Motor Nerve Electrophysiology

of Guillain-Barré Syndrome.

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Electrofysiologie van de motore zenuwen bij het Guillain-Barré syndroom

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PROMOTIECOMMISSIE:

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General introduction

Guillain-Barré syndrome (GBS) is a heterogeneous disorder regarding clinical manifestations, neurophysiology, treatment response and prognosis. This variability within GBS is only partly understood and may restrict accurate early diagnosis, subtyping, monitoring and prediction of outcome in individual patients with GBS.

Traditionally, nerve conduction studies (NCS) are used for confirming the diagnosis and determining the subtype of the GBS. However, conventional NCS have several drawbacks, making them less useful for early diagnosing, monitoring and prognostication. The need for alternative diagnostic markers and markers for subtyping and monitoring led to the development and exploration of a new generation of neurophysiological techniques, such as the compound muscle action potential (CMAP) scan and motor unit number estimation (MUNE) in relation to GBS, and is the main focus of this thesis.

In paragraph 1.1 of this chapter an overview is provided on the clinical aspects of GBS and the clinical unresolved issues that led to the research covered in this thesis. Paragraph 1.2 focuses on the principles, advantages and disadvantages of the conventional neurophysiological methods and of the more advanced neurophysiological techniques. In paragraph 1.3, the objectives of this thesis are defined.

1.1 Clinical aspects of GBS

Guillain-Barré syndrome
GBS is an acute immune-mediated disorder of the peripheral nerves and nerve roots (polyradiculoneuropathy). It is worldwide the most common cause of an acute flaccid paralysis.

Jean-Baptiste Octave Landry first described this clinical entity in 1869. Almost fifty years later (in 1916), three French neurologists, Georges Guillain, Jean-Alexandre Barré and André Strohl described two soldiers with an acute paralysis and absent deep tendon reflexes, followed by spontaneous recovery. They observed an increased cerebrospinal fluid (CSF) protein with a normal cell count ('*dissociation albumino-cytologique*') which was a differentiating feature from the then most common cause of an acute flaccid paralysis: poliomyelitis.[1]

GBS is considered a post-infectious disorder. The incidence of GBS in Western countries is reported to be 1 to 2 per 100,000 per year. In low-income countries with a higher background rate of infections such as Bangladesh, the incidence is approximately 2.5x higher, especially in children.[2-5] GBS can affect people of all ages, but the incidence rate increases with age.[6] Interestingly, men are affected about 1.5 times more often than women.

Clinical presentation and disease course

Although there is a wide clinical variability, generally patients have rapidly progressive ascending symmetric muscle weakness with a variable extent of sensory deficits.[7-9] Decreased or absent tendon reflexes typically develop early in the disease course, although approximately 10% of the GBS patients have normal or even increased reflexes in the early phase of the disease. GBS may also affect the cranial nerves, and autonomic dysfunction can occur. The majority of patients report pain throughout the disease course, and it can even precede the onset of weakness.[10]

By definition, muscle weakness should reach its maximal severity (nadir) within 4 weeks. However, in the majority of patients the nadir is reached within 2 weeks.[11] The severity of disease at nadir is highly variable; some patients have minor weakness and remain ambulatory, while others become fully paralyzed and require artificial ventilation. Monitoring during the acute phase of GBS is especially important to detect further deterioration. Especially when patients are ICU admitted and ventilated, clinical neurological investigation can be difficult. As yet, however, there are no alternative biomarkers available for diagnosing GBS and monitoring disease activity. We studied whether new neurophysiological techniques might aid in earlier diagnosing and monitoring during various phases of the disease.

The acute phase is followed by a plateau phase that can last for weeks or months. After the plateau phase, recovery begins. This recovery is often incomplete and may take years.

In general, GBS is considered to be a monophasic disease, although treatment-related fluctuations may occur.[12] Additionally, recurrences of GBS are reported in 2-5% of the patients.[13] Approximately 5% of the patients initially diagnosed with GBS are later diagnosed as having an acute onset chronic inflammatory demyelinating neuropathy (A-CIDP).[14] (Fig. 1)

Diagnosis

Even 150 years after the first case descriptions, GBS can still be difficult to diagnose. The diagnosis is currently based on a set of clinical characteristics that may require confirmation in additional investigations such as nerve conduction studies (NCS). This is especially the case when weakness is limited to the legs and other diseases (such as a spinal cord lesion) are considered. Examination of the CSF is important, mainly to exclude infections or other causes of an increased CSF cell count, although an elevated CSF protein and low CSF cell count are highly supportive for the diagnosis GBS. Both NCS and CSF examination however can be normal early in the course of the syndrome. As mentioned above, more sensitive diagnostic markers are required. The results of studies exploring the use of new neurophysiological methods in this context are described in this thesis.
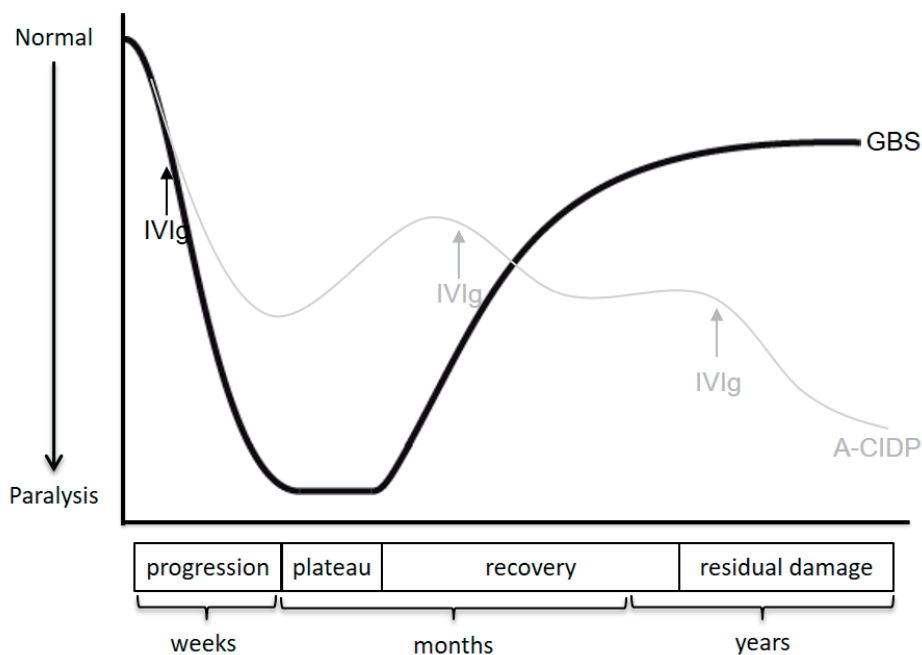
Figure 1. Disease course in GBS and A-CIDP. GBS = Guillain- Barré syndrome, A-CIDP= acute - chronic inflammatory demyelinating polyneuropathy; IVIg =intravenous immunoglobulins;

The first criteria for defining GBS (Table 1) were developed by Asbury and Cornblath in 1990 and are still commonly used in clinical practice and research.[15] The differential diagnosis of GBS is wide and depends on the clinical characteristics and abnormalities in additional investigations present in individual patients.[16, 17]

Children

As mentioned, GBS may affect persons of all ages, including children. Although the same diagnostic criteria for GBS are used for children as in adults, the clinical presentation and course may differ. Two-thirds of preschool children with GBS are initially misdiagnosed, resulting in a delay in diagnostic workup and adequate treatment.[18] This misdiagnosis may result in death, especially when early respiratory failure develops without adequate monitoring and support.[18, 19]

Clinical experience suggests that children show a faster recovery after GBS than adults. However, two thirds of children do have some residual symptoms after more than a year following the onset of GBS.[20] It is not known if the electrophysiology of the nerves completely normalizes over time or of it is still abnormal in these patients. In this thesis, changes of nerve excitability of patients who had GBS during childhood are described.

Table 1. Diagnostic Criteria for Guillain-Barre Syndrome[15]

Required features
Progressive weakness in both arms and legs
Areflexia or hyporeflexia
Exclusion of other causes
Features supportive of diagnosis
Progression of symptoms over days to 4 weeks
Relative symmetry
Mild sensory signs or symptoms
Cranial nerve involvement, especially bilateral facial weakness
Recovery beginning 2 to 4 weeks after progression ceases
Autonomic dysfunction
Absence of fever at onset
Typical CSF findings (albuminocytologic dissociation)
NCS showing characteristic signs of a demyelinating process in the peripheral nerves
Features casting doubt on the diagnosis
Asymmetrical weakness
Persistent bladder and bowel dysfunction
Bladder or bowel dysfunction at onset
>50 leukocytes/mm3
Distinct sensory level
Features that rule out the diagnosis
Hexacarbon abuse
Abnormal porphyrin metabolism
Recent diphtheria infection
Lead intoxication
Other similar conditions: poliomyelitis, botulism, hysterical paralysis, toxic neuropathy

Subtypes and clinical variants of GBS

Because of the heterogeneity, GBS is subdivided in distinct subtypes based on patho-logical and electrophysiological findings and in clinical variants. The subtypes of GBS are the acute inflammatory demyelinating polyneuropathy (AIDP), acute motor axonal neuropathy (AMAN), and acute motor sensory axonal neuropathy (AMSAN).[21] There is a remarkable difference in geographical distribution of these subtypes.[22, 23] The axonal variants seem more prevalent in Asia, while AIDP is predominant in Western coun-tries.[22, 24, 25] This geographical variation is unexplained, but may be related to differences in genetic and/or environmental factors.

In addition, various clinical variants of GBS have been described, including the Miller Fisher syndrome (MFS), paraparetic variant,[26] and bulbar and pharyngeal-brachial vari-ants, that may be quite dissimilar clinically from "classical GBS". MFS is characterized by the clinical triad of ophthalmoplegia, ataxia, and areflexia.[27] In typical cases of MFS, limb muscle strength is preserved and standard motor NCS are normal.[28] Yet, some patients with MFS may develop a rapidly progressive weakness of limb and respiratory muscles requiring ventilation and additional treatment. The existence of such an MFS-GBS-

overlap syndrome further suggests that GBS and MFS are part of the same continuum, although this has not been conclusively demonstrated. In this thesis, we added to this discussion by exploring the excitability of peripheral nerves of MFS patients.

Pathogenesis

GBS is a typical post-infectious disorder with a delay between the symptoms of the infection and the onset of weakness of days to several weeks. About two thirds of patients report preceding symptoms of an infection, usually of an upper respiratory tract or flu-like infection or of a gastrointestinal tract infection. Other patients with GBS have no symptoms of preceding infection, but additional diagnostic tests demonstrate evidence for a recent subclinical infection. The most frequently identified type of preceding infection in adult patients with GBS is the *Campylobacter jejuni,* a common bacterial cause of gastro-enteritis. In children with GBS the most common cause of infection is *M. pneumoniae*, a common cause of upper respiratory tract infection.[29] Other types of infections associated with GBS are cytomegalovirus (CMV), Epstein-Barr virus (EBV), *Haemophilus influenza*, *Hepatitis E virus* and Zika virus.[30, 31]

The short time window between infection and onset of weakness and the typical monophasic disease course indicate the importance of infections (and possibly vaccinations) as a main trigger in the pathogenesis of GBS. In addition, the type of preceding infection is associated with the clinical and electrophysiological subtype of GBS, as well as the prognosis.[7, 32, 33] Especially patients with preceding infections with *C. jejuni* have a distinct phenotype with frequently a severe pure motor form of GBS, with axonal involvement, and possibly have an unfavourable long-term outcome. However, whether *C. jejuni* exclusively results in an axonal GBS is subject of debate.[34] We studied whether this paradigm is valid for Dutch patients.

The mechanism by which infections may precipitate the occurrence of GBS is partly understood, and may differ between the type of infection and between individual patients. There is no evidence that these bacteria or viruses directly infect or damage the nerves. Instead, previous studies indicate that the immune system is involved in the injury of the peripheral nervous system. First, nerves and nerve roots from patients show depositions of immunoglobulins and activated complement factors, and infiltration of macrophages. Second, neurotoxic antibodies to peripheral nerve structures, including gangliosides, are found in the acute stage of GBS. Third, immunomodulatory therapies such as IVIg and plasmapheresis are effective in most patients.

Most knowledge on the immune-mediated pathogenesis of GBS comes from studies conducted in patients with a preceding *C. jejuni* infection. The immune response upon infection with *C. jejuni* in part is directed to the carbohydrate portion of the lipo-oligosaccharide structure in these bacteria. This carbohydrate structure is similar to

those in gangliosides that reside in human peripheral nerves. Because of this 'molecular mimicry', antibodies induced to *C. jejuni* may cross-react to gangliosides and bind to the peripheral nerves. [35-37]

This antibody binding to nerves may cause local complement activation and damaging of the nerves. In AMAN, this process first results in lengthening of the nodes of Ranvier, subsequently followed by axonal degeneration of motor fibers. In AIDP, the role of cross-reactive anti-ganglioside antibodies is less clear, but possibly subsets of antibodies bind to the surface of the Schwann cell, causing complement activation and demyelination. Damaging of the axon or Schwann cells in AMAN and AIDP, respectively, results in macrophage invasion and phagocytosis of axonal or myelin debris, as observed in patients who died of GBS (Fig. 2).
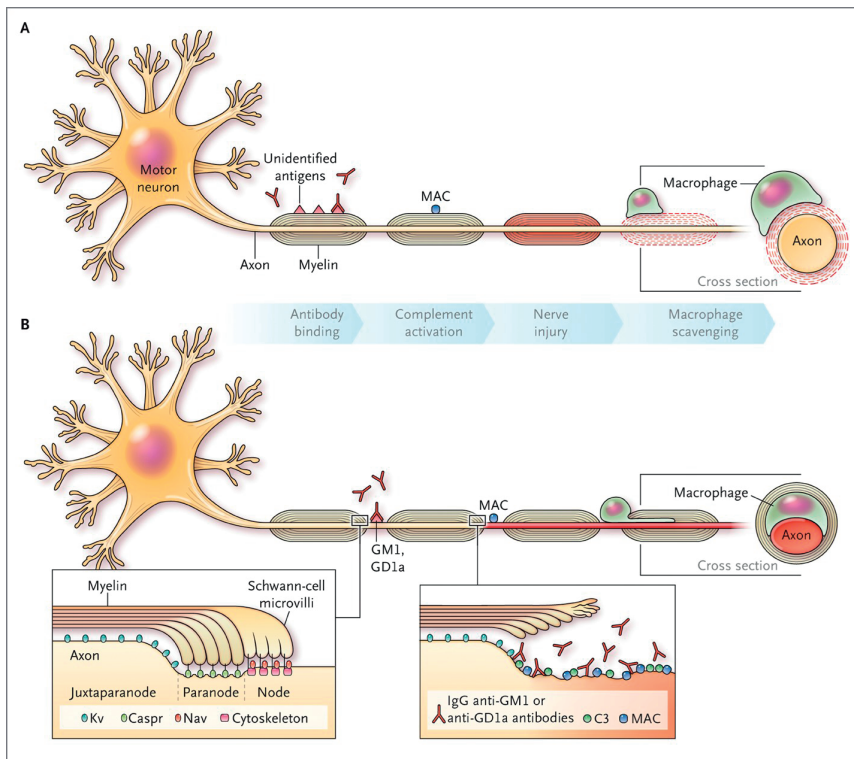
Figure 2. Schematic overview of the possible pathogenesis of Guillain-Barre syndrome. [8] A: possible pathogenesis of acute inflammatory demyelinating polyneuropathy. Binding of (not yet identified) autoantibodies to (not yet identified) myelin antigens, leading to activation of complement, followed by formation of membrane-attack complex (MAC), ultimately leading to nerve injury and disruption of action potential propagation. Later macrophages invade myelin to remove myelin fragments. B: possible pathogenesis of acute motor axonal neuropathy. Binding of autoantibodies to the nodal axolemma, activation of complement, followed by MAC formation. This is thought to result in disruption of the nodal axolemma causing nerve-conduction failure and muscle weakness and ultimately to axonal degeneration.

In about half of the patients with GBS, serum antibodies to gangliosides or ganglioside complexes can be found. These gangliosides have a different tissue distribution in the peripheral nervous system, explaining why the specificity of the antibodies to gangliosides is associated with the type of neurological deficits. For instance, patients with antibodies to gangliosides that are largely localized in motor nerves, such as GM1, GM1b, GD1a and GalNAc-GD1a, develop a purely motor and axonal variant of GBS. On the other hand, patients with antibodies to gangliosides that largely reside in cranial nerves involved in eye movements, like GQ1b, GD3 and GT1a, are associated with ophthalmoplegia and MFS.

In addition, anti-GM1 antibodies may also cause (reversible) nerve dysfunction by binding near voltage-gated Na^+ channels located at the nodes of Ranvier.[38] These voltage-gated Na^+ channels are important in the generation and propagation of action potentials. Binding of antibodies to GM1 near these Na+ channels may result in Na^+ channel dysfunction, blocking the conduction.

Despite the progress made in understanding the pathogenesis of GBS, many questions remain unsolved. For example, what triggers the onset of GBS in those 30% of patients without clinical symptoms or positive test result of a preceding infection? Or, with the above pathophysiological model in mind, how can it be explained that no antibodies to gangliosides are found in about 40% of patients with GBS? Third, we do not yet know why the rate of disease progression varies considerably in patients with the same type of preceding infection and antibodies, nor do we know the factors that modify this progression.

Treatment

The most important therapy for GBS is supportive care, generally requiring a multidisciplinary approach.[7] Monitoring, treatment, and support of respiratory and autonomic function are particularly important, as is prevention of secondary complications such as respiratory infection, deep vein thrombosis, and pulmonary embolism. At present, most patients with GBS that are unable to walk receive the same treatment regime - one course of intravenous immunoglobulin (IVIg) in a dosage of 0.4 g/kg for 5 consecutive days. A recent study showed that a second course of IVIg is not beneficial and might even be harmfull.[39] It is not clear if patients that are still able to walk, would also benefit from IVIg.[40] IVIg is similarly effective as plasmapheresis, but it is easier to administer, better tolerated and it has less complications. [41, 42]

Prognosis

GBS is still a life-threatening disorder, even with proper treatment and care. Mortality rates vary between 3% and 13%, being higher in low-income countries than high-

income countries, possible due to a lower standard of care (including absence of good ICU/ventilation facilities) and delay in diagnosis.[43-45]

Despite treatment, up to 30% of patients remain severely disabled even after years. About 35% of former GBS patients have a mild handicap and approximately 35% fully recover from their weakness. However, even when fully recovered from the weakness, patients frequently suffer from long-term effects. Approximately 38% of the patients still have pain one year after GBS,[10] and approximately 80% suffer from severe and persistent fatigue several years after GBS.[46] This fatigue may result in substantial impairment, which has a high impact on perceived health status.[46] Treatment of this disabling fatigue remains challenging. No adequate drug therapy exists.[47] Intensive physical training seems to have a long-term positive effect on fatigue.[48] The mechanisms underlying post-GBS fatigue are unclear and as a result, fatigue is sometimes regarded a symptom caused by psychological factors. To get a better understanding of fatigue after GBS, and to identify a possible underlying mechanism, we investigated the presence of electrophysiological differences between severely fatigued and non-fatigued former GBS patients.

1.2 Neurophysiological techniques

Together with the needle electromyographic (EMG) investigation, nerve conduction studies (NCS) comprise the electrodiagnostic examination of the peripheral nervous system. To demonstrate the presence of a (demyelinating) neuropathy, NCS are the golden standard.

Motor NCS: the basics

Motor NCS constitute a major element in the diagnosis of neuropathies. In motor NCS, various sites along the nerve are stimulated transcutaneously. This results in motor unit action potential generation in muscles that are innervated by the stimulated nerve. These action potentials are recorded by surface electrodes that are placed upon the muscle belly. The summed response of all action potentials generated by the muscle fibers is called the compound muscle action potential (CMAP).

The amplitude of the CMAP, i.e. the size of the recorded signal measured from baseline to peak, is an indicator of functionally intact nerve fibers and muscle fibers (Fig. 3).

The velocity with which an electrical pulse passes along motor nerve fibers is related to nerve fiber diameter and the width of the myelin sheath (as well as temperature and age). Because this diameter varies considerably between fibers, action potentials arrive non-synchronously at the muscle despite the fact that the fibers in a nerve are stimulated synchronously. Due to this temporal dispersion, a proximally elicited CMAP will be smaller than a distally elicited CMAP. In healthy persons, however, this decrease in amplitude is only small.

The onset latency of the CMAP, i.e. the time lapse between stimulus and response, represents the fastest conducting nerve fibers. When a pathological process only affects the conduction of a subset of fibers, the nerve conduction velocity (NCV) (which is calculated from the onset latency) and related parameters (such as distal motor latency and f-wave latency) remain normal as long as the fastest conducting fibers are unaffected.
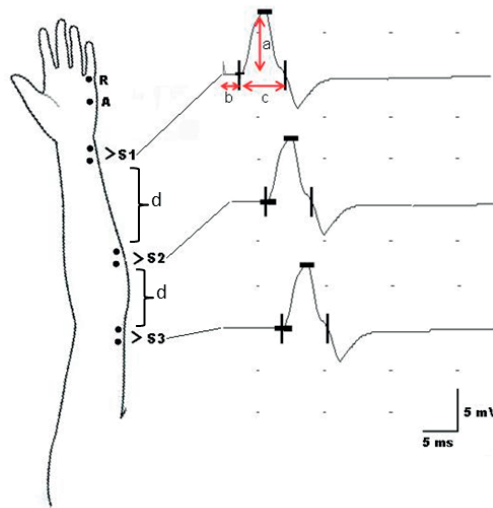
Figure 3. Example of motor nerve conduction study of ulnar nerve. Recordings are made from the hypothenar muscles. When the ulnar nerve is stimulated at ever more proximal sites (S1→S2→S3), Compound Muscle Action Potentials (CMAPs) of increasing latency are recorded. The letters indicate various CMAP parameters; a: CMAP amplitude, b: latency, c: CMAP duration, d: nerve conduction velocity (distance between stimulation points divided by the difference of onset latencies of the corresponding CMAPs).

Neurophysiology of GBS

Use of NCS in diagnosing GBS

To increase the sensitivity of the NCS, multiple nerves should be investigated. Although it there is no consensus on how many nerves should be investigated to form a reliable conclusion, it is common practice to examine at least four motor nerves and two sensory nerves.

In the first few days after symptom onset in GBS, NCS can be normal, even when patients have severe limb weakness. The optimal time to perform NCS is unknown and still a subject of debate. Some authors argue that the optimal time is at least 2 weeks after weakness onset [24]. Others argue that timing is less important, but that the clinical severity at nadir determines the success rate of electrophysiological diagnosis.[70] However, for clinical diagnostic purposes, waiting for two weeks or the clinical nadir, is often

too long, as treatment should preferably start as soon as possible. Therefore, in clinical practice NCS are often not used for diagnosing GBS, but mainly for confirmation of the clinical diagnosis, especially when there is an atypical clinical presentation. We explored whether advanced neurophysiological methods can detect nerve abnormalities in the first few days after disease onset. The results of these studies are described in chapter 3.

Use of NCS in subtyping GBS

NCS of motor nerves are used as a surrogate marker to identify features of demyelination and axonal degeneration and hence to distinguish demyelinating from axonal subtypes of GBS. Sensory studies help to differentiate between forms of axonal GBS (that is, AMAN from AMSAN). Classical features of demyelination include prolonged distal motor latency (DML), reduced NCV, prolonged F-wave latency, increased temporal dispersion and conduction block (Fig. 4a). Low sensory and motor amplitudes without features of demyelination are considered to be compatible with axonal pathology (Fig. 4b).

Unfortunately, there is no consensus about how to classify GBS patients in subtypes based on the electrophysiological findings. Various criteria sets exist,[24, 25, 49] yet no set is universally accepted, and no comparative studies with nerve biopsies are available.

To complicate matters, in the acute phase of GBS, the changes in pathology in both AIDP and AMAN patients are dynamic. Recent studies show that (reversible) conduction blocks[50] and other (reversible) 'demyelinating features' can also be found in the acute phase in AMAN patients.[38, 51] This is called reversible conduction failure (RCF). The 'demyelinating features' in AMAN patients are transient and generally normalize within 1-2 weeks. Yet in the early phase, patients may falsely be classified as AIDP instead of AMAN.

A study from Italy showed a change of classification in 24% of the patients when serial NCS were performed and advocate for serial electrodiagnostic testing to reliably distinguish AIDP from AMAN.[52] In clinical practice however, this is often not done because of logistic reasons and lack of clinical significance. A more recent prospective study contradicts this finding and shows that, whatever criteria set is used, in the majority of patients a 'stable' subtype classification can be reached with a single electrodiagnostic study.[49,53]

Currently, subtyping GBS has no clinical consequences; all subtypes receive the same treatment. However, when subtype specific therapies become available, GBS patients should be classified into the various subtypes as early as possible after onset of weakness. Furthermore, it is important to have a better understanding of the pathophysiology of the various GBS subtypes to aid the development of better therapies. New markers are needed to accomplish this. The results of studies analysing the use of new neurophysiological methods in this context are presented in this thesis.
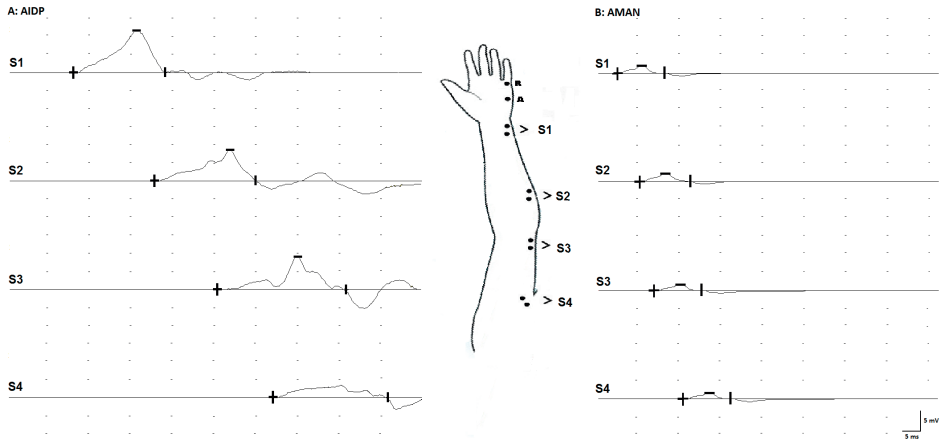
Figure 4. Ulnar motor NCS: examples of NCS findings in a patient with AIDP and a patient with AMAN.
[54] Motor NCS of the ulnar nerve, recorded from the abductor digiti minimi muscle.
A: patient with AIDP. There is a prolonged DML (S1: 8.2 ms), low NCV (S1→S2: 23.4 m/s, S2→S3: 13.3 m/s, S3→S4: 24.3 m/s), normal distal CMAP amplitude (S1: 11.1 mV), conduction block (CMAP amplitude S3: 8.8 mV, S4: 4.3 mV) and some temporal dispersion (CMAP duration S1: 10.9 ms, S2: 12.2 ms, S3: 15.6 ms, S4: 12.6 ms).
B: Patient with AMAN. There is a normal DML (S1: 2.7 ms), normal NCV (S1→S2: 62.5 m/s, S2→S3: 57.4 m/s, S3→S4: 54.1 m/s), low distal and proximal CMAP amplitudes (S1: 2.2 mV, S2: 2.2 mV, S3: 1.9 mV, S4: 1.9 mV), and no temporal dispersion (CMAP duration S1: 5.5 ms, S2: 5.7 ms, S3: 5.8 ms, S4: 5.8 ms).

Other neurophysiological techniques

Motor unit number estimation (MUNE)

A motor unit (MU) consists of an α-motor neuron in the spinal cord, its axon and the muscle fibers it innervates. Since the number of motor units decreases in diseases that affect motor axons (such as AMAN), this number is of obvious interest for diagnostic and monitoring purposes. However, it is not possible to actually count the number of motor units. Instead, neurophysiological techniques can be used to gain an estimate. In 1971, McComas developed and described a method for estimating the number of motor units.[55] Since then, various techniques for estimating the number of motor units have been published.[71]

MUNE is based upon the division of the maximal CMAP amplitude by an estimate of the mean motor unit potential (MUP) size. This mean MUP is calculated by averaging a number of individual MUPs that have been sampled.[56] In order to obtain a representative mean MUP size, a significant proportion of all MUPs have to be sampled. A larger MUP sample increases the accuracy of the estimate.[57, 58] Although it is not clear what proportion of all MUPs should be sampled to obtain a representative mean MUP, most investigators aim to obtain at least 20 MUPs. Furthermore, since the MUNE is derived from the MUP sample, it is essential that this sample is unbiased.

There are various techniques available to obtain an average MU size. Some techniques are based on electrical stimulation, such as the incremental technique (with multiple point stimulation), F-wave analysis or using statistical analysis. Other methods are based on needle EMG, such as the spike triggered averaging method. All methods have various drawbacks resulting in a limited global applicability. Furthermore, most methods require specialized software.

One of the major drawbacks of most currently available methods is the limited possibility of acquiring a representative MUP sample. By performing multiple point stimulation MUNE with high density surface EMG, this problem can be partly overcome.[58, 59]

Other techniques, that has become available in the recent years (after the start of the studies in this thesis), are the MScanFit MUNE[72] and MUNIX[73]. MScanFit MUNE is based on the Compound Muscle Action Potential (CMAP) scan (see below). It is a fast, sensitive and reproducible technique, that takes all MUs within a muscle into account to calculate a MUNE. MUNIX is a method that has become globally accepted in the recent years. It is a neurophysiological technique, based on surface interference patterns during voluntary contractions. It does not produce an absolute number of motor units but rather a value that is proportional to the number of motor units.

Motor unit number estimation (MUNE) and high density surface EMG (HDsEMG)
In MUNE with HDsEMG, MU responses to low-intensity electrical stimulation are recorded with an array of 126 densely spaced electrodes positioned over the muscle belly. Using these densely spaced electrodes results in spatiotemporal profiles ("fingerprints") of individual MUs (Fig. 5 & 6). Since the fingerprint of each MU is unique, it enables the detection and recognition of MUPs. In turn, this increases the number of MUPs that can be sampled compared to conventional single-electrode recordings [58, 59] and, hence, the accuracy of the MUNE is increased. By repositioning the stimulator along the nerve, different motor units can be recruited, which again increases the number of MUs that can be sampled. This technique is called multiple point stimulation and when used in combination with HDsEMG it allows approximately 20-30 MUPs to be sampled. From this sample the mean MUP is calculated, which is divided into the maximum CMAP to derive the MUNE. This technique has several advantages as mentioned above. However, it is time-consuming for both patient and operator, making it less applicable for clinical practice. MUNE with HDsEMG was used to study the association between residual severe fatigue as a long-term consequence of GBS and motor unit abnormalities to gain a better understanding of this fatigue.
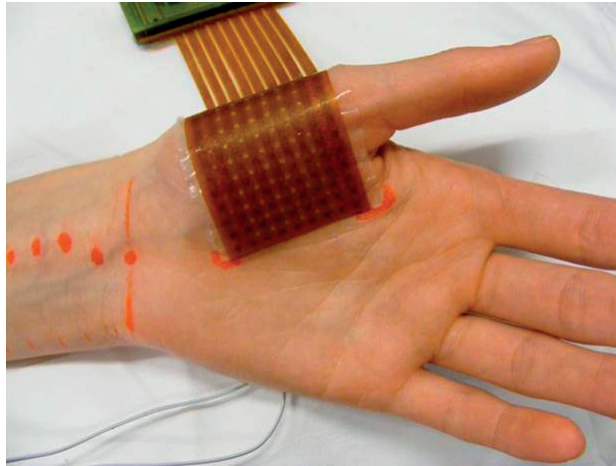
Figure 5. Example of a flexible high density grid with 9x14 electrodes
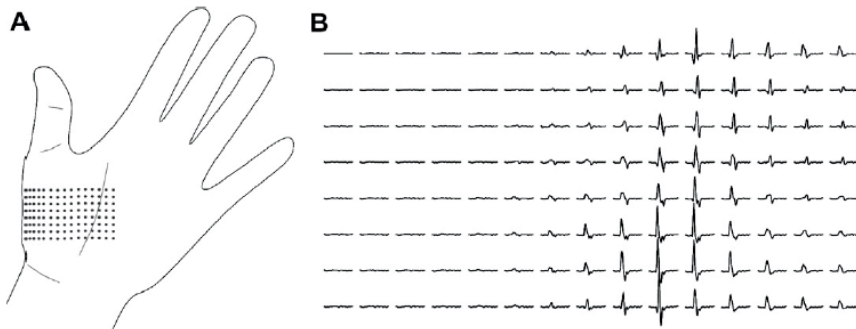
Figure 6. Example of a single motor unit potential recorded with high density surface EMG[60]. (A) High-density electrode array containing 120 densely spaced electrodes as applied over the thenar muscles. (B) Motor unit potential spatiotemporal profile ("fingerprint") as recorded with the high-density array in response to a single, low-intensity stimulus that activated only the lowest threshold motor unit.

