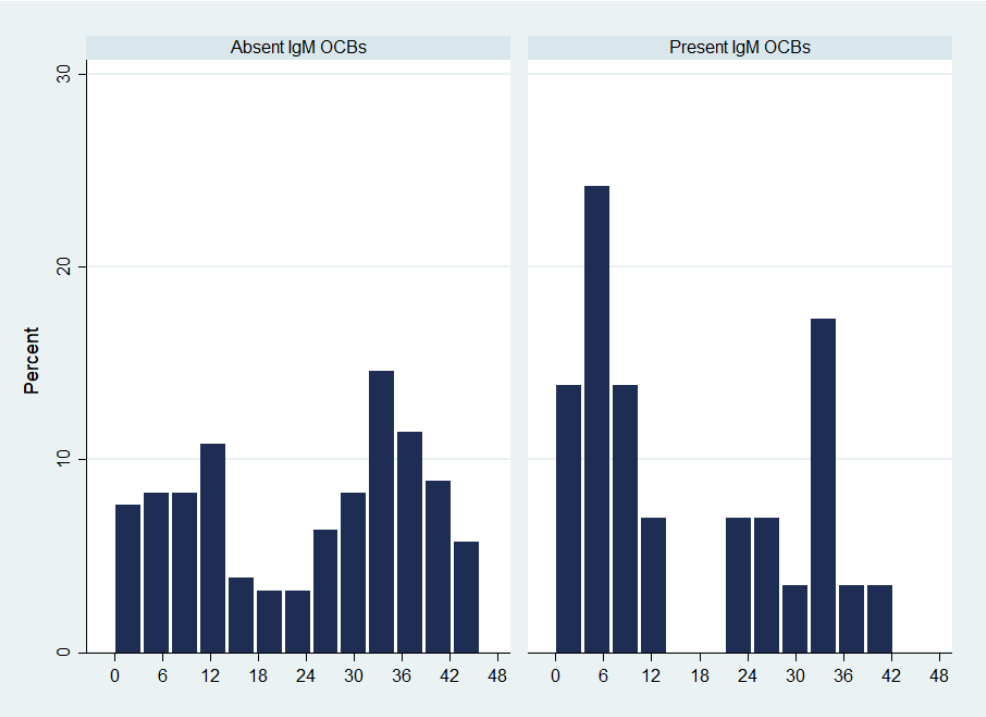


Figure 68: *distribution graph (%), inflammatory Rasch-build Overall Disability Scale (i-RODS) at admission.*

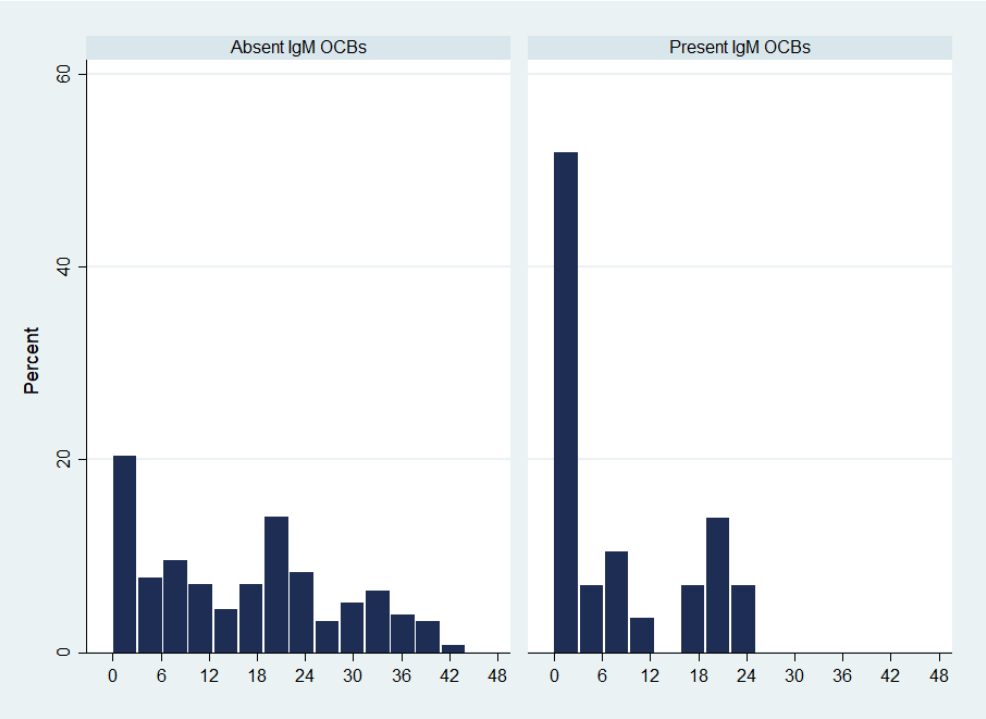


Figure 69: *distribution graph (%), inflammatory Rasch-build Overall Disability Scale (i-RODS) at nadir.*

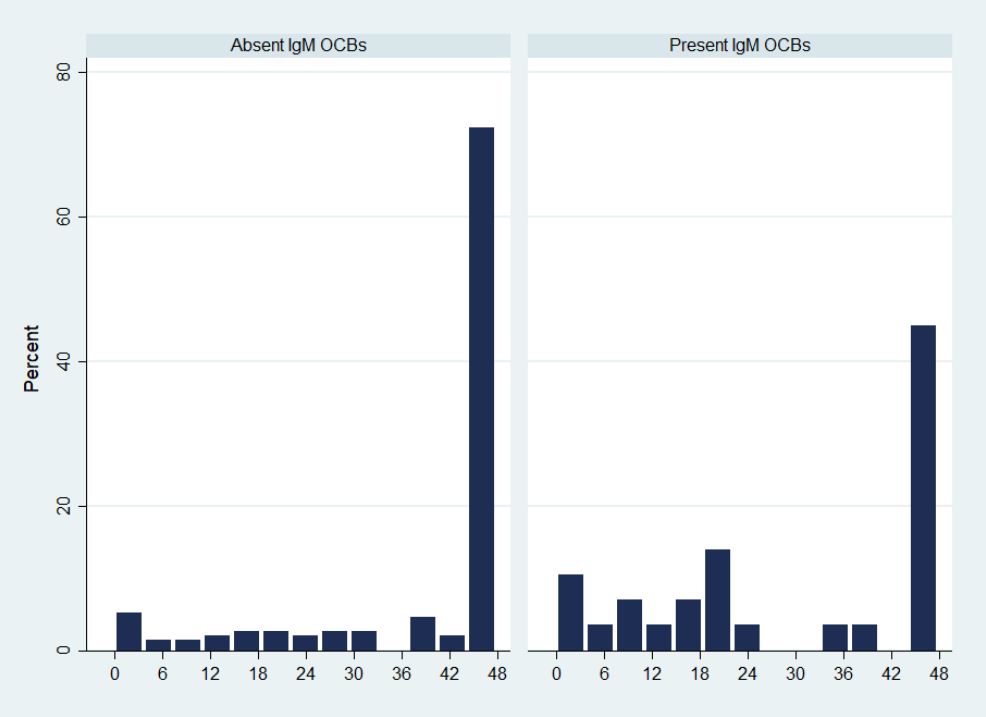


Figure 70: *distribution graph (%), inflammatory Rasch-build Overall Disability Scale (i-RODS) after 12 months of follow up.*

Altough a higher proportion of IgM OCBs positive patients was unable to walk unaided at hospital admission, such difference did not reach statistical significance at group comparison analysis (p=0.061). Nevertheless, considering the whole follow up, the percentage of patients who needed assistance in walking was 93% among IgM OCBs positive patients, significantly higher than that of negative patients (75%), with p=0.033. The proportion of unable-to-walk-unaided patients after 12 months of follow up was significantly higher among IgM OCBs positive patients (48% vs 17%, p<0.001) (**figure 71**). The median time between the onset of

neuropathy and this outcome was significantly shorter in the IgM OCBs positive group (1 day less than negative patients, p<0.001) (**figure 72**).

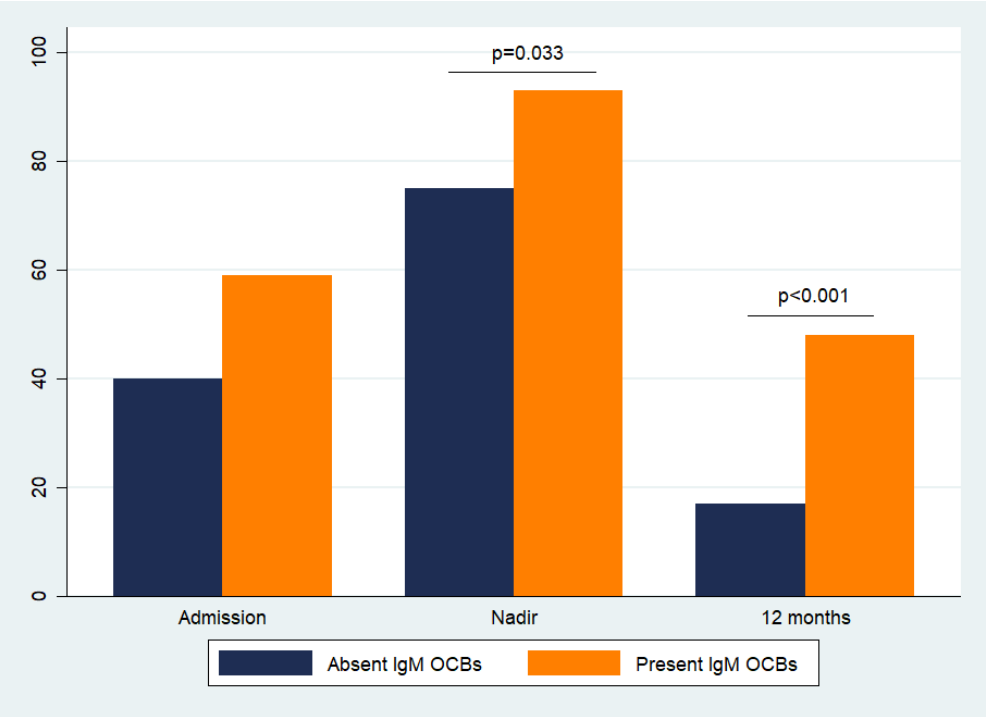


Figure 71: *bar graph (%), proportion of patients unable to walk unaided at admission, at nadir and after 12 months of follow up.*

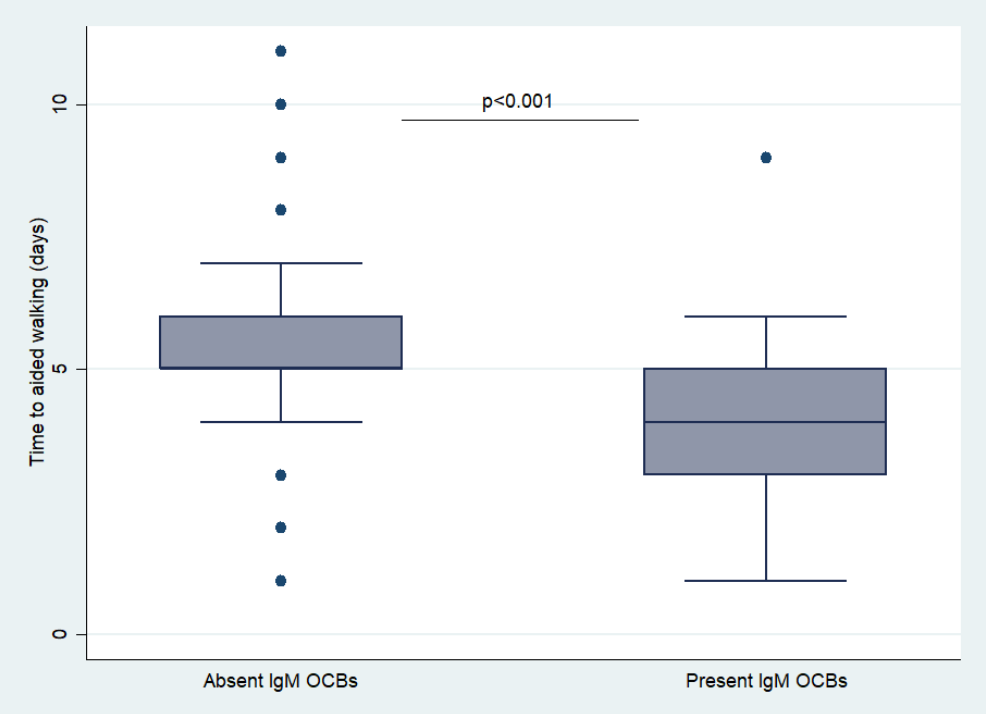


Figure 72: *box plot, median time from the onset of neuropathy to aided walking (days).*

The median time to reach nadir was significantly shorter among IgM OCBs positive patients (7 vs 9 days, p=0.002) (**figure 73**).

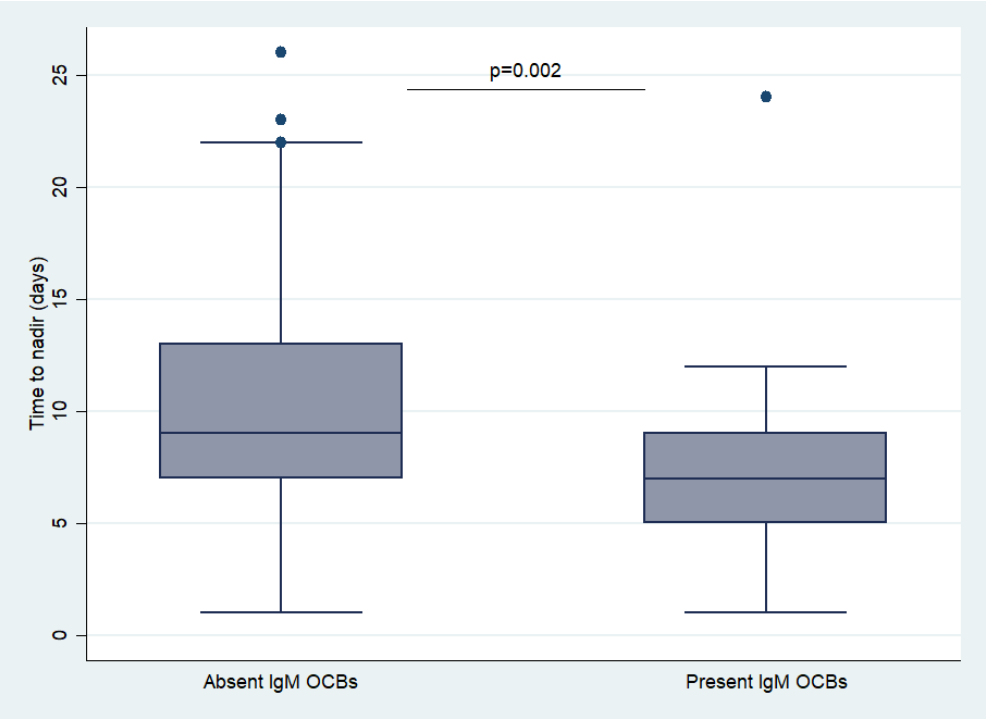


Figure 73: *box plot, median time from the onset of neuropathy to nadir (days).*

The proportion of bedridden patients was higher among IgM OCBs positive patients (66% vs 42%, p=0.022) (**figure 74**). Interestingly, the median time between the onset of neuropathy and confinement into bed was 4 days shorter among the IgM OCBs positive patients (p<0.001) (**figure 75**).

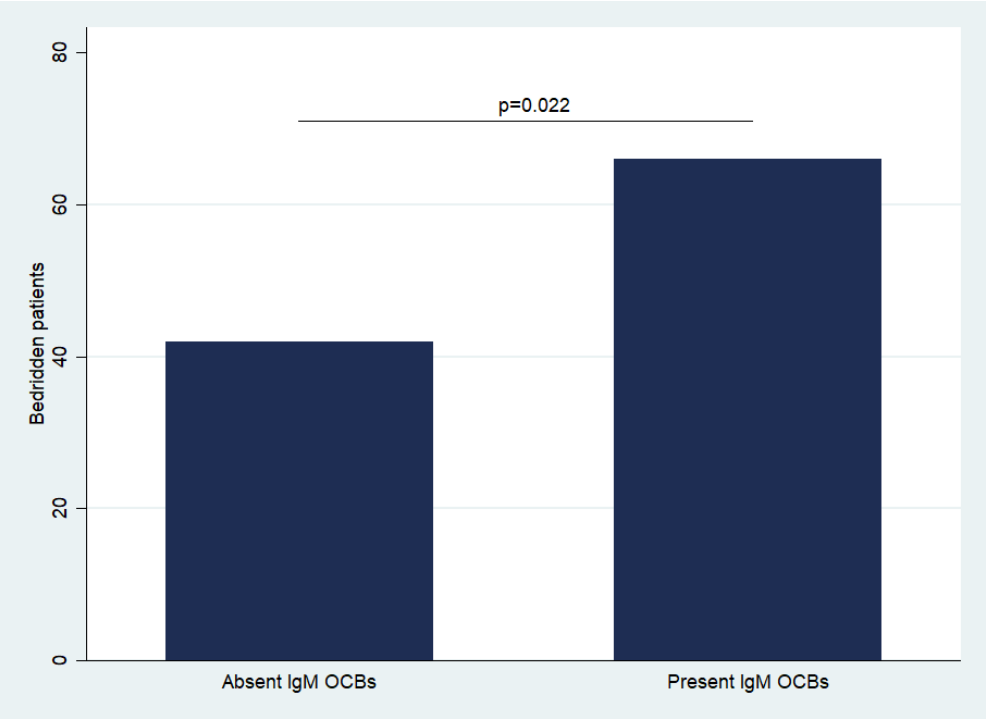


Figure 74: *bar graph (%), proportion of bedridden patients.*

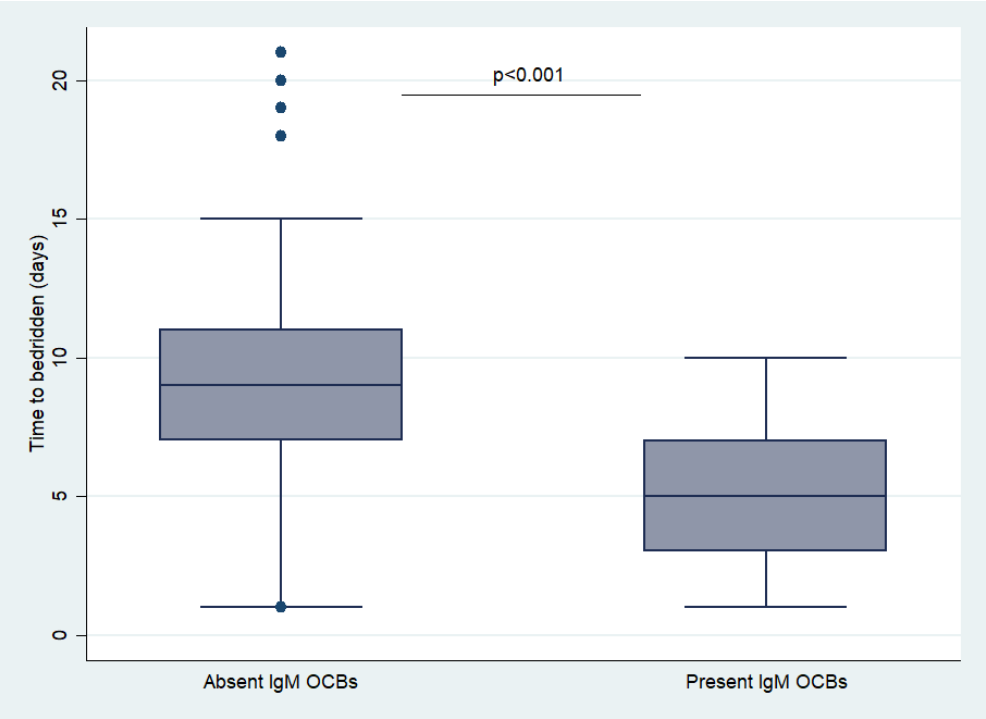


Figure 75: *box plot, median time from the onset of neuropathy to confinement into bed (days).*

The proportion of patients who needed assisted ventilation during the acute phase of the disease was two-times higher among IgM OCBs positive patients (28% vs 13%), but without reaching statistical significance (p=0.051). The same trend was seen for the proportion of died patients (10% vs 5%, p=0.267) (**figure 76**). The median time in days between the onset of symptoms and the start of mechanical ventilation was significantly shorter among IgM OCBs positive patients, i.e. 3 vs 7 days (p=0.027) (**figure 77**). On the contrary, no significant difference was found for the time from the onset of neuropathy and death.

