UNDERSTANDING THE PATHOGENESIS OF GUILLAIN-BARRÉ SYNDROME THROUGH NEUROPHYSIOLOGY AND SEROLOGICAL ANALYSES

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ABSTRACT

Guillain-Barré syndrome (GBS) is the leading cause of post-infectious flaccid paralysis worldwide. Over the years, research on the neurophysiological characteristics and serological profile of GBS patients has contributed towards our greater understanding of its pathogenesis. GBS can be classified into demyelinating and axonal subtypes, based on their neurophysiological features. In axonal GBS, antibodies against several glycolipids have been identified whereas target antigens in demyelinating GBS remain unknown. In this thesis, a series of published original work addressing the current limitations in GBS electrodiagnosis and serological associations with disease features are presented. In the first series of publications, prospective serial nerve conduction studies (NCS) in a cohort of multi-ethnic Malaysian GBS patients are described. We found that in order to make a true electrodiagnosis of GBS, at least two sets of NCS were required performed with the first 2 weeks of disease onset and 3 to 8 weeks later. Based on NCS, we also found an almost exclusive involvement of sensory fibres in patients with the GBS variant, Miller Fisher syndrome irrespective of their symptoms. The second series of publication investigated the presence of antibodies against glycolipid complexes in GBS patients from Asian and Western cohorts. The relationship between these antibodies and the clinical features as well as neurophysiological characteristics of GBS based on serial NCS was also investigated. Our studies provided robust evidence that antibodies to single glycolipids and glycolipid complexes are associated with axonal forms of GBS and not acute inflammatory demyelinating polyneuropathy. Future studies incorporating standardized methods of neurophysiological assessment and serological analyses in heterogeneous populations are required to better understand GBS pathophysiology. Existing on-going international research collaboration is one platform in which findings from the current work can be further validated.

ABSTRAK

Sindrom Guillain-Barré (GBS) adalah punca utama lumpuh lembik selepas jangkitan kuman di seluruh dunia. Selama ini, penyelidikan terhadap ciri-ciri neurofisiologi dan profil serologi pesakit GBS telah banyak menyumbang terhadap pemahaman kami mengenai patogenesis penyakit ini. GBS boleh dikelaskan kepada jenis "demyelinating" dan "axonal", berdasarkan ciri neurofisiologi pesakit. Dalam "axonal" GBS, antibodi terhadap beberapa glycolipid telah dikenalpasti manakala antigen yang bertanggungjawab dalam patogenesis "demyelinating" GBS masih tidak diketahui. Dalam tesis ini, dibentangkan siri penerbitan kerja-kerja asal yang mengatasi batasan semasa dalam elektrodiagnosis GBS serta perhubungan antara serologi dan ciri-ciri penyakit GBS. Dalam siri penerbitan pertama, kajian pengaliran saraf (NCS) dalam kohort pelbagai etnik pesakit GBS Malaysia dibincangkan. Kami mendapati bahawa untuk membuat elektrodiagnosis sebenar GBS, sekurang-kurangnya dua set NCS perlu dilaksanakan, dalam jangkamasa 2 minggu pertama permulaan penyakit dan 3 ke 8 minggu kemudian. Berdasarkan NCS, kami juga mendapati penglibatan hampir eksklusif saraf deria pesakit dengan varian GBS, sindrom Miller Fisher tanpa mengira tanda-tanda klinikal mereka. Penerbitan siri kedua pula menyiasat kehadiran antibodi terhadap kompleks glycolipid dalam pesakit GBS dari kohort Asia dan Barat. Hubungan antara antibodi tersebut dan ciri-ciri klinikal serta ciri-ciri neurofisiologi pesakit GBS berdasarkan kajian NCS yang berturutan juga dikaji. Kajian kami memberikan bukti kukuh bahawa antibodi terhadap glycolipid tunggal dan glycolipid kompleks adalah berkaitan dengan kelas "axonal" GBS dan bukan kelas "demyelinating". Kajian masa depan perlu menggabungkan kaedah taksiran neurofisiologi dan analisis serologi yang seragam dalam pelbagai populasi untuk lebih memahami patofisiologi penyakit GBS. Penyelidikan pergabungan antarabangsa yang telah sedia ada adalah salah satu platfom di mana hasil kajian yang dibentangkan dari tesis in dapat disahkan.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

AAN   :   Acute ataxic neuropathy

AIDP   :   Acute inflammatory demyelinating polyneuropathy

AMAN   :   Acute motor axonal neuropathy

AMCBN   :   Acute motor conduction block neuropathy

AMSAN   :   Acute motor sensory axonal neuropathy

AO   :   Acute ophthalmoplegia

BBE   :   Bickerstaff brainstem encephalitis

CMAP   :   Compound muscle action potential

CMV   :   Cytomegalovirus

CSF   :   Cerebrospinal fluid

Cst-II   :   *Campylobacter* sialyltransferase

EGOS   :   Erasmus Guillain-Barré syndrome outcome score

ELISA   :   Enzyme-linked immunosorbent assay

EMG   :   Electromyography

GBS   :   Guillain-Barré syndrome

GSC   :   Ganglioside complexes

IgG   :   Immunoglobulin G

LOS   :   Lipo-oligosaccharide

mEGOS   :   modified Erasmus GBS outcome score

MFS   :   Miller Fisher syndrome

MRC   :   Medical Research Council

NCS   :   Nerve conduction studies

PCB   :   Pharyngeal-cervical-brachial

SNAP   :   Sensory nerve action potential

LIST OF APPENDICES

Guillain-Barré syndrome (GBS) is an immune-mediated neuropathy characterized by acute onset of paralysis with loss or reduced reflexes. Since the near-elimination of poliomyelitis, GBS has become the most common cause of acute flaccid paralysis. Patients typically present with a history of antecedent illness up to four weeks prior to the development of their neurological symptoms, making GBS the prototype of post-infectious autoimmune diseases. GBS can be classified through neurophysiology into two major subtypes, acute inflammatory demyelinating polyneuropathy (AIDP) and acute motor axonal neuropathy (AMAN). There are also clinical variants of GBS such as Miller Fisher syndrome (MFS) where patients present with a triad of ataxia, ophthalmoplegia and areflexia; and Bickerstaff brainstem (BBE) where patients also develop altered levels of consciousness.

Understanding the pathophysiology of GBS is important as this will eventually lead to the development of better treatments. Molecular mimicry between self and microbial components has been proven to be the underlying pathogenesis in some presentations of axonal GBS. Some patients with AMAN have IgG autoantibodies to ganglioside, GM1 and molecular mimicry was demonstrated between GM1 and lipo-oligosaccharide of *Campylobacter jejuni* isolated from these patients. Disease models by sensitization of rabbits with GM1 and *C. jejuni* lipo-oligosaccharide have also been established. However, in other GBS subtypes, the pathophysiology remains uncertain.

One of the key diagnostic tests in establishing a diagnosis of GBS is nerve conduction studies (NCS) which help to confirm the presence of neuropathy as well as classify the patterns into demyelinating or axonal. The current neurophysiology criteria

were derived in the mid to late 1990's and rely on one set of NCS to provide a snapshot of the underlying neuropathy. Recent studies have suggested that there are flaws to the criteria especially when applied at the early stages of disease. Establishing the true pattern of neuropathy is important as it is likely that autoantibodies in GBS target antigens that reside either at the myelin sheath or axon.

In the current series of published works, the studies are focused on refining the neurophysiology approach to the diagnosis of GBS and its various subtypes. The relationship between IgG antibodies against gangliosides and the patterns of neurophysiology and clinical features are also investigated as both neurophysiology and serological profile of GBS patients are likely to provide further insight into the pathophysiology of GBS.

The study methodology employed was prospective recruitment of GBS patients presenting to University Malaya Medical Centre, Kuala Lumpur, Malaysia who fulfilled the predetermined diagnostic criteria of GBS. Serial NCS were performed at three time periods which included time at admission, 3 to 8 weeks after disease onset and 8 to 12 weeks after disease onset. Acute sera prior to immunotherapy was obtained from each patient and frozen at -20°C until ready for analysis through enzyme-linked immunosorbent assay (ELISA) technique. The studies were approved by the University Malaya Medical Centre Medical Ethics Committee.

The first original work from the current series is titled "Serial nerve conduction studies provide insight into the pathophysiology of Guillain–Barré and Fisher syndromes" (Shahrizaila, Goh, Kokubun, Abdullah, & Yuki, 2011). This paper was a proof of concept study in which serial NCS was performed prospectively in a small number of patients with GBS and MFS. In this study, we presented a case of GBS where initial NCS demonstrated demyelinating features in keeping with AIDP.

However, a second NCS revealed that the findings of "demyelination" had rapidly reversed and along with this, the patient also demonstrated rapid recovery. The final electrodiagnosis of this patient was acute motor conduction block neuropathy which is a less extensive variant of AMAN. In support of this was his serological profile which was positive for IgG against multiple gangliosides, GM1, GD1a, Gal-NAc-GD1a and GD1b. We went on to perform serial NCS in a further five patients and the results are presented in this paper which demonstrated that true AIDP patients would have persistent demyelinating features on serial studies. In contrast, patients with axonal neuropathy may show pseudo-demyelinating features at the first study which would typically reverse as is seen in AMAN and MFS patients. Calls for larger prospective studies and clarity on the timing of NCS were made.