Compound Muscle Action Potential (CMAP) scan

The various drawbacks of conventional NCS led to the search for new neurophysiological diagnostic methods. Almost simultaneously, researchers from Erasmus MC (dr. J.H. Blok) and from the Royal Brisbane and Women's Hospital, Brisbane, Australia (dr. R.D. Henderson) published about a new neurophysiological tool, which was later named the CMAP scan.[61][62]

The CMAP scan is a non-invasive neurophysiological method, which records the electrical activity of a muscle in response to repetitive transcutaneous stimulation of the motor nerve.[61,62] It is based on differences in stimulation thresholds of MUs, i.e. the differences with respect to the stimulus intensity that is required to activate them. If the stimulus intensity is gradually increased from subthreshold to supramaximal values,

all MUs in the muscle are successively activated. Plotting the size of the elicited CMAPs against the stimulus intensity (SI) normally results in a smooth, sigmoid curve: the CMAP scan (Figure 7). This stimulus-response curve can be used to study excitability properties of peripheral nerves.[63] For that purpose, stimulus intensity (plotted on the abscissa) generally provides the most information. Changes in SI parameters reflect changes in axonal excitability in very basic terms: the more current that is required to elicit a CMAP of certain size, the greater the threshold change that is necessary to activate the axons that together generate this CMAP, and the lower their excitability.[64] Therefore, a shift of the curve towards the right implies a decreased excitability.
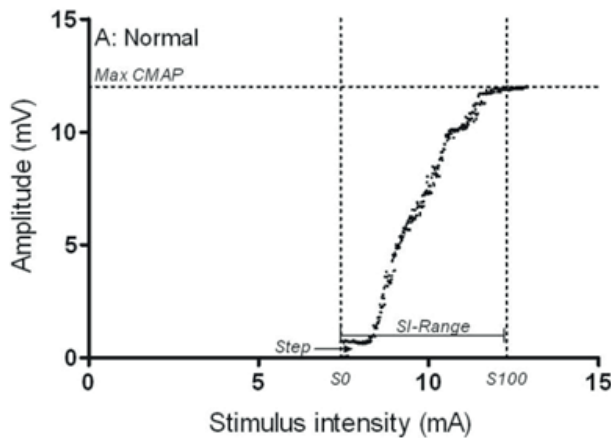
Figure 7. Normal CMAP scan. Each dot in the curve represents the amplitude of a single submaximal CMAP recorded from the thenar muscles after stimulating the median nerve at the wrist. The stimulus intensity (SI) is gradually increased, resulting in a stimulus-response curve. Key variables of the CMAP scan are the maximum CMAP amplitude, S0 (SI at which the first motor unit is activated), S100 (SI at which all motor units are activated), SI-range (S100-S0) and the presence of steps.

Henderson et al. were the first to suggest that other characteristics of the stimulus-response curve provide information on single motor units.[62] In their high-resolution plots, they could identify steps, i.e., clearly visible size differences between consecutive stimuli, which often result from the presence of large motor units. They also showed that the curve differs between normal subjects and patients with amyotrophic lateral sclerosis with respect to the presence of steps. Blok et al. subsequently described how various properties of the curve may provide clinically relevant information regarding MU number, MU size, and axonal excitability.[61] This has been validated in later studies, especially in patients with motor neuron diseases.[65-68]

The CMAP scan is well tolerated and quick to perform, and can be used as a monitoring tool in motor neuron disease.[65, 67-69] It is unknown if the CMAP scan is also of value for diagnostic purposes in neuropathies such as GBS. However, since basic changes in

nerve excitability are easily measured with the CMAP scan, it might be useful in those types of diseases. Since the CMAP scan provides information on both excitability and axonal damage, it might be better suitable than conventional NCS for diagnostic and monitoring purposes. This technique forms the basis of various studies in this thesis.

1.3 Objectives and outline of this thesis

Objectives of this thesis

GBS is a heterogeneous disorder, in which an immune response leads to a disturbed propagation of action potentials in peripheral nerves and nerve roots, causing weak-ness and/or sensory symptoms. Neurophysiological techniques can detect and measure disturbed action potential propagation. The traditionally used NCS however, have various drawbacks. The development of more advanced neurophysiological techniques, that mitigate some of the shortcomings of conventional NCS, created opportunities to address various clinical issues in GBS that still remain open.

The objectives of the studies in this thesis were:

1. to examine whether the CMAP scan can be of additional value in diagnosing, subtyp-ing, and monitoring patients with GBS, and

2. to determine whether peripheral nerve physiology normalizes in patients recovered from GBS, and whether any remaining abnormalities are related to long-term clinical outcome, such as weakness or fatigue.

3. to study whether a preceding *C. jejuni* infection can also induce a demyelinating form of GBS.

Outline of this thesis

The CMAP scan, especially the nerve excitability properties of the CMAP scan, plays an important role in this thesis. To be able to use the CMAP scan as a diagnostic and moni-toring tool, its intra- and interobserver variability had to be established first. Therefore, in **chapter** 2 we first describe the reproducibility of various parameters of the CMAP scan in healthy subjects.

In the next chapters, the articles are structured according to the disease phase for which they are relevant. Chapter 3 concerns the acute phase GBS and Chapter 4 the late phase of GBS. In **chapter 3.1** motor nerve excitability changes during the acute phase and subsequent recovery in patients with a classical MFS are described. Next, in **chapter 3.2**, changes in motor nerve excitability in acute phase GBS and the differences of these excitability properties between AMAN and AIDP patients are described. To ensure a sufficient number of both AIDP and AMAN patients, this study was performed in The Netherlands (with predominantly AIDP patients) and Bangladesh (with predominantly AMAN patients). The time course of the motor nerve excitability changes during follow-

up and its relation with clinical symptoms are described in **chapter 3.3**. Finally, based on a combination of immunological and neurophysiological data, in **chapter 3.4** the relation between Campylobacter infection and GBS subtype is described.

In **chapter 4.1**, the association between residual severe fatigue as a long-term consequence of GBS and motor unit abnormalities (as measured with MPS-HDsEMG) is examined. The study in **chapter 4.2** determines whether nerve function, as measured with the CMAP scan, normalizes in patients who suffered from GBS during childhood. Finally, in **chapters 5 and 6**, the results of the various studies in this thesis are discussed and summarized.

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2

Compound Muscle Action Potential (CMAP) scan

2.1 Reproducibility of the CMAP scan

2.1

Reproducibility of the CMAP scan

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Abstract

Introduction The CMAP scan is a surface EMG method based on the successive activation of motor units. It provides information about reinnervation processes, the number of functional motor units and nerve excitability. The CMAP scan has potential value as a follow-up tool in monitoring disease progression, recovery or aging of the peripheral nerves. In this study, we assessed its interobserver and different-day reproducibility.

Methods Two investigators recorded CMAP scans in ten healthy subjects, each on two different days. Intraclass correlation coefficients (ICCs) and coefficients of variation (CoVs) were calculated for the parameters extracted from the CMAP scan.

Results All CMAP scan parameters had a good different day (ICCs >0.8 and CoVs <15%) and interobserver reproducibility (ICCs >0.7 and CoVs ≤ 15%). Different-day reproducibility was better than interobserver reproducibility.

Conclusion CMAP scan test–retest variability is small, suggesting that as a follow-up tool it may be sensitive to fairly small (patho)physiological changes in the studied variables.

Introduction

The compound muscle action potential (CMAP) scan is a surface EMG method in which the build-up of the CMAP is visualized. It is based on the successive activation of motor units (MUs) through transcutaneous electrical stimulation. Each MU has a different stimulus intensity (SI) at which it can be activated. Therefore, a gradual increase in SI from threshold (the SI at which the MU with the lowest threshold is activated) to supramaximal (the SI that elicits a maximum CMAP) values will result in successive activation of all MUs in the muscle. Plotting the elicited CMAP amplitudes versus the corresponding SIs results in a stimulus–response curve (Fig. 1A). This curve is sometimes used to study excitability properties of peripheral nerves [1-3]

If made with many stimuli and, hence, a high resolution, the stimulus–response curve provides much information that is not available through conventional EMG methods [4-6]. For example, it allows the identification and quantification of steps. Steps are clearly visible size differences in the CMAP amplitude between consecutive stimuli. These amplitude differences are larger than the regular increases with stimulus intensity. They appear as abrupt jumps in the usually sigmoid curve and result from the firing of large, newly recruited motor unit potentials (MUPs) (Fig. 1A and B). The presence and properties of steps and CMAP variability throughout the curve differ significantly between normal subjects and patients with amyotrophic lateral sclerosis (ALS) [4]. Hence, various properties of the curve may provide clinically relevant information regarding MU number, MU size and stability, and axonal excitability [5]. Because this clinically useful information is made available through a quick assessment of the functional activity of all MUs in a muscle, we decided to refer to the high-detail stimulus response curve as "the CMAP scan".

The CMAP scan can be a valuable follow-up tool for monitoring disease progression or the speed and quality of nerve recovery in motor neuron disease, demyelinating diseases, or following trauma [7]. To be able to use the CMAP scan for this purpose, its reproducibility must be known. Assessing CMAP scan different-day reproducibility as well as interobserver reproducibility is, therefore, the purpose of the present study.

The clinical relevance of the interobserver and different day differences should be interpreted in perspective to the extent of CMAP scan changes that can be found in pathological conditions, which can be considerable. Although the latter is beyond the scope of this study, we illustrate these pathological changes by describing typical CMAP scans of five patients with amyotrophic lateral sclerosis (ALS) and neuropathies such as Guillain–Barré syndrome (GBS) and chronic inflammatory demyelinating neuropathy (CIDP).
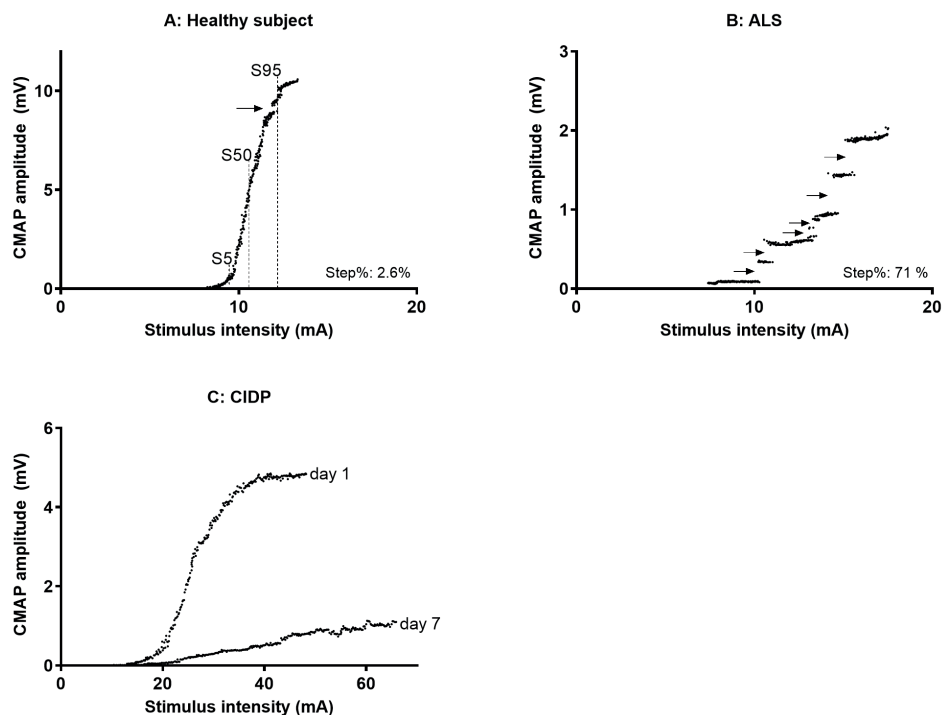
Figure 1. CMAP scans of healthy subject, ALS patient and CIDP patient.
(A) CMAP scan of a healthy subject. The arrow indicates the presence of a step. This step has a size of 0.28 mV, which is 2.6% of the maximum CMAP amplitude (10.6 mV). (B) CMAP scan of an ALS patient (Patient 1). Note the presence of multiple steps, indicated by the arrows. The six steps have an absolute size of (from bottom to top) 0.23, 0.19, 0.09, 0.09, 0.45 and 0.38 mV, respectively. The summed step size is 1.43 mV, which is 71% of the total CMAP amplitude (2.02 mV), implying that step% = 71%. (C) Two serial CMAP scans of Patient 2 with acute-onset CIDP, made with a one-week interval. Neither shows steps. Note the high stimulus intensities that were needed to record the CMAP scans. Over this week, the patient deteriorated clinically and the variables in his CMAP scan worsened. The maximum CMAP amplitude decreased from 4.8 to 1.1 mV, and S5, S50, and S95 increased from 18, 26 and 36 mA to 20, 43 and 60 mA, respectively. Note the different scaling of the axes of the CMAP scans in (A–C).

Methods

Subjects and design

Ten subjects (three men, seven women, age 19–37 years) with no symptoms or signs of a neurological disease were included in this study. Carpal tunnel syndrome was excluded by means of conventional nerve conduction studies. CMAP scans were recorded on two different days by each of two investigators who were blinded for their own results of the previous measurement and for the results of the other investigator. The interval between the recordings ranged from 6 to 73 days. The experimental protocol was approved by the institutional Medical Ethics Committee. All subjects gave informed consent.

The illustrative patient data were collected from patients who participate in ongoing longitudinal studies on motor neuron disease and GBS/CIDP. Carpal tunnel syndrome

was excluded in all at the time of their inclusion. Patient 1 (53 years, female) was diagnosed with ALS 5 months before her first CMAP scan was recorded. Patient 2 (61 years, male) suffered from acute-onset CIDP and was recorded twice during the acute phase with a one-week interval between measurements. He clinically deteriorated (progressive weakness and sensory disturbances) between the first and second recording. The third patient (53 years, male) suffered from GBS five years prior to the measurement. Patient 4 (68 years, female) was diagnosed with ALS two years prior to the measurements and the last patient (60 years, female) was diagnosed with ALS 6 months prior to the CMAP scans. All patients had moderate weakness of their thenar muscles (grade 4 according to medical research counsel (MRC) scoring).

Recordings

CMAP scans were recorded using the novel CMAP scan application on a Viking Select EMG system (CareFusion, San Diego, CA). The CMAPs were obtained from the thenar muscles of the non-dominant hand using 10 mm diameter, silver–silver chloride cup electrodes. The active electrode was placed over the muscle belly at a position that optimized the size and a steep negative offset of the maximum CMAP. The reference electrode was placed on the interphalangeal joint of the first digit. The ground electrode (self-adhesive surface electrode) was placed on the dorsum of the hand. The median nerve was stimulated at the wrist with a bar stimulator consisting of two 6×20 mm rectangular felt pad electrodes with an interelectrode distance of 20 mm. The stimulator was strapped to the wrist at the point where the lowest SI was needed to stimulate the nerve. The thumb was taped to the side of the hand to prevent signal changes due to movement and subjects were asked to remain relaxed and silent during the recordings.

Recordings started with the determination of the lowest SI that elicited an all or nothing response from the lowest-threshold MU (S0) and the minimal SI at which the maximum CMAP could be recorded (S100). To check that all MUs were activated, the SI was turned up by another 50%. Next, 30 stimuli, with SI decreasing from S100 to S0 (downwards recording), were applied to ensure that S0 and S100 were correctly set. If necessary, they were adjusted. Subsequently, the CMAP scan was recorded downwards using 500 evenly spread stimuli, with a frequency of 2 Hz and stimulus duration of 0.1 ms. To ensure that no part of the CMAP scan was undersampled (which can occur in the steepest part of the CMAP scan) sometimes 50–75 additional stimuli were applied. The total procedure lasted 10–15 min, including optimal placement of the electrodes.

After the first recording, all electrodes were removed before the second investigator performed the CMAP scan. This entire procedure was repeated on the second day.

CMAP scan parameters

Data were exported to Excel 2003 (Microsoft, Redmond, WA) and subsequently imported in Matlab (version R2009b; The MathWorks, Natick, MA) for quantitative analysis using a program that was developed for this purpose. The parameters that were extracted from the CMAP scan were: the maximum CMAP, the SIs that elicited 5%, 50%, and 95% of the maximum CMAP (S5, S50, and S95, respectively), the absolute SI range (S95–S5), the relative SI range ((S95–S5)/S5) and step percentage (step%; see below).

The step analysis was semi-automated: after manual identification of the steps, the program determined their sizes (in mV). We defined steps as clear gaps in the CMAP scan that were bounded by plateaus at the upper and lower end of the gap, each of which consisted of at least three consecutive responses of about the same size (i.e., disregarding noise). The step% variable is defined as the sum of the step sizes of all steps in a CMAP scan, relative to the maximum CMAP amplitude. For the SI parameters we used S5 and S95 rather than S0 and S100 to minimize the influence of noise. The maximum CMAP, S5, S50, S95 and the SI ranges were automatically determined by the program.

Statistical analysis

SPSS (version 15.0.1; SPSS Inc., Chicago, IL) was used for statistical analysis of the data. Data were tested for normality with Kolmogorov–Smirnov tests. Since all parameters were normally distributed, parametric tests were used. The 20 paired observations (ten on two days per investigator) were used to assess interobserver reproducibility by means of the coefficients of variation (CoV) and intraclass correlation coefficients (ICC) calculated with a two-way random effects model with single measure (model: 2, 1). Paired t-tests were used to test for significant differences between the two investigators. A p-value <0.05 was considered to be statistically significant. Finally, we determined how far apart (on average) two paired recordings were, disregarding whether investigator 1 or investigator 2 obtained the larger value. For this purpose, the mean of the absolute values of the difference between the paired measurements was calculated.

Absolute and relative different-day reproducibility in the ten subjects was tested using the ICC, the CoV and upper limits of differences based on Bland–Altman ($\bar{d}$ + 2S$_d$; $\bar{d}$ = mean of the differences, S$_d$ = standard deviation of the differences)[8]. The CoVs were calculated for all parameters except for the step percentage, since several individual values of step percentage equaled zero.

Results

In total, 40 CMAP scans were recorded in the ten healthy subjects. All were of good quality (adequately sampled, good signal-to-noise ratio and no movement artefacts). Fig. 2A shows the four CMAP scans recorded in one of the healthy subjects. The scans in black were performed by Investigator 1 and those in grey by Investigator 2. The CMAP scans

of Investigator 2 are shifted slightly more towards the right as a result of systematically higher SI values for Investigator 2 (see below).
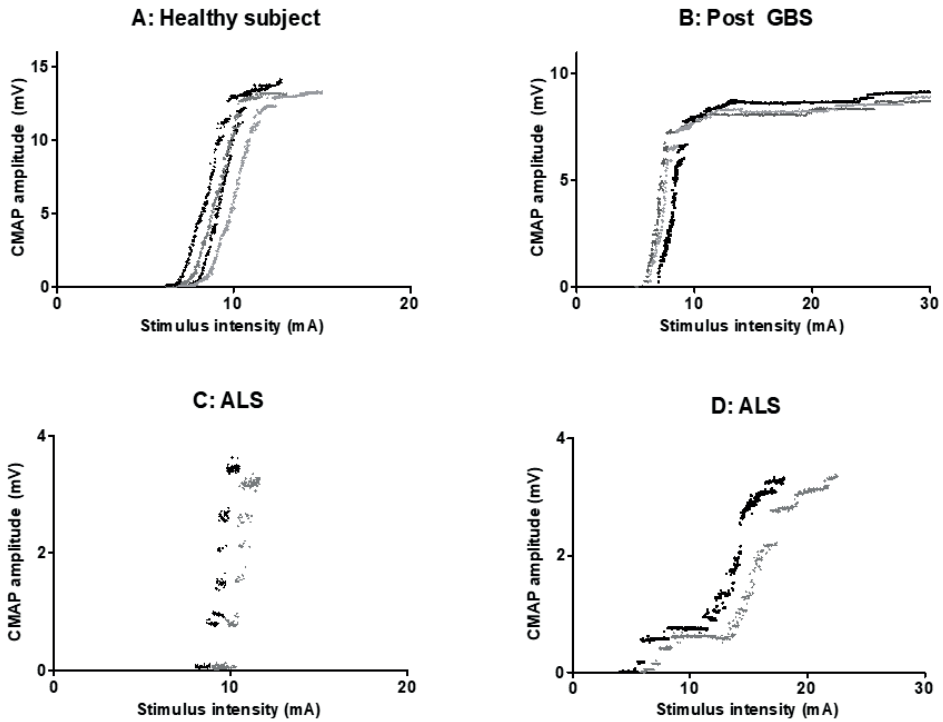
Figure 2.
(A). Four CMAP scans (two per investigator) of a healthy subject. The CMAP scans in black are performed by Investigator 1 and those in grey by Investigator 2. The CMAP scans of Investigator 2 are shifted slightly more towards the right as a result of the systematically higher SI values for Investigator 2. Despite this slight variation, the shapes are very similar. Step% ranged from 12% to 15% between the four CMAP scans. (B) Example of three repeated CMAP scans (black, grey, light grey; same day) made by Investigator 1 in Patient 3 who had suffered from GBS five years earlier and was now stable. Note the broad shape of the CMAP scan (high SI range). (C and D) Examples of two repeated CMAP scans in two ALS patients (Patients 4 and 5). The CMAP scans were performed on the same day by Investigator 2. Shapes are similar and the steps reproduce well, with step% of 75% for both CMAP scans in (C) and 41% for both CMAP scans in (D). Note the different scaling of the axes of the CMAP scans in (A–D).

The CMAP scan properties of the 20 CMAP scans per investigator are summarized in Table 1. There was a small but significant difference in the SI parameters S5, S50, S95, and absolute range between the two investigators, with consistently higher values for Investigator 2. The largest difference was found for S95 (mean difference 1.9 mA). The relative range, maximum CMAP and step percentage did not differ significantly between the investigators. The ICCs for the interobserver reproducibility varied between 0.72 (for S95) and 0.87 (for relative range), the CoVs between 6% (for maximum CMAP) and 15% (for range).

Table 2 presents the upper limits of differences, the CoVs and the ICCs for the different-day reproducibility of Investigator 1. The results for Investigator 2 were similar. The lowest ICC was 0.83 for S95, the highest was 0.94 for relative range.

Figure 1 and Figure 2 present the CMAP scans of the five patients. The ALS patients (Fig. 1 C, Fig. 2C, and Fig. D) showed a higher step% (71%, 75% and 41%, respectively) than the healthy subjects (Fig. 1 and Fig. 2A; 2.6% and 12%, respectively) and patients with a demyelinating neuropathy (Fig. 1 and Fig. 2B; 0% and 24%, respectively). The latter patients showed increased SI ranges ('broader' CMAP scans), a finding that probably reflects decreased nerve excitability.

Table 1. Interobserver effects on CMAP scan parameters

Parameter	Mean (SD) Investigator 1	Mean (SD) Investigator 2	Mean difference*	p-value	CoV (%)	ICC
Maximum CMAP (mV)	12.8 (2.9)	12.4 (2.9)	1.3	0.28	6	0.83
S5 (mA)	7.3 (2.0)	8.3 (2.3)	1.3	<0.01	10	0.80
S50 (mA)	8.6 (2.0)	9.9 (2.2)	1.5	<0.01	11	0.76
S95 (mA)	9.6 (2.3)	11.6 (2.5)	1.9	<0.01	13	0.72
Range (mA)	2.7 (1.1)	3.4 (1.4)	0.7	<0.01	15	0.80
Relative range	0.4 (0.3)	0.5 (0.4)	0.1	0.11	7	0.87
Step percentage (%)	3.9 (4.0)	4.5 (4.2)	1.9	0.24	NT[#]	0.84

[#] NT = not tested because several individual values equal 0
*Mean of the absolute values of the differences between the paired measurements.

Table 2. Different-day effects on CMAP scan parameters (for Investigator 1)

Parameter	Upper limit of difference	CoV (%)	ICC
Maximum CMAP (mV)	3.0	8	0.91
S5 (mA)	2.6	8	0.84
S50 (mA)	2.5	8	0.84
S95 (mA)	3.1	8	0.83
Range (mA)	1.4	3	0.87
Relative range	0.2	10	0.94
Step percentage (%)	4.7	NT[#]	0.86

[#]NT = not tested because several individual values equal 0

Discussion

This study shows that, in healthy subjects and with two well-trained investigators, the interobserver reproducibility and the different-day reproducibility of all investigated variables are good. They appear to be in the same range as the reproducibility of other electrophysiological variables, such as CMAP amplitude, nerve conduction velocity, and distal motor latency. [9-11]. Fig. 2 illustrates that not only the parameters of the CMAP

scan reproduce well, but also their overall shapes. Furthermore, the upper limits to the differences found with different-day recordings are small compared to the pathophysiological changes that can often be observed in serial CMAP scans in patients.

In this study we have shown typical examples of CMAP scans, recorded from five patients with ALS, GBS or CIDP (Fig. 1 and Fig. 2). These are in concordance with the few clinical studies on the CMAP scan that thus far have been published. Henderson et al., 2006 have reported that ALS patients had more and larger steps in their CMAP scans than healthy subjects, as a sign of a decrease in MU number and/or increase in MU size. We have previously presented clear abnormalities in stimulus intensity variables (similar to those in Fig. 1C and Fig. 2B) in CMAP scans of acute-phase and late-phase GBS patients. [7] Typical numerical values for the variables in these and other pathologies have been published as supplementary material [5] and agree with values presented in this study. We conclude that the day-to-day variability of the CMAP scan in healthy subjects is relatively small compared to the pathophysiological changes that can be found in ALS and demyelinating neuropathies. This makes the CMAP scan a potentially sensitive tool for follow-up studies.

Interobserver reproducibility

Neither the maximum CMAP nor the step percentage differed significantly between the investigators. This indicates that the positioning of the recording electrodes is not very sensitive to subjective factors. The interobserver ICCs for S50 and S95 were, however, relatively low, probably because of a small systematic difference in these variables between the two investigators. Considering that the SIs depend strongly on the relative location of the stimulus electrodes to the axons in the nerve trunk, the most likely explanation for this bias is that the stimulus electrode positioning of Investigator 2 was slightly less optimal. For longitudinal studies that aim to detect small changes in SI parameters, this finding implies that it is preferable for CMAP scans to be recorded by the same investigator. Finally, the relative range corrects for the abovementioned bias and, therefore, showed no significant difference between the investigators. However, compared to the direct measures S5, S50, S95 and absolute range, the relative range is less easy to interpret and possibly less sensitive.

Different-day reproducibility

All parameters had a good different-day reproducibility with ICCs >0.8 and CoVs <15%. Different-day reproducibility of the SI parameters was better than the interobserver reproducibility, confirming that reproducibility increases when recordings are performed by the same investigator. That the reproducibility is good also appears from the low upper limits of differences, particularly those of the SI parameters (Table 2). These upper limits represent the maximum difference between measurements that can be expected

as a result from normal variability in healthy subjects. Changes beyond these limits are likely to result from pathological changes or recovery processes. In this context it should be emphasized, however, that these small limits can only be reached with careful optimal placement of the stimulation electrodes, which requires some time and experience of the investigator.

Generalisability

Although this study provides evidence for a good reproducibility of all parameters in healthy subjects, this does not necessarily imply that our findings are also valid for patients. Nevertheless, with respect to the variability in the SI variables, we believe that our current findings pertain at least roughly to pathological conditions as well, for the following reasons. First, we noted that in healthy subjects the variability in SI variables mainly depends on minor changes in (sub)cutaneous tissue impedance, the distance between the stimulator and the nerve, and possibly on nerve diameter. It is not likely that these factors are greatly influenced by the diseases of the investigated patients. Second, repeated recordings in patients suggest a similar variability as in healthy subjects. For example, Fig. 2B shows repeated CMAP scans made by Investigator 1 on a single day in a stable patient who had suffered from Guillain–Barré syndrome a few years earlier.

The variability in the steps mainly depends on noise and alternation. Because steps are larger in patients undergoing reinnervation processes (Fig. 1B), the signal-to-noise (step-to-noise) ratio may be expected to go up in these conditions, and when fewer motor units are present, alternation would be reduced. Hence, we anticipate that the reproducibility of step percentage is at least as good in patients as in healthy subjects [4]. The examples in Fig. 2C and D support this notion. However, the upper limit of differences in step percentage as presented in Table 2 may not apply for patients, because this percentage tends to be very low in healthy subjects. In this context, we would like to add that it is not uncommon for steps to be found in CMAP scans of healthy subjects. These steps mostly occur in the very high or low end of the scan, where there is little alternation. The all-or-nothing response of a single MU to a stimulus then causes an easily noticeable 'jump' in the CMAP amplitude. Steps that are found in healthy conditions may also be due to the physiological existence of a few large MUs and/or the normal ageing process. [12].

We conclude that the CMAP scan reproducibility is good. Our results suggest that the CMAP scan as a follow-up tool is suitable to detect (patho)physiological changes in the studied variables.

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3

Neurophysiological changes in acute phase GBS

3.1

Limb motor nerve dysfunction in Miller Fisher syndrome

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Abstract

Typical Miller Fisher syndrome lacks limb muscle weakness, but some patients may unpredictably progress to severe Guillain-Barré syndrome. The compound muscle action potential scan is a recently developed non-invasive, painless and reproducible method for detecting early changes in motor nerve excitability. This technique was used to monitor subclinical limb motor nerve dysfunction during disease course in typical Miller Fisher syndrome. Three Miller Fisher patients with preserved limb muscle strength and normal routine nerve conduction studies were included. Frequent serial compound muscle action potential scanning of the median nerve was performed during acute phase and follow-up and related to clinical course and outcome. All patients showed an abnormal increase in the range of stimulus intensities at the day of hospital admission, indicating reduced motor nerve excitability already at the earliest stage of disease. Median nerve dysfunction progressed in parallel or even before clinical deterioration, and improved with clinical recovery. Our study shows that typical Miller Fisher syndrome is a more general neuropathy, affecting peripheral motor nerves even in patients with preserved limb strength and conduction velocity. Compound muscle action potential scanning is a sensitive technique for early detection of subclinical motor nerve dysfunction and for monitoring disease activity in immune-mediated neuropathies.

Introduction

Miller Fisher syndrome (MFS) is an immune-mediated neuropathy, characterized by the clinical triad of ophthalmoplegia, ataxia, and areflexia.[1] In typical cases of MFS, limb muscle strength is preserved and standard motor nerve conduction studies (NCS) are normal.[2] Some patients with MFS may develop a rapidly progressive weakness of limb and respiratory muscles requiring ventilation and additional treatment.[3] The existence of such an MFS-Guillain-Barré syndrome (GBS)-overlap syndrome suggests that GBS and MFS are part of the same continuum.[4] Disease progression is probably related to the neurotoxic effects of antibodies to the ganglioside GQ1b, which are frequently found in patients with MFS and in patients with GBS and respiratory insufficiency.[5,6]

We speculated that in MFS these antibodies induce an initially subclinical dysfunction of limb motor nerves that in some patients progresses to overt limb weakness. The compound muscle action potential (CMAP) scan is a recently developed neurophysiological method based on CMAP recordings induced by a range of stimulus intensities.[7] Previous studies showed that the CMAP scan detects early and reversible motor nerve excitability changes in patients with GBS.[8,9] This prompted us to apply the CMAP scan to determine limb motor nerve dysfunction during the course of disease in patients with typical MFS.

Materials and Methods

In this prospective study three consecutive patients with typical MFS were included within a week of symptom onset. During a follow-up of at least one year, we determined clinical neurological deficits by standard neurological investigation, including muscle strength testing of hypothenar and thenar muscles. In addition, we used more standardized scores, such as the MRC sum score, GBS disability score and overall disability sum score (ODSS).[10,11] Serial standard NCS were performed of median, ulnar, peroneal, tibial, and sural nerves, including F-waves and tibial nerve H-reflexes.[12] NCS were performed on the non-dominant side. All patients showed preserved limb muscle strength and no abnormalities in motor NCS (normal latencies, nerve conduction velocities, minimal F-wave latencies, and CMAP amplitudes in all tested nerves). At admission serum was obtained to determine anti-GQ1b antibodies by ELISA.[13] Patients gave written informed consent. The study was approved by the local medical ethics committee.

Serial CMAP scans were performed using a standard Viking Select electromyography machine (CareFusion, San Diego, CA). CMAP scan recordings were obtained from the thenar muscles after stimulating the median nerve at the wrist. CMAP scans were performed on the same side as the NCS. Nerves were stimulated with gradually increasing stimulus intensities (SIs), ranging from subthreshold to supramaximal values, resulting in increasing CMAP amplitudes depending on the excitability of individual motor units. Key characteristics are the maximum CMAP amplitude, SI activating the first motor unit

(S0), SI at which 50% of the maximum CMAP amplitude is generated (S50), SI activating all motor units (S100), and SI-range (S100-S0). Although informative, these parameters are somewhat arbitrary. Changes in SI parameters reflect changes in axonal excitability. A shift of the curve towards the right implies a decreased excitability. A derived, relatively sensitive but aspecific characteristic of the CMAP scan is its 'steepness', which is calculated by dividing the maximum CMAP amplitude by the SI range.
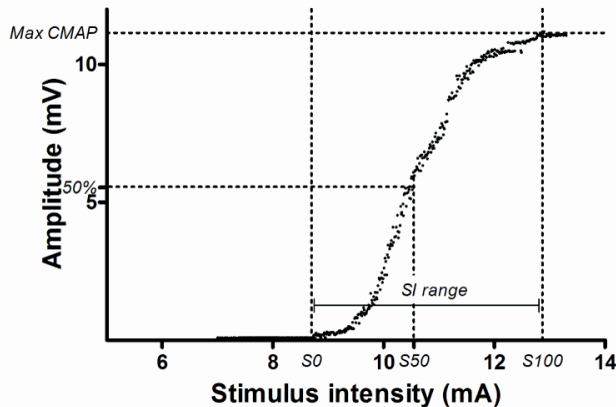
Figure 1. Compound muscle action potential (CMAP) scan of a healthy control subject. Each dot in the curve represents the amplitude of a single submaximal CMAP recorded from the thenar muscles after stimulating the median nerve at the wrist. The stimulus intensity (SI) is gradually increased, resulting in a stimulus–response curve. Key variables of the CMAP scan are the maximum CMAP amplitude, S0 (SI at which the first motor unit is activated), S50 (SI at which 50% of the maximum CMAP amplitude is generated), S100 (SI at which all motor units are activated), and SI range (S100–S0). The steepness of this CMAP scan is 2.6mV/mA (11.1 mV/4.2mA).