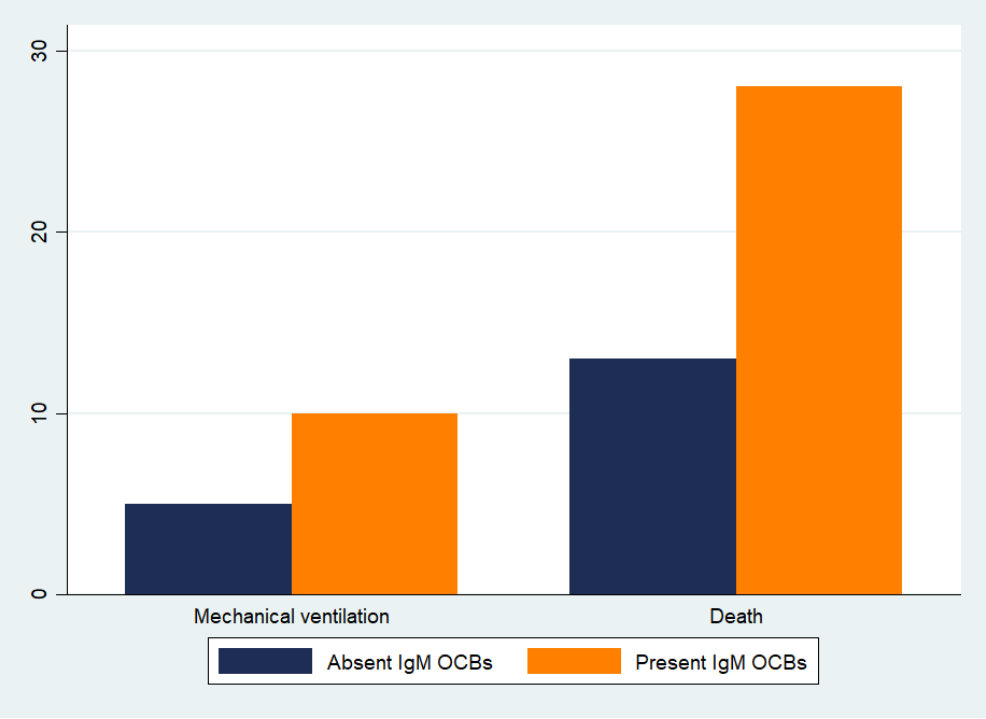


Figure 76: *bar graph (%), proportion of ventilated and dead patients.*

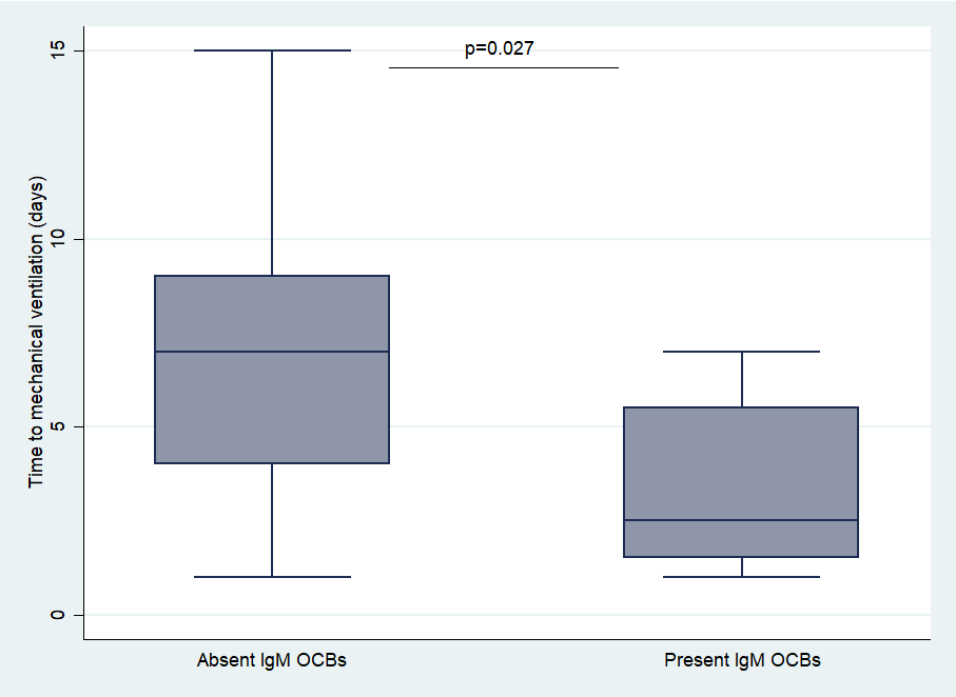


Figure 77: *box plot, median time from the onset of neuropathy to mechanical ventilation (days).*

Among the 146 patients who lost their ability to walk unaided during the acute phase of the disease, only 48% of those with IgM OCBs were able to walk without assistance again, against the 77% of IgM OCBs negative patients (p=0.002) (**figure 78**). The median time necessary to reach this outcome was 2 weeks longer among IgM OCBs positive patients, with p<0.001 (**figure 79**). Finally, the complete recovery from all symptoms was significantly less frequent for IgM OCBs positive patients (45% vs 72%, p=0.004) (**figure 78**). In this group of patients, the median time that was necessary to reach complete recovery was significantly longer (44 vs 29 weeks), with a highly significant P-value (p<0.001) (**figure 80**).

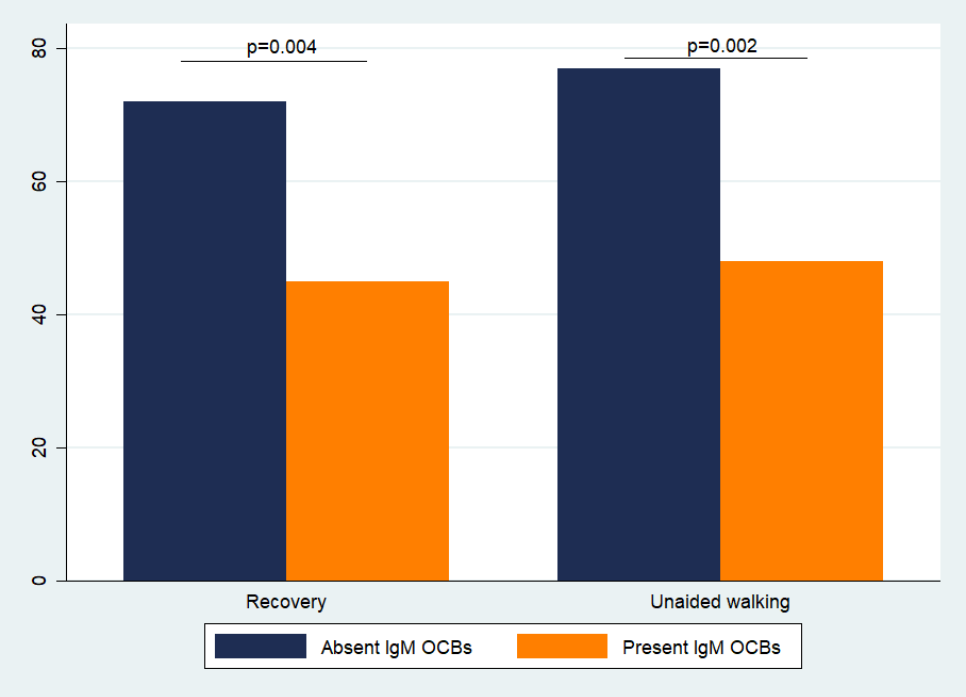


Figure 78: *bar graph (%), proportion of patients with complete recovery and who regained the ability to walk unassistedly.*

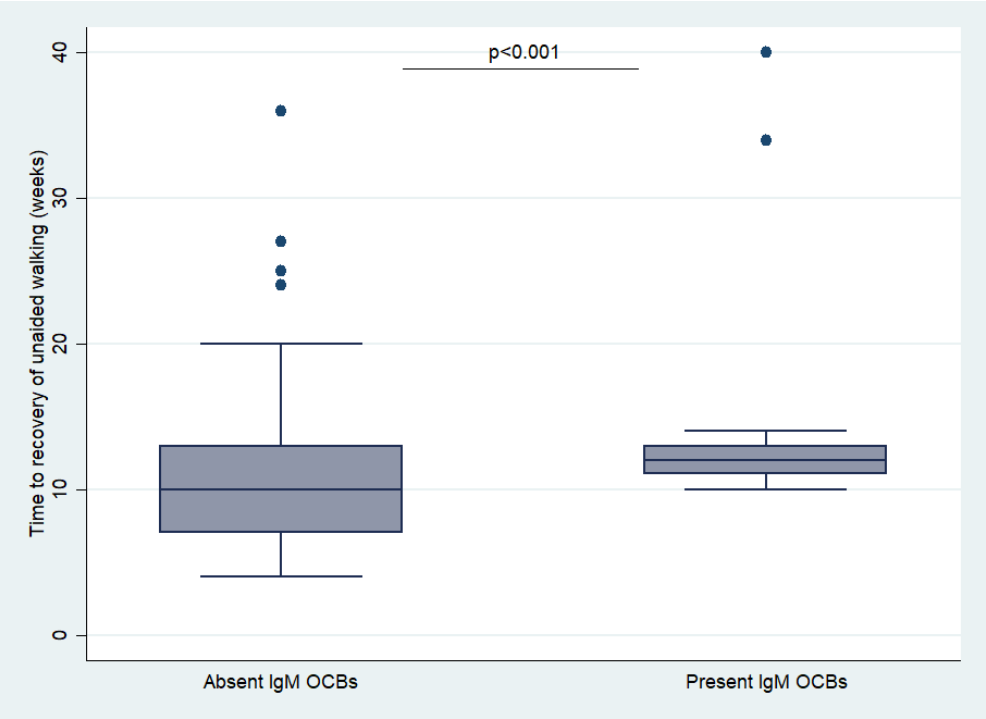


Figure 79: *box plot, median time from the onset of neuropathy and the ragined ability of walk unaided (weeks).*

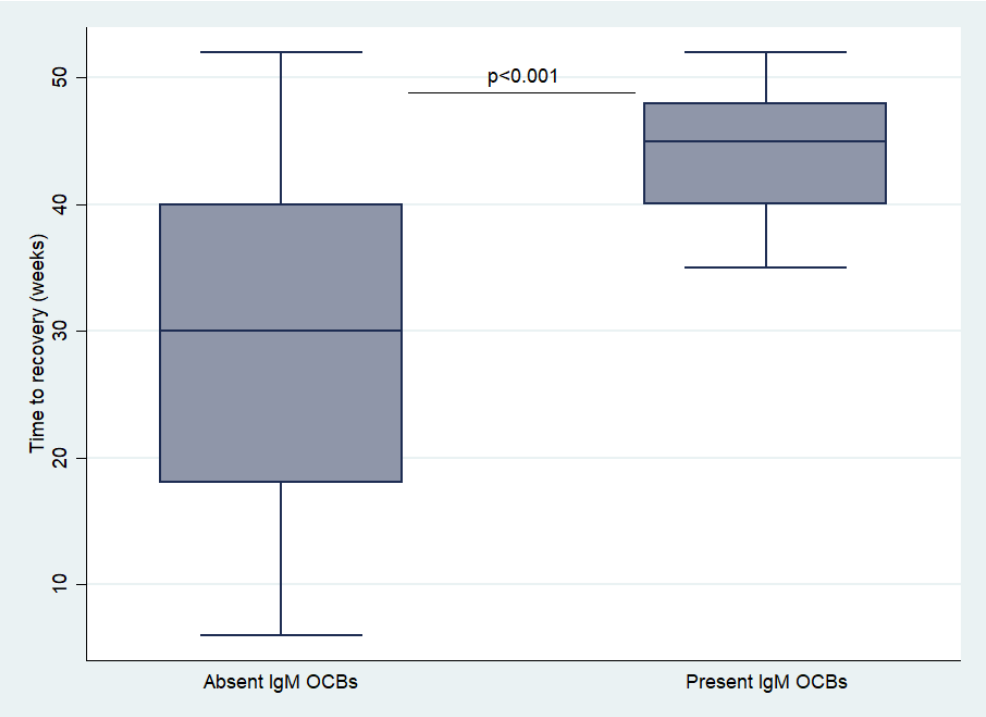


Figure 80: *box plot, median time from the onset of neuropathy and complete recovery (weeks).*

In conclusion, considering the trends observed for the different outcome measures, it clearly emerges the tendency towards more severe clinical involvement, lower chance of satisfactory recovery, as well as longer time to reach positive outcomes among patients with IgM OCBs in CSF.

3.3 Regression analysis

The next three tables show the results of univariate regression analysis for clinical, neurophysiology and laboratory analysis in association with the presence of IgM OCBs in CSF (**tables 17, 18, 19**). The following two tables (**tables 20, 21**) report the univariate and multivariate regression analysis for the different considered outcomes. Linear, ordinal or logistic regression analysis were performed for continuous, ordinal or dichotomic variables and the correlation measures are expressed as coefficient (linear and ordinal regression analysis) or Odds Ratio (OR: logistic regression analysis). P-value was significant if inferior than 0.05 for univariate regression analysis and inferior than 0.01 for multivariate regression analysis after Bonferroni's correction. Significant results were reported in bold.

3.3.1 Regression analysis – clinical variables

Table 17 reports the results of univariate regression analysis for clinical variables.

Variable	Coeff/OR	95% CI	p
Male sex	0.80	0.357 - 1.792	0.587
Age at onset of neuropathy	3.75	-3.369 - 10.873	0.300
Age ≥ 60 years	1.31	0.589 - 2.929	0.506
Infective prodromes (overall)	1.93	0.807 - 4.628	0.139
Gastrointestinal	1.12	0.462 - 2.736	0.797
Airways	1.61	0.689 - 3.745	0.273
Other	1.40	0.370 - 5.320	0.618
Antecedent vaccinations	**8.02**	**2.011 - 31.989**	**0.003**
Gangliosides (overall)	**0.26**	**0.074 - 0.888**	**0.032**
GM1	1.29	0.445 - 3.731	0.641
GD-GQ-GT	0.15	0.019 - 1.117	0.064
Clinical involvement			
Sensory	0.56	0.235 - 1.357	0.201
Bulbar	**3.06**	**1.352 - 6.947**	**0.007**
Ataxia	0.66	0.213 - 2.021	0.462
Autonomic	**3.25**	**1.371 - 7.708**	**0.007**
Clinical phenotype			
Classic	0.73	0.330 - 1.632	0.447
Pure motor	**2.79**	**1.083 - 7.170**	**0.034**
Miller-Fisher syndrome	0.54	0.119 - 2.463	0.428
Regional variants	0.75	0.209 - 2.708	0.664
mEGOS at hospital admission	**0.94**	**0.234 - 1.652**	**0.009**
EGRIS at hospital admission	**1.01**	**0.286 - 1.740**	**0.006**
Treatment (overall)	0.54	0.054 - 5.398	0.601
IVIG	0.73	0.078 - 6.750	0.779
PE	1.62	0.625 - 4.172	0.322
Both therapies	1.69	0.653 - 4.385	0.278
IgG monoclonal component in serum	0.67	0.081 - 5.566	0.711

Table 17: *univariate regression analysis, clinical characteristics. Results are reported as coefficient (linear or ordinal regression) or Odds Ratio (OR, logistic regression). CI: confidence interval. Statistical significance (p<0.05) is highlighted in bold.*

Univariate regression analysis confirmed the strong correlation between the presence of IgM OCBs and antecedent vaccinations (OR 8.02, p=0.003), bulbar and autonomic symptoms (OR 3.06 and 3.25 respectively, with p=0.007 for both variables) and higher scores at mEGOS (coefficient 0.94, p=0.009) and EGRIS (coefficient 1.01, p=0.006) at hospital admission.
A significant but weaker correlation was found between IgM OCBs and the absence of anti-gangliosides antibodies (OR 0.26, p=0.032) and a purely motor clinical phenotype (OR 2.79, p=0.034).
Age at onset, sex, presence of prodromes and type of treatments were not significantly associated with the presence or absence of IgM OCBs in CSF.

3.3.2 Regression analysis – neurophysiological variables

Table 18 reports the results of univariate regression analysis for neurophysiological variables.

Variable	Coeff/OR	95% CI	p
Time from onset to first study (days)	-0.08	-2.388 - 2.224	0.944
Second NCS study	1.25	0.535 - 2.937	0.602
AIDP	**0.20**	**0.084 - 0.470**	**<0.001**
AMAN	**4.67**	**1.854 - 11.766**	**0.001**
AMSAN	2.92	0.688 - 12.424	0.146
Axonal NCS	**5.02**	**2.127 - 11.862**	**<0.001**
Abnormal F waves	**8.45**	**2.457 - 29.058**	**0.001**
Prolonged F waves latency	1.80	0.808 - 3.997	0.150
F waves absence	**3.58**	**1.531 - 8.387**	**0.003**
Sensory involvement	1.09	0.489 - 2.445	0.829

Table 18: *univariate regression analysis, neurophysiology characteristics. Results are reported as coefficient (linear or ordinal regression) or Odds Ratio (OR, logistic regression). CI: confidence interval. Statistical significance (p<0.05) is highlighted in bold.*

Regarding NCS characteristics, at univariate regression analysis the presence of IgM OCBs in CSF was significantly associated with AMAN subtype of GBS (OR 4.67, p=0.001) and, more generally, with an axonal pattern of neural damage (OR 5.02, p<0.001). Specularly, demyelinating features at NCS consistent with electrodiagnostic criteria for AIDP are associated with the absence of IgM OCBs in serum (OR 0.20, p<0.001).
A specific damage of nerve roots expressed as an overall abnormality of F waves was strongly associated with the presence of IgM OCBs in CSF (OR 8.45, p=0.001). Furthermore, IgM OCBs in CSF were strongly related to axonal nerve roots damage (OR 3.58, p=0.003).
The other NCS variables were not significantly associated with IgM OCBs presence in CSF: in particular, the time from onset to the first NCS study, the necessity of a second NCS study and the presence of sensory involvement.