The second original work presented here is titled "Two sets of nerve conduction studies may suffice in reaching a reliable electrodiagnosis in Guillain–Barré syndrome" (Shahrizaila, Goh, Abdullah, Kuppusamy, & Yuki, 2013). In this study, we expanded on our initial work on serial NCS with the aim of elucidating when and how frequent NCS can be performed to reflect disease pathophysiology. We prospectively recruited GBS patients, documenting the initial and final electrodiagnoses following serial NCS performed at three time intervals: 1–2 weeks, 3–8 weeks and 8–12 weeks. A total of twenty-one patients were recruited over a period of 2 years. Electrodiagnosis within 2 weeks revealed 17 AIDP, two AMAN and two unclassified. After 12 weeks, the final diagnoses had been revised and there were now 12 AIDP, seven AMAN and two unclassified. NCS performed within the 3–8 week period reflected the true electrodiagnosis. Patients with AIDP had persistent demyelination features at the 8–12 week nerve conduction studies. Based on these studies, we were able to establish that two sets of NCS performed within the first 2 weeks and between 3–8 weeks of disease onset is likely to suffice in elucidating the true electrodiagnosis of GBS. The study was

of significance as there have been calls within the GBS community to revise the existing electrodiagnostic criteria and serial NCS was felt to be integral to this revision. However, the timing of the studies remained less clear. Findings from the current study could be incorporated into a much-needed revision of the existing GBS electrodiagnostic criteria. In this study, we also investigated the natural history of our GBS population, focusing in particular on the predicted prognostic outcome of patients based on a recently established prognostic scale, modified Erasmus GBS Outcome Score. This prognostic scale was previously validated in the Dutch population and thus, it was important to study its validity in other patient populations. Interestingly, we found variability between the predicted outcome and actual outcome in patients suggesting that other ancillary studies such as NCS and electromyography were important in prognosticating GBS patients who have a heterogeneous presentation.

The third original work in this series of published works is titled "Sensory nerves are frequently involved in the spectrum of Fisher syndrome" (Shahrizaila, Goh, et al., 2014). In this study, we specifically investigated patients with the GBS variant, MFS. MFS is rare, and few series have incorporated prospective serial studies to define the natural history of NCS in MFS spectrum of disease. Interestingly, we found that in our cohort of GBS patients, almost 50% were within the spectrum of MFS which represents a higher percentage than that described in the Western population (5%). As a result, we were in a better position to describe the pattern of disease seen in this group of patients. We recruited 17 patients with MFS. Serial NCS detected significant abnormalities in SNAP amplitude in 94% of patients associated with 2 patterns of recovery—non demyelinating reversible distal conduction failure and axonal regeneration. Similar changes were seen in motor nerves of 5 patients. We thus concluded that patients with MFS spectrum of illness have significant sensory involvement, which may only be evident with serial neurophysiological studies. In this study, we also described the

serological profile of our MFS variant patients. In other cohorts, IgG against GQ1b ganglioside has a high sensitivity (between 86%-99%) and specificity for MFS. In our cohort, we detected IgG against GQ1b in 53% of patients. 40% of patients were seronegative suggesting that these patients are likely to harbour IgG antibodies against other unidentified target antigens. In this work, we also described the natural history of our typical MFS patients who made complete recoveries regardless of whether they had received immunotherapy. These findings are important as it highlights the good prognosis and has implications in resource-poor countries where immunotherapy comes at a high cost to patients, thus limiting its availability.

The fourth original work presented here is titled "Association of antibodies to ganglioside complexes and conduction blocks in axonal Guillain-Barré syndrome presenting as acute motor conduction block neuropathy" (Creange, Shahrizaila, Salhi, Lefaucheur, & Yuki, 2014) This study was done in collaboration with the French neurologists. In this study, we investigated the presence of antibodies against single glycolipid and glycolipid complexes in patients with axonal GBS who had conduction block present on NCS. Axonal GBS has been linked to IgG against single glycolipids but recent studies have found that some patients who are seronegative for IgG against single gangliosides might have antibodies against ganglioside complexes. Ganglioside complexes are thought to represent new clustered epitopes that are recognized by antibodies that would normally not recognize epitopes of a single glycolipid. One study in particular had demonstrated that Ig G against anti-GM1/GalNAc-GD1a complex was associated with acute motor conduction block neuropathy. We hypothesized that conduction block at the early phase of axonal GBS would also be associated with these antibodies. Seven patients were identified to have this pattern of axonal conduction block on neurophysiology. Serological testing failed to detect IgG against GM1/GalNAc-GD1a complex in our cohort. However in some patients, antibodies

against other ganglioside and ganglioside complexes were present. Interestingly, a reduced reaction against GM1/GalNAc-GD1a complex was seen in 3 patients. The study demonstrated that this particular antibody is not always seen in axonal conduction blocks.

The fifth original work included in this published works is titled "Antibodies to single glycolipids and glycolipid complexes in Guillain-Barré syndrome subtypes" (Shahrizaila, Kokubun, et al., 2014). In this study, we comprehensively investigated the serological association of antibodies against single and complex ganglioside with neurophysiological and clinical features of GBS. This work involved collaborations with other centres in Singapore and Japan. Previous smaller studies have suggested that some patients with AIDP have IgG against these ganglioside complexes indicating that these complexes are potential myelin targets. However, we believed that the studies demonstrating these associations were flawed by the singular NCS performed in patients. As previously discussed in our previous works, serial NCS are crucial in making a true electrodiagnosis of GBS. We performed comprehensive serological analyses using ELISA of IgG antibodies to glycolipids including gangliosides, neutral glycolipids and glycolipid complexes in a large number of GBS patients (n=199) and analysed their association with different GBS subtypes based on serial NCS as well as specific clinical features. To our knowledge, this is the first published work that investigates the association of these antibodies in such a comprehensive manner. Based on serial NCS, the electrodiagnoses were as follows: 69 demyelinating subtype, 85 axonal subtype, and 45 unclassified. Significant associations were detected between AMAN subtype and IgG antibodies to GM1, GalNAc-GD1a, GA1, or LM1/GA1 complex. Reversible conduction failure was significantly associated with IgG antibodies to GM1, GalNAc-GD1a, GD1b, or complex of LM1/GA1. No significant association was demonstrated between AIDP and any of the glycolipids or ganglioside complexes.

Antiganglioside complex antibodies alone were detected in only 7 patients (5 axonal subtype). We were able to conclude that the study provided Class II evidence that antibodies to glycolipids are increased in patients with acute motor axonal neuropathy and acute motor conduction block neuropathy but not acute inflammatory demyelinating polyneuropathy. We also investigated the association of these antibodies to certain clinical features including ophthalmoplegia, facial weakness, bulbar palsy, sensory impairment and the need for artificial ventilation. We found that the presence of ophthalmoplegia and bulbar palsy was associated with IgG anti-GQ1b antibodies, in keeping with previous reports. Patients who lacked sensory impairment (indicating a predominant motor form of GBS) were significantly associated with IgG antibodies to GM1, GalNAc-GD1a, and GA1 as well as IgG antibodies to LM1/GA1, GM1/ GalNAc-GD1a, and GM1b/GA1. The presence of facial palsy was associated with a diagnosis of AIDP without significant serologic associations. Instead, the presence of IgG anti-GM1, -GalNAc-GD1a, -GD1b, and -GA1 antibodies was less likely to result in the development of facial palsy. What was also interesting to note was that IgG antibodies against ganglioside complexes alone were positive in only 7 patients who were seronegative for single ganglioside.

In this summary, a synopsis of five key publications in this body of work on GBS has been presented. The candidate is the principal author if not also, the corresponding author in all five publications. The publications represent studies that have focused on improving the electrodiagnostic criteria of GBS as well as establishing the serological associations of various GBS subtypes. Work presented here was done and published over a period of four years (2010-2014). The candidate is the primary researcher directly involved in the study design concept, acquisition of data and analysis as well as drafting of the initial drafts along with subsequent drafts for submission. However, the

contribution from other authors and collaborators are acknowledged as the publications would not have been possible without their input.

In addition, the original work described here was preceded by comprehensive literature reviews of various aspects of GBS including the molecular mimicry theory in GBS, MFS and its variants, the role of anti-ganglioside antibodies in GBS and the role of immunotherapy in GBS. These works listed in Appendix A have been published in peer-reviewed journals and as book chapters, and the candidate is the primary author. Although they have not been included as part of main thesis, they represent published works undertaken by the author during this period of time. Other published works include editorial commentaries that have had impact on work in this field by proposing strategies on how research within this field can move forward.