The CMAP scan has a good reproducibility and is presented as a stimulus-response curve showing the SIs and corresponding CMAP amplitudes (Fig 1).[8,14] Normal values were obtained in 14 healthy controls from the same age category as the patients. CMAP scan plots and SI parameters were calculated via Matlab (version 2009b; Mathworks, Natick, MA).

Results

Patient 1, a 42-year-old previously healthy male, presented with tingling of hands and feet and double vision since three days. Neurological examination revealed left abducens nerve paralysis, sensory disturbances of hands and feet, ataxic gait, and limb hypo-/areflexia. Additional studies demonstrated serum anti-GQ1b IgG antibodies (titer 1: 400) and absent tibial nerve H-reflexes. The CMAP scan showed motor nerve abnormalities, including an increased SI-range (Fig 2B, Table 1). In parallel with clinical

recovery, the CMAP scan improved in the first week (Fig 2B). Because of the mild clinical course the patient received no intravenous immunoglobulins (IVIg). After eight days he was discharged with mild diplopia and hyporeflexia. Six months later no neurological deficits were observed, although the CMAP scan showed further motor nerve recovery during a follow-up of 2 years.

Patient 2, a 59 year-old male with a history of myocardial infarction, presented with double vision and unstable gait on awakening. Neurological examination showed paresis of left oculomotor, abducens and facial nerves, ataxic gait, and limb hyporeflexia. A brain CT-scan was normal, serum contained anti-GQ1b IgG antibodies (titer 1: 102,400) and tibial nerve H-reflexes were absent. The S0, S50, S100, and SI-range were above the upper limits of normal. The ataxia, bilateral cranial nerve involvement, GBS disability
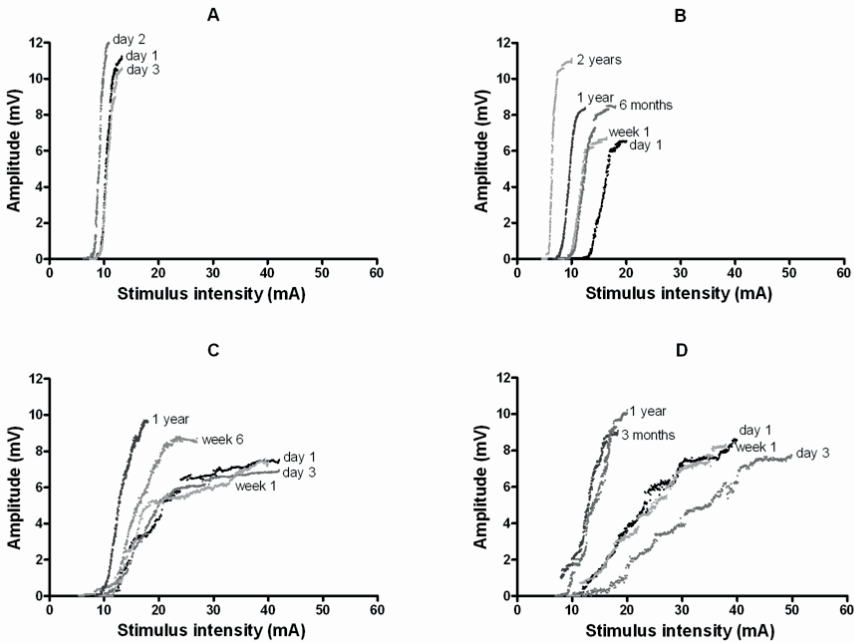
Figure 2. Serial compound muscle action potential (CMAP) scans in healthy control subject and three Miller Fisher patients. Serial CMAP scans of thenar muscles after stimulation of the median nerve at the wrist in one healthy control (A) and three patients with Miller Fisher syndrome (MFS) (B–D). All MFS patients had normal limb strength and motor nerve conduction studies. Time of follow-up after hospital admission is indicated. (A) Reproducibility of the CMAP scan in a 42-year-old healthy control. (B) MFS patient 1: increased S50 and stimulus intensity (SI) range on day 1, with improvement at 1 week and normalization after 2 years, indicating a transiently disturbed nerve excitability in the acute phase. Although the other parameters are within normal values at day 1, they improve during follow-up. (C) MFS patient 2: increase in all SI parameters in the first week, with improvement at 6 weeks and normalization at 1 year. (D) MFS patient 3: a decrease in nerve excitability (shift to higher SI) from days 1 to 3 paralleling clinical deterioration, and subsequent improvement of nerve excitability with general clinical recovery.

score and ODSS deteriorated the same day. In parallel, the CMAP scan abnormalities progressed (Fig 2C, Table 1). He received IVIg (2g/kg body weight) and improved gradually over the next six weeks. In parallel, the SI-range decreased from 28.5 to 20.5 mA. The patient was discharged with mild ophthalmoparesis, ataxia, and areflexia. One year later no residual symptoms were found, but the CMAP scan showed further improvement to normal.

Table 1. CMAP scan characteristics in healthy controls and three MFS patients in relation to clinical course.

Subject	Disability score (0-6)	ODSS (0-12)	S0 (mA)	S50 (mA)	S100 (mA)	SI-range (mA)	Steepness (mV/mA)	Max CMAP (mV)
Healthy controls								
Mean (SD)	0	0	8.0 (1.2)	10.9 (1.9)	14.5 (2.8)	6.5 (1.5)	2.1 (0.8)	10.3 (1.7)
ULN/LLN			10.4	14.7	20.1	9.5	0.5	6.9
MFS patient 1								
Day 1	1	2	7	15.5	19	12	0.5	6.5
Week 1	1	1	9	11.5	16.5	7.5	0.9	6.6
6 months	0	0	9	12	16.5	7.5	1.1	8.5
1 Year	0	0	7	9.5	12.5	5.5	1.5	8.4
2 years	0	0	5.5	6.5	10	4.5	2.5	11.1
MFS patient 2								
Day 1	2	4	11	19	42	31	0.3	7.5
Day 3	3	5	10	17.5	42	32	0.2	7.0
Week 1	3	4	11.5	16.5	40	28.5	0.3	7.5
Week 6	1	3	6.5	15.5	27	20.5	0.4	8.7
1 year	0	0	8.5	12.5	18	9.5	1.0	9.6
MFS patient 3								
Day 1	3	7	11	22	40	29	0.3	8.6
Day 3	4	8	10	30	50	40	0.2	7.7
Week 1	4	8	11.5	22	38	26.5	0.3	8.3
3 months	2	4	8	12.5	18	10	0.9	9.0
1 year	1	2	8	14	20	12	0.9	10.2

Normal values for CMAP scan variables were derived from 14 healthy male controls (age 44–67 years) and are presented as means (and standard deviation between brackets). These data were used to calculate for S0, S50, S100, and SI range the upper limits of normal (ULN, mean+2SD) and for the steepness and maximum CMAP amplitude the lower limits of normal (LLN, mean – 2SD). Columns show GBS disability score (0=normal, 1=minor disability, 2=unable to run, 3=unable to walk independently, 4=bed or wheelchair bound, 5=requiring assisted ventilation, 6=dead), overall disability sum score (ODSS, higher scores reflect greater disability), SIs at which the first motor unit is activated (S0), at which 50% of the maximum CMAP amplitude is generated (S50) and at which all motor units are activated (S100), the SI range (S100–S0), the steepness (max CMAP/SI range), and the maximum CMAP amplitude (max CMAP).
CMAP, compound muscle action potential; MFS, Miller Fisher syndrome; SI, stimulus intensity.

Patient 3, a 62 year-old previously healthy male, presented with unsteady gait and slurry speech since one day. He showed bilateral ptosis, ophthalmoplegia and facial

palsy, bulbar dysarthria, ataxia, sensory disturbances of all modalities and limb areflexia. Additional studies revealed serum anti-GQ1b IgG antibodies (titer 1: 25,600), absent sural sensory response and tibial nerve H-reflex, and an increased S0, S50 S100 and SI-range (Fig 2D, Table 1). He received IVIg (2g/kg body weight) and reached clinical nadir at day 4 and started to improve at day 7. The SI-range improved several days prior to onset of clinical recovery (Fig 2D). He was discharged 3 weeks later with mild ptosis, ophthalmoplegia, ataxia, sensory disturbances, and limb areflexia. After one year he had minor sensory disturbances of the feet. The CMAP scan parameters were improved but still abnormal.

Discussion

Our study shows that typical MFS is a more general neuropathy like GBS, affecting peripheral motor nerves even in patients with preserved limb strength and normal standard NCS during follow-up. The CMAP scan demonstrated a reduced excitability of median nerve motor fibers in three MFS patients already on the day of hospital admission. The nerve dysfunction demonstrated by the CMAP scan progressed in parallel with the initial clinical deterioration of cranial nerve palsy or ataxia. Improvement of motor nerve excitability preceded the onset of clinical recovery in two patients. These changes in nerve excitability were larger than can be expected in normal nerves.[14] The CMAP scan also showed residual motor nerve dysfunction after a follow-up of one to two years in patients without residual neurological deficits. This involvement of limb motor nerves confirms that typical MFS is a true variant of GBS. Furthermore, these findings indicate that CMAP scanning is a sensitive method for early detection of changes in clinical disease activity and of residual nerve damage in MFS.

The CMAP scans showed an increase in SI-range and S100 rather than S0 and S50. This pattern suggests a mild to major affection of some axons, resulting in increased thresholds, but sparing of other axons. Some affected axons became inexcitable, resulting in a reduction of the maximum CMAP amplitude. Although some of the parameters in the CMAP scan can also be derived from standard NCS, the visualisation of all parameters into a single graph is a new and very informative method, especially in serial measurements and follow up of patients with peripheral nerve disease.

Nerve excitability highly depends on the density and characteristics of voltage-gated sodium channels at the nodes of Ranvier.[15,16] Anti-GQ1b antibodies may reduce the channel density, as previously demonstrated for anti-GD1a antibodies.[17] Another possible mechanism in MFS might be related to the presence of endoneurial edema. Pathological studies in GBS patients [18] found edema to be the earliest change in peripheral nerves, followed by swelling and irregularity of the myelin sheaths. This edema might result in a short-circuiting of the applied current, and, hence, result in higher SIs. It

is also conceivable that other morphological factors such as demyelination or exposure of paranodal K-channels affected axonal excitability.

Reduced motor nerve excitability may represent an initial step in nerve dysfunction, a process that results in muscle weakness only when a critical proportion of nerve fibers becomes inexcitable, as found in patients with GBS.[9] This threshold for developing overt weakness was not reached in these three MFS patients, although the CMAP in all patients increased with clinical recovery.

GBS has a highly variable clinical course and patients may require individualized treatment.[19] Serial recordings by CMAP scan appear to be a useful tool in clinical practice to monitoring (subclinical) disease activity, because it is a non-invasive, painless, fast and well-tolerated technique and has a high reproducibility and sensitivity for detecting early changes in motor nerve dysfunction.

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3.2

Changes in motor nerve excitability in acute phase Guillain-Barré syndrome

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Abstract

Background: The most common subtypes of Guillain-Barré syndrome (GBS) are acute inflammatory demyelinating polyneuropathy (AIDP) and acute motor axonal neuropathy (AMAN). In the first days after the onset of weakness, standard nerve conduction studies (NCS) may not distinguish GBS subtypes. Reduced nerve excitability may be an early symptom of nerve dysfunction, which can be determined with the compound muscle action potential (CMAP) scan. The aim of this study was to explore whether early changes in motor nerve excitability in GBS patients are related to various subtypes.

Methods: Prospective case-control study in 19 GBS patients from The Netherlands and 22 from Bangladesh. CMAP scans were performed within 2 days of hospital admission and NCS 7-14 days after onset of weakness. CMAP scans were also performed in age- and country-matched controls.

Results: CMAP scan patterns of patients who were classified as AMAN were distinctly different compared to the CMAP scan patterns of the patients who were classified as AIDP. The most pronounced differences were found in the stimulus intensity parameters.

Conclusions: CMAP scans made at hospital admission demonstrate several characteristics that can be used as an early indicator of GBS subtype.

Introduction

The Guillain-Barré syndrome (GBS) is a subacute disorder of the motor and sensory nerves and nerve roots with a heterogeneous pathophysiology and clinical course [1]. GBS can be divided into distinct subtypes depending on the extent of the peripheral nerve demyelination or axonal degeneration. In clinical practice patients are classified by standard nerve conduction studies (NCS) into acute inflammatory demyelinating polyneuropathy (AIDP), and acute motor axonal neuropathy (AMAN) [2,3].

NCS parameters have been related to the risk of developing respiratory insufficiency and final outcome, which is highly variable in GBS [4,5]. Standard NCS provide information on nerve conduction velocity and axonal loss. However, NCS abnormalities need to deviate significantly from the normal range before the AIDP/AMAN distinction can be made.[6] In the first week after symptom onset NCS might show only minor abnormalities [7]. Furthermore in this period reversible conduction failure can occur, mimicking signs of demyelination, in patients who are later classified as AMAN [8]. Reduced nerve excitability may be the first electrophysiological manifestation of GBS [9] and can be assessed by the compound muscle action potential (CMAP) scan [10]. This is a non-invasive, fast and reproducible electrophysiological method [11].

In the current study, we investigated early changes in motor nerve excitability by CMAP scan in GBS patients and studied if this can be used as an early subtype discriminator.

Methods

Patients and controls

A prospective case-control study was conducted in GBS patients and age- and country matched healthy subjects enrolled via Erasmus Medical Center, Rotterdam, The Netherlands, and Dhaka Medical College and Hospital (DMCH), Dhaka, Bangladesh. Inclusion criteria and protocols for collection of clinical and electrophysiological data were the same for both centers. All patients fulfilled the diagnostic criteria for GBS , Miller Fisher syndrome [12] or other GBS variants and were admitted to the hospital within two weeks of onset of weakness. The patients had no concomitant clinical conditions. Standardised clinical scores including the GBS disability score,[13] and Medical Research Council (MRC) sum scores [14] were determined for all patients at admission. CMAP scans were performed within 2 days after hospital admission by the same researcher. Standard NCS were performed 7-14 days after the onset of weakness.

A control was recruited for each patient. Controls were screened to ensure that they had no neurological symptoms or diseases. In Bangladesh the controls were mainly derived from the same family as the patient; for the Netherlands the controls originated from an existing database that included healthy controls of various ages. Routine NCS

was performed in all control subjects to exclude median neuropathy at the wrist. CMAP scans were performed in the control group using the same protocol as used in patients.

The study was approved by the local Medical Ethics Committee of the Erasmus MC, The Netherlands, and by the Institutional Review Board and the ethical committees at the International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh, Bangladesh. All subjects and/or legal representatives gave informed consent.

Standard nerve conduction studies

NCS and CMAP scans were performed on the non-dominant side. Standardised motor NCS were performed of the ulnar, median, peroneal and tibial nerves. Standardised sensory NCS were performed on the ulnar, median, and sural nerves [15]. If sensory potentials were present, patients were tested for a carpal tunnel syndrome (CTS), by comparing the sensory conduction velocity of the median nerve across the carpal tunnel to the sensory conduction velocity in the palm. For motor nerves, the distal and proximal baseline-peak CMAP amplitudes, distal motor latency, motor nerve conduction velocity, and F-wave latencies were determined. For sensory nerves, the baseline-peak sensory nerve action potential amplitude and sensory nerve conduction velocity were measured. Reference values were derived from Buschbacher et al. [15]. The NCS were classified according to the Hadden electrophysiological criteria for GBS [2].

All Dutch patients were all warmed with hot water blankets.[16] This was not possible in Bangladesh, due to limited resources. However the temperature inside the hospital was as high as the outside temperature.

CMAP scans

CMAP scans were recorded using the CMAP scan application on a Viking Select EMG system (CareFusion, San Diego, CA). The CMAPs were obtained from the thenar muscles of the non-dominant hand after stimulation of the median nerve at the wrist in all patients and controls. All CMAP scans were performed by the same investigator (JD). In CMAP scanning the nerve is stimulated with gradually increasing stimulus intensities (SIs), ranging from subthreshold to supramaximal values. With increasing SI the recorded CMAP will increase until supramaximal values are reached. Plotting the CMAP amplitudes against the corresponding SIs results in a dose-response curve which defines the CMAP scan. It provides, through its dependence on SI, information on nerve excitability [11]. The presence of multiple large steps points to underlying processes of axonal loss and reinnervation [17]. We defined steps as clear gaps in the CMAP scan that were bounded by plateaus at the upper and lower end of the gap, each of which consisted of at least 3 consecutive responses of about the same size (disregarding noise).[11] The key parameters of the CMAP scan are provided in figure 1A. The entire procedure takes approximately 5-10 minutes.

Statistics

All data were tested for normality using Kolmogorov-Smirnov test. Since the data were not normally distributed, non-parametric tests were used for further analysis. Continuous variables were presented as medians and interquartile ranges (IQRs) and were compared using the Mann-Whitney-*U* test. Differences in proportions were determined using the Fishers exact test. Linear discriminant analysis was used to determine the independent factors that were associated with the GBS-subtypes. Data from controls were used to calculate the lower and upper limits of normal. Values <2.5 percentile and >97.5 percentile were considered abnormal. All calculations were performed using SPSS 17.0 (SPSS Inc, Chicago, IL). Two-tailed tests were used throughout, a p-value < 0.05 was considered to be statistically significant.
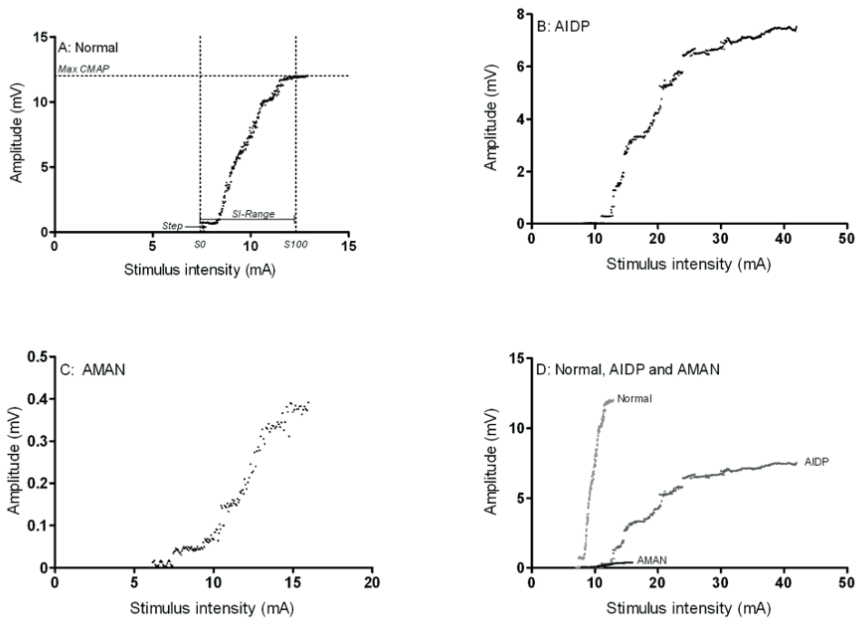
Figure 1. Compound muscle action potential (CMAP) scans of A: control, B: acute inflammatory demyelinating polyneuropathy (AIDP) patient, C: acute motor axonal neuropathy (AMAN) patient and D: control, AIDP and AMAN patient plotted in 1 figure. (A) Key variables of the CMAP scan that reflect excitability are: the stimulus intensity (SI) activating the first motor unit (S0), the SI that elicits 50% of the maximum CMAP (S50), the SI activating all motor units (S100), the SI-range (S100-S0) and the relative SI-range ((S100-S0)/S0). Other key characteristics of the CMAP scan are the maximum CMAP amplitude and the presence of steps, quantified as step percentage (Step%) [11]. The presence of multiple large steps points to underlying processes of axonal loss and reinnervation [17].

Results

Forty-one consecutive patients with GBS were included (32 males (78%), median age 38, range 9-77 years). Nineteen patients originated from The Netherlands and 22 patients from Bangladesh. Patients from Bangladesh were significantly younger than patients from the Netherlands (p<0.001).

The Dutch patients differed from the Bangladeshi patients with respect to electrophysiological GBS-subtypes based on the results of the standard NCS at two weeks according to the Hadden criteria [2] . GBS in most of the Dutch patients was classified as demyelinating, whereas it was classified as axonal in most patients from Bangladesh (Table 1).

Table 1. Demography, neurological deficits, and CMAP scan of GBS patients.

Parameter	Dutch GBS patients (n=19)	Bangladeshi GBS patients (n=22)	P-value
Demography			
Age	50 (38-64)	25 (17-35)	<0.001
Sex (male/female)	17/2	15/7	0.10
Neurological deficits			
Cranial nerve involvement	11 (58%)	10 (45%)	0.55
Sensory deficits	17 (89%)	3 (14%)	<0.001
MRC sum score at entry	50 (47-60)	25 (18-43)	<0.001
GBS disability score at entry	3 (2-4)	4 (4-4)	<0.001
GBS subtypes			<0.001
Demyelinating	14 (74%)	1 (5%)	
Axonal	0 (0%)	19 (86%)	
Equivocal	5 (26%)	2 (9%)	

Data are presented as medians (IQR) or number (percentages).

CMAP scan in controls

CMAP scans were performed in all control subjects. The CMAP scans from controls from Bangladesh and The Netherlands were first analysed separately (Supporting Information Table SS1, which is available online). No differences were found in CMAP scan characteristics between these two groups. The data were therefore combined and used as a single control group for the rest of the study. The upper and lower limits of normal for the CMAP scan variables were calculated based on the 2.5 percentile and 97.5 percentile and presented Supporting Information Table SS1.

CMAP scan in relation to GBS subtype

Based on the upper and lower limits of normal, 38 (93%) of the 41 patients showed abnormalities in the CMAP-scan. Of these 41 patients, 15 (37%) were classified as AIDP, 19

(46%) as AMAN, and 7 (17%) as equivocal. The AMAN patients were significantly younger than the AIDP patients (median 25 years and 50 years, respectively; p=0.001).

CMAP scans performed at hospital admission showed a difference in SI variables between AIDP and AMAN patients. Typical examples of the CMAP scans of the patients with AIDP and AMAN are provided in Figure 1b-d. The most pronounced differences were found in the S50, S100 and absolute SI-range (Table 2).

Linear discriminant analysis identified the combination of maximum CMAP amplitude and absolute SI-range as the parameters that best separate the different subgroups. Plotting the maximum CMAP-amplitude versus the absolute SI-range for the AIDP, AMAN, and controls resulted in distinct patterns for the three groups (Figure 2).

Table 2. Baseline characteristics and CMAP scan characteristics of subgroups and age matched controls.

Parameter	AIDP (n=15)	AMAN (n=19)	Controls (n=41)	p-value AIDP-AMAN	p-value AIDP-controls	p-value AMAN-controls
Baseline characteristics						
Age	50 (38-67)	25 (16-32)	36 (23-56)	0.001	0.07	0.02
Sex (males; n (%))	13 (87%)	13 (68%)		0.21		
Onset - CMAP scan (days)	4 (3-9)	8 (5-10)		0.06		
Onset – NCS (days)	13 (10-14)	13 (9-15)		0.70		
CMAP scan parameters						
Max CMAP (mV)	3.6 (1.1-6.9)	2.3 (0.7-4.3)	10.4 (9.7-12.4)	0.26	<0.001	<0.001
S0 (mA)	10.0 (8.5-12.9)	7.1 (5.9-9.0)	7.4 (5.5-8.4)	0.006	<0.001	0.77
S50 (mA)	16.7 (16.0-26.1)	9.9 (8.2-10.8)	10.5 (7.9-11.4)	<0.001	<0.001	0.82
S100 (mA)	29.0 (26.0-48.9)	13.3 (11.8-16.4)	12.9 (10.8-14.2)	<0.001	<0.001	0.24
Absolute SI-range (mA)	20.5 (14.2-27.8)	6.0 (4.9-8.5)	5.4 (4.4-6.8)	<0.001	<0.001	0.11
Relative SI-range	2.0 (1.2-2.4)	1.0 (0.6-1.2)	0.8 (0.6-1.0)	0.001	<0.001	0.16
Step %	8.1 (0.6-14.4)	6.5 (0.0-14.9)	1.7 (0.7-3.6)	0.63	0.01	0.03

Data are presented as median (IQR) or as numbers (percentage).
CMAP: compound muscle action potential. AIDP: acute inflammatory demyelinating polyneuropathy. AMAN: acute motor axonal neuropathy. NCS: nerve conduction studies. S0: stimulus intensity (SI) at which the first motor unit is activated. S50: SI at which 50% of the CMAP amplitude is generated. S100: SI at which all motor units are activated. Absolute SI-range: S100-S0. Relative SI-range: (S100-S0)/S0. Step %: percentage of the CMAP scan that consists of steps

CMAP scans in equivocal patients

Seven patients were classified as equivocal based on NCS. Two showed the 'axonal pattern' (low amplitudes, normal SI-ranges; patients 6 & 7 in Figure 2). These two patients came from Bangladesh and were classified as equivocal because they had conduction blocks in combination with an otherwise axonal NCS. Two other equivocal patients had CMAP scans that showed the 'demyelinating pattern' (normal amplitudes, high SI-ranges; patients 3 & 4). These were both Dutch patients with a classical Miller Fisher syndrome (ophthalmoplegia, ataxia, areflexia). In addition to absent H-reflexes, their standard NCS were normal. The 3 remaining equivocal patients (patients 1,2 & 5) had a 'normal CMAP scan pattern'. Patient 1 and 2 were Dutch patients with hyporeflexia and cranial nerve paresis. Patient 5 was a Dutch patient with ptosis, mild limb weakness and areflexia.
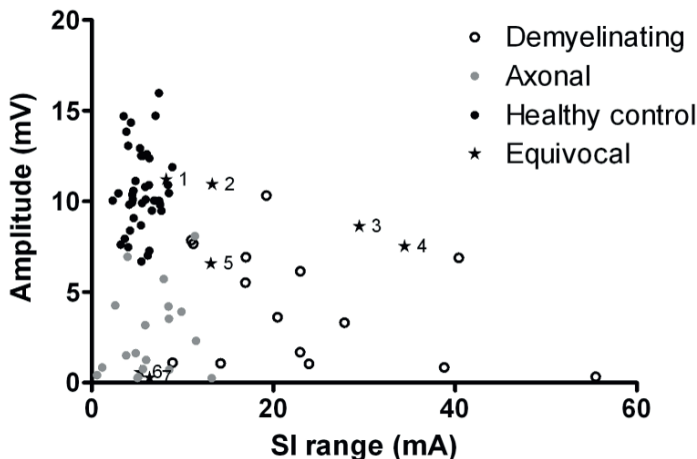
Figure 2. Maximum CMAP amplitude versus SI range of patients with NCS classified as demyelinating, axonal, and equivocal, and of controls. Equivocal (stars) patients 1 and 2 are Dutch patients with hyporeflexia and cranial nerve paresis. Patients 3 and 4, Dutch patients with classical Miller Fisher syndrome. Patient 5, Dutch patient with ptosis, limb weakness, and areflexia. Patient 6, patient from Bangladesh with severe limb weakness. Patient 7, patient from Bangladesh with severe limb weakness and cranial nerve paralysis

Discussion

In this study using the CMAP scan within the spectrum of patients with GBS, we show that the majority of patients already have electrophysiologically demonstrable nerve dysfunction at hospital admission. In this very early stage of disease, 93% of the patients show various types of abnormalities in the CMAP scan. In this stage of GBS - which is important for early diagnosis, monitoring and start of treatment – abnormalities in nerve electrophysiology may support clinical decision making. Furthermore, the results in the

current study show that the CMAP scan may also be used as a first and rapid screening technique, that might aid early distinguishing between different subtypes of GBS.

CMAP scan differences between AIDP and AMAN

The CMAP scan patterns of patients who were classified as AMAN were distinctly different compared to the CMAP scans patterns of the patients who were classified as AIDP. The division into the 'demyelinating' and 'axonal' subgroups was primarily based on differences in SI variables. Probably, these differences in the excitability of peripheral nerves reflect the variation in underlying pathophysiology between these subtypes of GBS.

The mechanism of conduction failure and excitability changes in AIDP is not well understood. One possible mechanism in the early phase of demyelinating GBS might be related to the presence of edema. Pathological studies found edema to be among the earliest changes in peripheral nerves in GBS, followed by swelling and irregularity of the myelin sheaths [18]. This edema might result in a shunting of the applied current away from the Ranvier nodes and, hence, result in higher stimulus intensities needed to depolarize the axon.

If only a proportion of the axons are involved, this will lead to a high S100 (the diseased axons are less excitable) in the CMAP scan, with a normal S0 (determined by the healthy axons) and an increased SI range (difference between SIs needed to activate the most healthy axon (S0) and the least excitable axons (S100)). If all axons are involved, this could result in an increase of all stimulus intensity parameters. Further experimental studies, preferably combined with pathology, are required to elucidate these mechanisms.

For 'axonal' GBS patients the presumed mode of action is mediated by antibodies to various types of gangliosides or ganglioside complexes [19], which leads to a complement-mediated disruption of voltage-gated sodium (Nav) channel clusters at the Ranvier nodes [20]. Dysfunction of the Nav-channels results in blockage of the action potential independently of the applied current. Such an explanation is consistent with both the reduced maximum CMAP amplitude and normal SIs in the CMAP scans of axonal patients.

The current classification of GBS patients as AMAN or AIDP is based on findings in NCS. Multiple sets of electrophysiological criteria have been developed to identify demyelination [3,2,7,21]. Yet, no set is generally accepted and the optimal time to perform NCS is still debated. Furthermore, various studies have demonstrated the existence of reversible conduction failure and conduction blocks in presumably axonal patients, which makes the differentiation between primary demyelinating GBS and primary axonal GBS even more difficult [2,3,22]. Indeed, two of our patients from Bangladesh were classified as equivocal because they had conduction blocks in combination with otherwise axonal

NCS. The CMAP scans of these two patients showed the 'axonal' pattern. The predominantly axonal NCS gives reason to believe that in these patients the 'axonal pattern' in the CMAP scan truly results from an 'axonal' GBS.

Study limitations

For the discrimination between AMAN and AIDP, NCS data collected and analyzed at 2 weeks were used as a golden standard for subtyping. However, we did not have an independent method such as pathological data to confirm a definitive subtype diagnosis. Furthermore, since we did not compare CMAP scans at admission with NCS at admission, it is unknown if NCS performed at admission would also have been able to discriminate between AMAN and AIDP at that time point.

For the purpose of the present study, we wished for patients with AIDP and AMAN to be represented equally. Because of the geographical spread of these subtypes, we decided to include patients from Bangladesh and The Netherlands [23]. Bias might have been introduced at this point. Most 'axonal' patients originated from Bangladesh, and most 'demyelinating' patients came from the Netherlands. Furthermore, the patients differed with regard to various demographic characteristics including age. However, since we found no differences between the CMAP scans of the younger Bangladeshi controls and the older Dutch controls, we cautiously conclude that the differences between our patients are not a result of just a geographical or age difference.

Due to infrastructural factors in Bangladesh, the time interval between symptom onset and hospital admission in the AMAN patients was longer than in the Dutch AIDP patients. Thus, the time between symptom onset and first CMAP scan is longer for the AMAN patients, although this difference was not statistically significant. Future studies should preferentially include AMAN and AIDP patients from the same country and also incorporate serial NCS performed at the same time as the CMAP scan, and after at least 2 weeks, since classification of the GBS subtype may change over time. This was not feasible in the current study. However, all of the AIDP patients had sensory deficits, making it unlikely that they were erroneously classified as AMAN. It cannot be excluded that they might have been classified as an AMSAN in a later stage, however AMSAN is rare.

Although in healthy subjects the reproducibility of the CMAP scan is good[11], this has not been tested in patients with GBS or other neuropathies. Studies on the reproducibility of the CMAP scan in patients with GBS and other neuropathies are needed. The CMAP scan is performed only in the distal part of one nerve and in GBS the pathological process is initially often segmental. Despite this limitation, the CMAP scan is a promising, very easy and quick method for determining the GBS subtype, at least in a subset of patients.

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Supporting Information Table SS1. CMAP scan parameters of Dutch and Bangladeshi controls and definition of normal values.

Parameter	Dutch controls (N=19)	Bangladeshi controls (N=22)	P-value	Combined set of controls (N=41)	ULN/LLN
Age	50 (39-64)	25 (17-35)	<0.001	36 (23-56)	
Max CMAP (mV)	9.9 (9.1-11.9)	10.6 (10.0-12.5)	0.17	10.4 (9.7-12.4)	6.7
S0 (mA)	7.8 (5.5-8.6)	7.1 (5.0-8.4)	0.27	7.4 (5.5-8.4)	11.8
S50 (mA)	10.6 (7.6-11.8)	10.2 (8.1-10.9)	0.30	10.5 (7.9-11.4)	14.6
S100 (mA)	12.8 (10.1-16.2)	13.0 (11.0-13.8)	0.46	12.9 (10.8-14.2)	18.5
Absolute SI-range (mA)	5.3 (4.5-7.4)	5.6 (4.2-6.5)	0.71	5.4 (4.4-6.8)	8.9
Relative SI-range	0.8 (0.6-0.9)	0.9 (0.6-1.0)	0.53	0.8 (0.6-1.0)	1.5
Step % (%)	1.8 (0.8-5.3)	1.4 (0.0-3.0)	0.32	1.7 (0.7-3.6)	20.0

Data are presented as medians (IQR). S0: stimulus intensity (SI) at which the first motor unit is activated. S50: SI at which 50% of the CMAP amplitude is generated. S100: SI at which all motor units are activated. Absolute SI-range: S100-S0. Relative SI-range: (S100-S0)/S0. Step %: percentage of the CMAP scan that consists of steps

3.3

Serial CMAP scans in Guillain-Barré syndrome to monitor disease activity, treatment response and clinical outcome

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Abstract

Objective: The compound muscle action potential (CMAP) scan is a fast, non-invasive and reproducible electrophysiological technique, which is well-tolerated and can be performed at bedside on a daily basis. The CMAP scan can be used to detect early signs of motor unit loss and reinnervation, and changes in nerve excitability. In this study we explored if serial CMAP scans can be used to monitor changes in peripheral nerve physiology in patients with Guillain-Barré syndrome (GBS) during the acute and later phases of disease, and if these changes relate to or even predict clinical outcome in individual patients.