3.3.3 Regression analysis – CSF variables

Table 19 reports the results of univariate regression analysis for CSF variables.

Variable		Coeff/OR	95% CI	p
Time from onset to lumbar puncture (days)		-1.36	-6.033 - 3.304	0.565
Albumin-cytological dissociation		**7.68**	**1.008 - 58.481**	**0.008**
Protein level in CSF (mg/dl)		**92.44**	**63.124 - 121.759**	**<0.001**
Cells in CSF (n/µl)		1.45	-0.424 - 3.320	0.129
BBB damage index		**3.94**	**2.989 - 4.881**	**<0.001**
	Abnormal	**6.81**	**1.561 - 29.750**	**0.011**
CSF IgG level (mg/dl)		**12.48**	**8.260 - 16.695**	**<0.001**
Serum IgG level (mg/dl)		108.12	-87.926 - 304.168	0.278
CSF albumin level (mg/dl)		**50.26**	**33.367 - 67.145**	**<0.001**
Serum albumin level (mg/dl)		197.24	-85.423 - 479.909	0.170
Link index		0.11	-0.005 - 0.218	0.060
	Abnormal	1.98	0.988 - 5.271	0.052
Reiber index		-0.45	-1.848 - 0.933	0.519
	Abnormal	1.53	0.468 - 4.974	0.498
Presence of mirror pattern IgM		1.95	0.867 - 4.406	0.101
Presence of mirror pattern IgG		1.88	0.786 - 4.511	0.143

Table 19: *univariate regression analysis, laboratory characteristics. Results are reported as coefficient (linear or ordinal regression) or Odds Ratio (OR, logistic regression). CI: confidence interval. Statistical significance ($p<0.05$) is highlighted in bold.*

Speaking about CSF laboratory characteristics, the presence of IgM OCBs was strongly associated with all the markers of a more severe BBB damage.

In particular, the CSF of IgM OCBs positive patients showed higher a higher concentration of overall proteins (coefficient 92.44, $p<0.001$), albumin (coefficient 50.26, $p<0.001$) and IgG (coefficient 12.48, $p<0.001$).

As a consequence, the presence of IgM OCBs in CSF was significantly related to albumin-cytological dissociation (OR 7.68, $p<0.008$), BBB damage index alteration (OR 6.81, $p<0.011$) and a higher value of BBB damage index (coefficient 3.94, $p<0.001$).

On the other hand, there was no significant association between IgM OCBs in CSF and the levels of albumin and IgG in serum.

Indexes of intrathecal IgG productions were unrelated with IgM OCBs in CSF, as well.

Finally, there was no significant association between IgM OCBs in CSF and the time from onset to lumbar puncture.

3.3.4 Regression analysis – outcome measures

Table 20 and **table 21** report the results of univariate and multivariate regression analysis for the considered outcome measures, respectively.

Variable		Coeff/OR	95% CI	p
Hospital admission				
	MRC scale	**-5.65**	**-9.433 - -1.817**	**0.004**
	GB-DS	0.65	-0.083 - 1.383	0.082
	i-RODS	**-7.35**	**-12.747 - -1.954**	**0.008**
	Unable to walk unaided	2.14	0.955 - 4.777	0.062
Nadir				
	MRC scale	**-10.24**	**-16.415 - -4.068**	**0.001**
	GB-DS	**1.00**	**0.281 - 1.716**	**0.006**
	i-RODS	**-8.41**	**-12.935 - -3.894**	**<0.001**
	Time to nadir (days)	**-2.83**	**-4.728 - -0.923**	**0.004**
	Unable to walk unaided	4.42	1.006 - 19.458	0.049
	Time to aided walking (days)	**-1.79**	**-2.659 - -0.924**	**<0.001**
	Bedridden patients	**2.58**	**1.127 - 5.907**	**0.025**
	Time to bedridden (days)	**-3.90**	**-5.880 - -1.924**	**<0.001**
12 months follow up				
	MRC scale	**-8.58**	**-14.705 - -2.454**	**0.006**
	GB-DS	**1.21**	**0.497 - 1.933**	**0.001**
	i-RODS	**-10.37**	**-16.304 - -4.441**	**0.001**
	Unable to walk unaided	**4.53**	**1.959 - 10.467**	**0.001**
	Recovery of unaided walking	**0.27**	**0.114 - 0.649**	**0.003**
	Time to recovery of unaided walking	**14.56**	**6.818 - 22.299**	**<0.001**
	Complete recovery	**0.31**	**0.139 - 0.705**	**0.005**
	Time to complete recovery	**13.11**	**7.433 - 18.790**	**<0.001**
Death		2.16	0.538 - 8.692	0.303
	Time (weeks)	-2.28	-6.455 - 1.902	0.284
Mechanical ventilation		2.49	0.976 - 6.330	0.067
	Time (weeks)	**-3.97**	**-7.189 - -0.752**	**0.016**

Table 20: *univariate regression analysis, outcome measures. Results are reported as coefficient (linear or ordinal regression) or Odds Ratio (OR, logistic regression). CI: confidence interval. Statistical significance ($p<0.05$) is highlighted in bold.*

Variable	Coeff/OR	95% CI	p
Hospital admission			
MRC scale	**-5.33**	**-9.097 - -1.566**	**0.006**
i-RODS	**-7.27**	**-12.683 - -1.866**	**0.009**
Nadir			
MRC scale	**-9.64**	**-15.736 - -3.539**	**0.002**
GB-DS	**0.89**	**0.168 - 1.618**	**0.008**
i-RODS	**-7.54**	**-11.862 - -3.216**	**0.001**
Unable to walk unaided	4.28	0.940 - 19.500	0.060
Bedridden patients	2.62	1.059 - 6.467	0.037
12 months follow up			
MRC scale	**-8.08**	**-14.057 - -2.103**	**0.008**
GB-DS	**1.18**	**0.441 - 1.921**	**0.002**
i-RODS	**-9.35**	**-15.037 - -3.659**	**0.001**
Unable to walk unaided	**5.08**	**2.029 - 12.699**	**0.001**
Recovery of unaided walking	**0.22**	**0.083 - 0.563**	**0.002**
Complete recovery	**0.30**	**0.124 - 0.715**	**0.007**

Table 21: *multivariate regression analysis, outcome measures. Results are reported as coefficient (linear or ordinal regression) or Odds Ratio (OR, logistic regression). CI: confidence interval. Statistical significance ($p<0.01$) is highlighted in bold, after Bonferroni's correction.*

Many of the considered outcomes showed a statistically significant trend related to the presence of IgM OCBs in CSF. As already seen in the previous section, regression analysis confirm that, as a general rule, IgM OCBs presence in CSF is related to worse performance at clinical outcome measures.

The presence of IgM OCBs in CSF was associated with lower MRC and i-RODS scores at hospital admission, confimed at the multivariate analysis (coefficient -5.33 and -7.27, p=0.006 and p=0.009, respectively). GB-DS score and the loss of unaided walk at admission did not reach statistical significance at the univariate analysis.

At nadir, patients with IgM OCBs in CSF performed significantly worse in terms of MRC score (coefficient -9.64, p=0.002), GB-DS score (coefficient 0.89, p=0.008) and i-RODS score (coefficient -7.54, p=0.001), as confirmed by the multivariate analysis. Other outcomes, such as the incapacity of walking without aid and the confinement into bed were statistically significant at the univariate analysis, but did not reach statistical significance at the multivariate analysis.

All of the considered outcomes at the end of the follow up (i.e. 12 months) performed significantly worse among IgM OCBs positive patients in both univariate and multivariate regression analysis. MRC score was significantly lower (coefficient -8.08. p=0.008) as well as i-RODS score (coefficient -9.35, p=0.001), while GB-DS score was significantly higher (coefficient 1.18, p=0.002) in these patients. The presence of IgM OCBs in CSF was also associated with the persistent loss of unaided walk after 12 months (OR 5.08, p=0.001) and with lower probability of recovery such ability among those patients who were not able to walk unaided during the acute phase of the disease (OR 0.22, p=0.002). Finally, the complete recovery from all neuropathic symptoms after 12 months of follow up was significantly and negatively related with the presence of IgM OCBs in CSF (OR 0.30, p=0.007).

Even though the Odds Ratio for death or need of mechanical ventilation was higher in case of presence of IgM OCBs in CSF (2.16 and 2.49, respectively), both outcomes did not reach statistical significance at the univariate regression analysis.

3.4 Survival analysis

In the following tables (**tables 22, 23**) are reported the results of univariate and multivariate Cox regression analysis for survival. Results are expressed as Hazard Ratio (HS) related with 95% Confidence Interval (95% CI). Significant P-value was <0.05 for univariate analysis and <0.001 for multivariate analysis after Bonferroni's correction. Bold character highlights significant associations.

Variable	HR	95% CI	p
Unable to walk unaided	**2.47**	**1.616 - 3.768**	**<0.001**
Bedridden patient	**2.44**	**1.460 - 4.062**	**0.001**
Recovery of unaided walking	**0.42**	**0.232 - 0.745**	**0.003**
Complete recovery	**0.39**	**0.222 - 0.700**	**0.001**
Death	2.10	0.557 - 7.915	0.273
Mechanical ventilation	**2.41**	**1.066 - 5.439**	**0.034**

Table 22: *univariate Cox regression analysis. Results are reported as coefficient (linear or ordinal regression) or Odds Ratio (OR, logistic regression). CI: confidence interval. Statistical significance (p<0.05) is highlighted in bold.*

Variable	HR	95% CI	p
Unable to walk unaided	**2.38**	**1.556 - 3.646**	**<0.001**
Bedridden patient	**2.59**	**1.543 - 4.330**	**<0.001**
Recovery of unaided walking	**0.40**	**0.221 - 0.710**	**0.002**
Complete recovery	**0.38**	**0.215 - 0.687**	**0.001**
Death	2.33	0.587 - 9.216	0.230
Mechanical ventilation	2.17	0.948 - 4.955	0.067

Table 23: *multivariate Cox regression analysis. Results are reported as coefficient (linear or ordinal regression) or Odds Ratio (OR, logistic regression). CI: confidence interval. Statistical significance (p<0.01) is highlighted in bold, after Bonferroni's correction.*

Loss of unaided walk and confinement into bed were significantly associated with the presence of IgM OCBs in CSF at the multivariate survival analysis, with Hazard Ratios of 2.38 and 2.59 respectively and a P-value <0.001 for both (**figures 81, 82**).

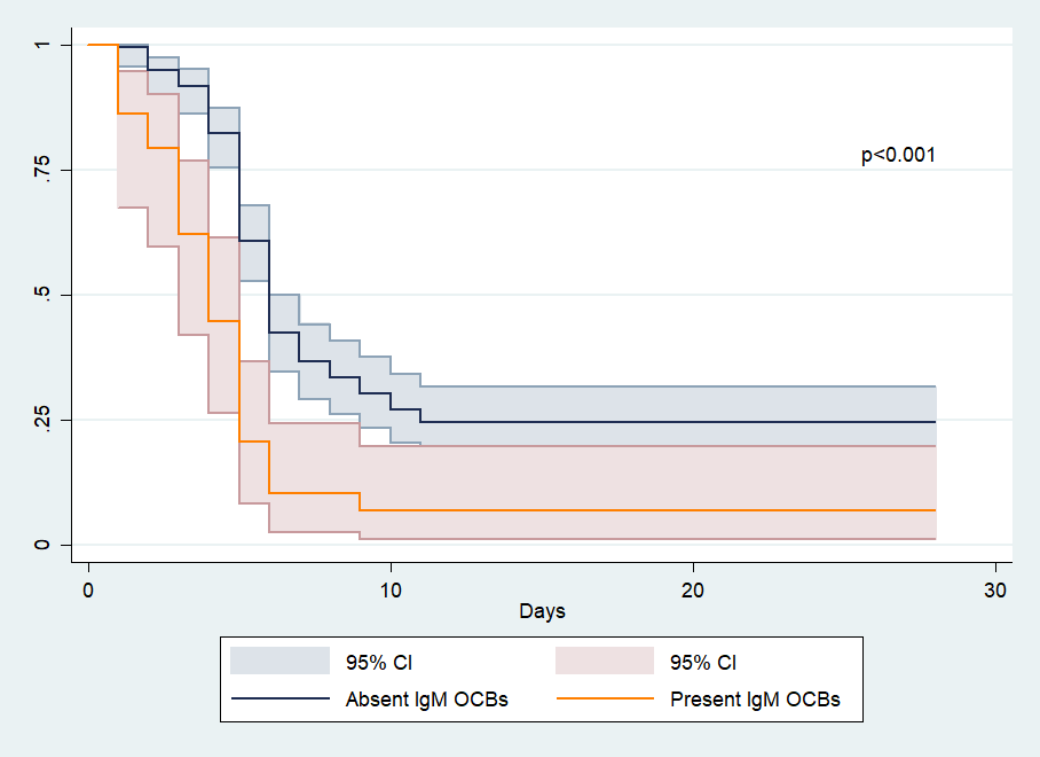


Figure 81: *Kaplan-Meier survival estimates. Outcome: loss of unaided walking.*

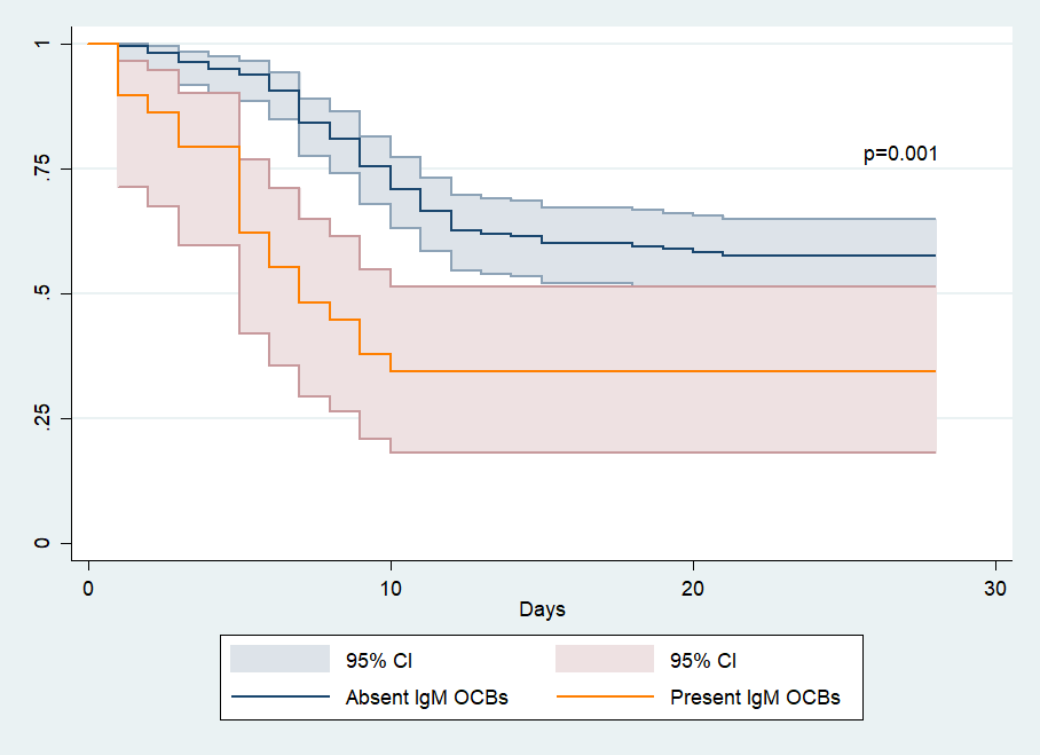


Figure 82: *Kaplan-Meier survival estimates. Outcome: confinement into bed.*

Conversely, recovery of unaided walking and complete recovery from neuropathy were negatively associated with IgM OCBs presence in CSF (HR 0.40, p=0.002 and HR 0.38, p=0.001, respectively) (**figures 83, 84**).