CHAPTER 1: INTRODUCTION

1.1 Description of research issues investigated

Guillain-Barré syndrome (GBS) can be broadly divided into two subtypes, acute inflammatory demyelinating polyneuropathy (AIDP) and acute motor axonal neuropathy (AMAN). (Uncini & Yuki, 2009) Miller Fisher syndrome (MFS) is widely accepted as a variant of GBS presenting with its own set of unique clinical features of ataxia and ophthalmoplegia. In recent years, our understanding of AMAN has improved in leaps and bounds with the identification of the serological markers (IgG antibodies against GM1, GM1b, GD1a or GalNAc-GD1a) and proof of molecular mimicry with *Campylobacter jejuni* in some cases of AMAN. (Yuki & Kuwabara, 2007) In contrast, serological markers in AIDP remain elusive and the search for pathogenic antigens in AIDP is ongoing. (Shahrizaila & Yuki, 2011a) There continues to be some debate as to whether MFS is a demyelinating or axonal disease. (Fross & Daube, 1987; Jamal & Ballantyne, 1988) MFS is strongly associated with IgG anti-GQ1b antibodies, and *C. jejuni* isolates from MFS patients carry GQ1b epitope. (Yuki, 2009) The ganglioside-like lipo-oligosaccharide (LOS) in *C. jejuni* strains are synthesized by *Campylobacter* sialyltransferase (Cst-II) encoded by *cst-II*. The genetic polymorphism of *cst-II* influences the ganglioside-like LOS that is expressed, which in turn produces either AMAN or MFS in susceptible patients suggesting that the pathophysiology of MFS is not demyelinating, but axonal.

Two of the key research areas in the understanding of GBS pathophysiology are through the electrodiagnosis of GBS and serological profiling of GBS patients specifically IgG antibodies against glycolipids.

1.2   Electrodiagnosis of GBS

NCS is performed in GBS patients to confirm the presence of neuropathy as well as elucidate the involvement of primarily axons or myelin. The commonly used electrodiagnostic criteria of Ho et al. and Hadden et al. (Hadden et al., 1998; Ho et al., 1995) were derived in the mid to late 1990's. However, in recent years, it has been demonstrated that there may be flaws in these criteria especially when applied at the early stages of the disease. (Uncini & Kuwabara, 2012) Early changes on NCS can mimic demyelination in certain axonal subtypes such as acute motor conduction block neuropathy. (Kokubun et al., 2010) Serial NCS are advocated when establishing the electrodiagnosis of GBS. However, it is less clear as to when and how frequent subsequent NCS should be performed to allow a more accurate electrodiagnosis of GBS to be made.

In the first series of studies included in this thesis, (Shahrizaila et al., 2013; Shahrizaila et al., 2011; Shahrizaila, Goh, et al., 2014) the role of serial NCS was investigated in GBS and its variants, including defining time-points and frequency of studies that can best reflect the true electrodiagnosis of GBS.

The study methodology employed were prospective recruitment of patients presenting with GBS or any of its variants to University Malaya Medical Centre, Kuala Lumpur, Malaysia who fulfilled the predetermined diagnostic criteria of GBS. (Asbury & McKhann, 1997) Serial NCS were performed at three time periods which included time at admission, 3 to 8 weeks after disease onset and 8 to 12 weeks after disease onset. The studies were approved by the University Malaya Medical Centre Medical Ethics Committee.

NCS were performed using the Medelec™ Synergy EMG machine. At least two limbs were assessed; four motor nerves and three sensory nerves as well as F wave latencies. Nerve stimulation and recorded compound motor action potentials (CMAP) were as follows: median nerve was stimulated at the wrist and elbow, recording over abductor pollicis brevis muscle; ulnar nerve was stimulated at the wrist, below elbow and above elbow, recording over abductor digiti minimi muscle; tibial nerve was stimulated at the ankle and popliteal fossa, recording over the abductor hallucis muscle. Sensory studies of the median and ulnar nerves were performed by using the orthodromic method of stimulating the index finger and little finger respectively and the sensory nerve action potentials (SNAP) recorded over the wrist crease. The radial and sural nerves however, were recorded using the antidromic method. The radial nerve was stimulated at the forearm and recorded over the anatomical snuffbox whereas sural nerve was stimulated at the calf and recorded below the lateral malleolus. Reference values were derived from NCS performed on normal patients at our laboratory. The electrodiagnosis of AIDP or AMAN was made based on the electrodiagnostic criteria set by Ho et al. (Ho et al., 1995) The criteria for abnormal sensory nerve studies were derived from the criteria for acute motor and sensory axonal neuropathy in which a reduction in SNAP amplitude to 50% of the lower limit of normal in at least 2 nerves was considered abnormal. (Feasby et al., 1993) When the initial sensory NCS were normal, any changes in the SNAP amplitude of 45% for median nerve, 49% for the ulnar nerve, or 60% for the sural nerve would be considered abnormal. (Uncini, Manzoli, Notturno, & Capasso, 2010) Motor conduction block was classified according to the American Association of Electrodiagnostic Medicine criteria as follows: (a) definite—presence of at least 50%, or 60% in the tibial nerve, reduction of proximal vs. distal CMAP amplitude in the nerves of the upper and lower limbs, respectively, with minimal temporal dispersion (<30% increased CMAP duration); or (b) probable—

presence of either at least 40% or 50% reduction of proximal vs. distal CMAP amplitude in the nerves of the upper and lower limbs, respectively, with minimal temporal dispersion (see above), or at least 50% or 60% reduction of proximal vs. distal CMAP amplitude in the nerves of the upper and lower limbs, respectively, with moderate temporal dispersion (31–60% increased CMAP duration). (Olney, 1999) Reversible distal conduction failure was defined as a decrease in distal CMAP amplitude that resolved without abnormal temporal dispersion (duration increase >30%), or other demyelinating features. (Kuwabara, Yuki, et al., 1998)

1.3 Serological analyses of IgG against glycolipids and glycolipid complexes

There is robust evidence that IgG anti-ganglioside antibodies are associated with the pathogenesis of AMAN whereas the target antigens in AIDP remains elusive. (Shahrizaila & Yuki, 2011a) In 2004, antibodies to ganglioside complexes (GSC) were reported in patients with GBS. (Kaida et al., 2004b) The patients who were seronegative for antibodies to single gangliosides were found to have anti-GSC antibodies. The authors have since described further associations between anti-GSC antibodies and variants of GBS. This includes antibodies to LM1 and its complexes in AIDP, (Kuwahara, Suzuki, Takada, & Kusunoki, 2011) to complex of GM1 and GalNAc-GD1a (GM1/GalNAc-GD1a) in AMCBN (Kaida et al., 2008) and to complexes of GD1a/GD1b and GD1b/GT1b in GBS patients requiring artificial ventilation. (Kaida et al., 2007) However all these studies refer to the electrodiagnosis of GBS was based on a single study. Given our current understanding that the neurophysiological findings in GBS can rapidly change in the early stages of the disease, we believe that the diagnosis of AIDP was likely to have been overestimated. Thus, this calls into question the validity of the serological profiling described in these studies.

The second series of studies included in this thesis (Creange et al., 2014; Shahrizaila, Kokubun, et al., 2014) aimed to investigate the relationship between anti-GSC antibodies and specific clinical features of GBS as well as the electrodiagnostic subtypes of GBS; the latter based on serial NCS in a large cohort of patients from different geographical locations.

Acute sera prior to immunotherapy was obtained from each patient and frozen at -20°C until ready for analysis through enzyme-linked immunosorbent assay (ELISA) technique. Sera samples were measured for IgG antibodies single glycolipids including gangliosides (LM1, GM1, GM1b, GD1a, GalNAc-GD1a, GD1b, GT1a, GT1b, and GQ1b) and a neutral glycolipid, asialo-GM1 (GA1), using ELISA method. In brief, serum samples diluted to 1:500 were placed in separate microtiter plate wells. The mean value for triplicate reference wells without antigen was subtracted from the mean value for triplicate wells of each sample, and the optical density assessed. An optical density of more than 0.5 was judged to be positive. Using the strict cut-off value, sera from patients with acute transverse myelitis (n=9), acute disseminated encephalomyelitis (n=46) and multiple sclerosis (n=44) were negative for those anti-ganglioside antibodies. Patients' sera were also assessed for IgG antibodies to ganglioside complexes, which were tested with a mixture of individual glycolipids at 5 pmol/well each. Anti-glycolipid and anti-ganglioside complex antibodies were considered positive when the optical density was greater than 0.5 of the sum of antibodies to individual antigens. The latter tests were performed in quadruplicate and a mean of the optical density value was measured. The ELISA methodology employed in the study differs from those of other investigators within this field. (Kaida et al., 2004a) In comparison to previous studies, our serological analyses utilized a reduced amount of antigen (e.g. GM1, 7.5 ng vs 200 ng) and a higher serum and secondary antibody dilution (1:500 and 1:2000 vs 1:40 and 1:500 respectively). The optical density value for seropositivity also

differed (≥ 0.5 in the current study vs > 0.1 in single gangliosides and > 0.2 in GSCs in other studies). We believe the methodology adopted in our study would result in more specific findings.

1.4 The objectives of the study

1. To investigate the contribution of serial NCS to the final electrodiagnoses of GBS and its variants

2. To investigate the timing and frequency of serial NCS in elucidating the true electrodiagnosis of GBS

3. To investigate the relationship between IgG against glycolipid and glycolipid complexes in different GBS subtypes and their clinical features

1.5 Contribution towards ongoing research on GBS pathogenesis

The original works that have been included in this thesis have further emphasised the need for serial studies in the electrodiagnosis of GBS. However, two studies would suffice in confirming the patterns of neuropathy in GBS provided the studies were performed within the first 2 weeks of disease onset and repeated within 3 to 8 weeks of disease onset. Experts within this field are currently working towards defining new electrodiagnostic criteria for GBS and two sets of NCS within the time-frames advocated from these studies are being advocated. In MFS, findings from the studies employed have identified involvement of sensory fibres in almost all patients with MFS regardless of clinical symptoms. These findings were again made evident by serial studies in this cohort of patients.