Methods: In this prospective observational study, 14 patients with GBS and 28 healthy controls were included. Serial CMAP scans were performed (1) within 1 week after onset of neurological symptoms, (2) at least weekly in the acute phase and (3) at least 2 times during follow up. Conventional nerve conduction studies were performed at least once in the acute phase and during follow-up.

Results: All patients showed one or more abnormalities on their first CMAP scan. Deterioration and improvement of the CMAP scan, demonstrated by changes in stimulus intensity parameters and/or changes in CMAP amplitude, often paralleled the clinical deterioration and improvement. In one patient, changes of the CMAP scan (decrease of required stimulus intensities; indicative for recovery) preceded the clinical improvement, in another patient, changes of the CMAP scan (increase of stimulus intensities) preceded deterioration. Patients with CMAP scans that improved within 1 week after the first CMAP scan, generally had a shorter time to reach a GBS disability score ≤ 3 than the other patients.

Interpretation: This exploratory study indicates that serial CMAP scans have a potential to be a biomarker for disease monitoring in GBS.

Introduction

Guillain-Barré syndrome (GBS) is an immune-mediated polyradiculoneuropathy. It is a heterogeneous disease that consists of various subtypes that may differ in the underlying pathogenesis, clinical manifestations, electrophysiology, pathology and prognosis.[1,2] Although GBS generally has an acute and monophasic course, the severity of the symptoms may vary considerable between patients. Some GBS patients deteriorate rapidly, and approximately 20–25% of severely affected GBS patients require artificial ventilation. Especially in the first 2 weeks after disease onset, monitoring of the clinical condition of the patient, the potential treatment effect and possible subsequent clinical deterioration is essential. Some patients have treatment related fluctuations [3] and are likely to benefit from a second dose of immunoglobulins. Deterioration of a patient might warrant admittance in an intensive care unit. Conversely, when recovery starts, plans for rehabilitation become more important.

However, objective monitoring of the neurological condition of GBS patients can be extremely difficult, especially in those patients that are severely handicapped, admitted in the intensive care unit, ventilated and sometimes sedated. Clinical neurological investigation in those situations is often limited and serial conventional nerve conduction studies (NCS) hamper from a large variability, even under strictly controlled conditions[4]. Therefore, conventional NCS are not used for daily monitoring of the disease activity in GBS patients.

The compound muscle action potential (CMAP) scan is a fast, non-invasive and reproducible electrophysiological technique, which is well-tolerated and can be performed at bedside on daily basis.[5] The CMAP scan is mainly used to detect signs of motor unit loss and/or reinnervation in various diseases.[6-8] However, also changes in nerve excitability can be measured. [9-12]

The aim of this study was to explore if serial CMAP scans can be used to monitor changes in peripheral nerve physiology in patients with GBS during the acute phase and subsequent recovery and to investigate if these changes relate to the clinical course and outcome in individual patients.

Subjects and Methods

Patients and healthy controls

In this prospective observational study 14 patients diagnosed with GBS or GBS variants were included within 3 days after hospital admission (0-9 days after symptom onset). All patients fulfilled the diagnostic criteria for GBS [13] or Miller Fisher syndrome (MFS) [14] and were admitted to the hospital. One patient later on appeared to have acute onset CIDP (A-CIDP) and was lost to follow-up after 50 days. The follow-up duration of the remaining patients was at least three months. All patients underwent a full neurological investi-

gation at admission and weekly during hospital stay. In addition, standardized clinical scores including the GBS disability score [15], the overall disability sum score (ODSS) [16] and Medical Research Council (MRC) sum scores [17] were determined for all patients during the acute phase and follow-up . The GBS disability score ranges from 0 ("healthy") to 6 ("dead"). The overall disability sum score (ODSS) is composed of an arm and leg disability scale with a total score ranging from 0 ("no signs of disability") to 12 ("most severe disability score"). The MRC sum score is the sum of MRC scores of 6 bilateral muscle groups in arms and legs, that ranges from 0 (paralysis) to 60 (normal strength).

CMAP scans were performed (1) within 5 days after hospital admission, (2) at least weekly in the acute phase and (3) at least 2 times during follow up. In patients with a rapid clinical deterioration during the acute phase, CMAP scans were performed with a shorter time interval. Standard NCS were performed 7-14 days after weakness onset and at least once during follow up. Additionally, CMAP scans were performed in 28 healthy controls.

The study was approved by the local Medical Ethics Committee of the Erasmus MC, The Netherlands. All subjects and/or their legal representatives gave informed consent.

Standard nerve conduction studies

NCS and CMAP scans were performed on the non-dominant side. Standard motor NCS were performed of the ulnar, median, peroneal and tibial nerves. Sensory NCS were performed on the ulnar, median, and sural nerves. Stimulation and recordings sites were standardized.[18] For motor nerves, the distal and proximal baseline-peak CMAP amplitudes (dCMAP and pCMAP), distal motor latency (DML), motor nerve conduction velocity (mNCV), and F-wave latencies were determined. For sensory nerves, the baseline-peak sensory nerve action potential (SNAP) amplitude and sensory nerve conduction velocity (sNCV) were measured. If sensory potentials could be elicited, screening for a carpal tunnel syndrome was performed. If distal limb temperature was below 32 ^{0}C, the arms and legs were warmed for 30 minutes.[19] Reference values were derived from Buschbacher et al.[18] The NCS were classified as demyelinating, axonal, inexcitable, equivocal, or normal according to the Hadden criteria.[20]

CMAP scan recordings

The CMAP scan is a non-invasive electrophysiological tool that records the electrical activity of a muscle in response to repetitive transcutaneous stimulation of a motor nerve [9,21,22] CMAP scan recordings were obtained from the thenar muscles after stimulating the median nerve at the wrist. If the stimulus intensity (SI) is gradually increased from subthreshold to supramaximal values, all motor units (MUs) in the muscle are successively activated, creating CMAPs of various amplitudes. Plotting these CMAP amplitudes against the SI results in a curve and is called the CMAP scan. (fig1) The CMAP scan gives

an overview of the basic excitability of all MUs of the investigated nerve. Furthermore, the CMAP scan reveals the presence and size of large MUs without a sample bias (in contrast to needle electromyography). [6]

Key characteristics of the CMAP scan are the maximum CMAP amplitude, SI activating the first motor unit (S0), SI activating all motor units (S100), and SI-range (S100-S0). (figure 1A) Changes in SI parameters can be a reflection of changes in axonal excitability. A shift of the curve towards the right (high S0 en S100) implies a decreased excitability with involvement of all MUs: more current is needed to stimulate the nerve fibers. A broadening of the CMAP scan (normal S0 with a high S100 and high SI range) implies a more disseminated disease process: if only a proportion of the axons have an altered excitability, this will lead to high S100 (the diseased axons are less excitable), with a normal S0 (determined by the healthy axons) and thus, an increased SI range. (figure 1B) Other key characteristics of the CMAP scan are the maximum CMAP amplitude and the presence of steps. Steps are clear gaps in the usually smooth sigmoid CMAP scan. The presence of multiple large steps points to underlying processes of axonal loss and reinnervation. To quantify the presence of steps, we used step percentage (step%). The step% is defined as the summation of the absolute step sizes of all individual steps in a CMAP scan, relative to the maximum CMAP amplitude.

Changes relative to the first CMAP scan were analysed. An increase in amplitude and/or decrease in SI reflects an improvement of the CMAP scan. Conversely, a decrease in amplitude and/or increase in SI reflects a deterioration of the CMAP scan. (figure 1B)
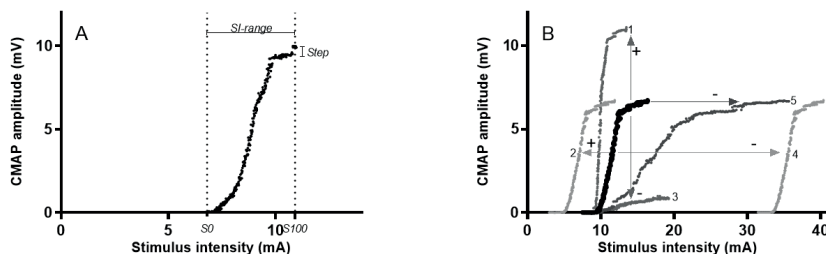
Figure 1. CMAP scan. A: Normal CMAP scan. Key characteristics are the stimulus intensity (SI) at which the first motor unit (MU) is active: S0 (6.8 mA), the SI at which all MUs are active: S100 (10.9 mA), the difference between S100 and S0: SI range (4.1 mA), maximum CMAP amplitude (10 mV) and step percentage (3%). B: potential changes of a hypothetical CMAP scan (in black); '+' indicates an improvement: increase in amplitude (1) and decrease in SI (2). '-' indicates deterioration: decrease in amplitude (3) and increase in SI (4&5) (shift of entire scan to the right (4) (caused by increase of all SIs (increased S0 and S100, relatively normal SI-range), or broadening of the CMAP scan (5) (normal S0, increase of S100, thus increase of SI-range))

Statistics

Data was tested for normality using the Shapiro-Wilk test. Data from the healthy controls were normally distributed, hence for continuous variables of the CMAP scan means and

standard deviations are presented. Upper limits of normal were defined as mean + 2SD for S0, S50, S100, and SI range. The lower limit of normal was defined as mean − 2SD for the maximum CMAP amplitude. Non-normally distributed data and ordinal data (CMAP scan data from patients and clinical scores) are presented as medians and interquartile ranges (IQR).

Non-normally distributed data and ordinal data were analysed with the Mann–Whitney U test. Paired measurements were calculated using Wilcoxon signed rank test for related samples. All calculations were performed using SPSS 25.0. Two-tailed tests were used throughout, a p-value of <0.05 was considered to be statistically significant, and when of multiple testing was performed, a p-value of < 0.005 was considered to be statistically significant.

Results

Healthy controls

The mean age of the 28 healthy controls was 48 years (range 30-75). In this group, no significant correlation was found between age, sex and the various CMAP scan parameters. The CMAP scan characteristics, including the upper limits of normal (ULN) for S0, S100, SI-range and step % and the lower limit of normal (LLN) for the maximum CMAP amplitude of the 28 healthy controls are presented in table 1.

Table 1. CMAP scan characteristics of healthy controls

CMAP scan parameter	Mean (SD)	ULN/LLN
Max CMAP amplitude (mV)	10.6 (2.0)	6.6
S0 (mA)	7.5 (1.7)	10.9
S100 (mA)	13.6 (2.8)	19.2
SI range (mA)	6.1 (1.7)	9.5
Step percentage (%)	3.2 (3.6)	10.4

Normal values for CMAP scan variables were derived from 28 healthy controls and are presented as means (and standard deviation between brackets). These data were used to calculate for S0, S100, SI range and step percentage the upper limits of normal (ULN, mean+2SD) and for the maximum CMAP amplitude the lower limits of normal (LLN, mean − 2SD). Max CMAP amplitude: maximum CMAP amplitude. S0: stimulus intensity (SI) at which the first motor unit is activated. S100: SI at which all motor units are activated. Absolute SI-range: S100-S0. Step %: percentage of the CMAP scan that consists of steps

Patients

Fourteen patients were included in the study, including 12 males and the mean age was 48 years (range 29-86). Four patients had a MFS, 9 GBS (including 7 classified as AIDP and 2 as equivocal) and 1 patient was initially diagnosed as GBS but later appeared to have A-CIDP. See supplemental table 1 for clinical scores at baseline.

CMAP scan

A total of 116 CMAP scans were recorded (range 5-21 CMAP scans per patient). (figure 2) All patients had one or more abnormalities in their first CMAP scan. The S0 was abnormal in 5 patients (36%), the S100 in 11 patients (79%), the SI-range in 13 patients (93%) the maximum CMAP amplitude in 6 patients (43%) and step% 4 patients (29%). An overview of the CMAP scan parameters of all patients is presented in table 2.

Table 2. Parameters of the first CMAP scan and GBS type of all patients

Patient nr	Age	GBS type	Max CMAP amplitude	S0	S100	SI-range	Step%	Time to GBS disability. score ≤3
1	38	AIDP	7.9	8.0	19.0	**11.0**	6.4	2 weeks
2	29	AIDP	**3.3**	**32.4**	**58.5**	**26.0**	0.0	4 weeks
3	70	AIDP	**0.9**	**60.0**	**99.0**	**39.0**	**15.0**	6 weeks
4	55	AIDP	**0.3**	**20.3**	**68.4**	**48.1**	0.0	6 weeks
5	86	AIDP	**1.0**	10.6	**23.9**	**13.4**	25.5	3 months
6	51	AIDP	**1.1**	**13.1**	**26.5**	**13.5**	3.2	6 months
7	66	AIDP	**3.6**	9.0	**29.0**	**20.0**	**19.0**	1.5 years
8	63	equivocal	8.6	10.0	**39.5**	**29.5**	7.8	2 weeks
9	36	equivocal	11.3	**12.0**	18.6	6.6	7.7	3 months
10	30	MFS	10.9	8.0	**21.0**	**13.0**	4.0	0 days
11	42	MFS	6.6	7.0	**20.0**	**13.0**	0.0	0 days
12	59	MFS	7.5	8.0	**42.0**	**34.0**	**36.0**	0 days
13	62	MFS	6.9	10.3	**26.0**	**15.7**	8.6	2 weeks
14[*]	42	A-CIDP	8.3	3.2	13.0	**9.8**	7.8	>50 days[*]

CMAP scan parameters, age, GBS type and time to reach GBS disability scale <3. MFS=Miller Fisher syndrome, AIDP= acute inflammatory demyelinating polyneuropathy, A-CIDP= acute chronic inflammatory demyelinating polyneuropathy. Max CMAP amplitude: maximum CMAP amplitude (mV). S0: stimulus intensity (SI) (mA) at which the first motor unit is activated. S100: SI (mA) at which all motor units are activated. Absolute SI-range: S100-S0 (mA). Step %: percentage of the CMAP scan that consists of steps. [*]Patient 14 was lost to follow-up after 50 days.

During follow-up, the CMAP scans of 13 patients improved. In 7 patients (patients 1,2,8,10,11,12,13) improvement was already visible within 1 week after the first CMAP scan. These patients also had a relative good clinical scores at baseline (suppl. table 1) and showed a good clinical improvement: all had a GBS disability score ≤ 3 within 1 month. In 6 patients (patients 3,4,5,6,7,9) the improvement of CMAP scan parameters was later. In one patient (patient 10), whose baseline CMAP scan was just borderline abnormal, the CMAP scan showed some variation during follow-up, without development to evident abnormal values. The CMAP scan of 1 patient (patient 14) showed fluctuations and later progression during the disease course. This patient was later diagnosed as A-CIDP.

In most patients, deterioration and improvement of the CMAP scan paralleled clinical deterioration and improvement (figure 2). In patient 7, the CMAP scan improved a week

prior to the clinical improvement and in patient 14 (ACIDP patient) deterioration of the CMAP scan was visible a week prior to the clinical deterioration (see the two illustrative cases below).
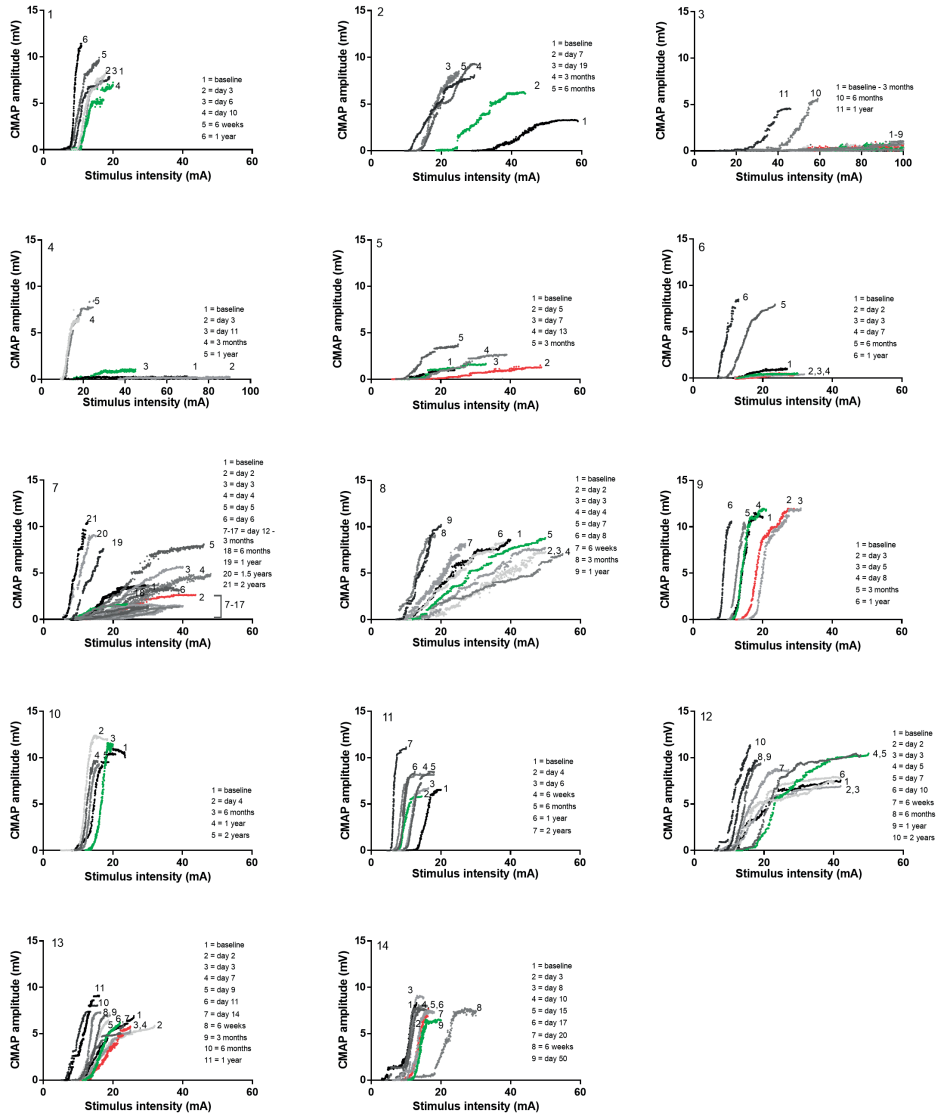
Figure 2. CMAP scans of all patients. 1= baseline CMAP scan, the other numbers indicate subsequent CMAP scans. CMAP scans in green indicate the timing of clinical improvement, CMAP scans in red indicate timing of clinical deterioration

Overall, on group level both CMAP amplitude and stimulus intensity parameters were worse at clinical nadir compared to the first CMAP scan and improved during follow-up. (Table 3). In 4 patients (patients 1,9,10,11) the last CMAP scan at follow-up completely

normalized. Patients 1, 10 and 11 showed a complete clinical recovery (MRC sum score 60, ODSS and GBS disability score 0). Patient 9 had an MRC sum score of 60, GBS disability score of 0 and an ODSS of 1 at last follow-up. S0 remained abnormal in 1 patient (7%), S100 in 6 patients (43%), SI range in 6 patients (43%), maximum CMAP amplitude in 3 patients (21%) and step% was abnormal in 8 patients (57%) (suppl. table 2).

Table 3. CMAP scan parameters of first CMAP scan, CMAP scan at nadir and last CMAP scan at follow-up

CMAP scan parameter	first CMAP scan	CMAP scan at nadir	last CMAP scan	p-value		
				first-last	first-nadir	nadir-last
Max CMAP amplitude	6.7 (1.1-8.4)	6.1 (1.2-7.9)	9.4 (7.7-10.7)	0.01	NS	0.006
S0	10.1 (8.0-14.9)	11.2 (8.0-17.9)	7.2 (5.5-9.7)	0.02	NS	0.002
S100	26.3 (19.7-46.1)	27.9 (19.9-55.8)	15.5 (11.8-23.5)	0.008	NS	0.002
SI range	14.6 (12.5-30.6)	16.4 (12.5-39.0)	8.5 (5.9-14.1)	0.007	NS	0.005
Step %	7.7 (2.4-16.0)	5.4 (0.0-24.1)	16.4 (3.1-24.5)	NS	NS	NS

Median (IQR) of CMAP scan parameters of the first CMAP scan and the last CMAP scan at follow-up. Max CMAP amplitude: maximum CMAP amplitude (mV). S0: stimulus intensity (SI) (mA) at which the first motor unit is activated. S100: SI (mA) at which all motor units are activated. Absolute SI-range: S100-S0 (mA). Step %: percentage of the CMAP scan that consists of steps. NS = not significant

Two illustrative cases

Patient 7

A 66-year old man presented with, since 1 day, rapidly progressive weakness of the limbs, without sensory symptoms. Neurological investigation showed a severe paraparesis of the limbs and areflexia. (MRC sum score 44, GBS disability score 2, ODSS 5). GBS was diagnosed and he was treated with IVIg (0.4 g/kg/day during 5 days). The CMAP scan showed an abnormal S100 (29 mA) and abnormal SI range (20 mA), with a normal S0 (9 mA). (figure 3A) During the IVIg course, the CMAP scan showed a clear, albeit short-lasting improvement. (figure 3B). At day 6, the CMAP scan deteriorated again (figure 3C). The clinical scores deteriorated (MRC sum score 8, GBS disability score 4, ODSS 12) and patient was admitted to the ICU. NCS 1 week after hospital admission showed a demyelinating polyneuropathy. The patient was intubated and was sedated for 2 days, clinical investigation was not possible. The CMAP scan however continued to deteriorate (figures 3D and E) Clinical scores remained bad (MRC sum score 5, GBS disability score 5, ODSS 12). NCS after 6 weeks showed a similar pattern as the NCS after 1 week, however with lower CMAP amplitudes. From day 54 to week 9 the CMAP scan started to improve (figure 3 F), the clinical scores started to improve 1 week later (at week 10). (MRC sum score 21, GBS disability score 5, ODSS 12). On follow-up the CMAP scan continued to improve (figures 3G and 3H), as were the clinical scores (figure 3I).
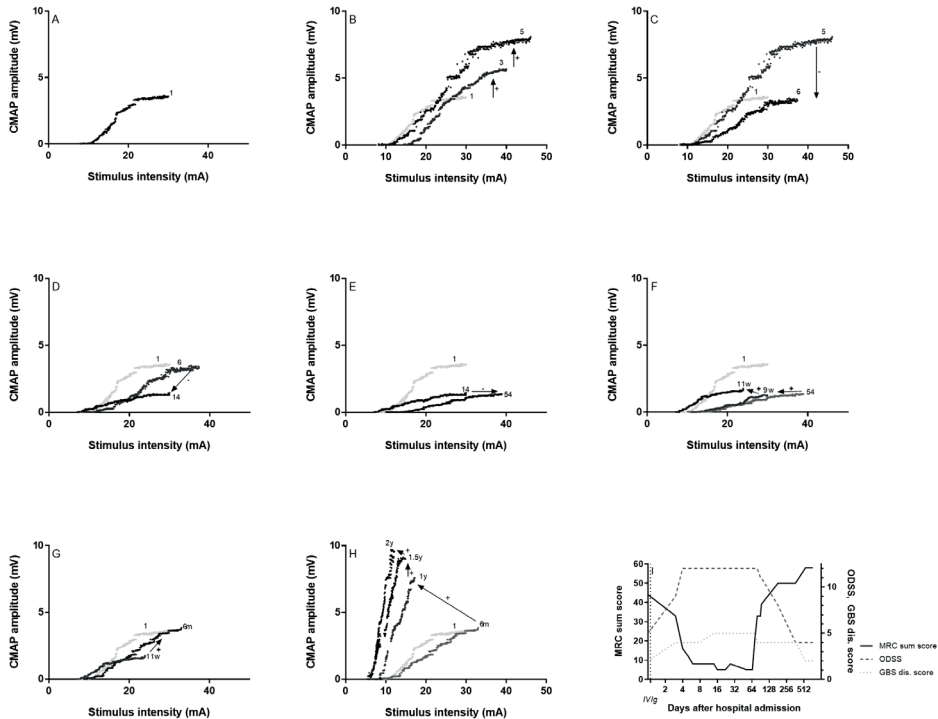
Figure 3. Serial CMAP scans of AIDP patient. An IVIg course (0.4 g/kg during 5 days) was started at after hospital admission (day 1). (A) CMAP scan on day 1 of hospital admission. (B) CMAP scans day 1, day 3 and day 5. The CMAP scan improves during the IVIg course, from day 1 to 3 and 3 to 5. (C) CMAP scans day 1, day 5 and day 6. The CMAP scan deteriorates from day 5 to day 6. (D) CMAP scans day 1, day 6 and day 14. The CMAP scan deteriorates from day 6 to day 14. (E) CMAP scans day 1, day 14 and day 54. The CMAP scan deteriorates from day 14 to day 54. (F) CMAP scans day 1, day 54, week 9 and week 11. The CMAP scans improve. (G) CMAP scans day 1, week 11 and 6 months. The CMAP scan improves further. (H) CMAP scans day 1, 6 months, 1 year, 1.5 years and 2 years. The CMAP scan improves further. (I) Time course of MRC sum score, ODSS and GBS disability score. Increasing values reflect an improvement for the MRC sum score and a worsening of the ODSS and GBS disability score. NB: the x-axes represents time on log2 scale.

Patient 14

A 43–year old man presented at the emergency department with progressive weakness of the limbs since 1 week. Neurological investigation revealed a paraparesis of the limbs and areflexia. (MRC sum score 52, GBS disability score 3, ODSS 6). GBS was diagnosed and he was treated with IVIg (0.4 g/kg/day during 5 days). NCS showed mild abnormalities and was classified as equivocal. The CMAP scan showed a slightly abnormal SI range (9.8 mA). After the IVIg, his MRC sum score improved slightly, but functional improvement was not obvious (MRC sum score 53, GBS disability score 4, ODSS 7). The CMAP scan showed a small, short-lasting improvement. (figure 4B) Approximately 1 week after hospital admission, the CMAP scan deteriorated again (figure 4C). One week later patient deteriorated also clinically (mainly sensory symptoms of the limbs, the other

scores remained stable (MRC sum score 52, GBS disability score 4, ODSS 7)) and also the CMAP scan continued to deteriorate further (figure 4D). He was treated with a second IVIg course and he was discharged to a rehabilitation centre. During follow-up, his CMAP scan further deteriorated (figure 4E), and also his MRC sum score decreased. Five weeks after the first hospital admission he was treated again with IVIg. NCS were repeated and showed a clear demyelinating polyneuropathy. The CMAP scan improved (figure 4F), as did his ODSS. The other clinical scores did not improve (figure 4G). He was later diagnosed as having an A-CIDP and was lost to follow-up after that.
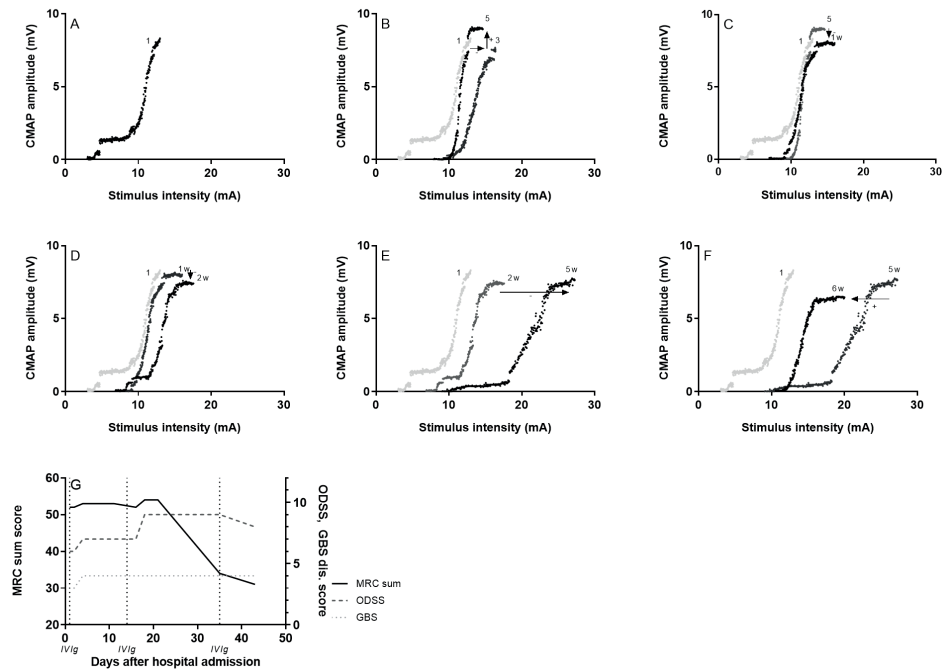
Figure 4. Serial CMAP scans of ACIDP patient. IVIg courses (0.4 g/kg during 5 days) were started on day 1, week 2 and week 5 after hospital admission. (A) CMAP scan on day 1 of hospital admission. (B) CMAP scans day 1, day 3 and day 5. The CMAP scan deteriorates from day 1 to 3 and improves from day 3 to 5. (C) CMAP scans day 1, day 5 and 1 week. The CMAP scan deteriorates from day 5 to week 1. (D) CMAP scans day 1, 1 week and 2 weeks. The CMAP scan deteriorates from week 1 to week 2. (E) CMAP scans day 1, 2 weeks, and 5 weeks. The CMAP scan continues to deteriorate. (F) CMAP scans day 1, 5 weeks and 6 weeks. The CMAP scan improves from week 5 to week 6. (G) Time course of MRC sum score, ODSS and GBS disability score. Increasing values reflect an improvement for the MRC sum score and a worsening of the ODSS and GBS disability score.

Discussion

This exploratory study provides first evidence that serial CMAP scanning reflects and possibly in some cases predicts the clinical progression and recovery in individual patients with GBS. The CMAP scan can be used as a tool to monitor nerve function in

patients with GBS. It is well-tolerated and quick and provides objective assessment of disease progression and can be performed at bedside on a daily basis independent of patients compliance. Especially in the acute phase of GBS, when clinical neurological investigation can be difficult due to artificial ventilation and sedation, the CMAP scan might be better feasible and more objective than clinical neurological investigation. Furthermore, most clinical scores are only an indirect measure for disease activity and peripheral nerve dysfunction. All investigated patients showed changes in the CMAP scan during follow-up. In most patients, these changes paralleled the clinical changes, indicating that the CMAP scan reflects the clinical condition. Interestingly, in 2 patients the changes in the CMAP scan preceded clinical changes, it preceded the clinical deterioration in one patient and clinical improvement in the other. The CMAP scans of all but one patient improved during follow-up and in 4 patients the CMAP scan completely normalized. The patient who's CMAP scan deteriorated during follow-up turned out to have A-CIDP. Although it is not clear whether all changes in the CMAP scan are clinically relevant, these cases indicate that the CMAP scan has the potential to be an objective biomarker for monitoring in GBS.

The parameters that remained most frequently abnormal during follow up were the S100 and SI range. This indicates that a subset of axons regained normal excitability (resulting in a normal S0), while a subset of axons still had a persisting altered excitability (resulting in an increased S100 and SI range). This finding is in line with the changes in CMAP scans of patients who had GBS during childhood. In 68% of these patients, the CMAP scans remained abnormal even years after the disease, and most persisting abnormalities were found in the stimulus intensity parameters.[23]

Also during follow-up more than half of the patients had an abnormal step%, while for most the maximum CMAP amplitude was normal. An abnormal step% with a normal CMAP amplitude probably indicates that reinnervation had occurred. Remarkably, 4 patients also had an abnormal step % at first CMAP scan as well. The cause of this increased step % at baseline is not clear, it might be caused by unknown prior nerve damage, but it might also be related to ephaptic coupling in demyelinated nerves at axonal level. Albeit not the scope of this study, the CMAP scan potentially provides more insight in the physiological processes occurring during the various phases of GBS and during treatment.

A short-lasting improvement in nerve excitability during IVIg treatment was seen in both above described cases. It is however currently unknown what the cause of this improvement is and if it is actually related to a clinical relevant improvement in nerve function. More advanced excitability studies in patients with CIDP also show early nerve excitability changes after IVIg treatment even when motor function did not yet improve.[24]

Whether the CMAP scan also has a prognostic value requires further evaluation in future prospective studies. Although in our cohort there was a relation between the timing of CMAP scan improvement and timing of clinical improvement, the numbers are too small to make a definite conclusion. Furthermore, 4 of the 7 patients in the group with quick recovering CMAP scans (and hence with a good prognosis) were diagnosed with MFS. Patients with MFS generally have a better prognosis than patients with GBS or GBS overlap syndrome. This might have influenced our results. Yet, 3 patients in the group with quick recovering CMAP scans were AIDP patients, and they also had a favorable prognosis.

Another shortcoming of our study is that the follow-up intervals, especially after patients were discharged from the hospital, were sometimes too large to define the precise timing of either clinical or electrophysiological improvement.

Another challenge remains to clarify what changes in the CMAP scan are clinically relevant. As with all physiological methods, CMAP scan measurements are subject to variability. In healthy subjects this variability is small [5] however, this has not been investigated in patients with GBS or other forms of neuropathy.

In conclusion, this exploratory study shows that the CMAP scan has a potential to be a quick and objective biomarker to monitor disease in patients with GBS. Further prospective studies in a larger cohort of patients with GBS are required to evaluate if the CMAP scan is an accurate predictor of clinical progression and recovery in individual patients.