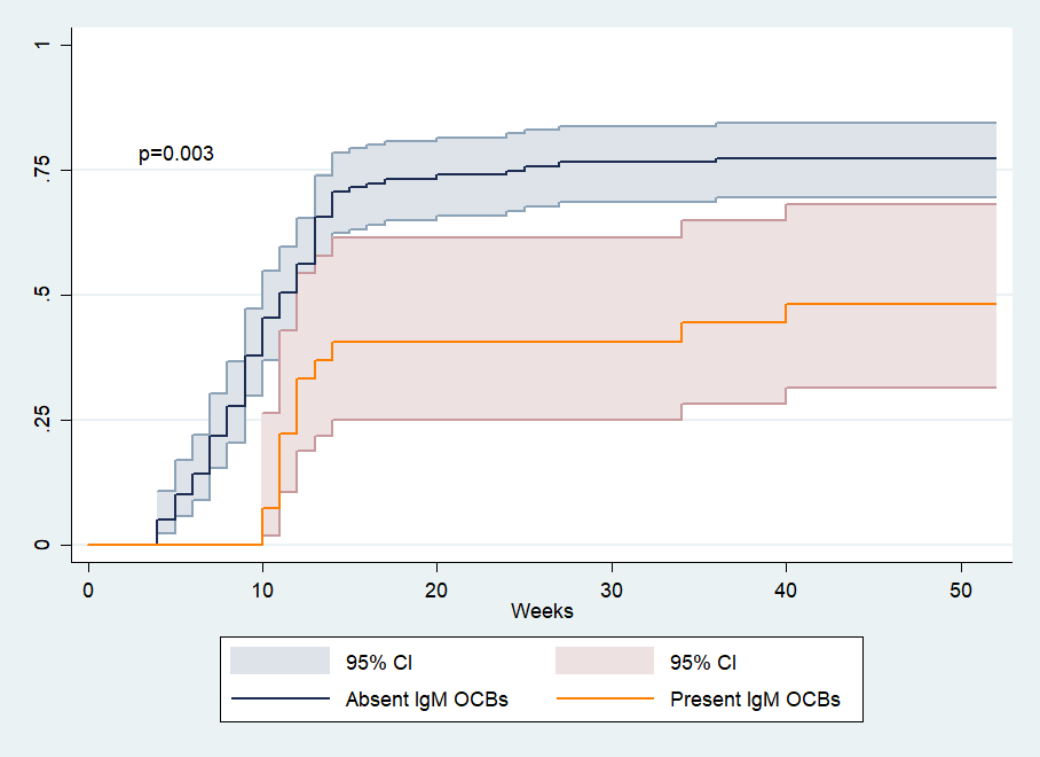


Figure 83: *Kaplan-Meier failure estimates. Outcome: recovery of unaided walking.*

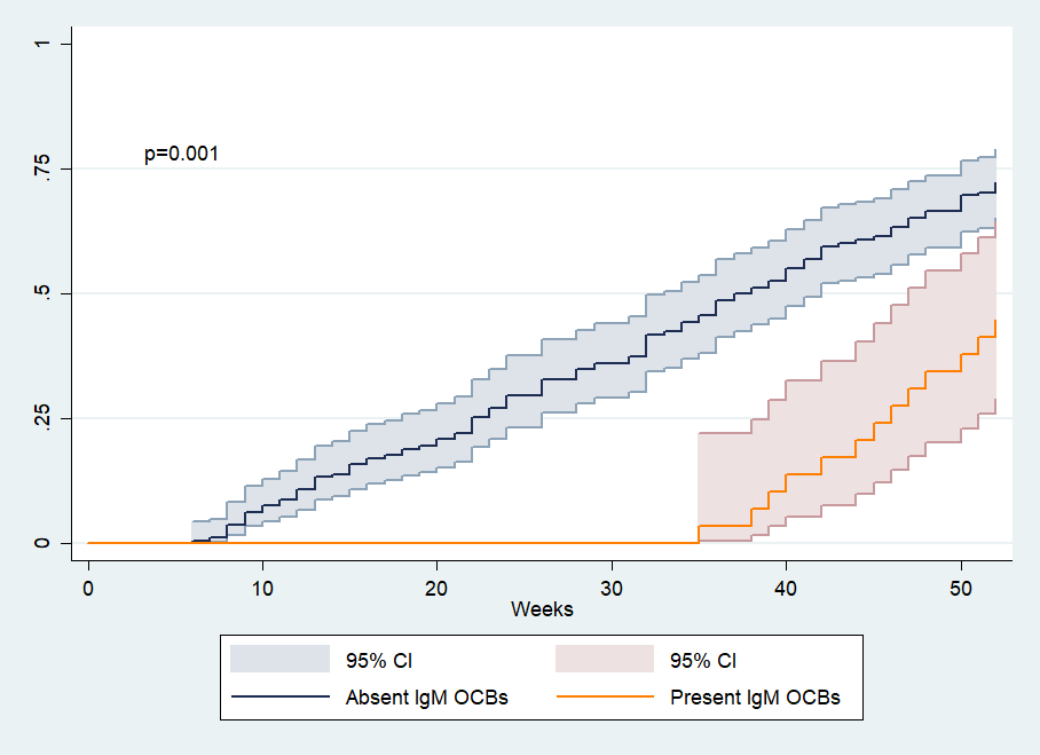


Figure 84: *Kaplan-Meier failure estimates. Outcome: complete recovery.*

As well as for regression analysis, death and need of mechanical ventilation did not reach statistical significance at the multivariate analysis, even though with HR of 2.33 and 2.17 respectively for IgM OCBs positive patients (**figure 85**).

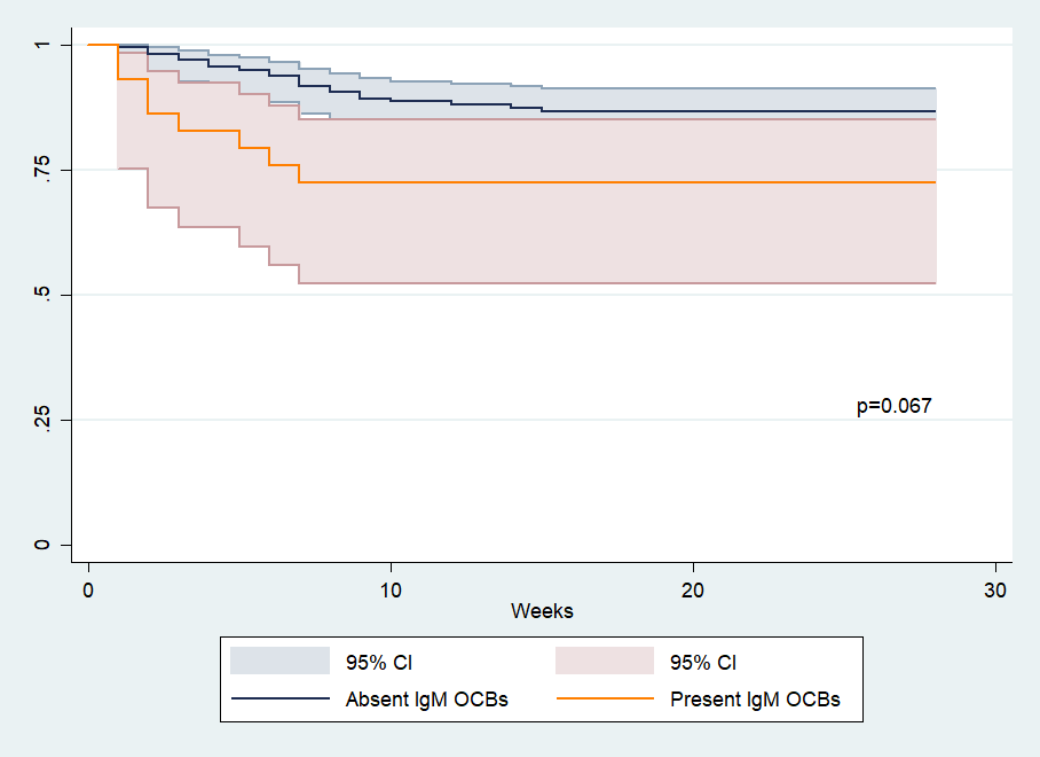


Figure 85: *Kaplan-Meier survival estimates. Outcome: mechanical ventilation.*

3.5 Receiving Operator Curve (ROC) analysis

The following figures (**figures 86-92**) shows the results of the compared Receiving Operator Curve (ROC) analysis for different outcomes. As represented in the figures, the presence of IgM OCBs in CSF always predicts the outcomes efficiently, showing the same positive or negative predictive value as some of the other known predictive variables (i.e. age at onset, dysautonomia, mEGOS or EGRIS scores). The multiple comparison for the equalities of AUCs between the selected variables (i.e. presence of IgM OCBs in CSF) and the other plotted variables results in a significant P-value for all the chosen outcomes ($p<0.001$). The Area Under the Curve (AUC) values, ranging between 0.55 and 0.70 for the different considered outcomes, are reported in the figures.

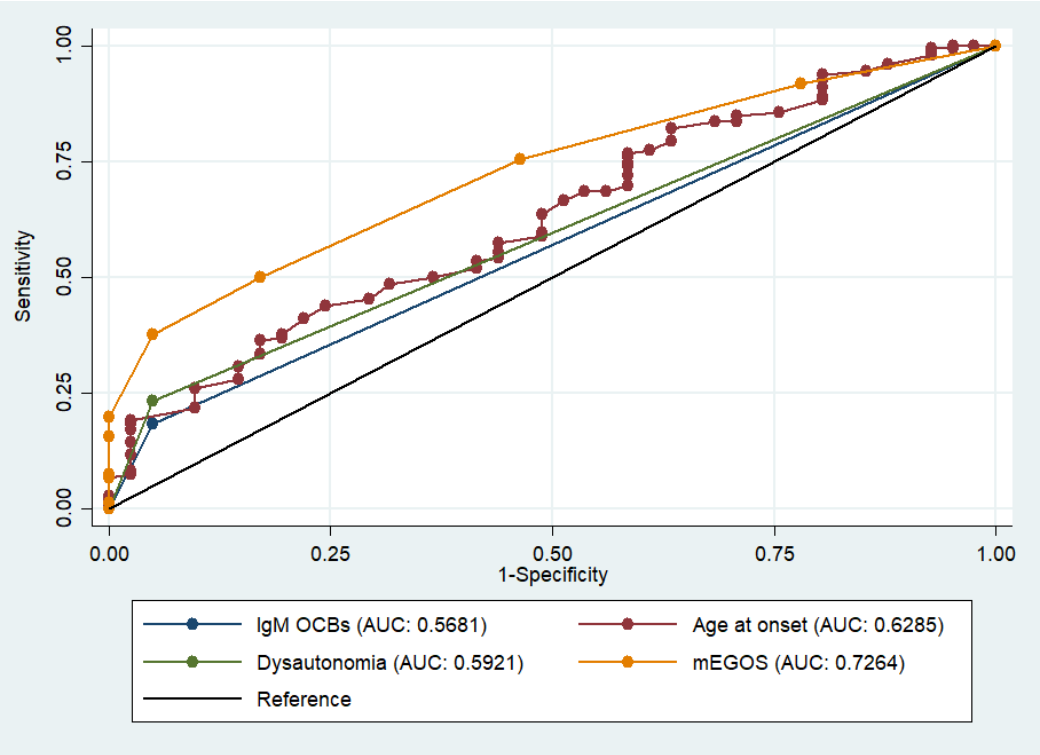


Figure 86: *multiple comparison of Receiving Operator Curve (ROC) analysis. Outcome: loss of unaided walking. Test for equality of Area Under the Curve (AUC) between IgM OCBs and the other plotted variables is statistically significant (p<0.001). AUC values are reported in the figure.*

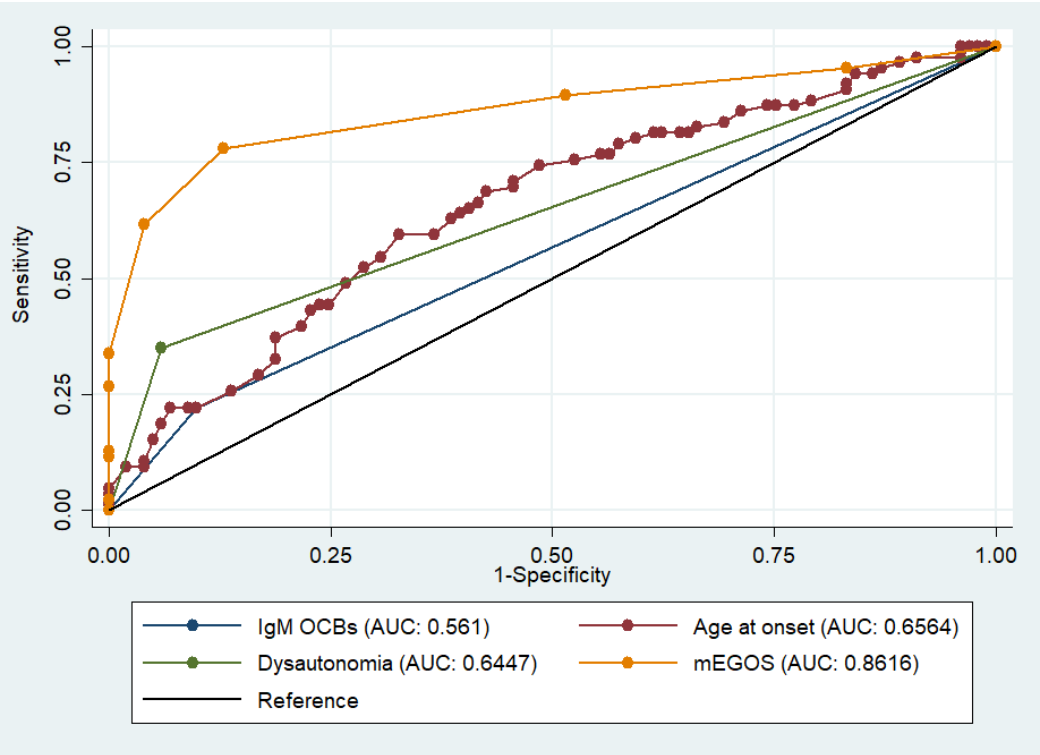


Figure 87: *multiple comparison of Receiving Operator Curve (ROC) analysis. Outcome: confinement into bed. Test for equality of Area Under the Curve (AUC) between IgM OCBs and the other plotted variables is statistically significant (p<0.001). AUC values are reported in the figure.*

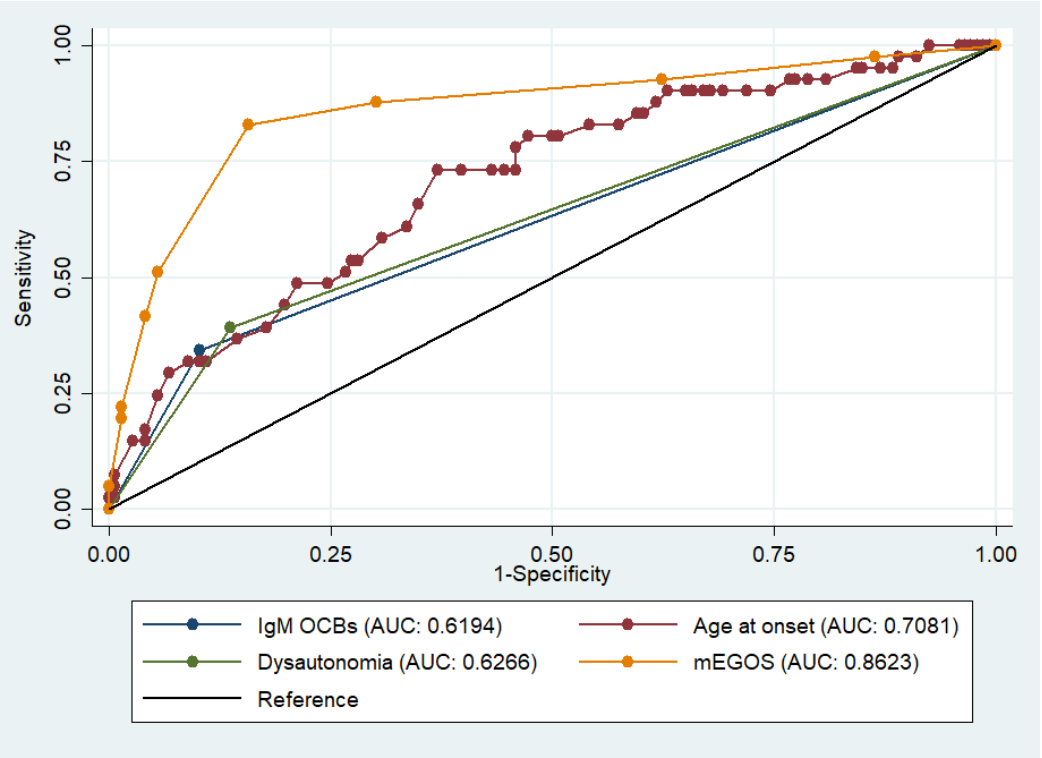


Figure 88: *multiple comparison of Receiving Operator Curve (ROC) analysis. Outcome: aided walking after 12 months. Test for equality of Area Under the Curve (AUC) between IgM OCBs and the other plotted variables is statistically significant (p<0.001). AUC values are reported in the figure.*

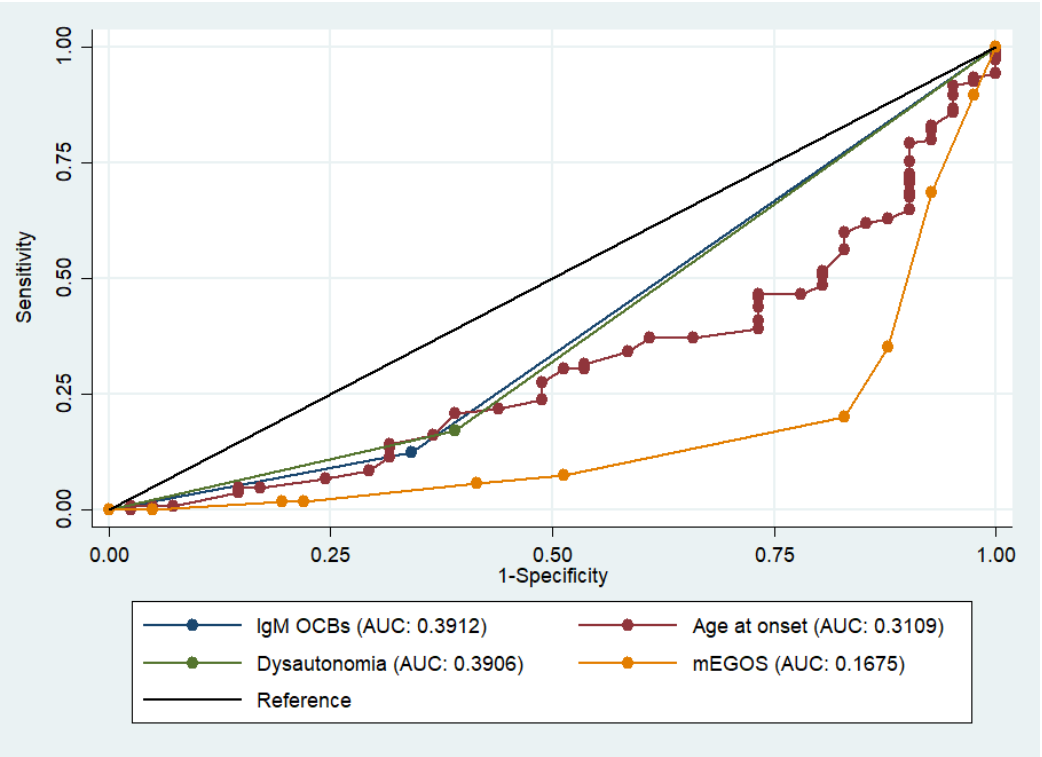


Figure 89: *multiple comparison of Receiving Operator Curve (ROC) analysis. Outcome: recovery of unaided walking. Test for equality of Area Under the Curve (AUC) between IgM OCBs and the other plotted variables is statistically significant (p<0.001). AUC values are reported in the figure.*