The latter study (Shahrizaila, Kokubun, et al., 2014) represents the largest cohort to date in which comprehensive serological analyses of IgG antibodies against glycolipids and GSC have been performed. The findings are robust and clearly demonstrate that antibodies against glycolipids are associated with axonal forms of GBS. In patients who were seronegative for single glycolipids, testing for antibodies against glycolipid complexes would yield a positive result in only a small number of patients. The study also demonstrates the need for standardised ELISA methodology in different cohorts. Further work is also required to elucidate true antigens in AIDP in order to develop more reliable disease models and improve future therapy.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

In 1916, three French neurologists, Guillain, Barré and Strohl described a clinical pattern of ascending weakness associated with albumin-cytological dissociation in their cerebrospinal fluid. (Guillain, Barré, & Strohl, 1916) This syndrome is now referred to as Guillain-Barré syndrome (GBS). GBS is an acute form of immune-mediated polyneuropathy. Since the near-elimination of polio worldwide, GBS has become the commonest cause of flaccid paralysis (Hovi & Stenvik, 2000; Olive, Castillo, Castro, & de Quadros, 1997). The disease typically follows a monophasic course and is also typically preceded by an infectious episode, such as a respiratory or gastrointestinal infection. The reported incidence of GBS ranges from 1-2 per 100000 population. (Chio et al., 2003; Govoni & Granieri, 2001; Van Koningsveld, Van Doorn, Schmitz, Ang, & Van der Meche, 2000) These studies have largely arisen from Europe and United States. There also appears to be a slight male preponderance and a linear rise of incidence with age. (Bogliun, Beghi, & Italian, 2004; Govoni & Granieri, 2001)

GBS can be further classified into two major subtypes based on the electrophysiological as well as pathological findings: acute inflammatory demyelinating neuropathy (AIDP) and acute motor axonal neuropathy (AMAN), involving the peripheral nerve myelin and axons respectively. (C. Hafer-Macko et al., 1996; C. E. Hafer-Macko et al., 1996) Based on the current electrophysiological criteria, (Hadden et al., 1998; Ho et al., 1995) AIDP appears to be the predominant subtype in the Western population, accounting for 95% of cases (Hadden et al., 1998) whereas AMAN occurs more frequently in studies of GBS in China, (McKhann et al., 1993) Japan, (Ogawara et al., 2000) and Central America, (Paradiso, Tripoli, Galicchio, & Fejerman, 1999)

presenting in 30-47% of GBS cases. More recent studies in Asia based on electrophysiology continues to show variable presentations of the two subtypes of GBS with AMAN presenting in 8% of 51 Indian patients, (Kalita, Misra, & Das, 2008) 22% of 41 patients in Israel, (Kushnir, Klein, Pollak, & Rabey, 2008) and 67% of 100 patients in Bangladesh. (Islam et al., 2010) There have since been more detailed electrophysiological studies suggesting that the current electrophysiology criteria may be insensitive at detecting other possible GBS subtypes such as AMAN with conduction block where the first electrophysiological presentation may mimic "demyelination" but subsequent electrophysiology convincingly shows an axonal pathology. (Kokubun et al., 2010)

Miller Fisher syndrome (MFS) is characterized by an acute onset of ataxia, areflexia and ophthalmoplegia (Fisher, 1956) and when there is associated disturbance of consciousness the condition is known as Bickerstaff brainstem encephalitis (BBE). (Bickerstaff, 1957; Bickerstaff & Cloake, 1951) Both disorders share many common features with each other and GBS, in particular the antecedent infection, the albuminocytological dissociation and also the presence of antiganglioside antibodies in certain cases. (Ito et al., 2008; Odaka et al., 2003) This suggests that GBS, MFS and BBE are in fact part of a spectrum of immune-mediated disorder involving the peripheral nerves at one end and the central nervous system at the other. Most epidemiology data on MFS have been gleaned from the description of MFS as a variant of GBS. The estimated annual incidence of MFS is 0.9/100000/year (Emilia-Romagna., 1998) and MFS is also reported to have an incidence of approximately 1-5% of GBS from Western countries but higher in Asian countries such as Taiwan (19%) (Lyu, Tang, Cheng, Hsu, & Chen, 1997) and Japan (25%) (Mori, Kuwabara, Fukutake, Yuki, & Hattori, 2001). There are no incidence data on BBE but clinical experience suggests that BBE has a lower incidence than MFS.

2.2   Clinical features

2.2.1   AIDP

At first presentation, it is often difficult to differentiate between AIDP and AMAN. Both present with flaccid paralysis, areflexia and cerebrospinal fluid (CSF) albuminocytological dissociation. Careful clinical assessment may suggest features that are supportive of AIDP. For instance, the clinical progression is longer in AIDP compared to AMAN (18.0 days *versus* 11.5 days). (Hiraga, Mori, Ogawara, Hattori, & Kuwabara, 2003) Facial weakness is more prominent in AIDP than in AMAN (71% *versus* 26%) (Kokubun et al., 2010) and there are descriptions of regional variants of AIDP referred to as "facial diplegia and paresthesias" where neurophysiology supports demyelination in the limbs. (Ropper, 1994) Other distinguishing features for AIDP patients include the more frequent need for artificial ventilation (Hiraga et al., 2005) and the presence of autonomic instability. (Asahina, Kuwabara, Suzuki, & Hattori, 2002)

Previous hypotheses of the pathogenesis of AIDP were largely based on the mice model, experimental autoimmune neuritis which resembles AIDP clinically and pathologically. Experimental autoimmune neuritis can be transferred to animals by T-cells sensitized to peripheral nerve proteins such as P2 protein. However, no investigators have shown conclusive evidence that such autoreactive T-cell response is seen in patients with GBS, indicating that experimental autoimmune neuritis is not a true model of AIDP. (Asbury & McKhann, 1997) In contrast, Hafer-Macko *et al.* in their autopsy cases of AIDP patients showed nerve injury that was mediated by complement activation. They speculated that epitopes on the outer surface of the Schwann cell trigger the binding of antibodies and the subsequent complement activation initiate the removal of myelin. (C. E. Hafer-Macko et al., 1996) This led to

the search for myelin and Schwann cell antigens. There have been postulations of anti-galactocerebroside antibodies (Kusunoki, Chiba, Hitoshi, Takizawa, & Kanazawa, 1995), anti-LM1 (Ilyas, Mithen, Dalakas, Chen, & Cook, 1992) (Susuki, Yuki, Hirata, & Kuwabara, 2002), anti-SGPG antibodies (Ilyas et al., 1992; Yuki, Tagawa, & Handa, 1996), anti-GM2 antibodies (Irie et al., 1996; Yuki & Tagawa, 1998) and anti-GD1b (Miyazaki, Kusunoki, Kaida, Shiina, & Kanazawa, 2001) as likely target autoantibodies in AIDP but studies have thus far been inconclusive.

2.2.2   AMAN

The typical AMAN presentation is distinct from AIDP with predominant motor involvement and little in the way of sensory findings. (McKhann et al., 1993)In the majority of cases, there is a history of antecedent respiratory or gastrointestinal illness. Examination reveals weakness in the upper and lower limbs. Deep tendon reflexes are typically diminished, although there have been reports of retained and even brisk reflexes throughout the course of illness in AMAN. (Yuki et al., 2012) In the AMSAN subtype, patients have sensory involvement on examination making it less distinct from AIDP and neurophysiology is crucial in making this distinction. (Capasso, Notturno, Manzoli, & Uncini, 2011) CSF analysis typically reveals a raised protein with normal cell count although a normal analysis can be seen when the test is done at an early stage.

Significant differences between the clinical course of AMAN and AIDP have been detected in observational studies. AMAN patients tend to reach plateau earlier and are less disabled at nadir in general in comparison to AIDP. Other studies have also observed that cranial neuropathies including facial palsies, autonomic involvement and the need for ventilation were less frequent in AMAN patients. In patients with AMAN

prognosis may hinge on whether axonal damage is predominantly distal axonal or proximal axonal in extent. (Shahrizaila & Yuki, 2014)

2.2.3   MFS

The classic triad seen in MFS is ataxia, ophthalmoplegia and areflexia. In the event there is associated alteration in the level of consciousness or hyperreflexia, a diagnosis of BBE is preferred as a reflection of the central nervous system involvement. In clinical practice and also in the original descriptions, there are other clinical symptoms and signs that can also be present in patients with MFS and BBE. The most common of these include ptosis, mydriasis, peripheral sensory disturbance and facial palsies (at times presenting as a delayed feature, after other features have started to improve). (Fisher, 1956; Mori et al., 2001)

2.3   Neurophysiological features

Nerve conduction studies in AIDP shows features consistent with demyelination and remyelination such as prolonged distal motor latencies, significantly reduced conduction velocities and temporal dispersion on proximal stimulation. (Albers, Donofrio, & McGonagle, 1985) Serial nerve conduction studies reinforces these findings by showing a progression in the distal motor latencies in the acute phase of illness and in cases where there is secondary axonal degeneration, nerves may become inexcitable.