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Suppl. table 1. Clinical characteristics

Patient nr	Age	MRC sum score	ODSS	GBS disability score	Time to GBS disability. score ≤3
1	**38**	**50**	**5**	**2**	**2 weeks**
2	**29**	**48**	**4**	**3**	**4 weeks**
3	70	50	11	5	6 weeks
4	55	50	9	4	6 weeks
5	86	38	9	4	3 months
6	51	47	4	3	6 months
7	66	44	5	2	1.5 years
8	**63**	**60**	**7**	**3**	**2 weeks**
9	36	59	6	3	3 months
10	**30**	**60**	**0**	**1**	**0 days**
11	**42**	**60**	**1**	**1**	**0 days**
12	**59**	**60**	**4**	**2**	**0 days**
13	**62**	**54**	**4**	**3**	**2 weeks**
14[*]	42	52	6	3	>50 days[*]

Clinical characteristics (age, MRC sum score, ODSS, GBS disability score) at baseline and time to reach a GBS disability score ≤ 3. [*]Patient 14 was lost to follow-up after 50 days. His GBS disability score at that time was 4. The patients in bold are the 7 patients with CMAP scan improvement within 1 week after the first CMAP scan.

Suppl. table 2. Abnormalities of 1st and last CMAP scan

Patient	Abnormalities 1st CMAP scan	Abnormalities last CMAP scan
1	SI-range	-
2	Max CMAP, S0, S100, SI-range	S100, SI-range, Step%
3	Max CMAP, S0, S100, SI-range, Step%	Max CMAP, S0, S100, SI-range, Step%
4	Max CMAP, S0, S100, SI-range	S100, SI-range, Step%
5	Max CMAP, S100, SI-range, Step%	Max CMAP, S100, SI-range, Step%
6	Max CMAP, S0, S100, SI-range	Step%
7	Max CMAP, S100, SI-range, Step%	Step%
8	S100, SI-range	S100, SI-range
9	S0	-
10	S100, SI-range	-
11	S100, SI-range	-
12	S100, SI-range, Step%	SI-range, Step%
13	S100, SI-range	Step%
14[*]	SI-range	Max CMAP, S100

[*]ACIDP patient. Max CMAP amplitude: maximum CMAP amplitude. S0: stimulus intensity (SI) at which the first motor unit is activated. S100: SI at which all motor units are activated. Absolute SI-range: S100-S0. Step %: percentage of the CMAP scan that consists of steps.

3.4

Guillain-Barré syndrome subtypes related to Campylobacter infection

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Abstract

Background: In Guillain-Barré syndrome (GBS) the diversity in electrophysiological subtypes is unexplained, but may be determined by geographical factors and preceding infections. Acute motor axonal neuropathy (AMAN) is a frequent GBS variant in Japan and one study proposed that in Japan *Campylobacter jejuni* infections exclusively elicit AMAN. In the Netherlands *C. jejuni* is the predominant type of preceding infection, yet AMAN is rare. This may indicate that not all Dutch GBS patients with *C. jejuni* infections have AMAN.

Objective: To determine if GBS patients with a preceding *C. jejuni* infection in the Netherlands exclusively have AMAN.

Methods: Retrospective analysis of preceding infections in relation to serial electro-physiology and clinical data from 123 GBS patients. *C. jejuni*-related cases were defined as having preceding diarrhoea and positive *C. jejuni* serology. Electrophysiological characteristics in *C. jejuni*-related cases were compared with those in viral-related GBS patients. In addition, eight GBS patients from another cohort with positive stool cultures for *C. jejuni* were analysed.

Results: Seventeen (14%) of 123 patients had *C. jejuni*-related GBS. *C. jejuni* patients had lower motor and higher amplitude sensory action potentials compared with viral-related cases. Nine (53%) *C. jejuni* patients had either AMAN or inexcitable nerves. However, three (18%) patients fulfilled the criteria for acute inflammatory demyelinating polyneuropathy (AIDP). Also, two (25%) of eight additional patients with a *C. jejuni*-positive stool sample had AIDP.

Conclusion: In the Netherlands *C. jejuni* infections are strongly, but not exclusively associated with axonal GBS. Some patients with these infections fulfil current criteria for demyelination.

Introduction

Guillain-Barré syndrome (GBS) is a post-infectious polyradiculoneuropathy with variable clinical and electrophysiological subtypes. GBS is commonly subdivided into acute inflammatory demyelinating polyneuropathy (AIDP), acute motor axonal neuropathy (AMAN), and acute motor sensory axonal neuropathy (AMSAN). There is a remarkable difference in geographical distribution of these subtypes.(1) The axonal variants abound in Asia, while AIDP is predominant in western countries.(1-3) This geographical variation is unexplained, but may be related to differences in genetic or environmental factors. Previous studies indicated that preceding infections may influence the electrophysiological subtype of GBS.(4-6) More specifically, a recent study proposed that in Japan infections with *Campylobacter jejuni* are exclusively related to AMAN.(7) The high incidence of *C. jejuni* infections in GBS patients in Japan would explain why axonal variants predominate there.

Patients from western countries most frequently report symptoms of preceding respiratory tract infections. Indeed, serological studies have yielded evidence for infections with cytomegalovirus (CMV), Epstein-Barr virus (EBV) and *Mycoplasma pneumoniae*.(5) Nevertheless, in these regions *C. jejuni* is the predominant type of infection preceding GBS, that may affect >30% of patients.(8, 9) AMAN in western countries is rare (<10%),(3, 10) however, suggesting that *C. jejuni* in western patients may also induce other GBS subtypes. Here, we defined what subtypes of GBS may occur after *C. jejuni* infection in the Netherlands. *C. jejuni* infections were defined by specific techniques, including those used in Japanese studies, to exclude methodological bias. Patients with *C. jejuni* infections were assessed in serial electrophysiological studies and compared with patients with preceding viral infections.

Patients and methods

Patients

Patients in our study were derived from a cohort of 147 GBS patients who participated in a randomised trial comparing the therapeutic effect of intravenous immunoglobulins (IVIg) versus plasmapheresis.(11) All patients fulfilled the diagnostic criteria for GBS, were admitted to the hospital within two weeks of onset of weakness, and were unable to walk ten meters independently (GBS disability score > 2).(12) During a follow-up of six months, patients underwent neurological assessments at standardised time points according to a predefined protocol. During these visits, clinical parameters such as sensory abnormalities, cranial nerve involvement, GBS disability scales, and MRC sum scores were determined.(13, 14) Informed consent was obtained from all patients and the study was approved by the local medical ethics committee. Data from serial electrophysiological studies and pretreatment serum samples were available from 123 (84%)

of the 147 patients. These patients were not significantly different from the excluded 24 cases in terms of baseline and clinical characteristics.

To confirm our findings in patients with positive *C. jejuni* serology in this cohort, we analysed patients with positive *C. jejuni* stool cultures from a second cohort who participated in a trial studying the effect of combined IVIg and methylprednisolone treatment. (15) For this later cohort, stool specimens were available, allowing isolation of *C. jejuni* bacteria directly from these specimens (the gold standard for identification of a recent *C. jejuni* infection). In this second cohort, eight (4%) of 225 GBS patients were identified with a positive stool culture for *C. jejuni*. All eight patients of this second cohort fulfilled the diagnostic criteria for GBS, underwent an extensive follow-up, and had an EMG within three weeks after randomisation.(15)

Electrophysiology

Electrophysiological findings vary considerably in the acute stage of GBS. This dynamic situation may lead to changes in classification of patients depending on the timing of the EMG. Therefore, in this study, serial EMG studies were performed at three standard time points: within two days of hospital admission, after one week, and after one month. (10) Kuwabara et al. categorized their EMGs based on the time elapsed since the onset of weakness rather than the time of admission.(7) To be able to compare our study with this previous study, we chose to analyse the EMGs that were made in the second week after onset of weakness (median 11 days, 95% CI 10-12 days) and more than 4 weeks after onset of weakness (median 33 days, 95% CI 32-38 days).

Motor and sensory nerve conduction studies were performed on the ulnar and median nerves. Stimulation and recordings were performed at standardised sites.(16, 17) Recordings from motor nerves were quantified using peak-to-peak distal and proximal compound motor action potential (CMAP) amplitudes, distal motor latency (DML), motor nerve conduction velocity (mNCV), and F-wave latencies. For sensory nerves, the peak-to-peak sensory nerve action potential (SNAP) amplitude and sensory nerve conduction velocity (sNCV) were measured. Needle EMG was performed in the anterior tibial muscle, abductor pollicis brevis muscle or the abductor digiti minimi muscle to determine the presence of denervation activity (fibrillation potentials and positive sharp waves). In all investigations, the electromyographers were blinded to the results for infection serology. The EMGs were classified as demyelinating, axonal, inexcitable, equivocal, or normal according to the criteria of Hadden and colleagues.(3):

Normal: no abnormalities in any single nerve.
Primary demyelinating: at least one of the following in each of at least two nerves, or at least two of the following in one nerve if all others are inexcitable and distal CMAP (dCMAP) >10% of lower limit of normal (LLN): DML > 110% upper limit of normal (ULN)

(120% if dCMAP < 100% LLN); mNCV < 90% LLN (85% if dCMAP < 50% LLN); F-wave latency >120% ULN; Conduction block (CB): CMAP/dCMAP ratio ≤ 50% (if dCMAP ≥ 20% LLN).

Primary axonal: no demyelinating features (except one feature allowed if dCMAP <10% LLN) and dCMAP <80% LLN in 2 or more nerves.

Inexcitable: dCMAP absent in all nerves (or present in 1 nerve with dCAMP<10% LLN).

Equivocal: does not fit criteria for any other group.

Nerves with a dCMAP < 10% LLN did not change the classification.

Reference values were defined previously.(17) The temperatures of the recording- and stimulation sites were registered.

Serological studies

Pretreatment serum samples were examined for evidence of a recent *C. jejuni* infection using tests from both Japanese and Dutch laboratories (Dokkyo Medical University in Tochigi, Japan, and SSDZ, Delft, The Netherlands).(18, 19) Both tests were based on an ELISA, and have been described in detail previously.(20, 21) In the Japanese laboratory, serological evidence of a recent *C. jejuni* infection was defined as anti-*C. jejuni* IgG titres of 2,000 or more; in the Dutch laboratory *C. jejuni* infection was defined as the presence of anti-*C. jejuni* IgM or IgA antibodies at higher levels than an internal standard.(8) Differences between the Japanese and Dutch tests have been described previously.(19) Both tests were used in the current study to exclude the possibility that the serology method introduced a bias. Patients were regarded *C. jejuni*-positive if they: (1) suffered from diarrhoea in the three weeks preceding the onset of weakness, and (2) tested positive for *C. jejuni* serology in either one of the two independent laboratories. The *C. jejuni*-related patients were compared with a group of GBS patients from the same trial cohort who had a positive serology for a recent infection with CMV or EBV according to previously defined criteria.(8) Furthermore, the pretreatment serum samples were tested for IgM and IgG antibody reactivity against GM1 and GD1a (in The Netherlands), and against GM1, GM1b, and GalNAc-GD1a (in Japan) using ELISA as previously described.(22, 23)

Statistical analysis

Normally distributed data were analysed using the two-sided *t*-test for independent samples. Ordinal data and not normally distributed data were analysed using the two-sided Wilcoxon rank sum score test. The X^2 test or Fisher's exact test was used for categorical data depending on sample size. Data are presented as means and standard deviations (SD) or as medians and 95% confidence intervals (95% CI). For categorical variables, frequencies and percentages are given. All calculations were performed using SPSS 15.0 (SPSS Inc, Chicago, IL). A *p*-value of < 0.05 was considered to be statistically significant.

Results

Comparison of patients with C. jejuni- versus CMV/EBV-related GBS

The electrophysiology of the patients with positive *C. jejuni* serology was compared with that of patients with a positive CMV or EBV serology. Seventeen (14%) of the cohort of 123 GBS patients met our strict criteria for a recent infection with *C. jejuni* (diarrhoea and positive *C. jejuni* serology). In the same cohort of patients 24 (20%) had a positive serology for either CMV or EBV. The electrophysiological characteristics of the CMV- and EBV-related patients were very similar; therefore, both groups were combined for further analysis. Mean age of the *C. jejuni*-related patients (56 ± 17 years) was significantly higher than of the CMV/EBV-related patients (36 ± 15years) ($p < 0.001$). Sensory deficits were less frequent in the *C. jejuni*-related patients (41%) compared to the CMV/EBV-related patients (78%) ($p = 0.02$). The median MRC sum score after six months was lower in the *C. jejuni*-related patients (44, 95% CI 31-58) than in the CMV/EBV-related patients (58, 95% CI 52-60) ($p = 0.004$). The median GBS disability score at six months was significantly higher for the *C. jejuni*-related patients (3, 95% CI 1-4) compared to the CMV/EBV-related patients (1, 95% CI 1-2) ($p = 0.02$), indicating a poorer outcome. Anti-ganglioside antibodies were present in 88% of the *C. jejuni*-related patients and in 4% of the CMV/EBV-related patients ($p < 0.001$).

The two groups differed considerably with respect to electrophysiological features and subtypes, as shown in Table 1. The *C. jejuni*-related patients had significantly lower CMAP amplitudes and lower DMLs, but higher SNAP amplitudes. These differences were found in both the ulnar and median nerves, although the differences in the ulnar nerves were more pronounced.

The frequency of denervation activity (fibrillation potentials and/or positive sharp waves) in the acute phase and at 4 weeks did not differ significantly between the two groups. Within 2 weeks, 20% of the *C. jejuni* patients and 21% of the CMV/EBV patients showed denervation activity (p=0.95). After 4 weeks, 69% of the *C. jejuni* patients versus 56% of the CMV/EBV patients showed active denervation (p=0.44).

In five patients from the CMV/EBV-related group, an EMG was performed only within two weeks and not after four weeks. At four weeks, the majority of EMGs in the *C. jejuni*-related group were classified as axonal or inexcitable, but two patients (12%) had a demyelinating type of EMG. In contrast, at that time 85% of the EMGs of the CMV/EBV-related patients were classified as demyelinating.

The distal temperature during all EMG registrations was not decreased, except for two patients. Patient number 11 in the *C. jejuni*-related group had a temperature at the wrist of 29 °C and was classified as axonal in the first EMG and as equivocal in the second EMG. One patient from the CMV/EBV-related group had a temperature at the wrist of 29 °C and had normal electrophysiology at both time points.

Table 1. Electrophysiological findings in the ulnar nerve of GBS patients with positive C. jejuni serology versus GBS patients with positive CMV/EBV serology.

	C jejuni (n=17)	CMV–EBV (n=24)[*]	p Value
EMG <2 weeks			
Ulnar CMAP (mV)	0.3 (0.0–3.0)	4.0 (1.9–5.0)	0.01
Ulnar SNAP (µV)	10.0 (3.6–18.5)	1.9 (0–8.0)	0.02
Ulnar mNCV (m/s)	52 (46–60)	47 (42–53)	0.08
Ulnar DML (ms)	3.4 (2.7–4.6)	4.6 (3.9–5.7)	0.04
Classification (n(%))			<0.001
Demyelinating	2 (12)	21 (88)	
Axonal	5 (29)	1 (4)	
Inexcitable	4 (24)	0 (0)	
Equivocal	6 (35)	1 (4)	
Normal	0 (0)	1 (4)	
EMG >4 weeks			
Ulnar CMAP (mV)	0.4 (0.0–3.5)	5.4 (2.0–10.0)	0.01
Ulnar SNAP (µV)	16.5 (4.0–26.0)	2.5 (0.0–6.3)	0.01
Ulnar mNCV (m/s)	49 (37–59)	44 (39–50)	0.12
Ulnar DML (ms)	3.6 (2.8–5.0)	5.6 (4.3–7.1)	0.003
Classification (n (%))[‡]			<0.001
Demyelinating	2 (12)	16 (85)	
Axonal	5 (29)	0 (0)	
Inexcitable	4 (24)	1 (5)	
Equivocal	6 (35)	1 (5)	
Normal	0 (0)	1 (5)	

Values for the ulnar CMAP, SNAP, DML and mNCV are presented as medians (95% CI). EMGs were made within 2 weeks (median 11 days) and after 4 weeks (median 33 days) following weakness onset.

[*] EMG at >4 weeks was missing in five (21%) of 24 CMV-EBV related GBS patients.

[†] Classification criteria according to Hadden *et al* (3): *Normal*, no abnormalities in any single nerve; *Demyelinating*, one of the following in two nerves (or two of the following in one nerve if all others are inexcitable and dCMAP >10% LLN): DML >110% ULN (120% if dCMAP <100% LLN); mNCV <90% LLN (85% if dCMAP <50% LLN); F wave latency >120% ULN; conduction block: CMAP/dCMAP ratio ≤50% (if dCMAP ≥20% LLN); *Axonal*, no demyelinating features (except one feature if dCMAP <10% LLN) and dCMAP <80% LLN in two or more nerves; *Inexcitable*, dCMAP absent in all nerves (or present in one nerve with dCAMP <10% LLN); *Equivocal*, does not fit criteria for any other group. Nerves with a dCMAP <10% LLN did not change the classification.

CMAP, compound motor action potential; CMV, cytomegalovirus; dCMAP, distal CMAP; EBV, Epstein–Barr virus; DML, distal motor latency; GBS, Guillain–Barré syndrome; LLN, lower limit of normal; mNCV, motor nerve conduction velocity; SNAP, sensory nerve action potential; ULN, upper limit of normal.

Classification of patients with positive C. jejuni serology

Nine (53%) of the 17 patients with positive *C. jejuni* serology tested positive in both the Dutch and Japanese assay; the other eight patients tested positive in the Dutch assay only. These two subgroups were indistinguishable in terms of clinical and electrophysiological characteristics and, therefore, were combined for further analysis. Table 2

provides an overview of the electrophysiological and serological data for all *C. jejuni*-serology positive patients.

Table 2. Electrophysiological classification of GBS patients with diarrhea and positive C. jejuni serology.

Patient No	C jejuni serology		Antiganglioside antibodies		EMG classification[*]	
	Dutch	Japanese	Dutch	Japanese	<2 weeks	>4 weeks
1	+	+	GM1	GM1	Axonal	Axonal
2	+	+	GM1	GM1, GM1b, GalNAc-GD1a	Axonal	Axonal
3	+	+	−	GM1b, GalNAc-GD1a	Axonal	Equivocal
4	+	+	GM1, GD1a	GM1, GM1b, GalNAc-GD1a	Equivocal	Axonal
5	+	+	GD1a	GM1, GM1b, GalNAc-GD1a	Equivocal	Equivocal
6	+	+	GM1	GM1, GM1b	Equivocal	Demyelinating
7	+	+	GM1	GM1, GM1b	Inexcitable	Inexcitable
8	+	+	GM1	GM1, GM1b, GalNAc-GD1a	Inexcitable	Inexcitable
9	+	+	GM1	GM1, GM1b	Inexcitable	Inexcitable
10	+	−	−	GalNAc-GD1a	Axonal	Axonal
11	+	−	GM1	GM1b, GalNAc-GD1a	Axonal	Equivocal
12	+	−	−	GM1b, GalNAc-GD1a	Equivocal	Axonal
13	+	−	−	−	Equivocal	Equivocal
14	+	−	−	GM1b	Equivocal	Equivocal
15	+	−	GM1	GM1, GM1b, GalNAc-GD1a	Demyelinating	Demyelinating
16	+	−	−	−	Demyelinating	Equivocal
17	+	−	GM1, GD1b	Not tested	Inexcitable	Inexcitable

C jejuni serology and antiganglioside antibodies were determined in two independent (Dutch and Japanese) laboratories. EMGs were made within 2 weeks (median 11 days) and after 4 weeks (median 33 days) following weakness.

* Classification criteria according to Hadden *et al (3)*: *Demyelinating*, one of the following in two nerves (or two of the following in one nerve if all others are inexcitable and dCMAP >10% LLN): DML >110% ULN (120% if dCMAP <100% LLN); mNCV <90% LLN (85% if dCMAP <50% LLN); F wave latency >120% ULN; conduction block: CMAP/dCMAP ratio ≤50% (if dCMAP ≥20% LLN); *Axonal*, no demyelinating features (except one feature if dCMAP <10% LLN) and dCMAP <80% LLN in two or more nerves; *Inexcitable*, dCMAP absent in all nerves (or present in one nerve with dCAMP <10% LLN); *Equivocal*, does not fit criteria for any other group. Nerves with a dCMAP <10% LLN did not change the classification.

CMAP, compound motor action potential; dCMAP, distal CMAP; DML, distal motor latency; LLN, lower limit of normal; mNCV, motor nerve conduction velocity; ULN, upper limit of normal.

Three of these 17 patients with positive *C. jejuni* serology fulfilled the electrophysiological criteria of Hadden et al for demyelination at one or both time points (Table 3). Four weeks after the onset of weakness, when most patients have reached a stable state, two of these three patients fulfilled the criteria for demyelination. The first patient (study number 6) also had high titres of IgG antibodies to GM1 in the Japanese and Dutch assays, normal SNAPs, and a purely motor form of GBS. Needle EMG did not show any signs of axonal damage in the form of fibrillation potentials and/or positive sharp waves. The second patient (study number 15) had high titers of IgG antibodies to GM1 in the Dutch assay and to GM1, GM1b and GalNAc-GD1a in the Japanese assay. This patient

Table 3. Patients positive for *C.jejuni*-serology or -culture fulfilling the criteria for a demyelinating form of GBS.

	C jejuni serology positive						C jejuni stool culture positive	
	Patient No 6		Patient No 15		Patient No 16		A	B
	<2 weeks	>4 weeks	<2 weeks	>4 weeks	<2 weeks	>4 weeks	19 days	12 days
Median nerve								
mNCV (m/s)	50 (98%)	**38 (75%)**	**43 (83%)**	*	59 (111%)	53 (100%)	**38 (81%)**	**37 (70%)**
DML (ms)	3.2 (73%)	3.7 (84%)	**4.6 (124%)**	*	**11.2 (255%)**	**7.6 (173%)**	4.8 (102%)	**6.0 (136%)**
F wave (ms)	–	–	*	*	**54.9 (164%)**	37.3 (113%)	–	**39.5 (128%)**
Abnormal SNAP	–	–	+	+	+	+	+	+
CB	–	–	–	–	–	–	–	–
Ulnar nerve								
mNCV (m/s)	58 (112%)	**37 (71%)**	**43 (83%)**	**28 (54%)**	64 (123%)	56 (108%)	**43 (83%)**	54 (102%)
DML (ms)	3.3 (89%)	**5.0 (135%)**	**4.6 (124%)**	3.6 (97%)	**4.3 (116%)**	4.0 (108%)	3.3 (89%)	**4.1 (111%)**
F wave (ms)	–	–	–	**44 (130%)**	–	36.1 (110%)	39.7 (117%)	33.9 (112%)
Abnormal SNAP	–	–	+	+	+	+	+	–
CB	–	–	+	+	–	–	–	–

Electrophysiological findings in patients with positive *C jejuni* serology (patient Nos 6, 15 and 16) or stool culture (patients A and B). Absolute values of NCV, DML and F wave latency are shown (with percentages for LLN for mNCV and ULN for DML and F waves in parentheses). Normal values (and related LLN and ULN) depend on sex, age and length. Abnormal SNAP amplitude and presence of CB are indicated by '–' and '+'. At the time points in bold typeface (second row), EMGs were classified as demyelinating. Numbers in bold typeface represent the abnormalities in the 'demyelinating range'.(3) Nerves with a dCMAP <10% did not change the classification.
* dCMAP <10% LLN.

CB, conduction block; CMAP, compound motor action potential; dCMAP, distal CMAP; DML, distal motor latency; LLN, lower limit of normal; mNCV, motor nerve conduction velocity; SNAP, sensory nerve action potential; ULN, upper limit of normal.

had abnormal SNAPs. Needle EMG also showed signs of axonal involvement (fibrillation and positive sharp waves). The third patient (study number 16), tested negative for anti-ganglioside antibodies, had abnormal SNAPs, and no denervation potentials in needle EMG.

Classification of patients with positive C. jejuni-stool culture

Two (25%) of the eight patients from the second cohort with a stool sample that was positive for *C. jejuni* fulfilled the demyelination criteria (Table 3). Patient A was a 63-year-old man who was admitted with a progressive burning sensation and paraesthesias in hands and feet and, one day later, progressive muscle weakness. His EMG was performed 19 days after weakness onset and showed prolonged DMLs in combination with low NCVs, a conduction block, and absent SNAPs. Needle EMG showed no denervation activity. He had a positive serology for IgG anti-GM1 antibodies. Patient B was a 36-year-old female, with pronounced muscle weakness and mild sensory disturbance. Her EMG was performed 12 days after weakness onset and showed prolonged DMLs in median, ulnar and peroneal nerves, an abnormal mNCV in the median nerve, and abnormal SNAPs of median and ulnar nerves. Needle EMG showed no denervation activity. No serum antibodies to gangliosides were found.

Discussion

The current study has confirmed the results of the previous study of Kuwabara et al. that in GBS patients, a preceding infection with *C. jejuni* is frequently followed by AMAN, even in western countries where this axonal subtype is relatively rare.(3) The majority of Dutch patients with a preceding *C. jejuni* infection developed a variant with inexcitable nerves or features of axonal degeneration in serial EMG studies. In contrast, Japanese and Dutch patients with a preceding EBV or CMV infection rarely develop AMAN, indicating that preceding infections influence the electrophysiological subtype of GBS. However, even when very strict clinical and serological criteria for a recent infection with *C. jejuni* were applied, three (18%) of 17 GBS patients with a *C. jejuni* fulfilled the electrophysiological criteria for AIDP. Furthermore, in another cohort, *C. jejuni* was cultured from the stools of two patients with a typical demyelinating form of GBS. These findings demonstrate that at least in Dutch patients, *C. jejuni* infections do not exclusively elicit the AMAN variant of GBS. This finding is in accordance with the relatively high frequency of both *C. jejuni* infections and AIDP in the Netherlands.

To be able to clarify which GBS subtypes can be elicited by a *C. jejuni* infection, our first priority was to prevent the inclusion of false-positive *C. jejuni* cases. For this reason, an extensively validated serological assay was used, which has a sensitivity of 96% and a specificity of 93% for detecting a recent *C. jejuni* infection in patients with GBS.(18) To further increase the specificity, we added the criterion of presence of preceding

diarrhoea. This combination of strict criteria explains the low frequency of *C. jejuni*-related cases compared with previous studies.(5, 22, 24) The specificity of the *C. jejuni* serology may differ among assays.(2, 19) To make our results as comparable as possible with previous studies from Japan,(7) serological testing for *C. jejuni* was performed in the Netherlands as well as in Japan. The findings that anti-ganglioside antibodies were almost exclusively found in patients with positive *C. jejuni* serology, lend further support to a high specificity of the assays. Furthermore, in two GBS patients with a positive stool culture for *C. jejuni*, the gold standard for identifying a recent infection, demyelinating forms were identified. Taken together, it is highly unlikely that the observed coincidence of *C. jejuni* infection and AIDP in five out of 25 *C. jejuni*-positive patients can be explained by false-positive cases of *C. jejuni*.

The second issue that needs to be addressed in this context is whether the electrophysiological features observed in these five patients can be attributed rightly to the process of demyelination. In the current study we used the criteria of Hadden and colleagues.(3) These criteria were developed in Caucasian GBS patients, but are similar to the criteria of Ho and colleagues (2), that were used in the previous study from Japan. (7) All patients in the current study that were classified as AIDP with Hadden's criteria, including the two *C. jejuni*-culture positive patients, also fulfilled the criteria for AIDP of Ho and colleagues. Over the years, multiple sets of electrophysiological (research) criteria have been developed to identify demyelination in GBS patients.(2, 3, 10, 25-27) No set is universally accepted, however, and no comparative studies with nerve biopsies are available for GBS including the current study. Furthermore, a few studies have shown the existence of conduction blocks, conduction slowing, and increased DMLs in AMAN patients.(28, 29) These phenomena were presumably caused by alteration of the resting membrane potential and sodium channel inactivation. Along the same lines, it should be noted that three of the five *C. jejuni*-positive patients with AIDP in our study had high titres of serum IgG antibodies to GM1 or other gangliosides. Only one of these patients also had signs of axonal involvement in the form of fibrillation potentials and positive sharp waves on EMG. Usually, these antibodies are strongly associated with axonal degeneration.(22, 30) Possibly, these findings point to the existence of an as yet unrecognised subtype, which merges axonal and demyelinating characteristics on EMG. Within the limits of the currently available criteria (whether those of Hadden, Ho or others), however, the EMGs of the five patients unmistakably showed primary demyelinating features. Moreover, the majority had marked sensory abnormalities on the EMG, which are not very common in AMAN.(29)

The current study is based on the EMGs of median and ulnar nerves, the only two motor nerves that were studied in this patient cohort systematically. In the study of Kuwabara et al, four motor nerves were examined. (7) The current criteria require the presence of a demyelinating feature in at least two nerves, which enabled us to classify

patients with AIDP. Performing EMG in a third (or fourth) nerve will only increase the sensitivity of detecting demyelination.

A third potential confounder for the subtyping of GBS patients is the timing of the EMG, since the electrophysiological findings vary considerably in the acute stage of GBS. (26) This dynamic situation may lead to changes in the classification of patients during follow-up, as was found in our study as well as in the Japanese study.(7) Four weeks after weakness onset, the situation may be expected to have stabilised. Even at that time, some of our patients fulfilled the electrophysiological criteria for demyelination. In this context, it should also be noted that none of our *C. jejuni* patients with a demyelinating EMG had the previously reported quickly reversible conduction blocks and increased DMLs that normalise within two weeks after weakness onset.(7)

More extensive studies are required to establish the relations between the geographical and ethnic origin of the patient, type of preceding infections, and subsequent electrophysiological and clinical phenotype. If the patient's origin indeed influences the phenotype of GBS after infection with *C. jejuni*, this raises the question of which factors could explain such an association. Previous studies showed that the type of *C. jejuni* strain and related ganglioside mimicry or additional virulence factors highly influence the subsequent neurological deficits.(31) Possibly, these characteristics vary between *C. jejuni* strains derived from different geographical areas. In addition to these pathogen factors, host factors may co-determine the phenotype by influencing the specificity and type of immune response triggered by a preceding *C. jejuni* infection. Alternatively, the antigenic makeup of the peripheral nervous system or accessibility of these targets to the immune system may depend on the origin of the host. Various genetic factors are associated with disease occurrence and severity.(32-34)

Extensive international collaboration and comparisons of large and standardised cohorts of GBS patients from various geographical and ethnic origins are required to resolve these issues. In addition, the current classification into demyelinating and axonal subtypes based on EMG characteristics may be too crude to account for the diversity in clinical, serological, and electrophysiological findings. A more refined classification of patients may lead to more targeted and individualised therapies.

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4

Neurophysiological changes in late phase GBS

4.1

Residual fatigue in Guillain-Barré syndrome is related to axonal loss.

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Abstract

Objective: To determine the occurrence of residual loss of peripheral nerve axons by motor unit number estimation (MUNE) and conventional nerve conduction studies (NCS) in patients with and without severe fatigue.

Methods: 39 patients at a median of 8 years (range 1-23 years) after diagnosis of Guillain-Barré syndrome (GBS) were neurologically examined and divided in two sub-groups based on the presence of severe fatigue (defined as a fatigue severity score ≥5). All patients were investigated with standard NCS, and motor unit number estimation (MUNE). Normal values for MUNE were collected in 14 healthy controls.

Results: MUNE of the thenar muscles was lower in the 15 patients with severe fatigue (median 125, IQR 65-141) compared to the 24 patients without severe fatigue (median 258, IQR 120-345) (p=0.002). In the healthy controls, MUNE was 358 (245-416). Severe fatigue was also related to lower sensory nerve action potential (SNAP) amplitude of the median (p=0.01) and ulnar nerve (p=0.03). The two subgroups did not differ regarding neurologic deficits, disability and the remaining conventional motor NCS.

Conclusion: This study demonstrates that severe fatigue after GBS is related to more pronounced axonal loss, represented by lower MUNEs and lower sensory nerve action potentials.

Introduction

Guillain-Barré syndrome (GBS) is a subacute peripheral neuropathy that may cause severe weakness[1, 2]. Despite recovery of this weakness in the majority of patients, approximately 80% have severe fatigue. This fatigue is more severe than in healthy persons and may result in substantial disability, with a high impact on perceived health status.[3] The mechanisms underlying post-GBS fatigue are unclear.[4] Previous research has shown that severe fatigue after GBS is not related to recovery of muscle strength and sensory deficits, nor to the type of antecedent infection.[5] As a result, fatigue is sometimes attributed to stress or psychological factors.

In other neuromuscular disorders more severely affected patients experience a higher level of fatigue than less affected patients.[6] This may suggest a relation between experienced fatigue and axonal loss. Yet, nerve conduction studies (NCS) in GBS patients have not shown a relation between the occurrence of fatigue and axonal loss.[7] A follow-up study in GBS patients demonstrated that residual axonal loss is present in a number of clinically well-recovered GBS patients.[8] However, whether this axonal loss is associated with fatigue is unknown.

Advanced electrophysiologic techniques such as motor unit number estimation (MUNE) might be more sensitive in detecting axonal loss than conventional electrophysiologic methods, because with conventional methods axonal loss is masked by reinnervation processes.[8] Hence, the aim of the present study was to use MUNE, a technique that provides information on the number of functional motor units, to investigate the relation between axonal loss and severe fatigue after GBS.

Methods

Subjects

Thirty-nine GBS patients (20 men, 19 women, age range 31-77 years) were included in this study. Patients were recruited from an existing database of GBS patients included in previous clinical studies [9-11]. From this database 129 patients were approached to participate in the current study and 40 (33%) agreed. Two patients were referred to us by neurologists from regional hospitals for the purpose of this study. Three of the 42 patients that were willing to participate in this study had concomitant diseases (pulmonary problems, diabetes, cancer) and were not included in this study. All patients fulfilled the diagnostic criteria for GBS[12] at the time of onset of disease, and were clinically stable for at least one year. The GBS was not accompanied by concomitant conditions either in the acute phase or at follow-up (e.g., no diabetes, malignancy, hypothyroidism, cardiac and pulmonary problems, chronic diseases or depression), and the patients did not use medication that might cause or influence fatigue. The median time between the onset of GBS and enrollment in this study was 8 years (range, 1-23 years). Fourteen healthy

subjects with a similar distribution of sex and age (7 men, 7 women, age 27-73 years) were recruited as controls. Before entering the study, all participants were clinically and electrophysiologically screened for carpal tunnel syndrome (CTS). The patients underwent a full neurologic investigation (using clinical scores as described below), standard NCS, and MUNE measurements. In the healthy subjects, only MUNE measurements were performed.