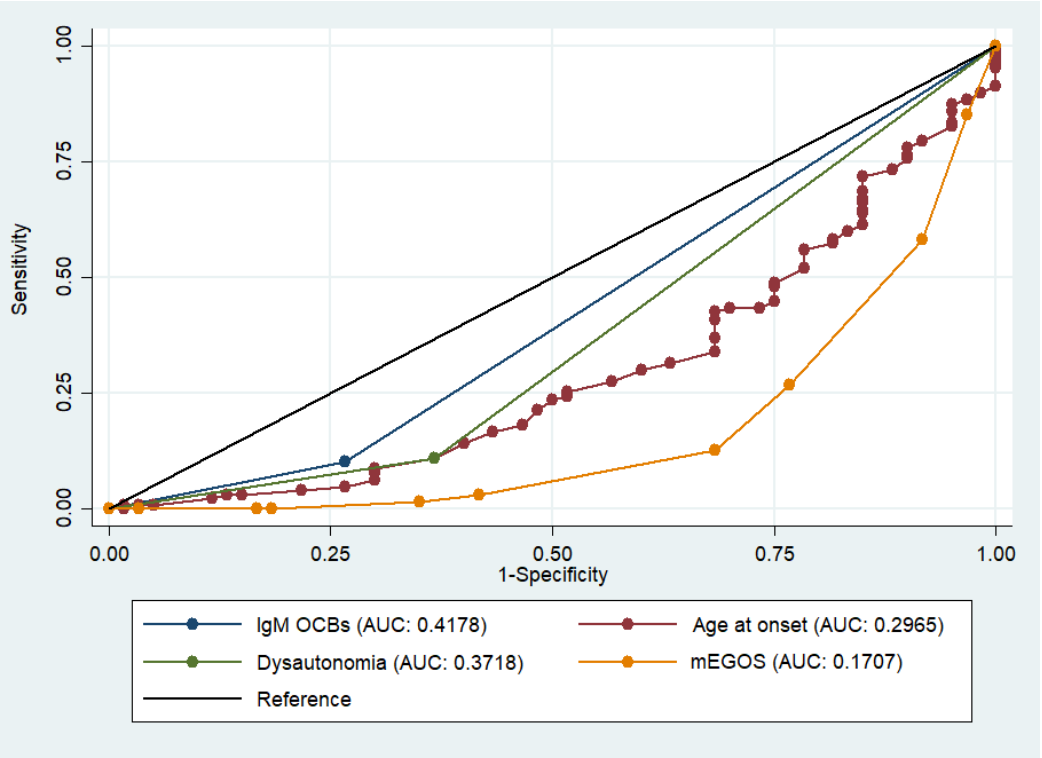


Figure 90: *multiple comparison of Receiving Operator Curve (ROC) analysis. Outcome: complete recovery. Test for equality of Area Under the Curve (AUC) between IgM OCBs and the other plotted variables is statistically significant (p<0.001). AUC values are reported in the figure.*

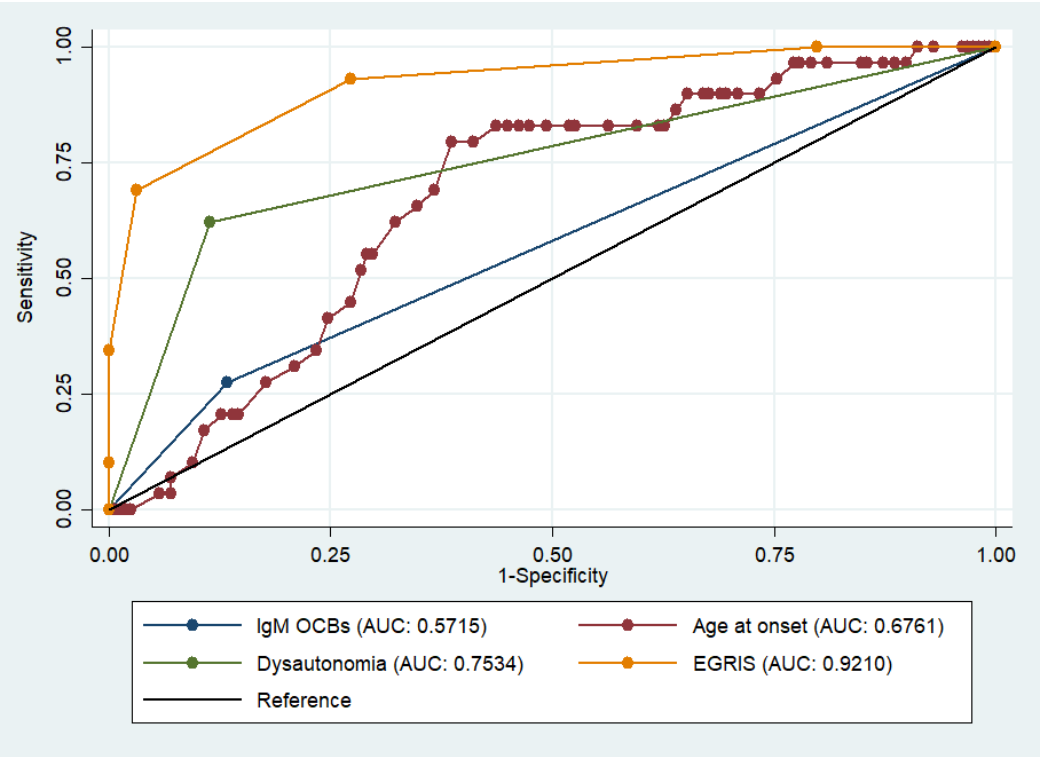


Figure 91: *multiple comparison of Receiving Operator Curve (ROC) analysis. Outcome: mechanical ventilation. Test for equality of Area Under the Curve (AUC) between IgM OCBs and the other plotted variables is statistically significant (p<0.001). AUC values are reported in the figure.*

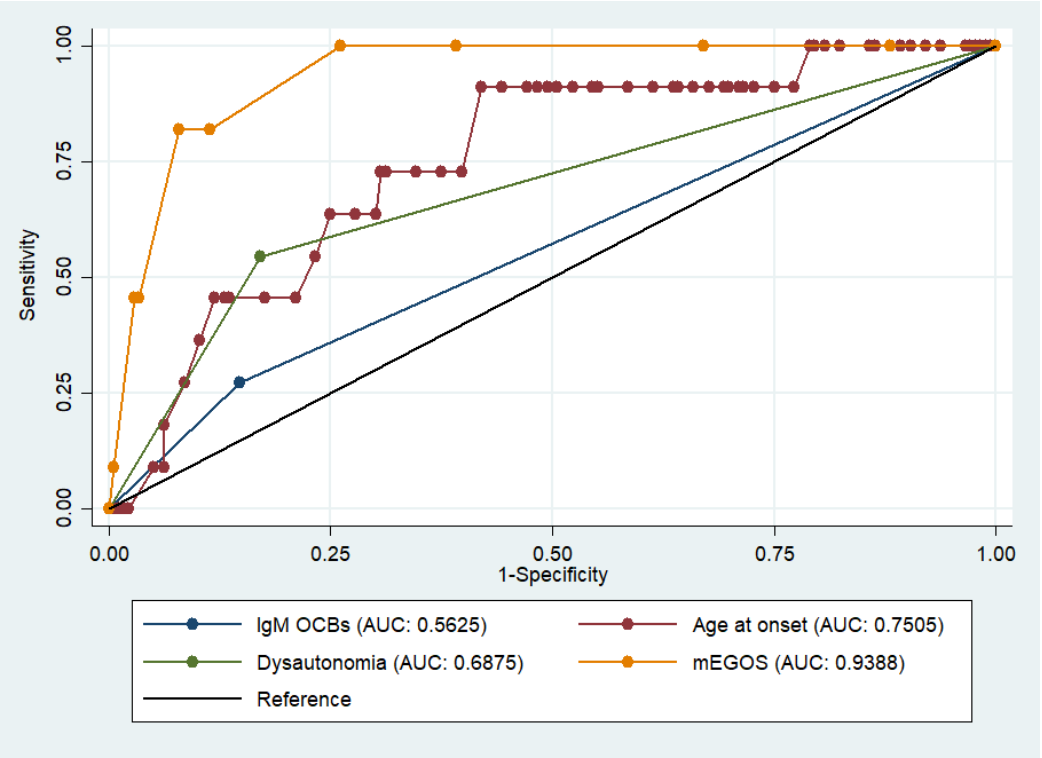


Figure 92: *multiple comparison of Receiving Operator Curve (ROC) analysis. Outcome: death. Test for equality of Area Under the Curve (AUC) between IgM OCBs and the other plotted variables is statistically significant (p<0.001). AUC values are reported in the figure.*

Section 4: discussion and conclusions

4.1 Data reproducibility: comparison between our cohort of patients and other GBS populations

One of the most important issue about this study concerns the representative value of our cohort of GBS patients and how results can be extended to general GBS population. In other terms, is our population representative of all GBS patients and therefore are our results reproducible? **Table 24** resumes the main characteristics of the population subject of this study and other cohorts of GBS patients reported in literature.[3,33,143-148] The most representative of these cohort of patients is the IGOS one, comprehensive of a vast number of subjects (925 in the original epidemiologic paper on regional variants,[3] 1500 in a more recent paper on CSF findings in GBS[33]). However, our group of GBS patients included almost only European caucasic patients, therefore the best representative population of reference might be the IGOS one, excluding 210 patients mainly from Bangladesh, South-Eastern Asia and Japan (715 patients left from Europe and USA only[3]). In fact, the most important differences from our population are evident for cohort coming from Bangladesh,[145] Japan[146,147] and China[148] (not shown in the table).

Variables	Present study (n=187)	IGOS study (n=925)	IGOS only Europe and America (n=715)	IGOS 1500 study (n=1500)	Italy (n=365)	Denmark (n=299)	Bangladesh (n=344)
Male sex	**63%**	60%	58%	60%	60%	58%	64%
Age at onset of neuropathy	**62 (45-74)**	51 (33-64)	55 (37-67)	50 (33-61)	62 (40-76)	55 (37-67)	33 (25-45)
Time from onset to admission (days)	**2 (1-3)**	3 (2-6)	3 (2-6)			5 (3-12)	10 (6-15)
Infective prodromes (overall)	**60%**	76%		79%	59%	70%	80%
Gastrointestinal	**26%**	27%		28%	18%	22%	50%
Upper airways	**26%**	35%	38%	39%	28%	38%	18%
Other	**8%**	14%				11%	12%
Clinical involvement							
Sensory	**78%**	59%	65%	57%		62%	22%
Bulbar	**39%**	50%	46%	48%		37%	60%
Autonomic	**19%**	25%	27%	24%		16%	30%
Clinical phenotype							
Classic	**62%**	61%	69%	61%	65%	77%	
Pure motor	**14%**	23%		23%	16%	14%	
Miller-Fisher syndrome	**11%**	10%	11%	11%	8%	7%	
Regional variants	**13%**	6%		5%	11%	2%	
Treatment (overall)	**98%**					78%	8%
IVIG	**97%**		86%	73%	79%	76%	6%
PE	**18%**			8%	19%	1%	2%
Both therapies	**17%**				7%		
Albumin-cytological dissociation in CSF	**81%**	67%			70%	67%	84%
Time from onset to lumbar puncture (days)	**8 (4-15)**	4 (2-8)		4 (4-5)		5 (2-11)	
AIDP NCS	**152 (81%)**	52%	55%			60%	32%
Axonal NCS	**35 (19%)**	10%				12%	53%
Time from onset to first NCS study (days)	**7 (5-10)**	7 (4-11)					

Table 24: *clinical, neurophysiological and CSF characteristics, comparison between our population and other cohorts of GBS patients reported in literature. Results are expressed as median (IQR) or %.*

The prevalence of male patients in our cohort is 63%, which is similar to the other described cohorts of GBS patients, ranging between 58% and 64%. On the other hand, the median age at onset is a bit higher when compared to the IGOS population or, most importantly, with Bangladesh and Japan populations. Actually, the difference is thinner considering only European and American patients of IGOS population or Denmark cohort. Interestingly, the median age of onset in our population of patients (i.e. 62 years) is exactly the same than a

previous multicentric Italian cohort of 365 patients. None of our patients was included in this study. Possibly, these data may reflect GBS epidemiology in an older general population such Italian one.
The median time interval between the onset of symptosms and hospital admission was 2 days, which is similar to the IGOS study (3 days) reflecting the highly accessible medical care in Italy, Europe and USA in opposition with the 10 days of Bangladesh study.
The prevalence of prodromic symptoms was 60%, which is very similar to previously described Italian and Danish populations (59% and 70%, respectively). Prevalence was a bit higher for IGOS population (76%), which considered a relevant proportion of Asiatic patients, for which a higher frequency of prodromes is reported (80% for Bangladesh and 82% for Japan). Infortunately, the prevalence of prodromic manifestations is not available for the subpopulation of European and American IGOS patients. The same trend can be seen when considering gastrointestinal and airways prodromes alone. Overall, our population showed similar prevalences as other European and Italian cohorts, while a much higher proportion of gastrointestinal prodromes was typical of Asiatic populations.
Regarding the clinical picture, sensory, bulbar and autonomic involvement was similar between our cohort and IGOS cohort, especially when considering European and American patients only. Actually, our cohort showed a slightly higher prevalence of sensory symptoms and a little bit lower prevalence of dysautonomia. Again, biggest variations are evident for Asiatic populations, with a much higher proportion of bulbar failure and very low frequency of sensory involvement.
Considering the distribution of clinical phenotype, there is a substantial coincidence between our cohort of patients and other populations of GBS patients. The classic and Miller-Fisher phenotypes account for 62% and 11% of patients respectively, which is identical to the IGOS cohort. The prevalence of pure motor phenotype (i.e. 14%) is the same as for other European populations (14% for Denmark, 16% for Italy). On the other hand, IGO population shows a slightly higher proportion of pure motor phenotype (23%), which is influenced by the high prevalence of AMAN reported among Asian patients (from 22% in Japan up to 65% in China).
Almost all of our patients have been treated with intravenous immunoglobulins and/or plasmapheresis. Even if it is rarely reported in other papers, a very high prevalence of immune-modulating treatment is common for European countries, USA and Japan. The proportion of treated patients heavily drops for low-income nations as, for example, Bangladesh: patients enrolled from this country have benn treated only in 8% of cases, thus potentially affecting the long-term outcome.
Little can be said about CSF findings, because in most studies the only reported parameter is albumin-cytological dissociation. In our cohort, 81% of patients had high levels of proteins and normal cell count in CSF. This is a higher proportion than for IGOS patients (67%), while it is similar to Bangladesh population. Such finding can be explained by the time interval from the onset of symptoms and lumbar puncture, which is higher for our cohort of patients (8 days versus 4 days for IGOS patients). It is well known that the probability of albumin-cytological dissociation increases in the first week after the onset of symptoms, with a sensitivity peaking at 85% after 7 days from the onset of symptoms.[1,9,33,149]
Finally, the neurophysiological classification of GBS reflects the different criteria, timing, reference values used in different studies, with a highly variable proportion of second NCS testing when the first one is equivocal or normal.In our study, we managed to classify all patients as demyelinating (AIDP) or axonal (AMAN/AMSAN) GBS, using specific criteria by Uncini and Kuwabara[38] and repeating NCS a second time after some weeks when necessary. After such neurophysiological study, the proportion of demyelinating patients was 81%, while 19% had an axonal form of GBS. All other epidemiologic studies classified GBS patients on the basis of a single NCS performed in the first days after the onset of symptoms. This explains the significant difference in proportion of demyelinating forms, which ranges from 50 to 60% of patients. About 30-40% of patients included in these studies are classified as “equivocal” or “normal” at NCS: most of the patients in this group could have been defined as demyelinating or axonal at a second neurophysiological study. The great variability of adopted criteria, reference values and timing of testing is today one of the biggest issues in GBS diagnosis.[39]

Table 25 shows the main data concerning outcomes of GBS in different populations reported in literature.[3,33,143-148] Regarding outcome measures and potential prognostic factors, there are only few data in literature, mainly referred to the IGOS study cohort of patients.

		Present study (n=187)	IGOS study (n=925)	IGOS only Europe and America (n=715)	IGOS 1500 study (n=1500)	Italy (n=365)	Denmark (n=299)	Bangladesh (n=344)
Hospital admission								
	MRC scale	**52 (46-56)**	46 (32-54)	48 (38-56)	47 (34-54)			24 (4-36)
Nadir								
	MRC scale	**46 (32-52)**	44 (25-53)	46 (30-54)				20 (4-34)
	GB-DS	**3 (3-4)**	4 (3-4)				3 (2-4)	
	Time to nadir (days)	**9 (7-12)**					10 (6-16)	5 (3-7)
	Unable to walk unaided	**78%**	79%	76%		67%	72%	94%
	Bedridden patients	**46%**	59%			47%	42%	85%
12 months follow up								
	MRC scale	**60 (53-60)**						
	GB-DS	**1 (0-2)**						
	Unable to walk unaided	**22%**						
	Recovery of unaided walking	**72%**	81%	83%				
	Time to recovery of unaided walking (weeks)	**10 (8-13)**		9 (4-26)				
	Complete recovery	**68%**						
	Time to complete recovery (weeks)	**32 (20-42)**						
Death		**6%**	7%	5%			3%	
	Time (weeks)	**4 (3-12)**	4 (2-12)					
Mechanical ventilation		**16%**	19%	17%	17%		13%	

Table 25: *outcome measures, comparison between our population and other GBS cohorts of patients reported in literature. Results are expressed as median (IQR) or %.*

The severity of muscle impairment at hospital admission and at nadir as measured with the MRC scale is similar between our study and the IGOS cohort of patients. As seen before, the Bangladesh study is characterized by a more severe form of diseases, with a dignificant difference in MRC scores at hospital admission and at nadir.
The same trend may be seen for disability measures. Our patients performed similarly to the IGOS ones in terms of GB-DS scale, bedridden patientst at nadir and proportion of patients unable to walk without assistance. Analogue proportions were seen also for Italian and Danish GBS populations. On the contrary, the worse clinical involvement of patients from Bangladesh is reflected by a significantly higher proportion of bedridden and unable-to-walk patients in this population. The time to reach nadir follows the same pattern, with longer time for our population and the IGOS one and shorter time for Bangladesh cohort.
The proportion of patients who recovered the ability to walk unaided after 12 months of follow up is slightly lower in our study than observed in IGOS population, but the time to this event is similar for the two cohorts of GBS patients. One of the explanations for this difference may be the older age at onset of neuropathy in our population if compared with the IGOS one. Actually, as demonstrated by different studies, age at onset is a well known prognostic factor, meaning that older patients are usually affected by more severe disease and tend to have lower recovery chance after neuropathic injury.[1,9,22,50-58,150]
Finally, the proportion of dead patients and the frequency of mechanical ventilation are roughly the same between the sample of patients considered in this study, the IGOS cohort and the Danish population.

Overall, we can say that the carachteristics of our cohort of patients are similar to those of the IGOS study, as well as in line with nation-wide studies performed for Italian and Danish populations. On the opposite, Asian population of GBS patient shows different epidemiological and clinical patterns, reflecting a likely distinct genetic predisposition or environmental

onfluences in aetiology. Outcomes are different in this population, as well. Globally, we can affirm that our cohort of patients appear to be representative of the European and American population of GBS patients, while our data might not be reproduced in other populations, such as Asian or African ones. In fact, our cohort of patients included almost only White Caucasian patients (98%), all of which lived in Italy at the moment of GBS onset.
In an indirect way, this comparison with large and multicentric cohorts such as the IGOS one is reassuring about the methodological conduction of our study in terms of selection of patients, collection of data and measurement of outcomes. Unfortunately, when requested, the Authors of the IGOS study did not provide us with the raw data about their cohort of patients, so it is impossible to elaborate a statistical comparison between groups with appropriate tests. As well, it is not possible to perform a formal external validation for outcome measures and prognostic factors in our cohort of patients. Nevertheless, the striking similarity between our cohort of patients and other described populations of analogue ethnicity strongly supports the validity of our findings obout IgM OCBs and their reproducibility at least for the population of the Western world.