Neurophysiology studies of AMAN have seen many changes in recent years. The earliest of the cases from China characterized the neurophysiology of AMAN by the reduction of compound motor action potentials (CMAP) without demyelinating features which were defined as prolonged distal motor latencies, reduced conduction velocities,

delays in F wave latencies and evidence of temporal dispersion. When there was concurrent reduction in sensory amplitudes of more than 50%, AMSAN was diagnosed. (McKhann et al, 1993) A study by Kuwabara et al of AMAN and AMSAN patients found axonal features in neurophysiology as well as rapid resolution of conduction block and conduction slowing at common entrapment sites that were inconsistent with demyelination or axonal degeneration. (Kuwabara, Yuki, et al., 1998) Capasso et al later noted conduction blocks across the intermediate forearm segments of AMAN patients with rapid resolution on repeat NCS and they proposed the term acute motor conduction block neuropathy (AMCBN) as a variant of GBS. (Capasso et al., 2003) In a study by Kokubun and colleagues, conduction block was detected in 12 of 18 AMAN patients. In seven of their patients, there was rapid resolution of conduction block and in two of these patients, the conduction blocks were at the intermediate forearm segments in keeping with AMCBN. (Kokubun et al., 2010) These studies raised the importance of performing serial nerve conduction studies to better classify the electrodiagnosis of GBS. **(Figure 1)**

More recently, Capasso et al demonstrated that sensory fibres are often involved in AMAN. (Capasso et al., 2011) They showed patterns of conduction failure in sensory as well as motor fibres in AMAN and AMSAN, suggesting that AMSAN is in fact part of the AMAN spectrum of disease rather than representing a separate disease entity. Neurophysiology plays an important role in the diagnosis of GBS as well as in the classification which differentiates AIDP and AMAN. Repeated neurophysiology studies are helpful as they provide further insight into the underlying pathophysiology in AMAN and can be utilised to characterise the subtypes within the AMAN spectrum such as AMCBN and AMSAN. **(Figure 2)**
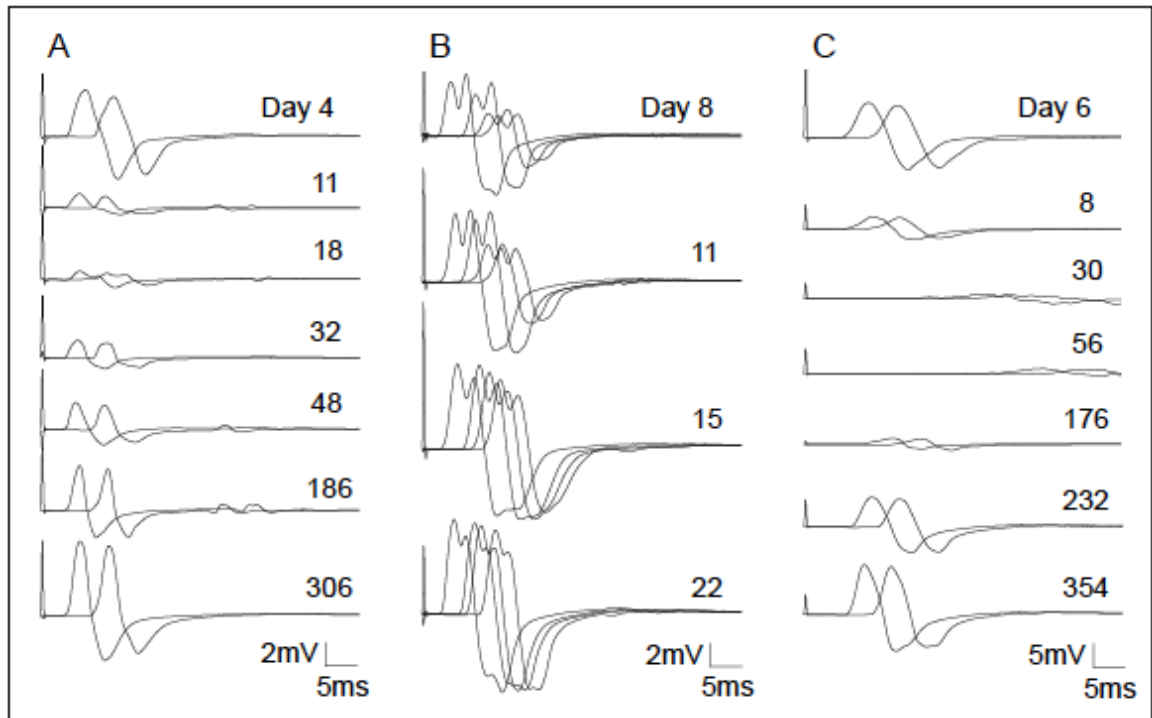
Figure 2.1: Serial nerve conduction studies in Guillain-Barré syndrome.

Panel A is the median nerve study in a patient with acute motor axonal neuropathy. The compound muscle action potential was markedly reduced at day 11 corresponding to his symptom nadir and started to show recovery at Day 32 to return to normal at day 304. Panel B is the ulnar nerve study of a patient with acute motor axonal neuropathy with conduction block across the elbow. The conduction block rapidly resolved by day 15 without progressing to axonal degeneration in parallel with clinical recovery. Panel C is the median nerve study of a patient with acute inflammatory demyelinating neuropathy. Although clinical nadir was reached at week 3, there was gradual prolongation of the distal latency with reduction in the motor amplitude reaching the slowest conduction at day 56. The patient had started to clinically recover after nadir and could walk unaided by day 56. The nerve conduction studies showed recovery on day 176 and continue to improve to normal by day 354. Figure reproduced from Shahrizaila, N., & Yuki, N. (2014). Acute Motor and Motor–Sensory Neuropathy (Axonal Subtypes of Guillain–Barré Syndrome), Immunology of. . In A. M. J. a. D. R.B. (Ed.), Encyclopedia of the Neurological Sciences, 2nd (Vol. 1, pp. 49-53): Oxford: Academic Press.
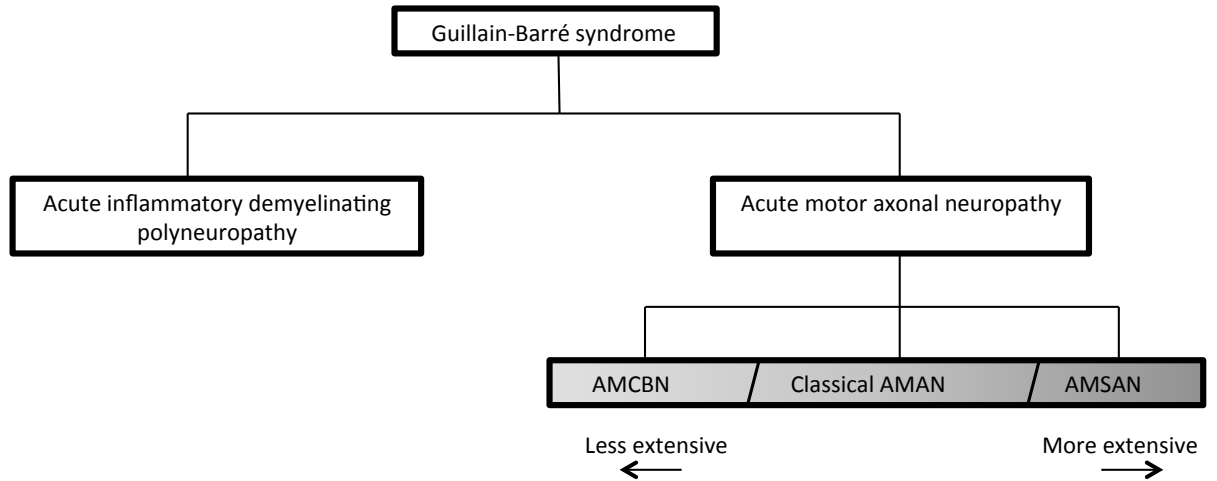
Figure 2.2: Proposed classification of Guillain-Barré syndrome.

AMCBN: acute motor conduction block neuropathy; AMAN: acute motor axonal neuropathy; AMSAN: acute motor-sensory axonal neuropathy

Figure reproduced from Shahrizaila, N., & Yuki, N. (2014). Acute Motor and Motor–Sensory Neuropathy (Axonal Subtypes of Guillain–Barré Syndrome), Immunology of. . In A. M. J. a. D. R.B. (Ed.), Encyclopedia of the Neurological Sciences, 2nd (Vol. 1, pp. 49-53): Oxford: Academic Press.

The true electrodiagnosis of MFS and its related conditions have been subject to differing opinions with studies supporting demyelination and axonal pathophysiology. (Fross & Daube, 1987; Jamal & Ballantyne, 1988) In one study, non-demyelinating reversible conduction failure was demonstrated in 6/15 (40%) of patients with MFS and its related conditions. (Umapathi, Tan, Kokubun, Verma, & Yuki, 2012) Although well-recognised as a GBS variant, MFS is rare and few prospective studies have comprehensively investigated this patient cohort.