Clinical scores

To determine the residual effects of GBS we recorded the GBS disability score,[13] overall disability sum score (ODSS),[14] and Medical Research Council (MRC) sum score.[15] The presence and severity of fatigue were assessed with the Fatigue Severity Scale (FSS).[16] The FSS is a validated self-reported fatigue questionnaire containing nine items, each of which is scored on a scale of 1 to 7. Severe fatigue was defined according to previously established criteria as a mean FSS score of 5.0 or more.[5] To exclude the potential effect of a (subclinical) depression on experienced fatigue, patients were additionally screened with the Hospital Anxiety and Depression (HADS) Scale.[17] The HADS contains 14 items and consists of two subscales: anxiety and depression. Each item is rated on a four-point scale, giving maximum scores of 21. On the depression subscale, a score of 0–7 may be considered normal.[17]

Standard nerve conduction studies

Standard motor NCS were performed of the ulnar, median, peroneal, and tibial nerves. Sensory NCS were performed of the ulnar, median, and sural nerves. If distal limb temperatures were below 32 °C, the arms and legs were warmed for 30 minutes.[18] Stimulation and recordings sites were standardised.[19] For motor nerves, the distal and proximal baseline-peak compound muscle action potential (CMAP) amplitudes, distal motor latency (DML), motor nerve conduction velocity (mNCV), and F-wave latencies were determined. For sensory nerves, the baseline-peak sensory nerve action potential (SNAP) amplitude and sensory nerve conduction velocity (sNCV) were measured. Reference values were derived from Buschbacher and Prahlow.[19]

Motor unit number estimation

Motor unit number estimation (MUNE) is based on the division of the maximal CMAP amplitude by an estimate of the mean motor unit potential (MUP) size.[20] This mean MUP is calculated by averaging a number of individual MUPs that have been sampled using one of a variety of approaches.[21] To obtain a representative mean MUP, a significant proportion of all MUPs have to be sampled. A larger MUP sample increases the accuracy of the estimate.[22, 23] In this study we determined the MUNE of the thenar muscles with the adapted multiple-point stimulation technique using high-density surface electro-

myography (HDsEMG).[23] In HDsEMG, the motor unit (MU) responses are recorded with an array of 126 densely spaced electrodes. Using such an array provides spatiotemporal profiles ("fingerprints") of individual MUs. Because the fingerprint of each MU is unique, it facilitates the detection of MUPs from single MUs. In turn, this increases the number of MUs that can be sampled compared with conventional single-electrode recordings.

The recordings commenced with stimulation of the median nerve at an intensity low enough to elicit a single, all-or-none MU response. Then, the stimulus intensity was gradually increased until another response, representing the co-activation of a second MU, was seen and then slowly to still higher intensities, activating ever more MUs, until the variation in the signal was too high to enable reliable offline analysis. The stimulus electrode was then moved to a slightly different location along the nerve and the process was repeated. Usually, this allowed the recording of responses from other MUs. The entire process was repeated until approximately 20 to 30 MUPs were sampled. From this sample the mean MUP was calculated, which was divided into the maximum CMAP to derive the MUNE. [23, 24]

Statistics

All data were first tested for normality using the Kolmogorov-Smirnov test. Normally distributed data (age and years since GBS diagnosis) were presented as means and standard deviations (SD) and non-normally distributed and ordinal data (MUNE, clinical scales and NCS parameters) as medians and interquartile ranges (IQRs). Normally distributed data were analysed using the two-sided t-test for two independent samples or the one-way ANOVA for three independent samples. Non-normally distributed data and ordinal data were analysed with the Mann–Whitney *U* test. The chi-square test was used for proportions. Correlations were tested with the Spearman test. Stepwise multiple regression analysis was performed to investigate confounding factors on FSS. All calculations were performed using SPSS 15.0 (SPSS Inc, Chicago, IL). A p-value < 0.05 was considered to be statistically significant.

Standard Protocol Approvals, Registrations, and Patient Consents

The local Medical Ethics Committee approved the experimental protocol. All subjects gave written informed consent.

Results

The 39 GBS patients were divided into two subgroups based on the FSS scores. Fifteen patients (38%) had an FSS score ≥ 5 and were considered severely fatigued. The mean age of the severely fatigued patients was 59 ± 10 years, the non-severely fatigued patients was 58 ± 11 years, and the healthy controls was 54 ± 13 years. These two subgroups of patients did not differ regarding distribution in age, sex and residual deficits

and disability (Table 1). One patient had an HADS score > 8. This patient belonged to the non-severely fatigued patient group. None of the patients had clinical and/or electro-physiologic signs of CTS.

The median MUNE of all GBS patients was 141 (IQR 107-277) and lower than the MUNE of healthy controls at 358 (245-416) (p<0.001). Furthermore, the MUNE differed between the severely fatigued patients and the non-severely fatigued GBS patients (125 (IQR 65-141) and 258 (120-345), respectively; (p=0.003), and between the two patient groups and the healthy controls (Fig. 1). The MUNE in GBS patients was correlated to the FSS (ρ=0.40, p=0.01), to age (ρ=-0.31, p=0.03), to the residual MRC sum score (ρ=0.67, p<0.001), GBS disability score (ρ=-0.36, p=0.03) and ODSS (ρ=-0.46, p=0.004). Although the MUNE of all GBS patients together correlated with age and clinical scores, there were no differences in age and clinical scores (MRC sum score, GBS disability score and ODSS) between the two patient groups (Table 1). Stepwise linear regression, including MUNE, age and MRC sumscore revealed MUNE as the only predicting variable (beta -0.36, p=0.02) for the FSS.

Table 1. Demography and residual clinical scores of GBS patients and healthy subjects.

	Non-severely fatigued patients (n=24)	Severely fatigued patients (n=15)	Control subjects (n=14)	p-value
Age (years)	58 (11)	59 (10)	55 (12)	ns
Sex (m:f)	13:11	7:8	7:7	ns
Years since diagnosis GBS	8.6 (6.0)	8.7 (6.7)	-	ns
GBS disability score	1 (0-2)	2 (1-2)	-	ns
MRC sum score	60 (59-60)	59 (51-60)	-	ns
ODSS	1 (0-2)	2 (0-5)	-	ns

Data are presented as means and standard deviation (single number) between brackets or as medians and interquartile range (two numbers) between brackets.
p-value calculated between non-severely and severely fatigued patients. ns: statistically not significant (p≥ 0.05).
GBS disability score ranges from 0 ("healthy") to 6 ("dead"). MRC sum score ranges from 0 (paralysis) to 60 (normal strength).
Overall disability sum score (ODSS) ranges from 0 ("no signs of disability") to 12 ("most severe disability").

Besides MUNE, the mean MUP size was also different between the two patient groups: 59µV (IQR 43-64) for the severely fatigued GBS patients and 29µV (IQR 19-44) for the patients who were not severely fatigued, p = 0.01. The mean motor unit potential size was correlated to the FSS (ρ=-0.35, p=0.03).

Conventional NCS showed no difference in motor nerve variables (DML, dCMAP, pCMAP, mNCV and F-waves) between the severely fatigued and non-severely fatigued patients (Table e-1 on the Neurology® Web site at www.neurology.org). Compared with the non-severely fatigued patients, the severely fatigued patients had lower SNAP ampli-tudes of the median nerve (median 17µV and 9µV, respectively; p=0.01) and ulnar nerve (median 14µV and 8µV, respectively; p=0.03) as well as lower sural sensory conduction

velocity. Stepwise linear regression (including MUNE, median SNAP amplitude and ulnar SNAP amplitude) identified MUNE as the only predictor for FSS score.
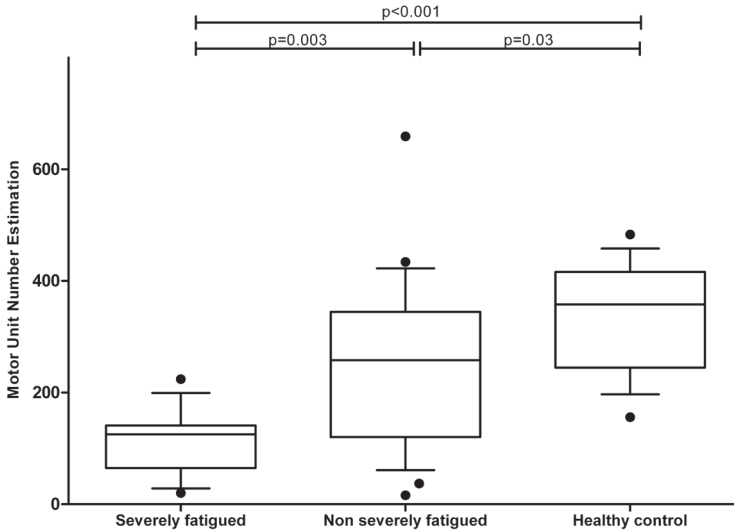

Figure 1. MUNE of GBS patients and healthy control subjects. The bottom and top of the box represent the 25th and 75th percentile, the band represents the median value, the whiskers represent the 10th and 90th percentile, and the dots represent the outliers of the motor unit number estimate (MUNE) of the severely fatigued GBS patients (n= 15), the non-severely fatigued GBS patients (n=24) and the healthy subjects (n=14).

Discussion

In this study, we found that severe fatigue in GBS is related to axonal loss. Axonal loss was detected by MUNE and not by clinical examination and conventional neurophysiologic measurements such as CMAP amplitudes. Severely fatigued GBS patients have, on average, lower MUNEs than non-severely fatigued GBS patients. Because the two patient groups did not differ regarding age and clinical scores, the difference in MUNE must be explained otherwise.

A low MUNE is an indication of pronounced axonal loss. Hence, this study provides the first direct evidence for a physiologic basis underlying experienced fatigue. Strikingly, the CMAP amplitudes did not differ between the two patient groups. This confirms our assumption that conventional electrophysiologic methods and clinical examination are not sufficiently sensitive to detect the presence of motor axonal loss, especially when reinnervation has occurred (resulting in preservation of the CMAP amplitude). A reduced MUNE in combination with a normal CMAP amplitude implies that in the severely fatigued patients the remaining MUs are larger (the same potential is produced by fewer motor units), a finding that is consistent with a previous study of MU size in

patients with GBS.[25] Indeed, analysis of the average mean MUP size reveals a larger mean MUP size in the severely fatigued group.

The question of how this axonal loss may lead to fatigue remains unanswered. Normally, upon voluntary activation of a muscle, the first MUs that are recruited are the smallest ones.[26] These allow for precise, fine movements. If more force is required, larger MUs are recruited. Possibly, this orderly recruitment mechanism has become disturbed in severely fatigued patients with GBS, because reinnervation made previously small MUs large. Early recruitment of these large MUs may then easily result in an overshoot of force. It is conceivable that this overshoot either directly or through compensatory processes results in increased fatigue. Furthermore, because after nerve injury the size-ordered organization of motor units properties can be restored after a period of time, large motor units that come from collateral reinnervation, fire fast and fatigue quickly, similar to normally large motor units. This possibly results in an increased clinical fatigue. [27]

In this study the severely fatigued patients had lower SNAP amplitudes than the non-severely fatigued patients, also a sign of axonal loss. In addition the sural NCV was lower in the severely fatigued patients. These differences might be attributable to chance caused by multiple testing. Stepwise linear regression analysis showed that the median and ulnar SNAP amplitudes were not predictors of the FSS score. It is likely that they reflect the axon loss in general and corroborate the results of the MUNE and motor fiber loss.

A possible limitation of this study might be that the presence of fatigue can be influenced by many factors. Although we excluded patients with known diseases that might influence fatigue, we did not screen for subclinical diseases (such as hypothyroidism and anemia).

To support our findings and hypothesis, longitudinal studies in patients with GBS are required as well as a similar study in patients with other peripheral neuropathies such as CIDP.

This study provides evidence that residual motor and sensory axonal loss is present in a substantial proportion of patients who recovered well clinically from a previous episode of GBS and that it is associated with fatigue in these patients. Although the mechanisms responsible for this fatigue are still unknown, these results provide a useful step toward their unraveling.

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Suppl Table e-1. Nerve conduction parameters of severely fatigued and non-severely fatigued GBS patients

	Severely fatigued patients (n=15)	Non-severely fatigued patients (n=24)	*p-value*
Motor NCS			
Median dCMAP (mV)	7.9 (5.4-9.6)	9.7 (7.0-11.3)	ns
Ulnar dCMAP (mV)	9.4 (5.7-11.5)	10.2 (8.9-11.4)	ns
Peroneal dCMAP (mV)	3.3 (2.7-7.7)	4.7 (3.3-8.3)	ns
Tibial dCMAP (mV)	6.5 (0.9-11.6)	10.4 (5.6-13.6)	ns
Median DML (ms)	4.3 (3.7-4.9)	3.9 (3.6-4.4)	ns
Ulnar DML (ms)	3.2 (2.9-3.7)	3.3 (2.8-3.7)	ns
Peroneal DML (ms)	4.3 (4.1-5.1)	4.8 (3.8-5.5)	ns
Tibial DML (ms)	4.4 (3.5-5.3)	4.0 (3.7-5.2)	ns
Median mNCV (m/s)	50 (43-53)	54 (48-58)	ns
Ulnar mNCV (m/s)	54 (49-60)	60 (54-64)	ns
Peroneal mNCV (m/s)	43 (38-47)	45 (41-48)	ns
Tibial mNCV (m/s)	41 (38-44)	44 (36-48)	ns
Median F-latency (ms)	31 (28-33)	28 (26-33)	ns
Ulnar F-latency (ms)	31 (28-33)	29 (26-33)	ns
Peroneal F-latency (ms)	56 (55-61)	51 (46-56)	ns
Tibial F-latency (ms)	58 (52-66)	55 (49-61)	ns
Sensory NCS			
Median dSNAP (µV)	9 (8-16)	17 (11-27)	0.01
Ulnar dSNAP (µV)	8 (5-12)	14 (9-17)	0.03
Sural dSNAP (µV)	7 (5-9)	10 (6-13)	ns
Median sNCV (m/s)	46 (41-51)	50 (43-55)	ns
Ulnar sNCV (m/s)	47 (44-54)	50 (45-54)	ns
Sural sNCV (m/s)	40 (38-45)	46 (40-49)	0.03

Medians and inter quartile range (between brackets) of motor and/or sensory nerve conduction variables of median, ulnar, peroneal, tibial posterior, and sural nerve. ns: statistically not significant

dCMAP = distal compound muscle action potential amplitude, DML = distal motor latency, mNCV = motor nerve conduction velocity, F-latency = minimum F-wave latency, dSNAP = distal sensory nerve action potential amplitude, sNCV = sensory nerve conduction velocity

4.2

Motor nerve excitability after childhood Guillain-Barré syndrome

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Abstract

Residual motor nerve dysfunction after pediatric Guillain-Barré syndrome (GBS) was determined in an observational cross-sectional cohort study in patients who previously developed GBS during childhood (<18 years). Ulnar motor nerve dysfunction was defined by compound motor action potential (CMAP) scan in patients after a follow up of at least 1 year compared with age-matched healthy controls, in relation to clinical course and outcome. A total of 37 persons previously diagnosed with GBS in childhood were included with a mean age at current examination of 20.6 years (4–39 years). The median time between diagnosis and follow-up was 11 years (range: 1–22 years). CMAP scanning indicated ulnar motor nerve dysfunction in 25 (68%) participants. The most frequent abnormality was a reduction in nerve excitability observed both in those with residual limb weakness and in the majority of those with complete recovery. CMAP scan characteristics were not related to prognostic factors or outcome. In conclusion, GBS in childhood results in residual motor nerve excitability disturbances, even in those completely recovered, probably reflecting altered physiology of regenerated peripheral nerves.

Introduction

Muscle weakness in the limbs is the predominant clinical symptom of the Guillain-Barré syndrome (GBS). Weakness in GBS shows a typical monophasic course with a rapidly progressive decline followed by a slow and often incomplete recovery.[1] Long-term follow up studies in GBS are scarce and usually focus on the clinical outcome in adult GBS patients.

GBS may affect children as well as adults. Previous studies have shown that the long-term prognosis of GBS in children is better than in adults, although approximately 20% may still suffer from residual complaints including fatigue and reduced quality of life. [2] Previously we investigated a cohort of persons who had GBS at childhood, after a follow-up period of at least one year. Residual clinical symptoms and complaints were defined in detail [3]. Residual peripheral motor nerve dysfunction was also determined in these children by the compound muscle action potential (CMAP) scan, a method based on CMAP recordings induced by a range of stimulus intensities.[4] The combination of high reproducibility and sensitivity for detecting clinical as well as subclinical changes in motor nerve dysfunction, makes the CMAP scan a valuable tool for investigating residual nerve damage.[5]

In the current study, we determined the utility of the CMAP scan for detecting changes in nerve physiology after clinical recovery of GBS and examined if these changes are related to the presence of residual neurological deficits and complaints.

Materials and Methods

A total of fifty-two patients were diagnosed with GBS at the age of 18 or less between 1987 and 2009 at the Sophia Children's Hospital.[6] Thirty-seven (71%) agreed to participate in the current study. All subjects previously fulfilled the diagnostic criteria of GBS [7] and did not have co-morbidity that could have influenced peripheral nerve function at the time of disease or at follow-up. Two of the 37 had a second episode of GBS. The time between the acute phase of the disease and follow up was at least one year (median 11 years, IQR 6-17 years, complete range 1-22 years). The clinical features of these patients have been presented in detail elsewhere. [3] At the time of conducting the CMAP scan follow up, 23 (62%) of the 37 patients had reached the age of 18 years. We performed the CMAP scan in 37 age-matched (within one year) healthy subjects. Informed consent was given by the parents and/or by the participants if 12 years or older. The study was approved by the Medical Ethics Committee of the Erasmus MC.

Information about the acute phase of disease was obtained regarding the main clinical prognostic factors (Erasmus GBS Outcome Score (EGOS score): preceding diarrhea, age and GBS disability score at 2 weeks after admission) [8], other neurological deficits, and routine electrophysiology. The electrophysiological subtypes were classified using the Hadden criteria. [9]

The clinical outcome of disease was defined by the GBS disability score, overall disability sum scale (ODSS), and Medical Research Council (MRC) sum scores. The presence of fatigue was assessed by the Fatigue Severity Scale (FSS).

CMAP scans were performed in all patients and healthy controls using the same standard protocol. The CMAP scan is basically a stimulus response curve in which the CMAP amplitude increases with the stimulus intensity (SI).[4] The CMAP scan is reproducible and well tolerated.[5] In all patients and controls, the ulnar nerve was stimulated at the wrist and the CMAPs were recorded from the abductor digiti minimi muscle. To ensure low skin impedance, stimulation and recording sites were rubbed with scrubgel and cleaned with alcohol. The stimulator was strapped to the wrist at the point where the lowest SI was needed to stimulate the ulnar nerve. The subjects were instructed to relax and not to move, since changes in electrode positioning can impact the stimulus intensity required to obtain a response. The active recording electrode was placed over the muscle belly at a position that optimized the size and a steep negative offset of the maximum CMAP. The reference electrode was placed on the interphalangeal joint of the fifth digit.

After electrode positioning, the lowest SI that elicited an all or nothing response from the lowest-threshold MU (S0) and the minimal SI at which the maximum CMAP could be recorded (S100) were determined.

Subsequently, the CMAP scan was recorded using 500 stimuli with a stimulus intensity decreasing from S100 to S0, with a frequency of 2 Hz and stimulus duration of 0.1 ms. To ensure that no part of the CMAP scan was undersampled sometimes 50–75 additional stimuli were applied.

The SIs required to activate motor units (MUs) differs from one MU to the other, implying that stimulating the nerve with gradually increasing SIs from subthreshold to supramaximal values, successively activates all MUs in the muscle. Plotting the elicited sub maximal CMAPs versus the corresponding SIs results in a curve: the CMAP scan (Fig. 1a).

The SIs reflect the excitability of the motor axons in very basic terms: the more current that is required to elicit a CMAP of certain size, the greater the threshold change that is necessary to activate the axons that together generate this CMAP, and the lower their excitability. Besides the SIs, the presence of 'steps' in the CMAP scan are determined. Steps appear in the CMAP scan as gaps and they result from the firing of large MUs. Step analysis was semi-automated: after manual identification of the steps, the program determined their sizes. Steps were defined as clear gaps in the CMAP scan that were bounded by plateaus at the upper and lower end of the gap, each of which consisted of at least three consecutive responses of about the same size (i.e., disregarding noise). Since after each stimulus a 100ms period is recorded, also F-wave latencies could be derived from the CMAP scan. The CMAP scan was performed using a program

implemented on an electromyography (EMG) machine (Viking Select; Cardinal Health, Dublin, OH, USA). CMAP scans were analyzed by one person, who was not informed of the clinical status of the patients.
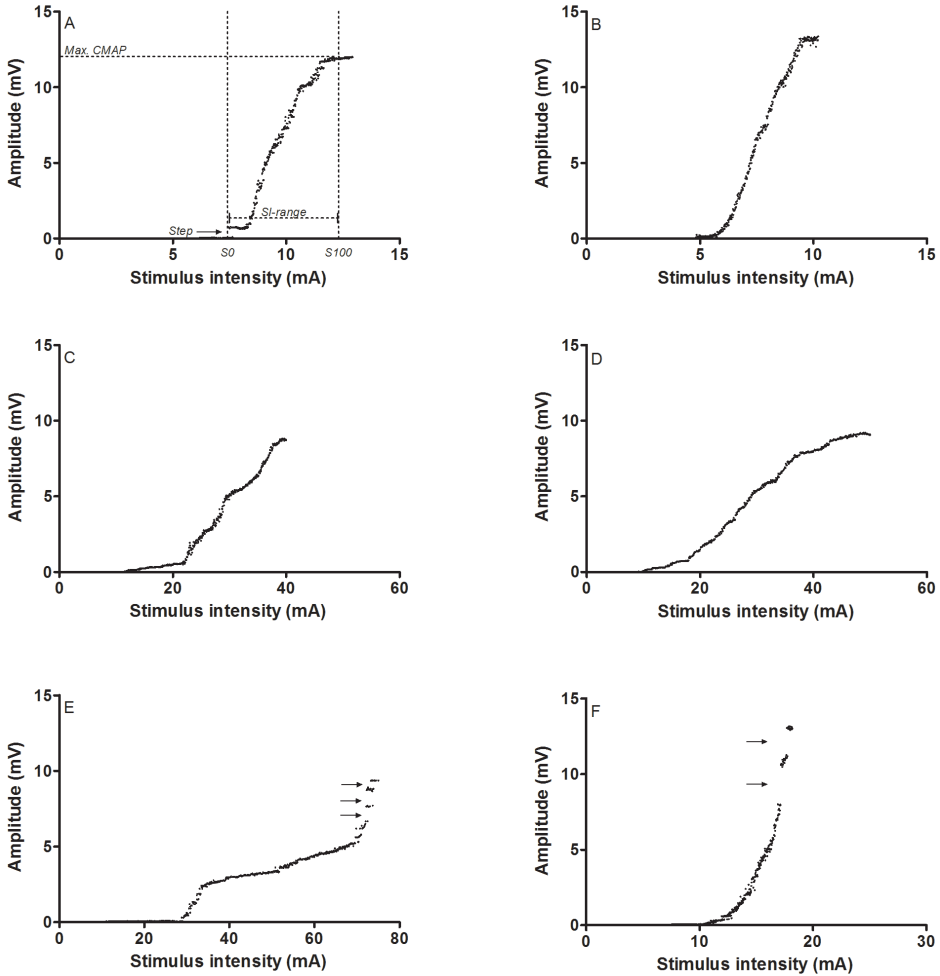

Figure 1. CMAP scans of healthy controls and former GBS patients. Examples of different CMAP scan patterns that are found in healthy controls and former GBS patients. A: CMAP scan of healthy control. The key characteristics of the CMAP scan are presented in the figure, including the maximum CMAP amplitude (mV), S0 (stimulus intensity at which the first motor unit is activated; mA), S100 (stimulus intensity at which the maximum CMAP is generated; mA), SI-range (S100-S0; mA), and steps (indicated with arrows). B: CMAP scan of an 18 year old female, 15 years after GBS episode. The CMAP scan pattern is normal. C: CMAP scan of a 39 year old male, 21 years after GBS episode. Note the increased SI-range (28 mA) D: CMAP scan of a16 year old female, 5 years after GBS episode. Note the increased SI-range (40 mA) E: CMAP scan of a 24 year old male, 14 years after GBS episode. Note the increased SI-range (46 mA) and the presence of steps (indicated with arrows). F: CMAP scan of a 26 year old female, 18 years after GBS episode. Note the presence of steps (indicated with arrows). Note that the X-axes of the various subcharts have different scales.

The CMAP amplitudes were plotted against SI and various quantitative measures were extracted: the maximum CMAP amplitude, the SI at which the first MU became active (S0), the lowest stimulus intensity at which all MUs were active (S100), the stimulus intensity range (S100-S0), the relative stimulus intensity range ([S100-S0]/S0) and the step percentage (step%, summed sizes of all steps relative to the maximum CMAP amplitude).

All variables, except age, were not normally distributed (tested with Kolmogorov-Smirnov test). To test age differences between the two groups, a t-test was performed. For all other variables the Mann-Whitney *U* test was used to compare medians between the healthy subjects and patients. The Spearman test was used to calculate correlations between clinical values and CMAP scan variables. Data from the healthy subjects were used to calculate the lower and upper limits of normal. Values <2.5 percentile and >97.5 percentile were considered abnormal. A two-sided p-value < 0.05 was considered statistically significant.

Results

The baseline characteristics of the 37 patients and age-matched healthy controls are provided in table 1. The median time between GBS diagnosis was 11years (range 1-22 years). Nerve conduction studies at time of diagnosis could be retrieved in 31 patients. Fifteen patients (48%) had the demyelinating subtype, 2 (6%) the axonal subtype, 13 (42%) had equivocal nerve conduction studies and 1 (3%) had normal electrophysiology.

At follow-up 3 (8.1%) patients had residual limb weakness indicated by an MRC sum score <60, 5 (14%) patients had an ODSS>1, and 9 of 23 (39%) patients had a FSS score>4 (table 2).

Table 1. Baseline characteristics in former GBS patients and matched healthy controls.

	Patients (n=37)	Healthy controls (n=37)	p-value
Sex (m:f)	19:18	19:18	1.0
Age at follow-up (years)[*]	20.6 (8.5)	20.7 (8.3)	0.95
Time between GBS diagnosis and study entry (years)[#]	11 (6-17)	-	
1-2 years after GBS	5 (13%)	-	
3-5 years after GBS	4 (11%)	-	
6-10 years after GBS	7 (19%)	-	
>10 years after GBS	21 (57%)	-	
Age at time of GBS diagnosis (years)[*]	8.8 (5.0)	-	
GBS disability score at nadir:			
5	5 (13%)	-	
4	20 (54%)	-	
3	5 (13%)	-	
2	7 (19%)	-	
1	0 (0%)	-	
0	0 (0%)	-	
Preceding diarrhea	11/35 (31%)	-	
Electrophysiological subtypes			
Demyelinating	15/31 (48%)		
Axonal	2/31 (6%)		
Equivocal	13/31 (42%)		
Normal	1/31 (3%)		

[*]mean (SD), [#]median (IQR)

Table 2. Residual deficits at follow-up (N=37).

Residual complaints	24 (65%)
Fatigue (FSS) ≥4	9/23 (39%)
Residual neurological deficits (total)	11 (30%)
MRC sum score <60	3 (8%)
ODSS	
0	24 (65%)
1	8 (22%)
≥2	5(14%)
GBS disability score	
0	33 (89%)
1	3(8%)
2	1(3%)

FSS, fatigue severity scale; MRC, Medical Research Council; ODSS overall disability sum scale.

All subjects underwent the CMAP scan examination without problems or discomfort. The criteria for an abnormal CMAP scan (based on 2.5 and 97.5 percentiles of healthy subjects) were: max CMAP < 5.8 mV, S0 > 11.9 mA, S50 > 19.9 mA, S100 > 22 mA, absolute range > 12.7 mA, relative range > 1.66 and step percentage > 6.1%. The CMAP scan parameters of the patients and age-matched controls are presented in Table 3. There were marked differences between patients and healthy controls, predominantly in the SI parameters. The minimal F-wave latencies did not differ between patients and healthy controls (median (IQR): 27ms (25-31ms) and 27 ms (26-28ms), respectively); p=0.53.

Table 3. CMAP scan parameters in former GBS patients and matched healthy controls

	Patients (n=37)	Healthy subjects (n=37)	p-value
Max CMAP (mV) [#]	9.4 (8.5-12.0)	11.2 (9.3-13.3)	0.07
S0 (mA) [#]	9.0 (7.0-11.3)	7.1 (5.9-8.9)	0.014
S50 (mA) [#]	16.3 (11.2-22.0)	10.5 (8.4-12.4)	<0.001
S100 (mA) [#]	22.5 (15.8-27.9)	13.8 (12.2-16.7)	<0.001
Absolute range (mA) [#]	13.2 (8.8-17.5)	6.5 (5.6-8.1)	<0.001
Relative range[#]	1.5 (1.2-1.8)	0.95 (0.79-1.12)	<0.001
Step percentage (%)[#]	3.0 (1.2-6.0)	0.0 (0.0-0.9)	<0.001
Abnormal CMAP scan	25/37 (68%)	(0%)	<0.001
Minimal F-wave latency	27 (25-31)	27 (26-28)	0.53
Abnormal max CMAP amplitude	0 (0%)	0 (0%)	
Abnormal S0	5 (14%)	0 (0%)	
Abnormal S100	20 (54%)	0 (0%)	
Abnormal SI-range	19 (51%)	0 (0%)	
Abnormal relative SI- range	10 (27%)	0 (0%)	
Abnormal Step%	10 (27%)	0 (0%)	

The key variables of the CMAP scan are: maximum CMAP amplitude (mV), S0 (stimulus intensity at which the first motor unit is activated; mA), S50 (stimulus intensity at which 50% of the CMAP amplitude is generated; mA), S100 (stimulus intensity at which all motor units are activated; mA), absolute range (S100-S0; mA), relative range (S100-S0)/S0 and step % (percentage of the CMAP scan that consists of steps). [#]median (IQR)

Correlation between age and the CMAP scan parameters was tested. There was no significant correlation between age and stimulus intensity parameters (maximum CMAP amplitude: ρ=0.20, p=0.23, S0: ρ=0.24, p=0.15, S50: ρ=0.13, p=0.44, S100: ρ=0.08, p=0.65, absolute range: ρ=0.02, p=0.89, relative range: ρ= -0.09, p=0.61).

Of 37 patients (70%) 26 had one or more abnormalities of the CMAP scan. Nine of those 26 patients (35%) had no residual weakness, no residual sensory abnormalities and no fatigue. Figure 1 shows different patterns that were seen in the CMAP scans of the patients (Fig. 1b-f).

All 3 patients with residual limb weakness (MRC sum scores <60) showed one or more abnormalities in the CMAP scan examination, mainly in the SI parameters. The first

patient was an 18 year old male, 12 years after GBS. His CMAP scan showed an abnormal S0, S50, S100 and absolute SI-range. The second patient was a 38 year old male, 22 years after GBS. His CMAP scan showed an abnormal S100 and relative SI-range. The last patient was a 23 year old male, 9 years after GBS. His CMAP scan displayed an abnormal S100 and absolute SI-range.

In addition, 23 (68%) of the remaining 34 patients without residual weakness showed one or more abnormalities in the CMAP scan. Again, these abnormalities were predominantly found in the SI parameters, predominantly in the S100 (20 patients). Of these 20 patients with an abnormal S100, 18 also had an abnormal SI range. Five patients had an abnormal S0; these five patients all had an abnormal S100 too. All patients had normal maximum CMAP amplitudes (table 3).

There was no correlation between CMAP scan parameters and disease onset and time between disease and follow-up. The CMAP scan variables were not associated with the prognostic factors defined in the early stage of disease including age, preceding diarrhea and GBS disability score at 2 weeks after admission. The CMAP scan variables were also not associated with the residual clinical disability defined by the ODSS, GBS disability score and FSS. (supplemental table) Interestingly, the patient with normal nerve conduction studies during time of diagnosis, had an abnormal CMAP scan, with markedly increased stimulus intensities. Also, the 2 patients with the axonal subtype had abnormal CMAP scans (both patients had an abnormal S100 and SI range, 1 patient (fig. 1e) also had an abnormal step percentage). The two patients with a recurrent GBS both had abnormal CMAP scans with increased S100 and SI range.

Discussion

Our study shows that the peripheral motor nerve function is permanently changed after GBS in the majority of patients, even after full clinical recovery and in absence of residual complaints. The predominant finding in the CMAP scan analysis conducted up to 22 years after diagnosis of GBS showed a reduced nerve excitability, reflecting the physiology of regenerated peripheral nerves. In this cohort of patients with GBS at childhood, almost 70% of the patients had one or more abnormalities in the CMAP scan of the ulnar nerve. The S0, S50, S100, absolute and relative range and step percentage were all significantly different in the group of GBS patients compared with age-matched healthy controls.

The finding that changes in the SI range and S100 rather than the S0 were more pronounced, suggests a diffuse pathophysiology. Apparently, in most patients some axons have retained their normal excitability and correspondingly low thresholds, representing the low end of the CMAP scan. Other axons in these patients have been more significantly affected, resulting in a permanent increase of the threshold. These changes in excitability might reflect the repair mechanism after GBS.