4.2 Presence of IgM OCBs in CSF

In our sample of GBS patients, IgM oligoclonal bands (OCBs) in (CSF) were found in the 15.5% of cases (29 patients, overall). Specifically, this means that the CSF of these patients showed the presence of clonal IgM antibodies which are not visible in serum, represented by non-corresponding bands on CSF and serum at gel electrophoresis (i.e. band present in CSF but absent in serum). This finding reveals the existence of primary and exclusive production of IgM clonal antibodies in the central nervous system in a subgroup of patients with GBS, directly inside the intrathecal space. Such result represents a unicum in literature, since it has never been reported before.

The role of IgM OCBs has been widely explored for multiple sclerosis (MS). Their role as marker of B cells and plasma cells activation directly inside the CNS have been proved in previous papers. In their study published in 2022,[108] Casanova et al demonstrated the significant correlation between the presence of IgM OCBs and neurofilament light chain (nFL) level in CSF in a cohort of 130 patients with MS. More specifically, nFL levels were higher among patients with IgM OCBs compared to those without IgM OCBs, independently from clinical or radiological activity of disease (i.e. clinical relapses or presence of gadolinium enhancing lesions of brain or spinal cord at magnetic resonance -MRI- study). Moreover, patients with MS but without IgM OCBs showed similar nFL levels when compared with a cohort of 124 voluntary healthy controls. Such findings confirm the independent role of IgM OCBs in CSF as a marker of active inflammation in MS, even without overt singns of disease activity at conventional radiological studies, and may explain the association with worse prognosis in these patients. Moreover, an association between IgM OCBs and axonal damage can be speculated, due to the absence of active demyelinating lesions at MRI study. Actually, such correlation had already been noted in previous studies from Villar et al.[151,152] In particular, the last of them, conducted on 127 consecutve MS confirmed patients and published in 2015,[152] a significant association between high CSF nFL levels, elevated CSF lymphocyte cell counts and intrathecal synthesis of IgM against lipids was found. These findings support a role for IgM OCBs as markers of axonal damage of CNS in MS, with is in turn related to disability and brain atrophy progression. As a consequence of these findings, Alvarez encouraged the search of IgM OCBs in clinical practice as well as their evaluation as biomarkers of pathogenesis and prognosis in an editorial appeared in the same volume of the European Journal of Neurology in 2015.[153] Overall, literature reports a plethora of studies that confirm the presence and the prognostic value of IgM OCBs in CSF of MS patients.[110-116] Furthermore, other studies confirmed the valuable meaning of IgM OCBs in clinically and radiologically isolated syndrome as predictors of disease activity, neurodegeneration and risk of progression to MS.[104,109]

If the presence and pathological role of IgM OCBs is well demonstrated for MS, the role of IgG and, most of all, IgM OCBs for immune neuropathies still has to be elucidated. A bunch of studies has been published in literature exploring the potential role of IgG OCBs in such diseases. Morevoer, some of these studies are isolated case reports and only very few of them focused specifically on GBS. Only a dozen of papers produced in the last 50 years deserve to be mentioned.

The first report about the potential presence of IgG OCBs in CSF dates back in 1975, when Link described the single case of a woman with GBS associated with long-term persistence of IgG OCBs in CSF.[124] Another single case report of a patient with GBS and IgG OCBs in CSF was reported by Grimaldi et al in 1986.[120] However, studies on larger populations never managed to demonstrate the significant, non casual presence of IgG OCBs in the CSF of GBS patients.

In 1979, Siden et al found specular IgG OCBs in CSF and in serum (i.e. mirror pattern IgG) of 17% of 27 screened patients with GBS, but none of them showed exclusive IgG OCBs in CSF without correspondence in serum.[125]

Similar results were obtained in 1981 by Kruger et al.[126] They examinated the CSF and seruma of 16 patients with GBS and found a mirror pattern IgG in 3 of them (19%), while 10 patients had IgG OCBs only in serum (63%). None of their patients had IgG oCBs in CSF.[126]

Another study conducted on a wider population of GBS patients was was conducted in 1985 by Vedeler et al.[127] Of the 80 tested CSF and serum, none showed IgG OCBs in CSF.[127]

In 1986, Segurado et al analized the CSF and serum of 71 patients with GBS and 7 patients with Miller-Fisher syndrome (MFS).[123] A mirror pattern IgG was found in 68% of them, mostly in a transient fashion, while the presence of CSF-restricted IgG OCBs was not reported. The Authors concluded that the presence of IgG OCBs in CSF may just represents the result of a transitory increase of permeability in the blood-brain barrier (BBB), with the passage of IgG from serum to the intrathecal space, without evidence of CSF primary production of antibodies.[123]

Such conclusions were reiterated by Harrington et al in their 1987 review.[129]

Again, in a 1993 review of 146 patients by Zeman et al,[128] none of the 16 patients diagnosed with GBS had IgG OCBs in CSF.[128]

Other confirmations of these findings were obtained in more recent years. In 2006, Matà et al studied th sera and the CSF of 73 patients with GBS and of 43 patients with CIDP.[130] Although the prevalence of a mirror pattern IgG was significant (10 patients, 9% of the total number) and the general indexes of BBB damage and IgG concentration in CSF were significantly higher than in the control group, none of the included patients had IgG OCBs in their CSF.[130]

In 2020, a study from Pannewitz-Makaj et al reported a prevalence of IgG OCBs in CSF in the 5% of 470 patients affected by neuropathy, without further specifications.[119] Similar frequencies were reported for other screened neurological conditions, such as neurodegenerative diseases, cerebrovascular conditions and epilepsy. A much higher frequency was found only for MS, as expected.[119] Unfortunately, the Authors did not perform a subgroup analysis for the different aetiologies of the "neuropathy" group of patient, with particular focus on immune-mediated neuropathies such as GBS or CIDP. In conclusion, the Authors' interpretation was that a low frequency of IgG OCBs positivity in CSF was non specific and possibly casual in many different neurological conditions.[119]

One year later, Ruiz et al confirmed similar data.[121] In their population, IgG OCBs were present in none of the 32 GBS patients and in only 1 of the 48 patients with CIDP (i.e. 2%). In contrast, mirror pattern IgG and abnormal indexes of BBB permeability were much more frequent, with a prevalence of 40% in GBS patients and 19% of CIDP patients.[121] Again, the Authors' concluded for the absence of significant evidence of IgG intrathecal production in such immune-mediated neuropathies.[121]

Another confirmation of the same results came from Tu et al in 2021.[122] In their cohort of 92 GBS patients compared with 67 patients, they found significant signgs of BBB damage (i.e. CSF/serum albumin quotient) but without respective increase of indexes of intrathecal IgG production (i.e. IgG index and IgG synthesis rate).[122]

As an overall conclusion, it can be affirmed that the presence of IgG OCBs in the CSF of GBS patients has to be considered as exceptional and limited to single specific instances, without any demonstrated causal link.

Differently from IgM OCBs in MS and IgG OCBs in GBS, there is just one study in literature reporting the potential presence of IgM OCBs in the CSF of GBS patients. In 2016, Ferraro et al assessed the frequency of EBV-specific IgG and IgM OCbs in CSF of 50 patients with clinically isolated syndrome (CIS) and 27 controls affected by GBS.[131] Quite surprisingly, six GBS patients (22%) showed EBV-specific IgG OCBs in both CSF and serum ("mirror pattern") while 3 patients (16%) had EBV-specific IgM OCBs both in CSF and serum (IgM mirror pattern, as well).[131] Analogue proportions were found in the CIS group of patients. No significant association with analyzed variables nor prognostic value was found at statistical analysis.[131] Even though in the form of mirror pattern and specifically EBV-related, this is the first and only study that reported the possible presence of IgM OCBs in the CSF of GBS patients. This observation was the starting point for our study, which aimed aimed to explore the presence and meaning of IgM OCBs in GBS patients.

Given these premises, the first question to answer is if the presence of IgM OCBs in our GBS patients could be casual or if it might represent the expression of specific pathogenic mechanisms, mainly of infective nature.

We found IgM OCBs in the CSF of 29 of our patients, which represents the 15.5% of our sample. Such frequency is strikingly similar to that described by Ferraro et al in their study of 2016 and represents a value which is too high to be considered as a merely casual association. Unfortunately, there is no other described population of GBS patients with specific focus on intrathecal IgM OCBs production, so it is not possible to compare our findings with expected epidemiological data based on literature. On the other way, as detailed in the previous section, the global epidemiological and clinical characteristics of our sample of patients strongly overlap most of the other described GBS population for Western countries. Therefore, macroscopic selection biases are unlikely to think for our study.
All of these 29 patients had a purely intrathecal production of IgM, with IgM OCBs strictly restricted to CSF without correspondent OCBs in the serum. In fact, we decided to exclude from the IgM OCBs positive group of patients all those who presented an IgM mirror pattern, i.e. the same IgM OCBs present both in serum and in CSF. The latter chance could represent the passive transfer of IgM from serum to CSF through a damaged and more permeable BBB, while we wanted to isolate specifically patients with primary intrathecal synthesis of IgM. For the same reason, patients with a IgM monoclonal component in serum were not considered for the study (for example, patients affected by monoclonal IgM gammopathies of undetermined origin -MGUS-, Waldenstrom macroglobulinaemia, multiple myeloma or IgM-related cryoglobulinaemia).

IgG or IgM OCBs has been reported in literature in association with Epstein-Barr Virus (EBV), Mycoplasma Pneumoniae (MP) and, recently, Sars-CoV-2.[121,131,154] It is postulated that, in these cases, the intrathecal production of IgM or IgG OCBs represents the specific immune response to bacterial and viral antigens. To exclude a link between these and other infections associated with GBS and/or OCB production, all patients were serologically screened for Campilobacter Jejuni (CJ), EBV, CitoMegaloVirus (CMV) and MP. Furthermore, since 2021, all GBS patient included in the study were tested for Sars-CoV-2 infection. Infectend and non-infected patients were equally distributed among both IgM OCBs positive and negative patients and statistical analysis showed no significant difference in IgM OCBs prevalence between patients with or without some of these infections. Moreover, patients with positive screening for such microbiological agents were way less numerous than IgM OCBs positive patients. In detail, 19 patients were positive for CJ (10%), 8 patients for EBV (4%), 5 patients for CMV (3%), 3 patients for MP (2%) and 3 patients for Sars-CoV-2 (2%).

From a clinical perspective, the prevalence and distribution of infectious prodrome did not significantly differ between IgM OCBs positive and negative patients, further confirming the absence of an evident causal link between IgM OCBs and antecedent infections in our sample. Differently, the proportion of antecedent vaccination differed significantly between the two groups, with a higher prevalence among IgM OCBs positive patients (17% vs 3%, p=0.001). However, the target of vaccinations were not reported to be causative of IgG or IgM production, being seasonal flu viruse, streptococcus pneumonia and varicella-zoster virus. It is more likely that the vaccination itself, with its strong immune stimulation, may have engaged the production of IgM OCBs rather than its specific antigen.
The production of IgG OCBs in GBS has been related by some Authors to the presence of antibodies against some gangliosides, especially GM1 and GD1a.[130] However, in our sample of patients, there was no significant difference in the distribution of anti-ganglioside antibodies among the two groups of patients, so there is no evident aetiologic link between IgM OCBs and a specific subset of anti-ganglioside antibodies. Actually, the anti-GD, anti-GQ and anti-GT subset of antibodies was proportionally slightly less frequent among IgM OCBs positive patients. Such finding is consistent with the evident predominance of motor axonal variants of GBS among those patients, which are less frequently associated with these anti-gangliosides antibodies, as described in the next section.
Finally, the presence of transient OCBs has been related to apheretic treatment, with a largely obscure underlying pathogenic mechanism.[155] In our study, however, plasma exchange was performed similarly in both groups of patients with and without IgM OCBs (24% vs 17%, p=0.319). Furthermore, in most cases lumbar puncture was performed before starting the treatment or during the first exchanges, so it lacks a clear temporal correlation. For these reasons, the role of plasma exchange as source of IgM OCBs in the CSF of our GBS patients appears improbable.

Regarding the laboratory testing for detection of IgM OCBs, we adopted the commonly used and widely validated method proposed by Villar et al in 2001 and replied by Ferraro et al in sequent studies.[140, 141] Up to date, this represents the gold standard for IgM OCB detection, with the highest sensitivity and specificity as confirmed in previous validation studies.[156,157] Of note, the adopted method shows the highest sensitivity and the best reproducibility when compared with other proposed tests, mainly due to three refinements: the alkalin reduction buffer with DiThioTheritol (DTT) instead of distilled water, the narrower applied pH range (5-8) and the elimination of the secondary antibody from the immunodotection stage. Further details are reported in the "Methods" section of this thesis. Even if it is quite complex and time consuming, it was performed by a trained and expert biologist, with an expertise of more than 20 years in performing gel electrophoresis. Result were read by two expert neurologists, blinded each other. Finally, to avoid prejudice biases, the result of the laboratory test was kept secret to the reviewer neurologist until the end of data collection and follow up time. Such methodological expedients should have reduced the risk of inconscious data manipulation, even if the desing of the study remains mainly retrospective.

In synthesis, our study demonstrate a consistent and significant presence of IgM OCBs in a subgroup of GBS patients. Our data do not suggest a relation between IgM OCBs and any of the known potential aetiologic sources in GBS patients. Thus, such finding is likely independent from the considered variables and may be the expression of a precocious and specifically intrathecal immune response in GBS, as further detailed in the following section.

4.3 Clinical significance of IgM OCBs in CSF

Given the presence of IgM OCBs in the CSF of a minority of GBS patients, the next question we tried to answer was if their presence might identify a specific subgroup of subjects, characterized by clinical or neurophysiological features.

As already said, IgM OCBs positive patients did not differ significantly from negative patients in terms of gender, age at onset, prodromes and underwent treatments. Regarding clinical picture however, the pure motor variant of GBS was significantly more frequent among IgM OCBs positive patients (28% vs 12%, p=0.028). Furthermore, the involvement of some specific districts such as bulbar and autonomic system is significantly more widespread in IgM OCBs positive patients, with an approximately two-fold prevalence compared with negative patients (p=0.006 for both). Not surprisingly, predicting scores such as the modified Erasmus GBS outcome score (mEGOS) and the Erasmus GBS respiratory insufficiency score (EGRIS) performed significantly worse among IgM OCBs positive patients (p=0.010 and p=0.007, respectively). In other words, the presence of IgM OCBs in CSF identifies a subgroup of GBS patients characterized by predominantly motor symptoms, with higher chance of bulbar involvement and dysfunction of the autonomic system.

Neurophysiology tests with analysis of nerve conduction study (NCS) confirmed the significantly higher proportion of motor and axonal forms of GBS among IgM OCBs positive patients, with a prevalence of the acute motor axonal neuropathy variant (AMAN) of 34% against 10% of negative patients (p<0.001). A specular trend was observed for the classical demyelinating form of GBS (acute immune demyelinating polyradiculoneuropathy, AIDP), which was significantly less frequent among IgM OCBs positive patients (55% vs 86%, p>0.001). No significant different of time-to-analysis was found between the two groups. These findings are of utmost importance, confirming that the presence of IgM OCBs in the CSF are associated with axonal nervous damage even from an instrumental point of view. Such result reflects what has been observed for multiple sclerosis, in which the presence of IgM OCBs is associated with axonal degeneration and brain atrophy, demonstrating disease activity even in the absence of demyelinating lesions.[151,152]

The analysis of CSF characteristics reveals some more important details. Most importantly, the timepoint of lumbar puncture in relation to the onset of symptoms was similar between the two grops, as well as the median concentration of cells in the CSF. First of all, GBS patients with IgM OCBs in their sera presented with a much higher grade of BBB damage and permeability, as expressed by a higher prevalence of albumin-cytological dissociation (97% vs 78%, p=0.022), higher median concentration of protein in CSF (164 vs 71 mg/dl, p<0.001), higher median concentration of albumin (87.5 vs 36.8 mg/dl, p>0.001) and of IgG (16.0 vs 5.8 mg/dl, p<0.001) in CSF and higher median index of BBB damage (2.8% vs 0.9%, p<0.001). On the contrary, none of our GBS patients had IgG OCBs in their serum and indexes of intrathecal IgG synthesis were not significantly different between the two groups. In other words, the presence of IgM OCBs in the CSF of GBS patients is strongly associated with damage and increased permeability of the BBB. Otherwise, as already noticed for multiple sclerosis (see previous section),[108-116] we confirm that even for GBS patients, the primary intrathecal production of IgM antibodies is distinct from IgG synthesis and that IgG OCBs are usually not present in the CSF of GBS patients.[119, 121-123, 125-130,158]

Given all these premises, the logical consequence is the correlation between IgM OCBs and a more severe clinical involvement in terms of muscle strength and disability. Actually, IgM OCBs positive patients performed worse at hospital admission both at the medical research council scale for muscle strength (MRC; median value 47 vs 53, p=0.002) and at the inflammatory Rasch-build overall disability scale (i-RODS; median value 10 vs 28, p=0.006). Even at nadir (i.e. the worse timepoint during the disease course) the same scores confirmed a more severe involvement for IgM OCBs positive patients (median MRC value: 32 vs 47, p=0.002; median i-RODS value: 3 vs 17, p<0.001). Similar results were confirmed with the GBS disability scale (GB-DS), whose median value was one point worse for IgM OCBs positive patients at nadir (p=0.006). In synthesis, the presence of IgM OCBs in CSF are associated with worse clinical involvement and higher disability since the onset of symptoms of neuropathy.

In conclusion, we can affirm that, in our population of GBS patients, the presence of IgM OCBs in CSF is related to a specific subgroup of patients, characterized by predominantly motor and axonal involvement, by higher chances of bulbar and autonomic dysfunction and by more severe damage of the BBB. As a consequence, patients with IgM OCBs in CSF present with worse clinical picture, being affected by greater muscular weakness and, therefore, higher grade of disability. All these characteristics coherently describe a well-defined group of GBS patients, grossly corresponding to the axonal motor variant of GBS (i.e. AMAN), which is known to identify a minority group of subjects affected by severe clnical picture, particularly prone to complications and generally with worse prognosis. This association with such a specific and homogeneous portion of GBS patients gives further consistence to the presence of IgM OCBs, making it more unlikely to be just a casual and unspecific finding.

4.4 Pathogenetic significance of IgM OCBs in CSF

As already observed in the previous sections, most of the information about the pathogenic and prognostic role of IgG and IgM OCBs in CSF come from studies about multiple sclerosis (MS), while nothing can be said about their respective roles in GBS. Therefore, in order to deduce the potential reasons and consequences of IgM OCBs presence in GBS it is necessary to examinate what we know about their role in MS.