2.4   Antiganglioside antibodies

Gangliosides are a large family of glycosphingolipids, predominantly distributed on the cell-surface membrane and anchored in the external leaflet of the lipid bilayer by a ceramide moiety. The sialylated oligosaccharides are exposed extracellularly. In GBS, anti-ganglioside antibodies were first reported in 1988 in five of 26 patients. (Ilyas et al., 1988) The possibility that antiganglioside antibodies were pathogenic in GBS became apparent following the report of two patients with *Campylobacter jejuni* enteritis who developed AMAN and had positive titres of IgG anti-GM1 antibodies which decreased with the clinical course of the disease. (Yuki, Yoshino, Sato, & Miyatake, 1990) In contrast, patients who had *C. jejuni* enteritis but developed no neurological disorder had no anti-GM1 antibodies. Further studies also found a similar association where patients with anti-*C. jejuni* and -GM1 antibodies were more likely to have axonal degeneration than those with neither antibody. (Jacobs et al., 1996; Rees, Gregson, & Hughes, 1995)
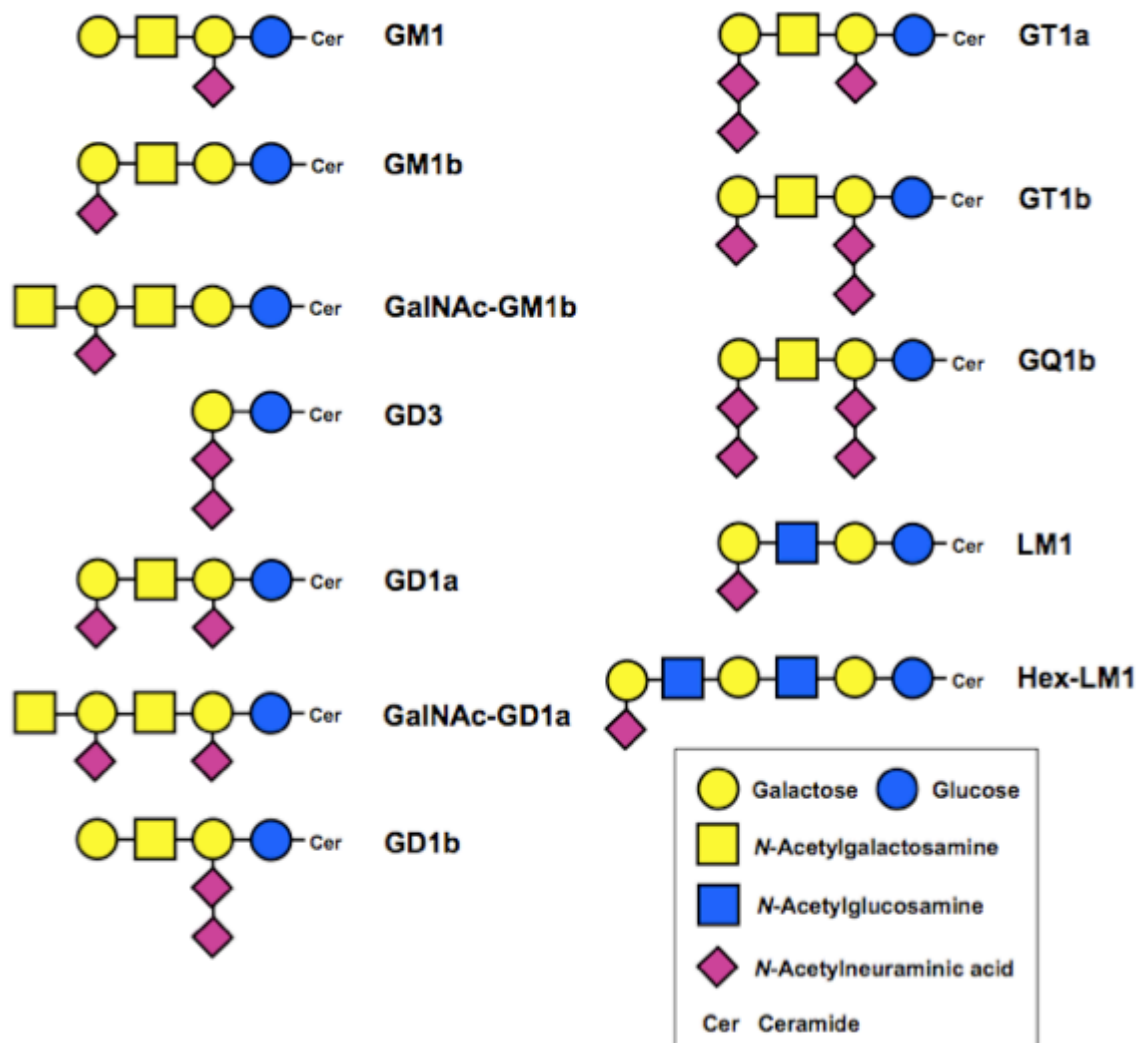
Figure 2.3: The chemical structures of gangliosides.

Reproduced from Yuki N. Ganglioside mimicry and peripheral nerve disease. Muscle Nerve 35(6), 691-711. Copyright © John Wiley&Sons (2007)

IgG anti-GD1a antibodies were detected in two AMAN patients in 1992. (Yuki, Yoshino, Sato, Shinozawa, & Miyatake, 1992) The presence of IgG anti-GD1a antibodies was later shown to be significantly associated with prolonged artificial ventilation and poor recovery after three months in 37 patients. (Yuki, Yamada, et al., 1993) In another large study of GBS patients and appropriate controls, 24% of 138 AMAN patients, but neither AIDP patients nor control subjects, had high IgG anti-GD1a antibody titers. (Ho et al., 1999) Anti-GD1a antibodies were found to be the most specific for AMAN amongst the antiganglioside antibodies tested which included GM1, GD1b, asialo-GM1 and GQ1b.

N-Acetylgalactosaminyl GD1a (GalNAc-GD1a) was recognised as the target molecule following the detection of these antibodies in the sera of some patients who developed AMAN subsequent to *C. jejuni* enteritis. (Kusunoki et al., 1994) Anti-GalNAc-GD1a antibody, detected in 14% of 132 cases, correlated with antecedent *C. jejuni* infection, a rapidly progressive and more severe course with predominantly distal weakness, and little sensory or cranial nerve involvement.

Autoantibodies to GM1b have also been shown to be a useful diagnostic marker of GBS (Kusunoki et al., 1996) associated *C. jejuni* enteritis. (Yuki, Tagawa, Irie, Hirabayashi, & Handa, 1997) Of 132 patients with GBS, 19% had anti-GM1b antibodies. (Yuki et al., 2000) Patients with anti-GM1b antibodies had a distinct clinical pattern with more rapidly progressive and severe weakness. Cranial nerve involvement and sensory deficits were less common and the presence of these antibodies was associated with slower recovery.

To clarify the relationship of AMAN to anti-ganglioside antibodies and *C. jejuni* infection, 86 GBS patients with the diagnosis of *C. jejuni* infection were studied.

(Ogawara et al., 2000) Electrodiagnostic criteria showed AMAN in 38% and AIDP in 36%. The most frequent antiganglioside antibodies were of the IgG class; against GM1 (40%), GD1a (30%) and GalNAc-GD1a (17%). Identified infections were *C. jejuni* (23%), cytomegalovirus (10%), *M. pneumoniae* (6%) and Epstein-Barr virus (3%). There was a strong association between AMAN and IgG antibodies against GM1, GD1a and GalNAc-GD1a. Those who had an antecedent *C. jejuni* infection frequently had AMAN or antiganglioside antibodies, although the patients with AMAN or antiganglioside antibodies were not always *C. jejuni*-positive. Ten patients who had anti-GM1b antibodies alone also frequently had AMAN (80%) preceded by *C. jejuni* infection. (Ogawara et al., 2003) These findings show that GM1, GM1b, GD1a and GalNAc-GD1a could be target molecules in AMAN subsequent to *C. jejuni* enteritis.

One of the major turning points in our understanding of MFS and BBE came with the discovery of the IgG anti-GQ1b antibody by Chiba and colleagues in six typical MFS patients (Chiba, Kusunoki, Shimizu, & Kanazawa, 1992). The authors closely followed this up by confirming the presence of anti-GQ1b in a further 18/19 typical MFS patients as well as in five patients with post-infectious ophthalmoplegia (AO) and five out of six GBS patients with ophthalmoplegia. (Chiba, Kusunoki, Obata, Machinami, & Kanazawa, 1993) Their immunohistochemical studies using the anti-GQ1b mouse monoclonal antibodies demonstrated prominent staining paranodal regions of the extramedullary portion of the oculomotor, trochlear and abducens nerve. Immunostaining of the dorsal and ventral roots were less remarkable. Other laboratories were also able to show a similar association between IgG anti-GQ1b antibodies and MFS; 83% were positive in 466 MFS patients (Ito et al., 2008) and all nine patients in the UK. (Willison & Veitch, 1994)

The identification of anti-GQ1b as a serological marker for MFS led the way in our understanding of other neurological syndromes that have also been closely associated with this antibody. At the time, BBE was considered distinct from MFS but this changed when IgG anti-GQ1b antibodies were detected in a comatose patient with acute ophthalmoplegia, ataxia and areflexia who made a complete recovery two months following the onset of her illness. (Yuki, Sato, Tsuji, Hozumi, & Miyatake, 1993) This unexpected finding led the authors to confirm anti-GQ1b antibodies in two other BBE patients. This common autoantibody profile in BBE and MFS supported a common autoimmune mechanism in both conditions. Other variants of MFS and BBE such as acute ophthalmoplegia (AO), acute ataxic neuropathy (AAN), MFS/pharyngeal-cervical-brachial (PCB), MFS/GBS and MFS/BBE have been associated with the anti-GQ1b antibodies. This has led to some researchers referring to this group of presentations collectively as the "anti-GQ1b syndrome" to consolidate their common serological profile.