The follow-up time was widely spread (ranging from 1 to 22 years). The time between the diagnosis of GBS and the CMAP scan might influence changes in excitability. However, there was no significant correlation between the follow-up time and CMAP scan parameters. This may indicate that excitability changes after a period of a few years have reached a steady state, in parallel with the persistence of residual clinical deficits after a few years. The 5 patients with a relatively short follow-up of 1 or 2 years showed no difference in CMAP scan patterns compared with the other subjects.

The step percentage was higher in patients than in control subjects. A higher step percentage indicates motor unit loss and/or reinnervation. This might be caused by axonal loss during the acute phase of the GBS. However, a compression ulnaropathy with secondary axonal damage during this phase cannot be excluded.

Previous studies have shown that signs of axonal loss in long term follow up after GBS is associated with permanent weakness and severe fatigue. [10,11] However, with these methods, more subtle nerve abnormalities are not investigated. This study shows, that even in patients without clinical symptoms, nerve function is not completely normalized.

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Supplemental table. Correlation between clinical parameters and CMAP scan parameters of the former GBS patients at disease onset and follow-up

	Maximal CMAP amplitude	S0	S50	S100	Absolute range	Relative range	Step percentage
Acute phase							
Age at time of GBS diagnosis	ns	ns	ns	ns	ns	ns	ns
GBS disability score time of GBS diagnosis	ns	ns	ns	ns	ns	ns	ns
GBS disability score at nadir	ns	ns	ns	ns	ns	ns	ns
Days of weakness before hospital admission	ns	ns	ns	ns	ns	ns	ns
Days between weakness onset and nadir	ns	ns	ns	ns	ns	ns	ns
Length of hospitalization	ns	ns	ns	ns	ns	ns	ns
Days until able to walk	ns	ns	ns	ns	ns	ns	ns
Prognostic factors (EGOS score)	ns	ns	ns	ns	ns	ns	ns
years between diagnosis and follow-ups	ns	ns	ns	ns	ns	ns	ns
Follow-up							
Age at follow-up	ns	ns	ns	ns	ns	ns	ns
MRC sum score at follow-up	ns	ns	ns	ns	ns	ns	ns
ODSS at follow-up	ns	ns	ns	ns	ns	ns	ns
FSS at follow-up	ns	ns	ns	ns	ns	ns	ns

ns: not significant ($p<0.05$)

5

General discussion

General Discussion

GBS is a heterogeneous disorder that consists of a spectrum of clinical variants, and pathogenic and electrophysiological subtypes with a highly variable clinical course and outcome. This diversity complicates the understanding of the pathogenesis of GBS, but also the diagnosis, treatment, monitoring and prognosis in daily clinical practice. Nerve conduction studies (NCS) have been used to delineate and support the diagnosis of GBS but have several drawbacks which are outlined in the introduction of this thesis. The general aim of the studies described in this thesis was to determine if both standard and more advanced electrophysiological techniques (CMAP scan[1] and MUNE) can be used in GBS to discriminate between subtypes, monitor the disease activity, predict the clinical course and define residual damage.

The CMAP scan is a quick, non-invasive and well-tolerated electrophysiological tool, which reflects basic changes in nerve excitability and motor unit morphology. MUNE can provide information on motor unit loss, and indirectly on reinnervation. These techniques are complementary to conventional NCS and provide additional information on various nerve function parameters. (Table 1)

Table 1. Comparison between clinical neurological investigation, conventional nerve conduction studies, EMG, CMAP scan and MUNE. MUNE and CMAP scan (in bold) provide additional information on various nerve function parameters which will be discussed in this chapter.
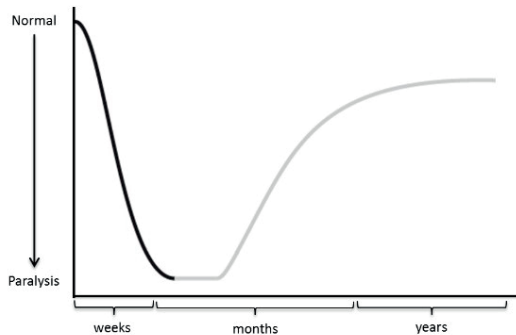
	Clinical neurological investigation	Conventional NCS	EMG	Threshold tracking	CMAP scan	MUNE
Quick	-	-	-	+	+	-
Non- invasive	+	+	-	+	+	+
Reproducible	+/-	+/-	+/-	+/-	+	+/-
Well tolerated	+	+/-	-	+/-	+	+
Easy to perform daily	+	-	-	-	+/-	-
Objective	+/-	+	+/-	+	+	+
Quantitative	-	+	-	+	+	+
Multiple nerves/muscles are tested	+	+	+	-	-	-
Provides information on nerve conduction slowing	-	+	-	-	-	-
Provides information on axonal loss	-	+/-	+	-	++	++
Provides information on reinnervation	-	-	+	-	+	+
Provides information on sensory nerves	+	+	-	-	-	-
Sensitive to nerve excitability changes	-	-		++	+	-

In this chapter the main findings of these studies are discussed, and suggestions for future research are made. The findings are structured according to the disease phase of GBS in which they are relevant: diagnostics and subtyping, monitoring, and residual damage.

5.1. Diagnosis and subtyping

5.1.1 Diagnosis

Neurophysiological studies are important to confirm that the observed clinical deficits are indeed caused by a polyneuropathy or polyradiculopathy, and to demonstrate the presence of a demyelinating or axonal subtype of GBS. However, as outlined in the introduction, conventional NCS have several limitations, especially because the main conduction parameters only represent the fastest conducting motor fibers and are relatively insensitive to MU loss. The CMAP scan has the advantage of measuring the contribution of all axons within the nerve. In the studies described in the thesis, it was investigated if the CMAP scan adds to the diagnosis and subtyping of GBS.

5.1.1.1 Can the CMAP scan be used as a diagnostic tool in GBS?
The CMAP scan can be considered an add-on to conventional NCS. The two techniques are similar in execution and both assess the more distal part of the peripheral nervous system. However, the CMAP scan also reflects the basic axonal excitability via a range of stimulus intensities. Higher stimulus intensities can indicate an increased threshold for stimulation, either of all axons within a nerve (then both S0 and S100 will be increased) or of a subset of axons within a nerve (then the S0 will be (near) normal, the S100 will be increased, and consequently also the SI-range will be increased). The physiological base for elevated stimulation thresholds however is not clear. An increased stimulation threshold might be caused by edema or thickening of the perineural tissue leading to an increased perineural capacitance. Also paranodal changes, changes in distribution of sodium channels or a combination of all above may lead to higher thresholds for stimulation. [2]

Furthermore, the CMAP scan provides a visual assessment of motor unit potentials contributing to a CMAP (Figure 1). This allows the identification and quantification of steps, which gives information about MU number and MU size. Such information is
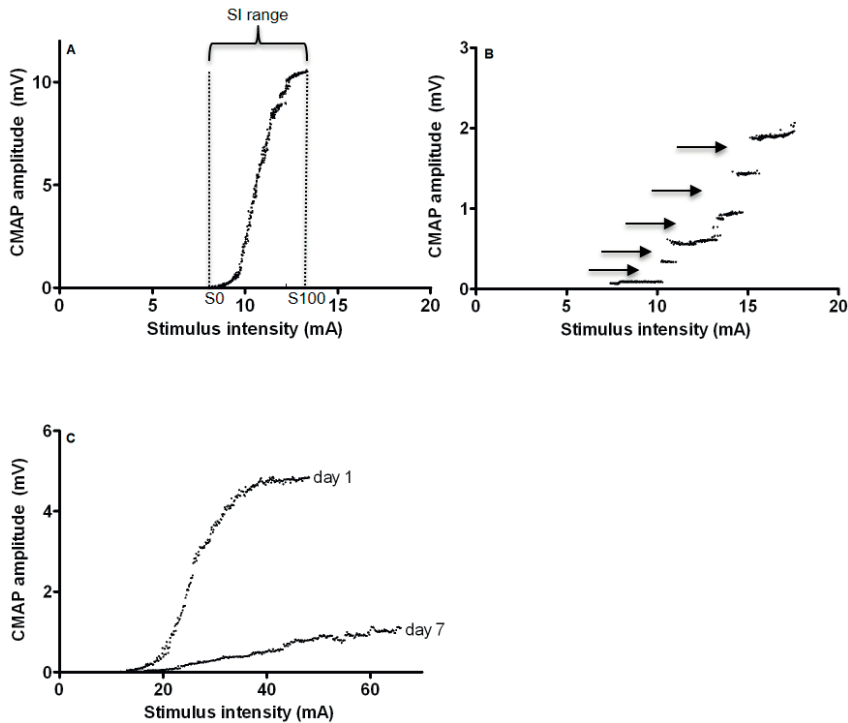
Figure 1. Different patterns that can be visualized by the CMAP scan[6] (A) CMAP scan of a healthy subject. S0 = 7.4 mA, S100 = 13,3mA, and SI range = 5.9 mA (B) CMAP scan of an ALS patient. Note the presence of multiple steps, indicated by the arrows. (C) Two serial CMAP scans of a patient with acute-onset CIDP, made with a one-week interval. Note the high stimulus intensities that were needed to record the CMAP scans. Over this week, the patient deteriorated clinically and the variables in the CMAP scan worsened. The maximum CMAP amplitude decreased from 4.8 to 1.1 mV, and S5, S50, and S95 increased from 18, 26 and 36 mA to 20, 43 and 60 mA, respectively. Note the different scaling of the axes of the CMAP scans in (A–C).

mainly of value in diseases where axonal loss and reinnervation processes are present, such as in ALS.[3-5] Enlarged MUs can also be identified with needle electromyography, but since not all MUs in the muscle are investigated with this technique it suffers from substantial sample bias.

In Chapter 3.1 is described that the CMAP scan can detect subclinical alterations in motor nerve function that are not detected by conventional NCS. In this study, three patients with Miller Fisher syndrome (MFS) underwent serial CMAP scans during the acute phase of their disease and during follow up. MFS is a considered to be a variant form of GBS and the diagnosis is based primarily on clinical characteristics of ophthalmoplegia, ataxia and areflexia. MFS can be difficult to diagnose, especially in case of a form fruste or when other causes are suspected. One of the MFS patients (patient 2 in Chapter 3.1) was initially diagnosed as having a stroke, received treatment accordingly and was admitted

at the stroke unit. However, his symptoms progressed during the day and he was eventually diagnosed with MFS. Already in a very early phase, the CMAP scan in this patient showed abnormal stimulus intensities (SIs) and SI ranges. In such cases, the CMAP scan might be helpful to discriminate between disorders of the central or peripheral nervous system, early in the course of the disease. Although we did not systematically investigate CMAP scans made in patients with diseases of the central nervous system, CMAP scans in 8 patients with an acute stroke showed no clear abnormalities (data not shown).

Conventional NCS in patients with MFS might show abnormal sensory responses[7, 8] and when serial motor conduction is scrutinized, features of reversible conduction failure can be found.[9] However, conventional NCS in general do not show abnormalities of the motor nerves in MFS. In contrast, and interestingly, the CMAP scan demonstrates that even in patients with a typical MFS, peripheral motor nerves have a reduced excitability, implying that MFS is a more widespread neuropathy than the clinical symptoms suggest. Reduced motor nerve excitability could represent the first step in nerve dysfunction.[10] Nerve dysfunction leads to muscle weakness only when a critical proportion of nerve fibers become inexcitable, as we found in patients with GBS.

5.1.2 Subtype classification

The various GBS subtypes are related to differences in pathogenesis, disease course, and prognosis [11-15] Yet patients with different subtypes currently receive the same medical treatment and supportive care. Hence, discrimination into the various subtypes is at present of little additional value for clinical practice. Subtyping is however, important in understanding the underlying pathophysiological processes in this heterogeneous disease. Furthermore, early classification and stratification in different subtypes creates opportunities to individualize treatment. For instance, GBS patients with anti-GM1 antibodies and C. *jejuni* infections, which are related to the axonal subtype, often have a more progressive and more severe pure motor variant of GBS. Their recovery is better after intravenous immunoglobulins than after plasma exchange.[16]

5.1.2.1 Is a preceding Campylobacter jejuni infection exclusively related to the axonal subtype?

In Asian countries, there is a high incidence of *C. jejuni* infections and axonal variants predominate in GBS patients - a strong correlation that is suggestive of a causal relation. There is an ongoing debate, however, whether preceding *Campylobacter jejuni* infection can induce a demyelinating GBS.[17] In Western countries, *C. jejuni* is also the predominant type of infection preceding GBS, affecting >30% of patients.[18] Yet, the axonal variant of GBS in these Western countries is rare. This suggests that *C. jejuni* in patients from Western countries may also induce other subtypes than axonal GBS. We contributed to this discussion by demonstrating in a well-defined cohort [19] and using very strict criteria

for defining a preceding *C. jejuni* infection (positive *C. jejuni* serology *and* preceding diarrhoea *and* no evidence for other preceding infections), that a clear demyelinating pattern can be found in *C. jejuni*-infected patients (Chapter 3.4). In another cohort, *C. jejuni* was cultured from stool samples (which is the golden standard for detection of a *C. jejuni* infection) of two patients with a typical demyelinating form of GBS. In combination, these results strongly suggest that *C. jejuni* can elicit the demyelinating form of GBS, a finding that may come to play a role in accurate subtyping and associated individualised treatment and prognosis. It may also contribute to the discussion whether it is sensible to divide GBS in the two main subtypes, or whether in reality GBS is more complex and consists of a continuum of subtypes. This is further discussed in chapter 5 (future remarks).

Preceding *C. jejuni* infections and axonal GBS are closely related to the presence of anti-ganglioside antibodies. In our study, anti-ganglioside antibodies were also present in patients classified as AIDP. This is in concordance with a recent study in which anti-ganglioside antibodies are found in approximately 30% of the patients classified as AIDP, irrespective of the used NCS criteria or timing of the NCS.[20]

It has been argued that NCS findings that appear most consistent with demyelination may in fact result from axonal dysfunction. Such axonal dysfunction, which is thought to lead to transient conduction slowing, is usually present within the first two weeks after weakness onset after which the conduction slowing improves [21]. It has been suggested that this feature might explain why some C. *jejuni* positive GBS patients are classified as a demyelinating subtype. However, in one of our *C. jejuni*-infected patients, the slowing was more pronounced 4 weeks after weakness onset than after 2 weeks, making a demyelinating pathology more likely.

Whether patients classified with the AIDP subtype always have an underlying demyelinating pathology, is subject of a separate debate (see next paragraph).

5.1.2.2 Shortcomings of the current classification criteria in GBS

Ideally, classification into different subtypes should not only accurately reflect the underlying pathophysiological process, but also the clinical features, physiology, and prognosis. Currently, differentiation into subtypes is solely based on a limited set of neurophysiological features, but this is often problematic for several reasons:

1. An important limitation for the development of accurate diagnostic criteria is the absence of gold standards for demyelination and axonal degeneration. In clinical practice nerve biopsies are not conducted to confirm the subtype. In addition, dysfunction of axons and myelin sheets may not necessarily be reflected in morphological changes.

2. There is as yet no consensus how to classify GBS patients based on the electrophysiological findings. Multiple classification sets of NCS findings are available, [12, 13, 20, 22-25] none of which is universally accepted. The classification into one of the subtypes is strongly influenced by the characteristics of the chosen criteriaset.[20]

3. The timing of the NCS might influence the classification, [17, 20, 21, 23, 26] since the electrophysiological findings may vary in the acute phase of GBS.[11] If NCS are performed in the acute phase, conduction properties might still be within the normal range.[27] Moreover, even after a few weeks, a definitive classification with standard NCS is often not possible, resulting in a high percentage of equivocal findings.

4. Changes in conduction properties measured with NCS that were originally attributed to demyelination can also be caused by processes unrelated to demyelination. AMAN was originally considered a 'simple' axonal polyneuropathy. However, recent studies show that in the AMAN subtype, changes at the nodal and paranodal areas of the motor axon occur, leading to reversible nerve conduction abnormalities. [28-32] This may lead to a misclassification of AMAN into AIDP when NCS are performed too early. It also may indicate that the AMAN subtype is not homogenous but consist of subgroups.

Some authors argue that serial NCS are necessary to overcome these problems.[33, 34] Others, however, show that a single NCS could suffice and that changes in electrodiagnostic subtype throughout the disease course are caused by suboptimal sensitivity and specificity of the current criteria or by disease progression.[20, 24, 35] Clinically, serial nerve conduction studies might be useful in cases of diagnostic uncertainty, especially if the first NCS findings are not informative.

Finally, the current NCS parameters that are used for classification may be too crude to account for the diversity in clinical, electrophysiological, and serological findings. These parameters are relatively insensitive to motor unit (MU) loss, and changes in most conduction properties only appear when the fastest conducting motor fibers are involved.[36]

Due to the abovementioned shortcomings, accurate classification into the various GBS subgroups purely based on conventional NCS seems unrealistic. The next paragraph discusses the potential of the CMAP scan as a tool for subtype classification.

5.1.2.3 Can the CMAP scan be used to discriminate between different GBS subtypes?

Reduced nerve excitability may be one of the first electrophysiological manifestations of GBS [10] , preceding the already mentioned changes in NCS properties. We further hypothesized that alterations in excitability properties might differ between the various subtypes and can be used in early classification of GBS.

To be able to investigate if CMAP scans can be used to distinguish demyelinating and axonal subtypes, it was required to evaluate this technique in a mixed study population. To reach sufficient statistical power in this study we tested the CMAP scan in patients with GBS originating from The Netherlands (where AIDP is the predominant subtype) and Bangladesh (where AMAN is the predominant subtype). We found that CMAP scans made at hospital admission predicted in 80% of the patients the ultimate classification as AIDP or AMAN, defined by the standard classification criteria, 2 weeks after disease onset. Two patients from Bangladesh were classified as equivocal because their NCS showed conduction blocks in combination with an otherwise axonal NCS. The CMAP scans of these two patients showed a clear 'axonal' pattern. This suggests that, with the CMAP scan, a distinction can be made in an early phase between 'axonal' and 'demyelinating' GBS patients, whereas with conventional NCS a significant number of patients are classified as equivocal even after two weeks. The division into the 'demyelinating' and 'axonal' subgroups is primarily based on differences in stimulus intensity (SI) variables, with higher stimulus intensities in the demyelinating patients. Probably, the differences in SI variables reflect the different underlying pathophysiological processes in the acute phase of the disease.

Possibly, the CMAP scan may, at least partially, overcome the problems with the existing classification criteria. This might especially be important when subtype specific therapies become available that requires early and accurate classification. Larger (international) studies with a more diverse patient population are needed to confirm these findings.

Strikingly, when nerve excitability is tested in an alternative and more advanced way, by means of threshold tracking, the most prominent abnormalities are found in patients with AMAN and not in those with AIDP.[37, 38] In the next paragraph, the differences and possible explanations of these apparent contradictory findings are discussed.

5.1.3 Advanced excitability testing: threshold tracking

Although not a subject of this thesis, there are other methods that study the excitability of human motor axons. A very elegant but complex method is the threshold tracking technique, first described by Prof. Joseph Bergmans [39] and further developed by Prof. Hugh Bostock.[40] Although both the CMAP scan and the threshold tracking technique explore nerve excitability, they do so in very different ways:

The CMAP scan reflects nerve excitability in a very basic way, i.e. the stimulus intensity that is required to depolarize a nerve and elicit an action potential. Threshold tracking is a more advanced test to define the nerve excitability. This technique provides in vivo information about axonal ion channel function and resting membrane potential.

In threshold tracking the resting threshold of a nerve (i.e. the SI required to generate a predefined proportion (often 40% of the maximum CMAP amplitude) is compared with the threshold of that nerve following changes in the environment. These changes are brought about by applying various electrical stimuli (ie hyperpolarising or depolarising currents, stimuli of various duration and stimulus trains with varying interval) on the nerve before determining the resting threshold again. Multiple nerve excitability variables can be recorded, all reflecting different aspects of axonal excitability.

5.1.3.1 CMAP scan versus threshold tracking

Threshold tracking has been used to study patients with chronic inflammatory demyelinating polyneuropathy (CIDP) and AIDP. In patients with CIDP, a characteristic pattern of abnormalities has been described [41-44], yet findings in patients with AIDP are less distinct. [37] In AIDP patients an increased threshold for 50% of the CMAP in the stimulus response curve has been described[44]. This corresponds with our findings that in AIDP, the CMAP scan shifts to the right.

So far, threshold tracking has no value in the diagnosis and monitoring in AIDP patients. However it is used for research purposes to gain more insight in the ongoing pathological changes as well as the effect of treatment.[42] As with the CMAP scan, threshold tracking measures excitability properties of the axonal membrane at the point of stimulation. Hence, only a small part of the nerve is investigated. Contrary to the CMAP scan, with threshold tracking only a proportion of axons is investigated. With threshold tracking, the changes in current required to produce a target potential of 40% of the maximal CMAP are analysed. With the CMAP scan, the stimulus intensities ranging from threshold intensity (generating 0% of the maximum CMAP) to maximum intensity (recruiting all excitable motor units and generating 100% of the maximum CMAP) are analysed.

There might be several reasons why threshold tracking does not show clear abnormalities in AIDP patients whereas the CMAP scan does. First, AIDP has a heterogeneous presentation. As presented in chapter 3, the CMAP scans show a wide variety of findings corresponding to this clinical diversity, ranging from normal CMAP scans to CMAP scans with clear excitability abnormalities. Furthermore, CMAP scan excitability parameters are very dynamic and may change even over the course of one day. However, in previous threshold tracking studies results of this heterogeneous group are averaged (resulting in a broad standard deviation). This makes it hard to find significant differences between the AIDP patients and for instance healthy control subjects. Especially when only small numbers of patients are investigated.

Second, demyelination in AIDP patients is disseminated, rather than a diffuse involvement of all axons within the nerve. This results in axons with intact nodes of Ranvier and axons with (paranodal) demyelination. With threshold tracking, only a proportion

of the axons are investigated. The axons that are studied are those that are most easily stimulated. In case of GBS, these are generally the healthiest axons. With the CMAP scan all axons are stimulated and thus investigated. If only a proportion of the axons have signs of demyelination, this will lead to high S100 (the diseased axons are less excitable) in the CMAP scan, with a normal S0 (determined by the healthy axons) and an increased SI range (difference between SIs needed to activate the most healthy axon (S0) and the least excitable axons (S100)). Threshold tracking however can be normal if a sufficient proportion of the axons is (still) healthy.
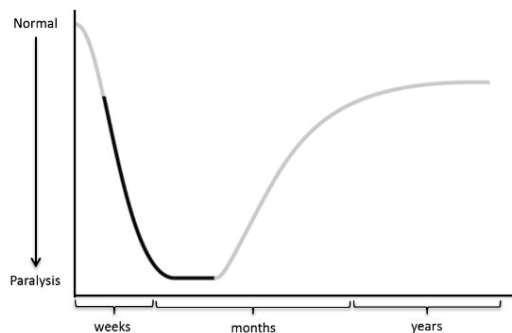
Third, with threshold tracking stimuli with a duration of 1.0 ms are applied to produce a stimulus response curve (which is basically a CMAP scan with less stimuli). With the CMAP scan the stimulus duration is ten times less (0.1 ms). With a short stimulus duration, the discriminative capacity is increased, i.e. a small increase in stimulus intensity with a short stimulus duration, will result in the excitation of only one or a few axons. In contrast, a small increase in stimulus intensity with a long stimulus duration will result in the excitation of much more axons at once.[45] This may also be the reason why in a previous study which examined stimulus response curves in GBS patients no differences were found between AIDP patients and healthy controls.[46]

Key points

> - The CMAP scan shows changes in motor nerve excitability already in the early and diagnostic phase of GBS and MFS.
> - Early CMAP scan abnormalities differentiate between demyelinating and axonal subtypes of GBS and may be more accurate than the current classification criteria.
> - C. *jejuni* can also trigger the demyelinating subtype of GBS, although there is a strong association with axonal subtypes.

5.2. Monitoring

To better understand and predict the disease course of GBS and to optimize the care and treatment, progression of disease activity needs to be closely monitored. Monitoring based on neurological examination of GBS patients is limited, especially in children and in patients that are severely affected, admitted to an intensive care unit, ventilated and sometimes sedated. Furthermore, the neurological examination has a moderate inter-

and intraobserver variability.[47-49] Conventional NCS are not used for monitoring nerve function in GBS. Although not studied in GBS, abnormalities in individual NSC parameters generally fail to predict clinical symptoms or neurological deficits.[50, 51] The question is whether the CMAP scan can be used for objective and reproducible monitoring of nerve function.

5.2.1 Can the CMAP scan be used to monitor nerve (dys)function?

The CMAP scan, in contrast to standard NCS, provides information about the excitability of all MUs within the nerve, and may therefore be a more sensitive tool to monitor nerve function in GBS. In Chapter 2, we have shown that the intra- and interobserver variability of the CMAP scan variables in healthy subjects is good and in the same range as the reproducibility of standard NCS variables. Besides the parameters of the CMAP scan, also the overall shape of the CMAP scan reproduces well. Even after a period of one-and-a-half years, the CMAP scan can look very similar in a healthy subject, when performed in a standardised way (figure 2). Also, the variation of the CMAP scan parameters between different-day recordings is small, especially compared to the changes of CMAP scan parameters that can be observed when serial CMAP scans are performed in patients with peripheral nerve diseases, such as GBS (see chapters 3.1 - 3.3).

Although a good reproducibility in healthy subjects does not necessarily imply that the reproducibility is also good in patients, we believe that our current findings also apply to pathological conditions as well. In healthy subjects, recording-related variability in SI variables mainly depends on the distance between the stimulator and the nerve and changes in tissue impedance. It is not likely that these factors are greatly
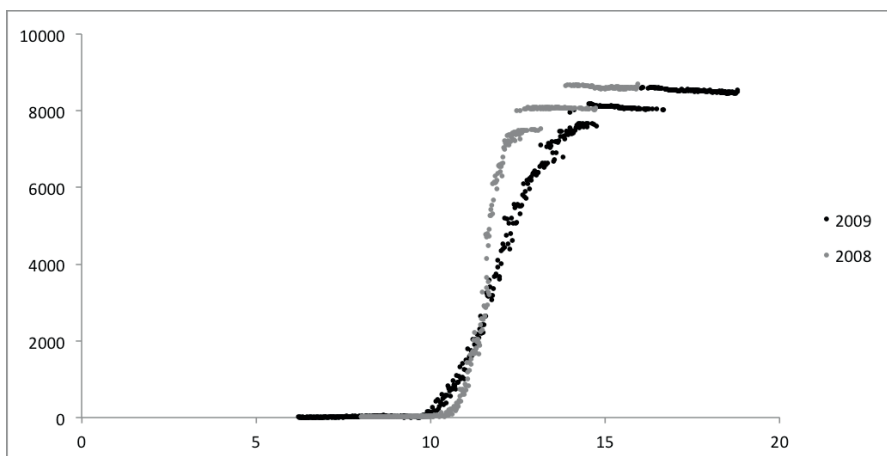

Figure 2. Two CMAP scans of the median nerve of J. Drenthen, performed as part of another study. The grey CMAP scan is made in March 2008, the black CMAP scan is made in October 2009. Notice the similarity of the overall shape.

influenced by the disease or its treatment. This line of thought is also supported by the fact that repeated recordings in stable GBS patients show a similar variability compared to healthy subjects. It should be highlighted, that this good reproducibility requires optimal placement of the recording and stimulation electrodes. This requires some training and expertise of the investigator.

In addition to being reproducible, a clinically useful monitoring technique should also be sensitive to detect small but significant changes. At present there are limited clinical features or biomarkers available that predict disease progression.[52] Although not validated in large groups of patients, Chapter 3.3 demonstrates that using the CMAP scan, it appears feasible to monitor nerve function at bedside. When performing serial CMAP scans, all patients showed abnormalities at their baseline scan. Clinical improvement or deterioration was often paralleled by progression or deterioration of the CMAP scan. In 2 patients these CMAP scan changes even preceded the clinical changes. For example, Chapter 3.3 describes a patient with an A-CIDP in whom the CMAP scan worsened 7 days before he clinically deteriorated. The deterioration might have been milder if subsequent treatment had started earlier, because the general opinion is that treatment should start as soon as possible to prevent irreversible (axonal) nerve damage.[53] Off course, the numbers are too small to make definite conclusions, however the CMAP scan shows promise of being an easy and reliable monitoring technique.

Monitoring during the acute phase of the disease, when various pathological processes are active, will possibly also give more insight in the ongoing pathological changes and the effect of treatment. For instance, in Chapter 3.3 we showed that the CMAP amplitude can increase dramatically within a few days, or even within a day. Such a very rapid increase of amplitude cannot be attributed to either reinnervation or remyelination because those mechanisms require more time. Other underlying processes, such as (transient) sodium channel blocking, must play a role, leading to reversible conduction failure.

Likewise, in Chapter 3.1 we showed that even in MFS patients without clinical signs of weakness in the extremities, the motor nerves had an abnormal excitability. Although these excitability changes were not as outspoken as the changes in AIDP patients, it does suggest that involvement of various peripheral nerves in MFS is more widespread than previously thought. Whether further decrease of the excitability predicts the development of a MFS-GBS overlap syndrome, would be very interesting, but needs to be studied in a larger cohort. However, this finding creates a new opportunity for in vivo examination of the nerves. This is relevant because in MFS conventional NCS hardly show any nerve conduction abnormalities besides low sensory action potential amplitudes and absent H-reflexes,[8] and the ocular nerves, which are clinically the most

affected nerves, are not accessible for NCS. Furthermore, there are no early clinical predictors for progression from MFS to MFS-GBS overlap syndrome.[52]

Key points

> - CMAP scan is highly reproducible in healthy subjects, when performed in a standardized way.
> - The CMAP scan can be useful to monitor disease activity and to predict the clinical course of disease, which is especially helpful when the possibilities for adequate neurological examination are limited.

5.3. Residual damage

5.3.1 Is there a physiological basis for long-term fatigue?

GBS is considered to be an acute and monophasic disease, yet many former patients report considerable residual effects for the rest of their lives. Severe fatigue is a serious consequence of GBS, that frequently has a major impact on their quality of life, even after an apparent full recovery from weakness and sensory deficits. As yet, however, the patho-

physiological mechanism underlying fatigue after GBS has not been elucidated.
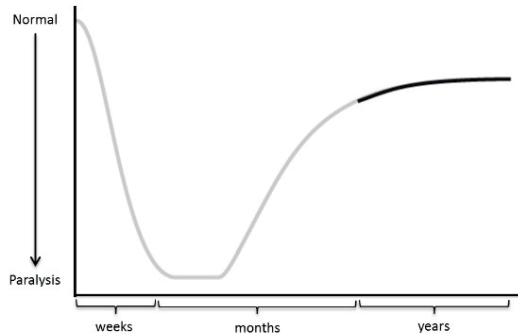
Previous research has shown that severe fatigue after GBS is not related to clinical recovery of muscle strength nor to antecedent infections [54, 55]. It is also not correlated with age, sex, or clinical scores that reflect residual deficits or disability [55]. The mechanism of fatigue in GBS and in neuromuscular diseases in general has not been clarified but it has been suggested that both neurological and psychological factors may play a role.[56,57]

Chapter 4.1 provides the first direct evidence that - apart from any psychological factors - there is also a physiological basis for long-term fatigue. We have shown that severely fatigued GBS patients have, on average, lower MUNEs than non-severely fatigued GBS patients, indicating pronounced axonal loss. Strikingly, the maximum CMAP amplitudes did not differ between the severely and non-severely fatigued patients. This demonstrates (again) that conventional NCS are not sensitive enough to detect motor axonal loss when reinnervation has occurred (resulting in preservation or recovery of the CMAP amplitude). A reduced MUNE in combination with a normal CMAP amplitude

implies that the remaining MUs are larger. (Figure 3) This finding is consistent with a previous study of MU size in GBS patients [58].

The question remains how axonal loss may lead to fatigue. Generally, upon voluntary activation of a muscle, MUs are recruited in order of smallest to largest as contraction increases. This is known as Henneman's Size Principle [59]. The small MUs allow for precise and fine movements, while the larger MUs are used if more force is required. It can be hypothesized that this orderly recruitment mechanism has become disturbed in severely fatigued GBS patients, because reinnervation made previously small MUs large. Early recruitment of these large MUs may then easily result in an overshoot of force. It is conceivable that this overshoot either directly or through compensatory processes results in reduced control of muscle activity and increased fatigue.