The presence of Ig OCBs in CSF is the most sensitive and specific marker of local, intrathecal synthesis of Ig antibodies.[92,93, 156,157] As for all Ig synthesis, it represents the clonal activation of maturating B-cells directly inside the CNS. Actually, B-cells has been demonstrated to reside inside the CNS, both in the meninges and in the parenchima.[158] However, only a small number of B-cells colones are present in the CNS, therefore any intrathecally-produced Ig can only ever be oligoclonal.[159] In particular, a considerable increase in CD5+ B lymphocytes was found in patients with IgM OCBs (**figure 93**).[151,160]

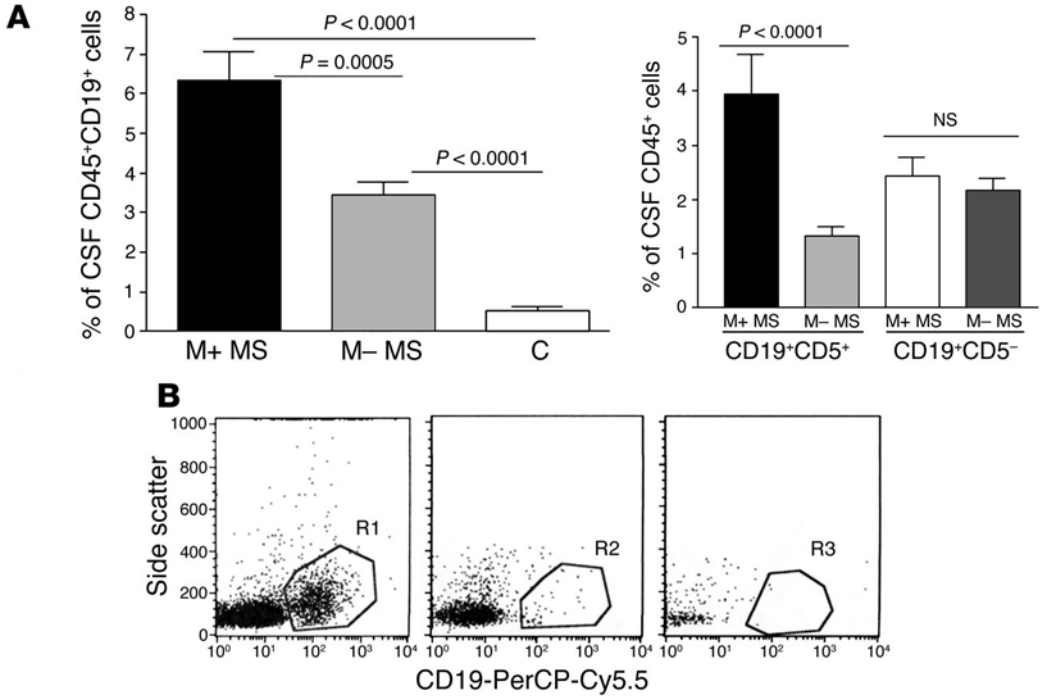


Figure 93: *study of B cellsin CSF of MS patients with and without IgM OCBs.* ***A:*** *IgM OCBs positive patients showed a higher percentage of CD19+ (left) and CD19+CD5+ (right) cells compared to negative patients.* ***B:*** *dot plot for CD19+ cells in CSF of IgM OCBs positive patients (left), negative patients (middle) and control patients (right). Reproduced from: Villar LM et al, Intrathecal synthesis of oligoclonal IgM against myelin lipids predicts an aggressive disease course in MS, J Clin Invest, 2005.*

Among the five different possible pattern of IEF analysis on agarose gel established in previous consensus statements,[161] pattern number two is specific for oligoclonal production of Ig directly inside the CNS (i.e. OCBs that are present in the CSF but not in the serum) (**figure 94**). This is the pattern we considered in our study.

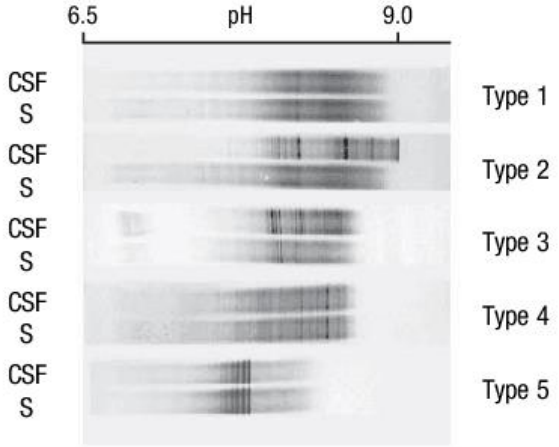


Figure 94: *five proposed patterns for immune electrofocusing of Ig OCBs in CSF and serum. Reproduced from: Freedman MS et al, Recommended standard of cerebrospinal fluid analysis in the diagnosis of multiple sclerosis: a consensus statement, Arch Neurol, 2005.*

IgM antibodies represent the first response of the innate humoral immune system. It is generically directed against lipids and lipoproteins of cellular membranes and it does not require a previous trigger to be initiated.[162] In the extracellular space, IgM antibodies aggregate in big pentameric complexes which are poorly soluble and tend to precipitate on biological surfaces. Due to their dimensions, pentameric IgM antibodies are less prone to pass through membranes, so most of their immunological effects develop in the site of production.[108,109] IgM antibodies are the most effective in activating the complement cascade of reactions, leading to the damage of cell membranes and to the chemotaxis of scavenger cells from the blood stream.[163] For all these reasons, IgM OCBs are considered a marker of precoucious and localized immune response inside the CNS, with a potential heavy burden of neural damage and BBB dysfunction.[108-116,141,151,152,160,164]

The target antigens of Ig OCBs are still undetermined. Clinical observation and experimental models point at some major constituent of myelin sheat, such as myelin basic protein.[94,95] Studies on recombinant cells revealed that lipid complexes cointaining sulfatide are the main target of part of IgG antibodies produced from single CSF plasma clones.[159] In particular, myelin-associated lipids have been consistently related to the presence of IgM OCBs in MS, phosphatidylcholine being the most frequently recognized lipid.[151] However, the oligo-clonal nature of OCBs suggests that the immune response is not entirely specific against one single antigen and that their presence reveals a diffuse activation of the humoral immune response in the CNS, possibly with different pathogenetic mechanisms involved.[94-96] Notably, an interesting study published in 2012 from Beltran et al demonstrated for the first time that IgM antibodies in CSF target neuronal surface antigens (**figure 95**).[162] Furthermore, the level of neuronal-recognizing IgM antibodies was directly correlated to brain atrophy in their population.[162] Actually, IgM antibodies are the most effective in activating the complement system and it has been reported that the acute axonal injury is complement-dependent:[163] therefore, as a conclusion, the Author suggest that IgM antibodies recognizing neuronal cell surface could be directly involved in complement-mediated axonal injury.

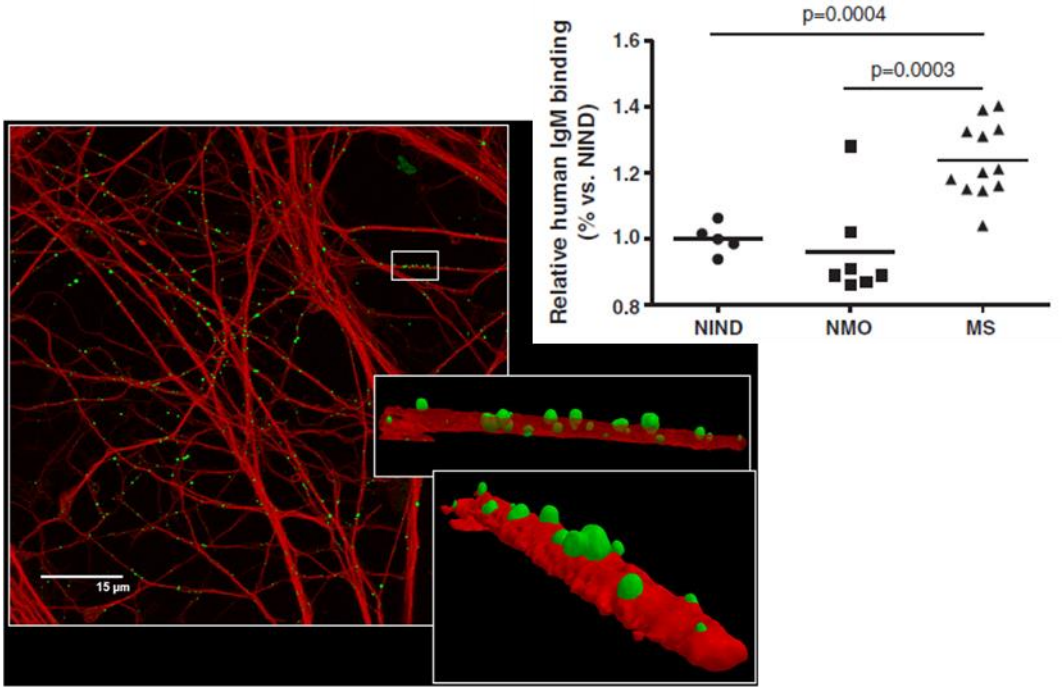


Figure 95: *immunofluorescent detection of IgM binding on neuroaxonal surface antigens in MS patients (left); graphical representation of relative IgM binding levels to neuroaxonal surface antigens for MS, neuromyelitis optica (NMO) and control (NINDS) patients (right). Reproduced from: Beltrán E et al, Neuronal antigens recognized by cerebrospinal fluid IgM in multiple sclerosis, J Neuroimmunol, 2012.*

As a matter of fact, of all patients with MS only about 40% presents IgM OCBs.[159,160] The reason of this is still unclear, but some studies tried to explore environmental factors or genetic predispositions associated with IgM intrathecal production. It has been reported that high CSF levels of CXCL13 and IL-6 correlate with higher B-cell number and immunoglobulin synthesis in the CNS. Their production is induced by TNF-alfa, which in turn has been associated with aggressive MS course and local IgM synthesis.[160] In 2014, Beltran et al demonstrated that IgM antibodies from the CSF of MS patients showed a high degree of somatic hypermutation, with an elevated content of mutations causing amino acid exchanges, especially in the complementarity determining regions of the IgM chains, which interact with unknown antigens.[165] These observations suggest that IgM antibodies undergo a process of antigen-driven maturation inside the CNS of MS patients, mediated inside B-cells by activation-induced cytidine deaminase, an enzyme normally produced in germinal centers and crucial for somatic hypermutation and class switch recombination.[165] However, in this study Authors did not find any evidence of intrathecal isotype switching from IgM to IgG.[165] In 2015, Delgado-Garcia et al found a possible explanation for this phenomenon, demonstrating the presence of a polymorphism of the IGHC locus that altering the isotype switching of Ig antibodies in MS population.[166] This findings could explain why the presence of IgM and IgG OCBs in CSF of MS patients is a distinct phenomenon, that remains stable in time usually without conversion from IgM to IgG production during the follow up of each single patient.[108,109,151,160] However, much has till to be learned about the different factors involved in IgM intrathecal production in MS patients and further studies are needed.

What has been discussed up to now is referred to the immune-pathogenic role of intrathecally produced IgM in MS. However, As we already seen in the sections before, a consistent presence of IgG OCBs in the CSF of GBS patients has never been demonstrated in previously published studies and the presence of IgM OCBs has never been evaluated before this study. Therefore, there is no available data about the role of the latter ones in GBS pathogenesis. Nevertheless, we can speculate that, given the actual presence of IgM OCBs in a particular subgroup of GBS patients, they can represent a precocious, partially specific, B-cell driven immune response

probably directed against lipid component of cellular membranes, as seen for MS. Actually, as already discussed in the "introduction" section, the central pathogenetic moment in GBS is molecular mimicry against unknown neuronal antigens.[9,12] The production of auto-antibodies in GBS is mainly sustained by B-cells and takes place in the first 2 weeks after the antecedent infectious event.[1,21,22] The best known trigger of molecular mimicry is Campilobacter Jejuni (CJ): in particular, it has been proved that specific strains of this bacterium carry lipopolysaccharides (LPS), that resemble gangliosides found in human nerves.[9,12] So, at least in this specific case, the triggering antigen is mainly lipidic in nature and this is consistent with a prevalently IgM-mediated immune response. Furthermore, we know that the antecedent CJ infection, as well as the major prevalence of GM1 gangliosides, are particularly related to axonal forms of GBS, in particular the so called AMAN variant.[167-172] Given this premises, the fact that in our population IgM OCBs are related with axonal and prevalently motor forms of GBS sounds not surprising, as a further possible confirmation of their active pathogenic role on these particular variants of GBS. From another point of view, we can consider that in patients with MS one possible target of intrathecal IgM is some neuronal surface antigen of lipidic nature and this finding explains the correlation between the presence of IgM OCBs and a wider axonal degeneration with brain atrophy even in absence of active demyelinating lesions.[151,159,162,163] The results obtained in our population of patients seem to confirm the same pathogenic role of intrathecal IgM antibodies even in GBS.

As seen for MS, the presence of intrathecal IgM antibodies is related to heavy complement activation and widespread BBB damage, consisting with the demonstration of increased permeability with passage of proteins and IgG antibodies from serum to CSF.[108,151,152,160,164] In our population, the presence of IgM OCBs was strongly associated with BBB damage. In detail, GBS patients with IgM OCBs in their sera presented higher concentration of protein, albumin and IgG in CSF compared with IgM OCBs negative patients. As a consequence, the prevalente of albumin-cytological dissociation was significantly higher, as well. Coherently, cell concentration was not significantly different between the two groups, as well as the performance of the indexes of intrathecal IgG synthesis. In summary, we found that the presence of IgM OCBs defines a similar inflammatory setting in MS and GBS patients, characterized by secondary BBB dysfunction but without private IgG production inside the CNS. Such findings are a further suggestion of the key role of IgM intrathecal antibodies in determining inflammation and, in turn, neural damage inside the subdural space.

Reasoning from an opposite perspective, we found no IgG OCBs in the CSF of our GBS patients, as a further confirmation of a widely demonstrated trend in literature.[119, 121-123, 125-130,158] As a consequence of previous considerations, we can propose at least three possible reasons to explain this finding in GBS patients.
The first reason takes into account the possible lipidic nature of the neuronal antigen identified by pathogenic antibodies, which favors an IgM-mediated response than an IgG-mediated one.[151]
Secondly, it is known that the immune response in GBS is mainly humoral, with B cells playing a primary role and secondary activation of cellular mechanisms. For what we have said before, IgM production represents a partially innate, B-cell dependent immune response which does not depend consistently on T-cells stimulus.[160] Specularly, IgG production is more specific, requires B-cells maturation and isotype class switching and is significantly dependent from cellular-based signaling.
The third factor to take into consideration is time. GBS is an acute disease, usually developing during the first 2 weeks from an antigenic stimulation that evokes molecular mimicry, sometimes recognized as an antecedent infection. Such period of time is consistent with a rapid, partially innate and quite aspecific immune response, leading to the production of IgM but inadequate to permit the production of IgG antibodies.[163] In addition, it must be considered that the collection of CSF samples by lumbar puncture in GBS is usually performed in the acute phase of the disease. In most of the studies, this procedure was made before 10 days since the onset of symptoms: this is true even for our study, in which the median time to lumbar puncture

was 8 days. Again, such a short time is not enough to guarantee a significant production of IgG antibodies, while IgM OCBs may be evident already.
Such considerations give even more support to the pathogenic role of IgM antibodies in the acute phase of GBS.

All these findings and the sequent reasonings are based on a foundamental supposition, that is the role of the intrathecal inflammatory milieu, especially mediated by IgM antibodies, in determining GBS pathogenesis. In fact, the presence of intrathecal IgM against gangliosides, especially GM1, has been demonstrated in past studies[173,174] and it is known that gangliosides are exposed not only on peripheral nerve, but also on neuronal surfaces inside the CNS.[130] In other words, is it possible that a polyradiculoneuropathy such as GBS finds its pathogenic *primum movens* inside the CNS and not outside of it, or at least at the interface between intra- and extra-thecal spaces? The answer to this dilemma might be found considering the nerve root, which is the most proximal region of the peripheral nerve, included between the emergency from spinal cord and the passage through neural foramina. In this tract, the nerve is still floating in CSF, contained inside the subdural space and wrapped by the dural meninges (**figure 96**). We supposed that the inflammatory activity of intrathecal IgM antibodies damages this specific region of the nerve in GBS.

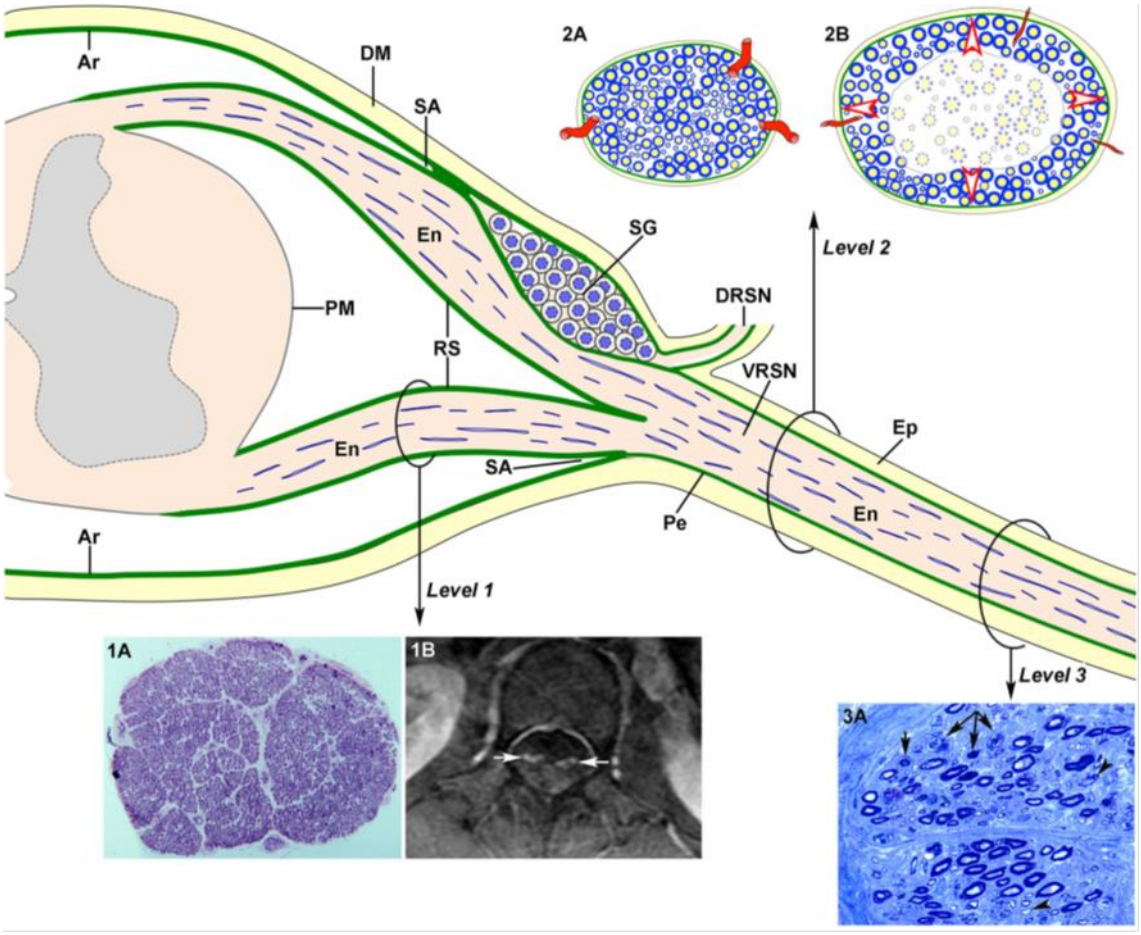


Figure 96: *Diagram of spinal root and spinal nerve microscopic anatomy. Proximal-to-distal, possible sites of early GBS inflammatory lesions are illustrated as follows: ventral lumbar root (level 1), spinal nerve (level 2) and sciatic nerve (level 3). Reproduced from: Berciano J et al, Proximal nerve lesions in early Guillain-Barré syndrome: implications for pathogenesis and disease classification, J Neurol, 2017.*

Actually, the preferential involvement of ventral nerve roots with inflammatory infiltrates and structural damage has always been a constant anatomopthological finding since the first description of the classical demyelinating form of GBS.[175,176] In 1996, Griffin et al described the

pathologic findings in 4 patients affected by axonal sensory-motor variant of GBS.[169] In 3 of these patients, autopsy was performed during the acute phase of the disease: all of them had marked spinal roots involvement (preferentially ventral roots), characterized by scattered lymphocytes, marked macrophagic infiltrates in the periaxonal space, wallerian-like degeneration of axons and scarce or absent signs of demyelination.[169] In contrast to spinal roots, wallerian-like axonopathy was occasionally seen in the peripheral nerves and nodal lengthening was not identified.[169] The fourth patient, who was examined 60 days after the onset of neuropathic symptoms, had extensive axonal loss both in spinal roots and in peripheral nerves.[169] Such findings identify the spinal roots of the nerve as the first structure to be involved in GBS pathogenesis.