2.5   Pathogenesis

2.5.1   GBS

Antecedent infections appear to play an important role in the development of GBS. Prospective studies have demonstrated *C jejuni* and cytomegalovirus infections to be significantly more frequent in patients with GBS as compared to controls. (Jacobs et al., 1998; Winer et al., 1988) Epidemiological association established between *C jejuni* infection and GBS noted that patients with an antecedent *C jejuni* infection had a more severe form of GBS with axonal degeneration. (Rees, Soudain, Gregson, & Hughes, 1995) The growing evidence suggests that microbial organisms are likely triggers of an

autoimmune response leading to the peripheral nerve injury seen in GBS. (Yuki et al., 2004; Yuki et al., 2001) The pathological processes underlying AIDP and AMAN are different, but the final pathway is common. In AIDP, there is complement activation and membrane attack complex formation on Schwann cell surface that lead to vesicular demyelination. (Hafer-Macko et al., 1996). In AMAN, antibodies against motor axons lead to complement-mediated membrane attack complex formation at the nodal axolemma and in severe cases, axonal degeneration of the motor axons develop (Hafer-Macko et al., 1996). In other words, GBS is a complement-mediated autoimmune disorder.

The target antigen in AIDP has yet to be identified. In contrast, research over the last 20 years has clarified some of the associated autoantigens in AMAN. There have been many studies that have reported the presence of various antiganglioside antibodies in patients with AMAN, namely IgG anti-GM1, -GM1b, -GD1a and -GalNAc-GD1a antibodies. (Kusunoki et al., 1994; Kusunoki et al., 1996; Yuki, Miyatake, Ichihashi, Sato, & Katagiri, 1992; Yuki et al., 1990) *C. jejuni*-related GBS is likely to be associated with AMAN. (Kuwabara et al., 2004) Molecular mimicry exists between gangliosides and the LOS of *C jejuni* isolated from an AMAN patient (Koga et al., 2006; Yuki, Taki, et al., 1993). Rabbits sensitized with GM1 ganglioside developed IgG anti-GM1 antibodies followed by acute flaccid paralysis, and pathological studies confirmed the characteristic features of AMAN. (Susuki et al., 2003) A replica of AMAN was also produced by sensitizing the rabbits with *C jejuni* LOS from the AMAN patient. (Yuki et al., 2004) Along with the epidemiological association between GBS and *C. jejuni* infection, (Rees, Gregson, et al., 1995) this sequence of events established GBS as the first autoimmune disorder to be triggered by molecular mimicry in humans (Shahrizaila & Yuki, 2011b).

Immunohistochemical studies performed on the peripheral nerves of AMAN rabbit models have successfully demonstrated the underlying mechanism of peripheral nerve injury in AMAN as follows. (Susuki et al., 2007) AMAN rabbits were studied at the acute progressive phase (a few days after onset), early recovery (2 weeks after onset) and late recovery. (4 weeks or more after onset) In the acute phase, there was lengthening of the nodes of Ranvier and IgG was noted to be deposited at some nodes where GM1 was expressed, as shown in AMAN patients. This binding of autoantibodies triggered complement activation at the nodes and eventually, the membrane attack complex formation at the nodal axolemma. (Susuki et al., 2007) This is followed by alteration of the sodium channel clusters due to the destruction of their stabilizing components which include the axonal cytoskeleton at nodes, Schwann cell microvilli and paranodal axo-glial junctions. This disruption would significantly lower the safety factor of impulse transmission causing muscle weakness in the acute phase of clinical illness. As the clinical course progresses into the early recovery phase, complement levels decreased but macrophage invasion was noted to be more prominent. This suggests that complement activation is crucial in acute nerve injury and macrophages are the scavengers that remove the injured nerve by-products. The sequential finding of complement activation followed by macrophage recruitment is compatible with the autopsy findings in AMAN patients. (Hafer-Macko et al., 1996) In severe cases, axonal degeneration can also occur.
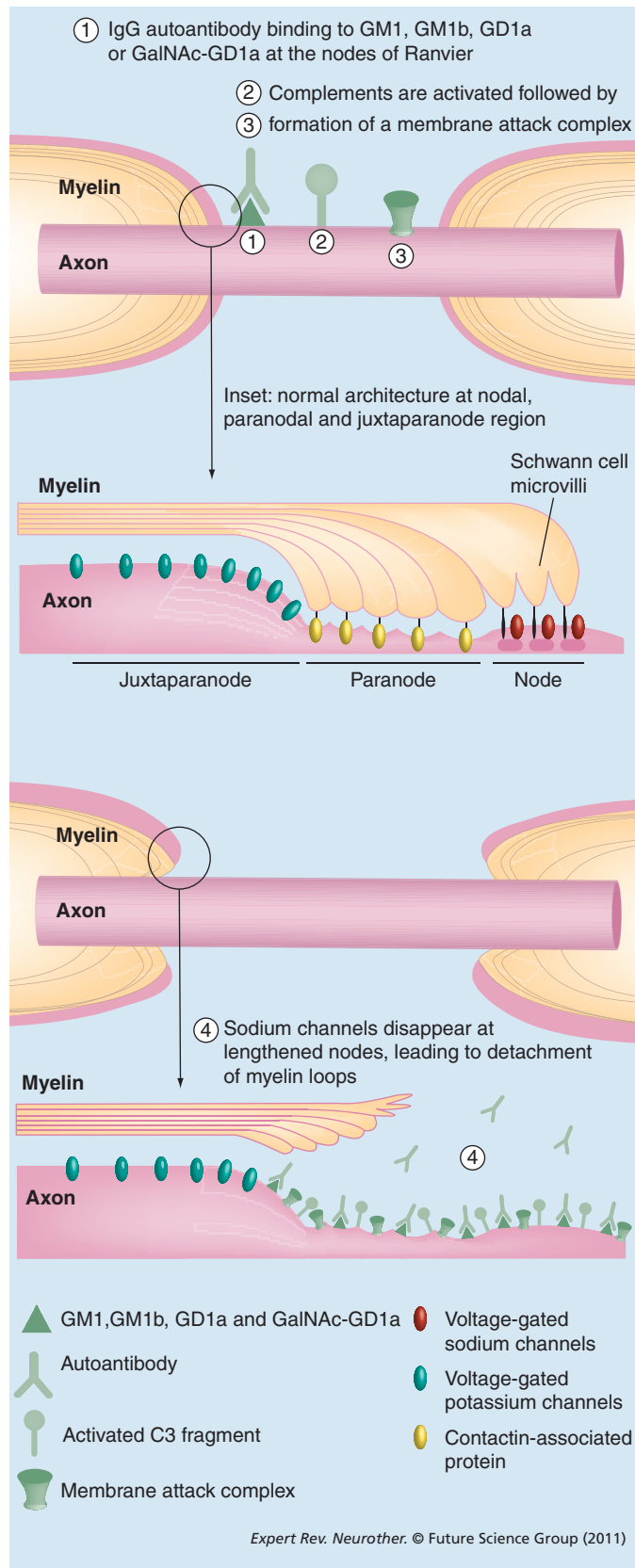
Figure 2.4: The immunopathogenesis of acute motor axonal neuropathy. Figure reproduced from Shahrizaila&Yuki. Antiganglioside antibodies in Guillain-Barré syndrome and its related conditions. *Expert Rev. Neurother*. 11(9), 1305–1313 (2011).

2.5.2   MFS and BBE

Chiba and his colleagues identified IgG autoantibodies against GQ1b in patients with MFS and proposed these autoantibodies as a diagnostic marker of MFS. (Chiba et al., 1992) In BBE, the association of IgG anti-GQ1b antibodies with BBE was first found in a patient with acute ophthalmoplegia, ataxia and areflexia who was initially comatose but made a complete recovery two months following the onset of her illness. (Yuki, Sato, et al., 1993)

Patients with MFS and BBE present with an antecedent episode prior to the onset of their neurological symptoms. Epidemiological association between *C jejuni* and *H influenza* infections have been established in patients with MFS (Koga, Yuki, Tai, & Hirata, 2001) and a study looking at the serological evidence of infection in BBE and MFS patients found *C jejuni* and *H influenza* to be the two most common in this group of patients. (Ito et al., 2008) Molecular mimicry whereby the LOS of *C jejuni* isolated from MFS or BBE patients mimic the GQ1b has been demonstrated. (Kimoto et al., 2006; Koga et al., 2005) Other mimics include the GQ1b-like lipo-oligosaccharide of *H influenza* isolated from an MFS patient. (Houliston et al., 2007) Therefore, it is likely that the infectious agents of patients with MFS or BBE carrying various GQ1b mimics induce the production of IgG anti-GQ1b antibodies leading to the development of the disease.