The axonal loss in severely fatigued patients is also reflected in lower sensory amplitudes. Normal physical activity relies on the integrity of motor and sensory systems. The level of physical activity is constantly regulated by feedback mechanisms. In healthy subjects, physiological fatigue is an important feedback mechanism to adjust this physical activity. Nerve impulses in the sensory systems regulate this feedback mechanism. It can be hypothesized that in patients with severe fatigue, the sense of normal fatigue is enlarged due to pathological changes in these feedback mechanisms, such as a dis-
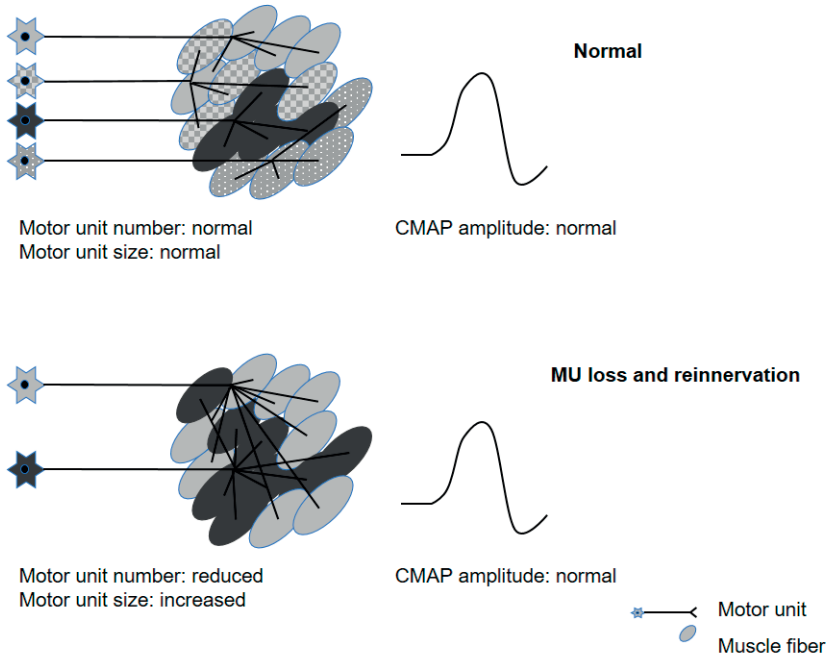

Normal

Motor unit number: normal
Motor unit size: normal

CMAP amplitude: normal

MU loss and reinnervation

Motor unit number: reduced
Motor unit size: increased

CMAP amplitude: normal

Motor unit
Muscle fiber

Figure 3. Schematic view of reinnervation. When motor unit loss occurs, reinnervation results in an increase in motor unit size of the remaining motor units. Conventional NCS are insensitive to this process; the remaining CMAP amplitude can remain normal

turbed sensory system. This could affect the level of physical activity and, hence, result in fatigue [56, 60].

To support our findings and hypotheses, the recruitment order of MUs in severely and non-severely fatigued patients should be investigated. This can be done by recording MUPs using a high-density surface EMG grid (to facilitate detection of various MUs) during light contraction of the muscle, ideally in combination with twitch force measurements of single motor units.[61] Furthermore, since our patient group was relatively small, our findings should be confirmed in larger, longitudinal studies in GBS patients as well as in patients with other peripheral neuropathies such as CIDP. In this thesis, only a small facet of the possibly involved components contributing to fatigue was studied.

5.3.2 Is nerve physiology altered after childhood Guillain-Barré syndrome?

GBS may affect persons of all ages, but GBS in children is considered to have a better prognosis than in adults. Nevertheless, approximately 20% of patients who had GBS during childhood have some degree of residual complaints [62], including fatigue [63]. Information about nerve function after GBS and possible alterations of this nerve function is lacking.

In Chapter 4.2, residual nerve dysfunction, determined by CMAP scan, after childhood GBS is described. The CMAP scan is easy to perform, even in small children (the youngest patient in this study was 4 years old) and is well tolerated by children. In a cohort of patients who suffered from GBS during childhood, we found that almost 70% had one or more abnormalities in the ulnar nerve CMAP scan performed 1-22 years after the onset of GBS. Even in most patients with full clinical recovery and no residual complaints, the CMAP scan showed abnormalities in the SI variables when compared to healthy age-matched controls. The abnormalities were mainly found in the SI range and S100, indicating reduced nerve excitability in a subset of axons (the S0 was less affected). These results help to further understand the physiology of nerve regeneration after GBS. Although the clinical consequences of these persisting nerve abnormalities are not known, it might be that the nerves remain more vulnerable for all kinds of other peripheral nerve disorders or GBS recurrences. Several studies report a tendency for neurological deficits to accumulate with increasing frequency of GBS recurrences [64,65].

Key points

> - The CMAP scan is quick and easy to perform and well-tolerated in children.
> - CMAP scanning demonstrates abnormal residual nerve function after childhood GBS implying that nerve physiology remains altered in a subset of patients.
> - Severe residual fatigue after GBS is associated with signs of axonal loss as demonstrated by MUNE.

5.4. Future perspectives

When we started the studies described in this thesis, the CMAP scan was still in its infancy and had to be further developed. Since then expertise has been gained, and it has proven to be a valuable addition to conventional NCS, not only in demyelinating neuropathies, but also in diseases of the motor nerves such as ALS[3, 66, 67,69]. However, work is still in progress. The CMAP scan was not commercially available until 2017, so not many researchers and clinicians were able to familiarize themselves with this electro-physiological tool. In the most recent version (2017) of The Viking and Synergy NCS EMG systems (Natus Medical Incorporated, California), the CMAP scan software application is incorporated and hence made available for centers worldwide. This standard feature will likely stimulate its use in clinical practice and in research in a wide range of diseases. Therefore, the diagnostic value of the CMAP scan will likely become more clear in the near future.

In addition to sheer availability, incorporation of the CMAP scan in routine clinical practice will probably also require that the underlying pathophysiological processes that lead to the changes in various CMAP scan parameters, are further elucidated. Future studies need to address not only how alterations in the CMAP scan arise, but more importantly what mechanisms may cause these abnormalities. It can be speculated that alterations in membrane properties, disruption of nodal sodium channels or internodal potassium channels, might play an important role, but so far we have no clear answers yet.

From a patient perspective, a major question remaining is how all these findings benefit the individual patient. Although currently all patients receive similar treatment, a better understanding of how the pathophysiological processes in each individual patient develop may in time open doors for a more individualized treatment when available. Since the CMAP scan seems to have the capability to detect early alterations in nerve excitability, it may become a quick and objective biomarker for disease progression. The CMAP scan can also play a role in subtype differentiation, either into differentiation between the current subtype paradigms (AMAN and AIDP), or perhaps in helping to develop a new subtype classification that actually better represents the differences in pathophysiology, prognosis and response therapy between patients.

I speculate that in the future we will no longer differentiate into the AIDP and AMAN subtype. I expect this terminology will change, since it does not reflect the underlying pathophysiology in the light of current knowledge. The findings on conventional NCS that we now call demyelinating (the "D" in AIDP) do not necessarily reflect an underlying demyelinating disease process. The term seems to originate from studies conducted in hereditary demyelinating diseases in which there is a profound and diffuse slowing of the nerve conduction due to defects of the myelin sheath. However, no systematic studies in human nerves combining pathology and nerve conduction have been performed

to see whether other pathological processes (such as AIDP) do or do not induce similar findings on NCS.

In future studies other parameters should be used to identify the various subgroups that differ regarding pathophysiology, prognosis, and possibly also response to various therapies. The latter will make proper subgroup differentiation essential. The way forward is to develop a large database with a wide variety of different GBS cases. Clinical, electrophysiological, serological, genetic and perhaps even nerve ultrasound parameters should be collected. By means of cluster analysis various subgroups may then be found, which possibly reflect differences in pathophysiology and prognosis. Such studies are already well on their way. The International GBS Outcome Study (IGOS) is a large international database study that collects various parameters at standardized time points in a standardized manner[68] and is well suited to perform 'big data' analysis.

It may well be that in the near future we will no longer speak of AIDP and AMAN. Although the terminology might change, the results in this thesis and the usefulness of the CMAP scan at the various phases of Guillain-Barré syndrome will still hold, since it will be the terminology that changes and not the underlying pathophysiology itself.

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6

Summary/samenvatting

Summery
Samenvatting

6.1 Summary

Guillain-Barré syndrome (GBS) is a heterogeneous disorder regarding clinical manifestations, neurophysiology, treatment response and prognosis. This variability within GBS is only partly understood and may restrict accurate early diagnosis, subtyping, monitoring and prediction of outcome in individual patients with GBS. Currently, conventional nerve conduction studies (NCS) are used for confirming the diagnosis and to determine the subtype. However, several drawbacks of the conventional NCS ensure that there is a need for alternative and more sensitive methods.

In this thesis, studies are presented that aimed to determine if both standard and more advanced electrophysiological techniques can be used in the discrimination between subtypes in an early phase, to monitor the disease activity, to predict the clinical course and to define residual damage. The compound muscle action potential (CMAP) scan, an alternative neurophysiological method, is an important part of this thesis. In several studies the relation between changes of the CMAP scan and clinical parameters, such as disease course and subtype are investigated and discussed.

Chapter 1 is the general introduction. **Chapter 1.1** provides background information about the clinical aspects of GBS, including clinical presentation, disease course, diagnosis, subtypes, pathogenesis, treatment and prognosis. In **chapter 1.2** various neurophysiological techniques that are used to assess the peripheral nerves are discussed. The drawbacks of the conventional NCS are pointed out, and more advanced neurophysiological techniques such as the CMAP scan and motor unit number estimation (MUNE) with high density surface EMG (HDsEMG) are introduced. In **chapter 1.3** the objectives of this thesis are presented. These objectives were:

1. to examine whether the CMAP scan can be of additional value in diagnosing, subtyping, and monitoring patients with GBS, and
2. to determine whether peripheral nerve physiology normalizes in patients recovered from GBS, and whether any remaining abnormalities are related to long-term clinical outcome, such as weakness or fatigue.
3. to study whether a preceding *C. jejuni* infection can also induce a demyelinating form of GBS.

In order to use the CMAP scan as a potential biomarker, the interobserver and different-day reproducibility should be adequate. In **chapter 2** the CMAP scan is further introduced and the interobserver reproducibility and test-retest variability was assessed. CMAP scans of ten healthy subjects were recorded by 2 different investigators, on 2 different days, resulting in a set of 40 CMAP scans. There was a small, but consistent difference in stimulus intensity parameters between the two investigators. Yet, the intraclass correlation coefficients for the interobserver reproducibility was >0.80 for all parameters and the coefficients of variation <15%, indicating a good reproducibility.

Besides the parameters, also the overall shapes of the CMAP scans reproduce well. This study showed that with two well-trained investigators, the interobserver reproducibility and the different-day reproducibility of various CMAP scan variables were good.

In the next chapters, chapters 3 and 4, the articles are structured according to the disease phase for which they are relevant. Chapter 3 concerns the acute phase GBS and Chapter 4 the late phase of GBS. In **chapter 3.1** motor nerve excitability changes during the acute phase and subsequent recovery in patients with a classical Miller Fisher syndrome (MFS) are described. Typically, MFS patients present with ophthalmoplegia, ataxia, and areflexia but have normal limb muscle strength and normal motor NCS of the upper and lower limbs. In chapter 3.1 serial CMAP scans of three patients with MFS are analyzed. The CMAP scans of all patients showed an abnormal increase in the stimulus intensity parameters at the day of hospital admission, indicating reduced motor nerve excitability already at the earliest stage of disease. Median nerve dysfunction progressed in parallel to clinical deterioration, and improved with clinical recovery. The CMAP scan appears to be a useful tool in clinical practice to monitoring (subclinical) disease activity.

This finding let to the investigation whether CMAP scans are also abnormal in the acute phase of GBS and if they differ between the various subtypes. In **chapter 3.2** early changes in motor nerve excitability by CMAP scan in GBS patients with different subtypes are described. Currently, differentiation the into acute motor axonal neuropathy (AMAN) subtype and acute inflammatory demyelinating polyneuropathy (AIDP) subtype is based on findings on NCS. However, NCS are often not reliable within the first 2 weeks after disease onset, due to reversible conduction abnormalities that can also be seen in presumably axonal patients. The axonal subtype is rare in western countries, while it is the most common subtype in Asian countries, such as Bangladesh. To be able to study both AMAN and AIDP subtypes, 22 patients from Bangladesh and 19 patients from The Netherlands were included. Fifteen patients (37%) were classified as AIDP, 19 as AMAN, and 7 as equivocal. In all patients, CMAP scans were performed within 2 days after hospital admission. Of these 41 patients, 38 showed abnormalities in this early CMAP scan. The CMAP scans performed at hospital admission already showed a highly discriminative difference in SI variables between AIDP and AMAN patients. Linear discriminant analysis identified the maximum CMAP amplitude and absolute SI-range as the strongest independent predictors for identification of the different subgroups. The CMAP scan may surpass the difficulties with subtype differentiation based on NCS abnormalities.

In **chapter 3.3** serial CMAP scans of 14 GBS patients are described. These patients had their first CMAP scan within 5 days after hospital admission, after which CMAP scans were performed at least weekly in the acute phase and at least 2 times during follow up. A total of 116 CMAP scans were performed (range 5-21 CMAP scans per patient). All patients had one or more abnormalities in their first CMAP scan, mainly in the SI

parameters. During follow up, all but one patient improved both clinically as well as with the CMAP scan. Patients with CMAP scans that improved within one week generally had a shorter time to reach a GBS disability score ≤ 3 (able to walk 10 meters), than patients with CMAP scans that improved later than one week or patients who had fluctuations in their CMAP scan. In 4 patients the last CMAP scan at follow-up completely normalized. The other patients still had 1 or more abnormalities in their last CMAP scan, either in SI parameters or in step percentage. The latter indicating that reinnervation had occurred. The patient that progressed during follow up turned out to have an acute chronic inflammatory demyelinating polyneuropathy (ACIDP). In most patients, deterioration and improvement of the CMAP scan paralleled clinical deterioration and improvement. In one patient the CMAP scan improved one week prior to the clinical improvement and in one patient (the ACIDP patient) deterioration of the CMAP scan was visible one week prior to the clinical deterioration. These two cases are described in more detail in this chapter. Although the number of patients in this study is low, the findings suggest that the CMAP scan has a potential to be a quick and objective biomarker to monitor disease in patients with GBS. Further prospective studies in a larger cohort of patients with GBS are required to evaluate if the CMAP scan is an accurate predictor of clinical progression and recovery in individual patients.

In GBS the diversity in electrophysiological subtypes is unexplained. Various studies indicate that preceding infections may influence the electrophysiological subtype of GBS. It is proposed that in Japan infections with *Campylobacter jejuni* are exclusively related to AMAN. The high incidence of *C. jejuni* infections in GBS patients in Japan would explain why axonal variants predominate there. However, in Western regions *C. jejuni* is also the predominant type of infection preceding GBS, yet AMAN in western countries is rare. In **chapter 3.4** we defined what subtypes of GBS may occur after *C. jejuni* infection in the Netherlands using a cohort of 147 GBS patients who participated in a randomised trial comparing the therapeutic effect of intravenous immunoglobulins (IVIg) versus plasmapheresis. Recent infection with *C. jejuni* was defined using very strict clinical and serological criteria, to exclude methodological bias. Three (18%) of 17 GBS patients with a *C. jejuni* fulfilled the electrophysiological criteria for AIDP. Furthermore, in another cohort, *C. jejuni* was cultured from the stools of two patients with a typical demyelinating form of GBS. These findings demonstrate that at least in Dutch patients, *C. jejuni* infections do not exclusively elicit the AMAN variant of GBS.

Despite good clinical recovery after GBS, approximately 80% of patients suffer from persistent severe fatigue, with high impact on quality of life. Little is known about the origin of this fatigue. In **chapter 4.1** thirty-nine former GBS patients are studied. These 39 patients were divided into two groups: patients who were severely fatigued and patients who were not. Fifteen of the 39 former GBS patients were considered severely fatigued based on their fatigue severity scale (FSS) scores. The two groups did not dif-

fer with respect to age, sex or clinical scores that reflect residual deficits or disability. However, the severely fatigued GBS patients had, on average, lower MUNEs than non-severely fatigued GBS patients. Also, they had lower sensory nerve action potentials. This indicates that residual axonal loss is present in a substantial proportion of patients who recovered well clinically from a previous episode of GBS and that it is associated with severe fatigue in these patients.

In **chapter 4.2** residual changes in motor nerve excitability after childhood Guillain-Barré syndrome are described. Thirty-seven persons who suffered from GBS during childhood were included in the study. Various clinical parameters were collected and CMAP scans were performed in all patients. The CMAP scan examination was well tolerated and feasible, also in children. Almost 70% of the patients had one or more abnormalities in the CMAP scan of the ulnar nerve, mainly in the SI parameters. Even in most patients with a full clinical recovery and no residual complaints, the CMAP scan showed changes of the normal peripheral nerve physiology. These findings indicate that the peripheral motor nerve function is permanently changed after GBS, irrespective of the clinical recovery.

Finally, in **chapter 5** the results of the various studies in this thesis are discussed.

6.2 Samenvatting

Het Guillain-Barré Syndroom (GBS) is een heterogene aandoening met betrekking tot klinische verschijnselen, neurofysiologie, behandel respons en prognose. Deze variabiliteit binnen GBS wordt slechts gedeeltelijk begrepen en kan het diagnostiseren, subtyperen, monitoren en voorspellen van de uitkomst bij individuele patiënten met GBS bemoeilijken. Momenteel wordt conventioneel zenuwgeleidingsonderzoek gebruikt om de diagnose te bevestigen en het subtype te bepalen. Verschillende nadelen van het conventionele zenuwgeleidingsonderzoek zorgen er echter voor dat er behoefte is aan alternatieve en gevoeligere methoden.

In dit proefschrift worden studies gepresenteerd die als doel hadden om te bepalen of zowel standaard als meer geavanceerde neurofysiologische technieken kunnen worden gebruikt om in een vroeg stadium een onderverdeling te maken tussen de verschillende subtypen, om de ziekteactiviteit te monitoren, om het klinische verloop te voorspellen en om restschade te definiëren. De compound muscle action potential (CMAP) scan, een alternatieve neurofysiologische methode, is een belangrijk onderdeel van dit proefschrift. In verschillende studies wordt de relatie tussen veranderingen van de CMAP scan en klinische parameters, zoals ziekteverloop en subtype, onderzocht en besproken.

Hoofdstuk 1 is de algemene inleiding. In **hoofdstuk 1.1** wordt achtergrondinformatie over de klinische aspecten van GBS, waaronder klinische presentatie, ziekteverloop, diagnose, subtypen, pathogenese, behandeling en prognose beschreven. In **hoofdstuk 1.2** worden verschillende neurofysiologische technieken besproken die worden gebruikt om eigenschappen van de perifere zenuwen te meten. De nadelen van het conventionele zenuwgeleidingsonderzoek worden besproken en meer geavanceerde neurofysiologische technieken zoals de CMAP scan en een methode om het aantal functionele motor units (Motor Unit Number Estimation (MUNE)) met behulp van multikanaals oppervlakte EMG (HDsEMG) worden geïntroduceerd. In **hoofdstuk 1.3** worden de doelstellingen van dit proefschrift gepresenteerd. Deze doelstellingen waren:

1. te onderzoeken of de CMAP scan van toegevoegde waarde kan zijn bij het diagnosticeren, subtyperen en monitoren van patiënten met GBS, en

2. om te bepalen of de perifere zenuwfysiologie normaliseert bij patiënten die hersteld zijn van GBS, en of eventuele resterende afwijkingen een relatie hebben met klinische symptomen, zoals zwakte of vermoeidheid.

3. te onderzoeken of een voorafgaande infectie met *C. jejuni* een demyeliniserend subtype van GBS kan veroorzaken.

Om de CMAP scan als potentiële biomarker te gebruiken, moet zowel de interobserver reproduceerbaarheid als de test-hertest betrouwbaarheid goed zijn. In **hoofdstuk 2** wordt de CMAP scan verder geïntroduceerd en wordt de interobserver reproduceer-

baarheid en test-hertest betrouwbaarheid onderzocht. CMAP scans van tien gezonde proefpersonen werden gemeten door 2 verschillende onderzoekers, op 2 verschillende dagen, wat resulteerde in een set van 40 CMAP scans. Er was een klein, maar consistent verschil in stimulus intensiteit parameters tussen de twee onderzoekers. Desondanks bleken de intraclass correlatiecoëfficiënten voor de interobserver reproduceerbaarheid boven 0.80 voor alle parameters en de variatiecoëfficiënten kleiner dan 15%. Dit wijst op een goede reproduceerbaarheid. Naast de verschillende parameters, was ook de vorm van de CMAP scans goed reproduceerbaar. Deze studie toonde aan dat bij twee goed getrainde onderzoekers de interobserver reproduceerbaarheid en test-hertest betrouwbaarheid van verschillende de CMAP scan variabelen goed waren.

In de volgende hoofdstukken, hoofdstukken 3 en 4, zijn de artikelen gerangschikt naar de ziektefase waarvoor ze relevant zijn. Hoofdstuk 3 heeft betrekking op de vroege fase van het ziektebeeld en hoofdstuk 4 op de latere fase. In **hoofdstuk 3.1** worden veranderingen in de prikkelbaarheid van de motore zenuwen tijdens de acute fase en het daarop volgende herstel beschreven bij patiënten met een klassiek Miller Fisher Syndroom (MFS). De trias aan symptomen van patiënten met een klassiek MFS zijn een ophthalmoplegie, ataxie en areflexie. MFS patiënten hebben normaal gesproken een normale spierkracht van de ledematen en een normaal motorisch zenuwgeleidingsonderzoek van de bovenste en onderste ledematen. In hoofdstuk 3.1 worden seriële CMAP scans van drie MFS patiënten geanalyseerd. De CMAP scans van deze patiënten vertoonde afwijkende stimulus intensiteits parameters op de dag van ziekenhuisopname, wat wijst op verminderde prikkelbaarheid van de motorische zenuw, al in een vroeg stadium van de ziekte. Verslechtering van de exciteerbaarheid van de nervus medianus trad op parallel aan de klinische verslechtering en de exciteerbaarheid verbeterde weer met klinisch herstel. De CMAP scan lijkt een methode te kunnen zijn om in de klinische praktijk (subklinische) ziekteactiviteit te monitoren. Deze bevinding gaf aanleiding om te onderzoeken of CMAP scans ook abnormaal zijn in de acute fase van GBS en of CMAP scans verschillen tussen de verschillende subtypen. In **hoofdstuk 3.2** worden vroege veranderingen in de prikkelbaarheid van de motorische zenuw, gemeten middels de CMAP scan, bij GBS patiënten met verschillende subtypes beschreven. Momenteel wordt het onderscheid tussen het axonale subtype (acute motor axonal neuropathy (AMAN)) en het demyeliniserende subtype (acute inflammatory demyelinating polyneuropathy (AIDP)) gebaseerd op bevindingen bij het zenuwgeleidingsonderzoek. Het zenuwgeleidingsonderzoek is echter soms niet betrouwbaar binnen de eerste 2 weken na het begin van de ziekte, onder andere vanwege reversibele stoornissen in de zenuwgeleiding, die ook voor kunnen komen bij patiënten die vermoedelijk het axonale subtype hebben. Het axonale subtype is zeldzaam in westerse landen, terwijl dit het meest voorkomende subtype is in Aziatische landen, zoals Bangladesh. Om genoeg patiënten met verschillende subtypes te kunnen onderzoeken, werden zowel patiënten uit Nederland als

uit Bangladesh geïncludeerd. Tweeëntwintig patiënten kwamen uit Bangladesh en 19 patiënten uit Nederland. Vijftien patiënten (37%) werden geclassificeerd als AIDP, 19 als AMAN en 7 als 'equivocal' (niet classificeerbaar). Bij alle patiënten werden er binnen 2 dagen na ziekenhuis opname CMAP scans verricht. Van deze 41 patiënten hadden er 38 vroege afwijkingen in deze vroege CMAP scan. Deze vroege CMAP scans toonden een duidelijk verschil in stimulus intensiteits variabelen tussen de AIDP en AMAN patiënten. Lineaire discriminantanalyse identificeerde de maximale CMAP amplitude en de absolute stimulus intensiteits range als de sterkste onafhankelijke voorspellers om de verschillende subgroepen te onderscheiden. De CMAP scan lijkt hiermee de problemen met subtype differentiatie op basis van bevindingen bij zenuwgeleidingsonderzoek te kunnen omzeilen.

In **hoofdstuk 3.3** worden seriële CMAP scans van 14 GBS-patiënten beschreven. Alle patiënten hadden hun eerste CMAP scan binnen 5 dagen na ziekenhuisopname, waarna er tenminste wekelijks in de acute fase en tenminste 2 keer tijdens de follow-up CMAP scans werden verricht. In totaal werden 116 CMAP scans verricht (5-21 CMAP scans per patiënt). Alle patiënten hadden één of meer afwijkingen in hun eerste CMAP scan, voornamelijk in de stimulus intensiteits parameters. Gedurende follow up toonden 13 van de 14 patiënten een verbetering, zowel klinisch als gemeten met de CMAP scan. Patiënten waarvan de CMAP scans binnen 1 week verbeterden, hadden over het algemeen een kortere tijd om een GBS disability score ≤ 3 te bereiken (in staat om 10 meter te lopen), dan patiënten met CMAP scans die later dan 1 week verbeterden of patiënten die variaties vertoonden in hun CMAP scan. Bij 4 patiënten was de laatste CMAP scan bij follow-up volledig genormaliseerd. De andere patiënten hadden nog één of meer afwijkingen in hun laatste CMAP scan, hetzij in de stimulus intensiteits parameters of in het percentage 'steps' in de CMAP scan. Dit laatste kan een teken zijn van reïnnervatie. De patiënt die tijdens de follow-up verslechterde, bleek een acute chronische inflammatoire demyeliniserende polyneuropathie (ACIDP) te hebben. Bij de meeste patiënten liepen de veranderingen in de CMAP scan, verslechtering en verbetering, parallel aan de klinische veranderingen. Echter, bij één patiënt verbeterde de CMAP scan één week voordat er een klinische verbetering zichtbaar was, en bij één patiënt (de ACIDP-patiënt) verslechterde de CMAP-scan één week voordat er een klinische verslechtering zichtbaar was. Deze twee casus worden in dit hoofdstuk in meer detail beschreven. Alhoewel het aantal patiënten in deze studie klein is, suggereren de bevindingen dat de CMAP scan een snelle en objectieve biomarker kan zijn om het ziektebeloop bij patiënten met GBS te monitoren. Verdere prospectieve studies bij een groter cohort met GBS patiënten zijn nodig om te evalueren of de CMAP-scan een nauwkeurige voorspeller is van klinische progressie en herstel bij individuele patiënten.

Het is niet duidelijk waarom de ene patiënt met GBS het axonale, en de andere patiënt met GBS het demyeliniserende subtype ontwikkelt. Uit verschillende studies

blijkt dat voorafgaande infecties het elektrofysiologische subtype kunnen beïnvloeden. Er wordt zelfs geopperd dat in Japan infecties met *Campylobacter jejuni* uitsluitend het axonale subtype veroorzaken. De hoge incidentie van *C. jejuni* infecties bij GBS patiënten in Japan zou verklaren waarom axonale varianten daar overheersen. In westerse landen is *C. jejuni* echter ook de meest voorkomende infectie voorafgaand aan GBS, echter AMAN in westerse landen is zeldzaam. In **hoofdstuk 3.4** hebben we onderzocht welke elektrofysiologische subtypen kunnen optreden na een *C. jejuni* infectie in Nederland. Hiervoor werd gebruik gemaakt van een cohort van 147 GBS-patiënten die deelnamen aan een gerandomiseerde studie waarin het therapeutische effect van intraveneuze immunoglobulinen (IVIg) versus plasmaferese werd vergeleken. Een recente infectie met *C. jejuni* werd gedefinieerd volgens zeer strikte klinische en serologische criteria om methodologische bias uit te sluiten. Drie (18%) van de 17 GBS patiënten met een voorafgaande *C. jejuni* infectie voldeed aan de elektrofysiologische criteria voor een AIDP. Bovendien werd, in een ander cohort, *C. jejuni* gekweekt uit de ontlasting van twee patiënten met een typische demyeliniserende vorm van GBS. Deze bevindingen tonen aan dat *C. jejuni* infecties in ieder geval bij Nederlandse patiënten niet uitsluitend de axonale variant van GBS veroorzaken.

Ondanks goed klinisch herstel na GBS, blijft ongeveer 80% van de patiënten last houden van ernstige vermoeidheid. Dit heeft een hoge impact op de kwaliteit van leven. Er is weinig bekend over de oorzaak van deze vermoeidheid. In **hoofdstuk 4.1** werden 39 patiënten onderzocht die een aantal jaar eerder GBS hebben doorgemaakt. Deze 39 patiënten werden verdeeld in twee groepen: patiënten die ernstig vermoeid waren en patiënten die dat niet waren. Vijftien van deze 39 GBS patiënten werden als ernstig vermoeid beschouwd op basis van hun scores op de Fatigue Severity Scale (FSS). De twee groepen verschilden niet wat betreft leeftijd, geslacht of klinische scores die resterende uitval of handicaps weerspiegelen. De ernstig vermoeide patiënten hadden echter gemiddeld een lagere MUNE dan de niet ernstig vermoeide patiënten. Tevens hadden zij lagere sensibele zenuw actiepotentialen. Dit betekent dat er bij een aanzienlijk deel van de patiënten, die klinisch goed herstelden na GBS, axonale schade aanwezig is, en dat dit axonaal verlies geassocieerd is met ernstige vermoeidheid bij deze patiënten.

In **hoofdstuk 4.2** worden persisterende veranderingen in de prikkelbaarheid van de motorische zenuwen beschreven bij patiënten die GBS tijdens de kinderleeftijd hebben doorgemaakt. Zevenendertig personen die tijdens hun kindertijd GBS hebben doorgemaakt, werden geïncludeerd. Verschillende klinische parameters werden verzameld en CMAP scans van de nervus ulnaris werden verricht in alle patiënten. Het CMAP scan onderzoek werd goed verdragen, ook door kinderen. Bijna 70% van de patiënten had één of meer afwijkingen in de CMAP scan, voornamelijk in de stimulus intensiteit parameters. Zelfs bij patiënten met een volledig klinisch herstel en zonder restklachten vertoonde de CMAP scan afwijkingen. Deze bevindingen wijzen erop dat bij een groot

deel van de patiënten de perifere motorische zenuwfunctie blijvend veranderd is na GBS, ongeacht het klinische herstel.

Tot slot worden in **hoofdstuk 5** de resultaten van de verschillende studies in dit proefschrift bediscussieerd.

A

Appendices

List of abbreviations

ACIDP	Acute chronic inflammatory demyelinating polyneuropathy
AIDP	Acute inflammatory demyelinating polyneuropathy
ALS	Amyotrophic lateral sclerosis
AMAN	Acute motor axonal neuropathy
AMSAN	Acute motor sensory axonal neuropathy
C. jejuni	*Campylobacter jejuni*
CIDP	Chronic inflammatory demyelinating polyneuropathy
CMAP	Compound Muscle Action Potential
CMV	Cytomegalovirus
CoV	Coefficient of variation
CSF	Cerebro Spinal Fluid
CTS	Carpal tunnel syndrome
dCMAP	Distal Compound Muscle Action Potential
DML	Distal motor latency
EBV	Epstein-Barr virus
EGOS	Erasmus GBS Outcome score
EMG	Electromyography
FSS	Fatigue Severity Scale
GBS	Guillain-Barré syndrome
HDsEMG	High density surface EMG
ICC	Intraclass correlation coefficient
IGOS	International GBS Outcome Study
IQR	Interquartile range
IVIg	Intravenous Immunoglobulins
LLN	Lower limit of normal
MFS	Miller Fisher syndrome
mNCV	Motor nerve conduction velocity
MPS	Multiple Point Stimulation
MRC	Medical Research Council
MU	Motor unit
MUP	Motor unit potential
MUNE	Motor unit number estimation
NaV	Voltage-gated sodium (channel)
NCS	Nerve conduction study
ODSS	Overall disability sum score
pCMAP	Proximal compound Muscle Action Potential
S0	SI that elicits the lowest-threshold MU
S50	SI at which the 50% of the maximum CMAP is elicited

S100	SI at which the maximum CMAP could be recorded
SI	Stimulus intensity
SI-range	Stimulus intensity range
SNAP	Sensory nerve action potential
sNCV	Sensory nerve conduction velocity
Step%	Step percentage
ULN	Upper limit of normal

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About the Author

Judith Drenthen was born on August 17th 1978 in Delft, The Netherlands. After finishing high school (Maerlant Lyceum, Den Haag and Preadinius gymnasium, Groningen) she started her training in medicine in 1996 at the University of Antwerp. In 1998 she continued the rest of her training at the Erasmus University, Rotterdam, where she obtained her medical degree in 2004. During her study she conducted research at the department of clinical neurophysiology, Erasmus MC and the Diving Medical Center, Royal Netherlands Navy Den Helder, about the effects of cerebral air embolism and subsequent hyperbaric oxygen therapy on brain metabolism and EEG monitoring. From 2004 to 2012 Judith was a resident neurology at Erasmus MC, Rotterdam (head Prof. dr. P.A.E. Sillevis Smitt). In 2007 she started her research underlying this thesis at the departments of neurology and clinical neurophysiology, Erasmus MC under supervision of Dr J.H. Blok, Dr. G.H. Visser, Prof. Dr B.C. Jacobs, and Prof. Dr. P.A. van Doorn. During this project she spent time in Dhaka, Bangladesh, where she collaborated with scientists and neurologists at the ICDDR,b to set up a research line on nerve conduction studies in GBS. Currently, Judith is working as a neurologist and clinical neurophysiologist at the Erasmus MC, Rotterdam. She lives with her partner Ruud and their daughter Anne-Sophie.

Portfolio

Description	Organizer	EC

Required

Description	Organizer	EC
Matlab Course (2007)		1.00
Erasmus MC - CC02 Biostatistical Methods I: Basic Principles (2008)		5.70
Erasmus MC - ESP01 Principles of Research in Medicine and Epidemiology (2008)		0.70
Erasmus MC - ESP03 Introduction to Data-analysis (2008)		1.00
Summer Programme (2008)		0.00
Nerve excitability Workshop (2008)	UCL institute of Neurology	3.00
SFEMG course (2009)		3.00
Erasmus MC - Biomedical Scientific English Writing (short) (2011)		2.00
Erasmus MC - BROK® (Basic course Rules and Organisation for Clinical researchers) (2015)		1.50
Deel BKO (2016)		0.00
Various Oral and poster presentations about research topic (2018)		5.00
Various international conferences (2021)		3.00
Various seminars and Workshops (2021)		3.00

Optional

Description	Organizer	EC
Jaarlijks onderwijs en practicum voor neuroscience (2021)		2.00
Begeleiden studenten masteronderzoek (7) (2021)		5.00
		------ +
Total EC		35.90