More recently, animal models of AMAN were developed by immunization of Japanese white rabbits with a bovine brain ganglioside mixture or with isolatd GM1 ganglioside.[171,177-179] These animals became seropositive for antibodies against GM1 and developed acute flaccid limb weakness.[171,177-179] In analogy with human cases, pathological findings in experimental models showed prominent axonal degeneration without demyelination, with marked involvement of ventral nerve roots and macrophage infiltration in the periaxonal space.[171,177-179]

In the last years, studies from Berciano and Coauthors resumed and delved into this typical characteristic of GBS.[180-183] During the acute phase of GBS, patients usually present with selective involvement of the proximal portion of nerves, i.e. nerve roots, spinal nerves and plexuses.[180-183] Pathological changes are predominant in proximal nerves, in particular at the passage from terminal spinal roots to beginning of spinal nerves, with a critical role played by the interface between peripheral nerve and intrathecal space.[180-183] In very early GBS, endoneurial and periaxonal inflammatory changes are the most evident abnormalities, especially in axonal variants (**figure 97**).[180-183]

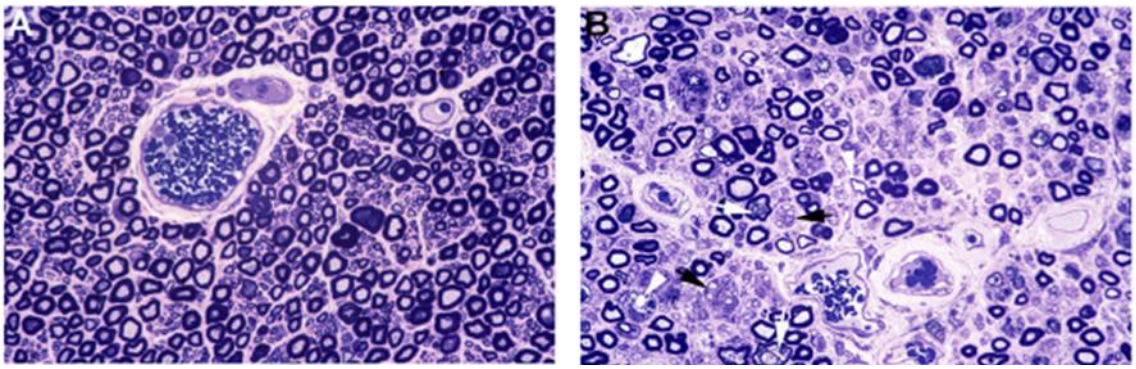


Figure 97: *AMAN pathology, transverse semithin section of L5 dorsal (A, left) and ventral (B, right) root. The density of myelinated fibers is preserved in dorsal root and reduced in ventral root. Reproduced from: Berciano J et al, Proximal nerve lesions in early Guillain-Barré syndrome: implications for pathogenesis and disease classification, J Neurol, 2017.*

Another curious clinical characteristic may be of interest in the evaluation of proximal nerve function in GBS. It is a common observation, confirmed by multicentric studies, that a small proportion of GBS patients may present with hyper-reflexia during the earliest stage of the disease.[184] Such characteristic has been associated both with demyelinatin and axonal variants of GBS.[184] The proposed mechanism is the alteration of the spinal inhibitory interneuronal network caused by antibodies that, in some GBS patients, may access the anterior spinal cord via inflamed anterior nerve roots.[185,186] This might be considered as a further, indirect proof of the presence and pathogenetic role of antibodies in the intrathecal space in GBS patients.

Such alterations of proximal nerve and, mostly, nerve roots, correspond to well known clinical findings at electrophysiology, magnetic resonance (MRI) and nerve ultrasound studies.

MRI imaging may show T2 hyperintensity and contrast enhancement of nerve roots, with selective involvement of ventral roots in AMAN variant.[180] One of the most important case series was reported in 1996 by Gorson and colleagues.[187] They prospectively collected

lumbosacral spinal MRI in 24 consecutive GBS patients. The exams were performed 2 to 42 days after the onset of symptoms (mean: 13 days). Twenty patients (83%) had cauda equine root nerve enhancement.[187] Analogue results were confirmed in later studies (**figure 98**).[188-190] In good correlation with pathological and MRI findings, erve ultrasound study frequently demonstrates enlargement and swelling of cervical nerve roots and plexus.[180,181-193]

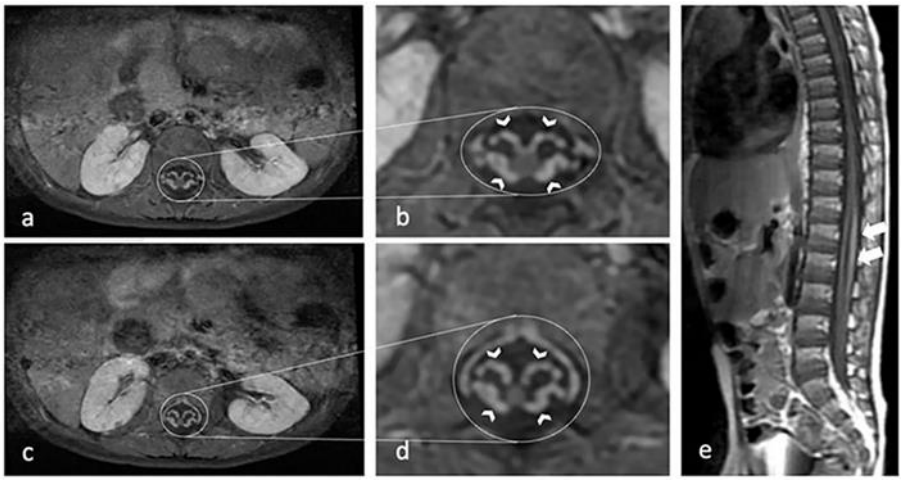


Figure 98: *Contrast-enhanced axial T1-weighted MR image shows marked enhancement of the anterior and posterior nerve roots (arrow heads) in the conus medullaris and cauda equina. Reproduced from: Pizzo F et al, Case report: Incidence and prognostic value of brain MRI lesions and elevated cerebrospinal fluid protein in children with Guillain-Barré syndrome, Front Neurol, 2022.*

Regarding neurophysiology, the earliest finding in nerve conduction studies (NCS) usually is late response abnormalities (i.e. F waves and H reflexes), which in most cases represent the first and only sign of GBS in a very precocious stage, while peripheral sensory and motor nerve conduction parameters are still normal.[180,194-196]

In 2011, Temucin et al explored specifically the motor conduction time of nerve roots, by means of the calculation of the latency difference of motor nerve conduction between NCS of ulnar nerve and cervical magnetic stimulation.[197] Results demonstrated that motor root condution time was altered in the 83% of GBS patients, with a significant difference when compared with the control group (**figure 99**).[197] In some patients, this was the only alteration at the first neurophysiological study, confirming that the involvement of nerve roots represents an early stage in GBS pathogenesis.[197]

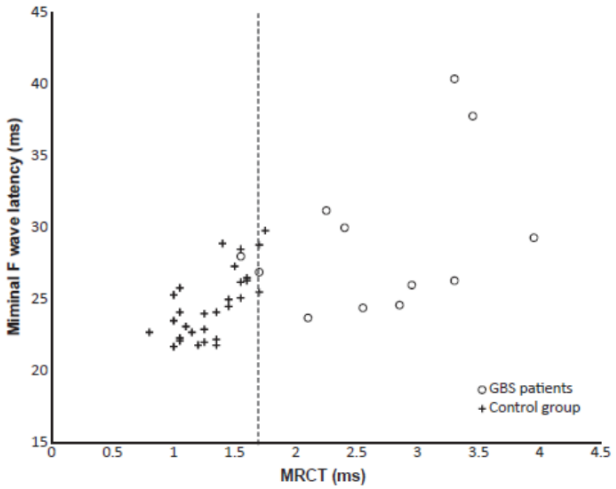


Figure 99: *minimal F-wave latency values and motor root conduction time in GBS patients and control group. Reproduced from: Temucin CM et al, Measurement of motor root conduction time at the early stage of Guillain-Barre syndrome, Eur J Neurol, 2011.*

In 2015, Gallardo et al described clinical, electrophysiological, ultrasonographic (US) and pathological findings in 6 consecutive early GBS patients, evaluated within 10 days from the onset of symptoms.[198] All the instrumental techniques converged identifying the involvement of ventral nerve roots as one of the first abnormalities in GBS patients, consisting mainly in inflammatory oedema with enlargement and blurring of nerve section at US study.[198] Corresponding electrophysiological finding was the alteration of later motor responses (i.e. F waves), mainly for the axonal variants of GBS, sometimes with normal peripheral NCS.[198]
The value of late motor responses in the electrophysiological evaluation of early stage GBS patient found further confirmation in a recent study from Rasera and Colleagues, published in 2021.[199] They studied retrospectively 36 patients with GBS and NCS study performed before 15 days from the onset of neuropathic symptoms.[199] The Authors found that the most frequent abnormal neurophysiological parameter was bilateral absence of H reflex (82%), followed by F waves abnormalities (i.e. prolonged latency or absence) in 64% of patients.[199] Most importantly, F waves abnormalities were present in all patients with AMAN variant of GBS. In contrast, "peripheral" nerve alterations were less frequent, with motor conduction involved only in 50% of patients and even less common reduction of sensory nerve action potential.[199] Notably, in this study patients were classified as demyelinating or axonal GBS according to the Uncini's neurophysiological criteria,[38] as we did in our study. The Authors' conclusion is that, in GBS patients, the alteration of late motor responses is the most sensitive and precocious electrophysiological sign and demonstrates the early involvement of the proximal tract of the peripheral nerves.[199]
In more recent studies, other alterations of late motor responses such as A-waves and repetitive F-waves have been proposed as early signs of GBS, with the same pathogenetic meaning of nerve roots precocious involvement during the first stages of the disease.[200]

Our neurophysiological results are in line with all these studies. We found abnormal F waves findings in 57% of our patients, with a significantly higher proportion among IgM OCBs positive patients (90% vs 51%, $p<0.001$). In particular, IgM OCBs patients showed a much higher frequency of F waves absence (41% vs 16%, $p=0.002$). If F waves abnormalities are one of the first and most sensitive elctrophysiological signs of nerve roots involvement in GBS, as demonstrated by previous studies, and if IgM OCBs are associated with a higher prevalence of these neurophysiological finding, as a consequence we can conclude that IgM OCBs are related to nerve roots damage in GBS. In particular, the absence of F waves in this group of patients could be related to axonal damage of nerve roots, which is consistent with the higher prevalence of axonal variants of GBS in IgM OCBs positive patients.

In conclusion, given the evidence of literature and the data emerging from our study, it is highly probable that IgM OCBs represent a primary mediator of inflammation and damage of nerve roots at least in a particular subgroup of GBS. They are the present in the early stage of the disease, cause increased permeability of BBB and determine an altered functioning of proximal nerves, as demonstrated at neurophysiological study. In particular, IgM antibodies seems related to axonal loss in nerve roots, therefore explaining their association with a particularly severe clinical picture. All considered, it appears even more unlikely that IgM OCBs may represent merely casual findings or innocent bystanders in GBS patients.

4.5 Prognostic value of IgM OCBs in CSF

As already seen before, many studies demonstrated a clear correlation between the presence of IgM OCBs and prognosis in MS (**figure 100**).[110-118,141,151,152] Given the striking clinical and pathogenetic similarities between MS and GBS patients with IgM OCBs in their CSF observed up to now, it would be legitimate to guess a parallel prognostic value for IgM OCBs in GBS patients. Such suggestion appears even more rightful when considering the strong association between such antibodies and axonal degeneration of proximal nerves with widespread damage

of BBB, which are well known markers of more severe pathology and, therefore, of worse prognosis.

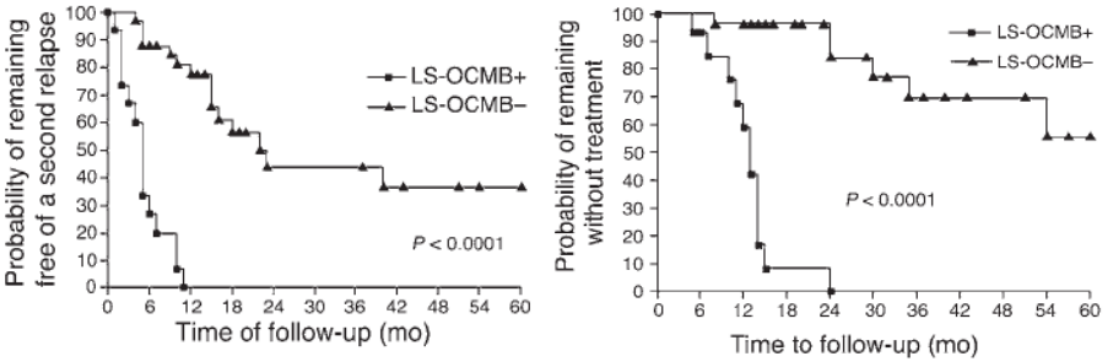


Figure 100: *association between IgM OCBs and prognosis in MS patients. Reproduced from: Villar LM, Intrathecal synthesis of oligoclonal IgM against myelin lipids predicts an aggressive disease course in MS, J Clin Invest, 2005.*

Actually, in our population of GBS patients, IgM OCBs have been associated with worse prognosis, independently from the considered measure of outcome or the statistical analysis performed (group comparison, regression analysis and survival analysis).
IgM OCBs positive patients were affected by more severe muscular weakness and higher level of disability at nadir, with higher proportion of non-independently walking or bedridden patients. In addition, the time from the onset of symptoms and clinical nadir was shorter among these patients, confirming the tendency to a more rapid and severe evolution of disease.
Data after 12 months of observation were not encouraging as well for IgM OCBs positive patients. Recovery in terms of muscular strength was significantly lower and the residual disability higher. Almost half of patients with IgM OCBs remaing dependent from some form of assistance during walking, with an odds ratio of 5 at multivariate regression analysis. Of those patients who lost the ability to walk unaided during the acute phase of the disease, the recovery of this skill was significantly less probable among IgM OCBs positive patients (48% vs 77%, p=0.002), with a 5-fold lower-than-one odds ratio. Similarly, the complete recovery from all symptoms was significantly less frequent among IgM OCBs patients (45% vs 72%, p=0.004), whit an odds ratio of 0.30.
Multivariate survival analysis confirmed such results, adding information about time to event. In particular, Kaplan-Meier estimates clearly show that positive outcomes such as recovery were not only less probable in the IgM OCBs positive group of patients, but also took much longer time to be reached.
Finally, the analysis with the Receiving Operator Curve (ROC) confirmed the same prognostic trends for IgM OCBs. In particular, it is possible to see how IgM OCBs describe an Area Under the Curve that is equally oriented and similar in extension when compared with other known negative prognostic factors, such as age at onset of neuropathy, autonomic involvement or performance at mEGOS score. This is a further hint to the non-casual association between IgM OCBs and GBS.

In conclusion, in our population of GBS patients the presence of IgM OCBs is a negative prognostic factor, being associated with more severe form of disease, more rapid evolution towards high disability, lower chance of satisfactory long-term improvement and longer time to recovery. Statistical analysis demonstrated how such value as a prognostic marker was independent from other variable and was similar to other known prognostic factors, such as age at onset. The prognostic role of IgM OCBs was expected and biologically consistent with the clinical and pathogenetic characteristics of IgM OCBs positive GBS patients, as described before (i.e. prevalent axonal damage of proximal nerves with severe damage of the blood brain barrier caused by intrathecal inflammatory environment).

All considered, in compliance with the most recent definitions,[201] we can affirm that the presence of IgM OCBs in CSF appears as a valid biomarker of diagnosis, pathogenesis and prognosis in GBS.

4.6 Limitations and further developments

Even though conducted on a conspicuous sample of patients, this study has intrinsic limitations since it is mainly retrospective and monocentric. Findings should be confirmed on larger cohort of patients, enrolled prospectively from different referral centers around the world to increase reproducibility.

To understand plainly if the presence of IgM OCBs is specific of GBS in the landscape of immune-mediated neuropathies, a process of external validation is needed by means of a comparison with control groups of patients selected among other immune neuropathies (e.g. CIDP), non-immune neuropathies (e.g. amiotrophic lateral sclerosis -ALS-, critical illness associated neuropathy) and healthy subjects.

The laboratory test for IgM OCBs has been already validated as a sensitive and specific method. However, it would be interesting to elaborate a quantitative methodology which could be useful to further stratify patients on the basis of the level of IgM antibodies in CSF. This might also be a potential measurement of how strong the inflammatory acitivity is inside the intrathecal space, which could represent an even more sensitive and specific biomarker.

Further analysis will be performed from the neurophysiological point of view. In particular, mixed methodologies of NCS and magnetic stimulation could be applied to describe with more sensitivity and specificity the characteristics of nerve roots involvement in IgM OCBs positive patients. With the same intent, the application of MRI and US technologies could be of greate value as well.

Finally, from a pathological perspective, the most intriguing and exciting development regards the research for a specific antigen (or more than one) as the target of IgM intrathecal antibodies in GBS patients presenting IgM OCBs. This would open new scenarios on the knowledge of aetiology and pathogenesis of GBS, which still remains a shadowy corner of the research about this disease, but with significant potential implications for prevention and treatment of patients.

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