Immunohistochemical studies show that GQ1b is highly expressed in the extramedullary regions of the human oculomotor, trochlear and abducens nerves. (Chiba et al., 1993) Neuromuscular junctions may be particularly vulnerable to autoantibody attack as they are outside the blood–nerve barrier, and monoclonal anti-GQ1b antibody has been shown to bind to motor endplates of human oculomotor muscles. (Liu, Willison, & Pedrosa-Domellof, 2009) Postural body sway analysis results suggest that

MFS patients also have a dysfunctional proprioceptive afferent system, and ataxia is caused by the selective involvement of muscle spindle afferents. (Kuwabara et al., 1999) These muscle spindles contain specialized muscle fibers, which have motor innervations enriched with sensory endings. It is likely that the neural components and intrafusal muscle fibers of these spindles are important targets in MFS because they have also been labeled by monoclonal anti-GQ1b antibodies in humans. (Liu et al., 2009) The muscle spindles are the likely underlying cause of ataxia experienced by MFS patients. This would also explain the good outcome seen in MFS and BBE patients who typically show recovery with no sequelae. (Ito et al., 2008; Mori et al., 2001)

In BBE, the characteristic distinguishing clinical feature is altered consciousness which is central in origin. Although the evidence is lacking, it is postulated that the breakdown of the blood-brain barrier at vulnerable sites such as the area postrema or blood-nerve barrier at the roots of the oculomotor cranial nuclei allow access to anti-GQ1b antibodies. This is followed by autoantibody binding to its related sites within the brainstem reticular formation resulting in altered consciousness.

To summarise, the possible mechanism underlying the pathogenesis of MFS and BBE is as follows: (i) infection by a micro-organism expressing GQ1b-like epitope triggers the production of IgG anti-GQ1b antibodies, (ii) these antibodies bind to GQ1b which are highly expressed on the oculomotor nerves and group 1a muscle spindles producing MFS, (iii) in some patients, these antibodies can also penetrate deficient sections of the blood brain barrier, binding to GQ1b that may be expressed in the reticular formation, thus causing BBE. (Yuki, 2009)

2.6   Treatment and Prognosis in GBS

Current evidence from clinical trials in GBS recommend that patients who present within 2 weeks of the onset of illness and are not able to walk unaided should receive either plasma exchange or intravenous immunoglobulins (IVIG), both of which will hasten their recovery and improve their outcome. (Shahrizaila & Yuki, 2011d) Despite the efficacy of both treatments, there continues to be an associated mortality and severe disability ranging from 9% and 17%. Studies have also suggested that patients with GBS associated with IgG anti-GM1 antibodies may do better with IVIG as compared to plasma exchange. (Jacobs et al., 1996; Kuwabara, Asahina, et al., 1998) The clinical presentations in GBS are heterogeneous and it is likely that there are situations when the existing recommendations could be modified to provide the best treatment options. Clinical prognostic scales in GBS have an important role in rationalising the treatment options to different cohorts of patients based on their prognosis.

The Erasmus GBS Outcome Score (EGOS) was the first validated prognostic scale in GBS which provided a simple clinical scoring system which could be applied to GBS patients at 2 weeks after hospital admission. (van Koningsveld et al., 2007) EGOS relied on the use of age, the presence of diarrhea and the disability functional score as variables that can accurately predict the chances of walking independently at 6 months. The Rotterdam group subsequently improved on their original scale to identify GBS patients with a poor prognosis at an even earlier phase of their illness. (Walgaard et al., 2011) The modified EGOS (mEGOS) assess GBS patients at admission as well as day 7 of hospital admission using similar predictive variables which are age, preceding diarrhea and disease severity. However, the disease severity is indicated by the Medical Research Council (MRC) sum score rather than the functional grading scale used in the original EGOS. The outcomes measured were also expanded to include functional abilities at 4 weeks, 3 months and 6 months. This is important in its application to

therapeutic trials of selective treatment modalities in patients predicted to have a poor prognosis. In turn, this could potentially impact our clinical approach and future management of this group of patients.

One of the potential drawbacks of mEGOS, which the authors acknowledge, is its utility in non-Caucasian populations. Studies have suggested that GBS subtypes differ between the Western and Asian populations. AIDP is more frequent in the Western population whereas AMAN predominates in Asia. However, there remains a possibility that the incidence of AMAN is underestimated in the West if serial nerve conduction studies are not performed to detect reversible forms of AMAN such as AMCBN. (Kuwabara, 2010) The mEGOS was derived from the Dutch Caucasian population and one could argue that its use may be restricted to the AIDP subtype. Studies of the mEGOS and EGOS in other GBS populations will be important to clarify its validity in these cohorts, including a retrospective look at its use in other existing GBS databases. (Shahrizaila & Yuki, 2011c)

There are other recognised prognostic factors in GBS. Nerve conduction studies form part of the diagnostic process in GBS and serial studies are important to further classify the GBS subtypes. Evidence of inexcitable nerves and axonal degeneration on neurophysiology are associated with a poor prognosis. (Hadden et al., 2001) Serological evidence of *C jejuni* or CMV infections have also been associated with a less favourable outcome. (Hadden et al., 2001; Visser et al., 1996) Patients with a poor prognosis also had positive IgG antibodies against GM1, GM1b, GD1a or GalNAc-GD1a. (Jacobs et al., 2008) In GBS patients who have received IVIG as part of their treatment, those who had a small rise in serum IgG levels had a worse outcome compared to those who had a significant rise in their IgG levels. (Kuitwaard et al., 2009). This group of GBS patients with poor prognosis may benefit from a second course or a higher dose of IVIG.

CHAPTER 3: PUBLISHED WORKS

In this chapter, five original works in which the candidate is the principal author are included. Each publication is in the original published format of the respective journals. Each publication is preceded by declaration of the contribution of the co-authors.

3.1 Publication 1

Serial nerve conduction studies provide insight into the pathophysiology of Guillain-Barré and Fisher syndromes. Shahrizaila N, Goh KJ, Kokubun N, Abdullah S, Yuki N. Journal of the Neurological Sciences. 2011 Oct 15;309(1-2):26-30.

3.1.1 Contribution of co-authors:

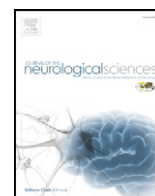|	
Design and concept of study	N Shahrizaila, N Yuki
Acquisiton of data	N Shahrizaila
Analyses of data	N Shahrizaila
Drafting of manuscript	N Shahrizaila
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Serial nerve conduction studies provide insight into the pathophysiology of Guillain–Barré and Fisher syndromes

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ABSTRACT

The electrodiagnosis of Guillain–Barré syndrome (GBS) can be broadly divided into acute inflammatory demyelinating polyneuropathy (AIDP) and acute motor axonal neuropathy (AMAN). Fisher syndrome (FS) is a variant of GBS, although the underlying neuropathy of FS has yet to be established. Serial nerve conduction studies (NCS) can provide further insight into the likely pathophysiology by further subtyping of GBS and FS. We present a patient with an initial diagnosis of AIDP in whom repeated NCS revealed the AMAN variant. This led us to investigate serial NCS in five patients with GBS, FS and FS/GBS overlap presenting over a period of a year. Three patients with AIDP showed a gradual increase in distal motor latencies during the acute phase of illness. NCS of two patients with FS and FS/GBS overlap showed no demyelinating features suggesting underlying axonal neuropathy in this group of patients. The importance of serial NCS in establishing the underlying pattern of neuropathy in GBS and FS is further emphasized in this study. Larger studies incorporating serial NCS are required to confirm the observations seen in our case series especially when pathological studies are often not justified in this group of patients.

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1. Introduction

Guillain–Barré syndrome (GBS) can be broadly divided into two subtypes, acute inflammatory demyelinating polyneuropathy (AIDP) and acute motor axonal neuropathy (AMAN) [1]. Fisher syndrome (FS) is widely accepted as a variant of GBS presenting with its own set of unique clinical features of ataxia and ophthalmoplegia. In recent years, our understanding of AMAN has improved in leaps and bounds with the identification of the serological markers (IgG antibodies against GM1, GM1b, GD1a or GalNAc–GD1a) and proof of molecular mimicry with *Campylobacter jejuni* in some cases of AMAN [2]. There continues to be some debate as to whether FS is a demyelinating or axonal disease [3,4]. FS is strongly associated with IgG anti-GQ1b antibodies, and *C. jejuni* isolates from FS patients carry GQ1b epitope [5]. The ganglioside-like lipo-oligosaccharide (LOS) in *C. jejuni* strains are synthesized by *Campylobacter* sialyltransferase (Cst-II) encoded by *cst-II*. The genetic polymorphism of *cst-II* influences the ganglioside-like LOS that is expressed which in turn produces either AMAN or FS in susceptible patients suggesting that the pathophysiology of FS is not demyelinating, but axonal.

The clinical features and electrodiagnosis of AMAN have seen a lot of change in recent years with reports of reversible conduction block [6,7]. Reports of patients with FS overlapped by GBS (FS/GBS overlap) have also facilitated in the better understanding of FS [8,9]. It is increasingly recognized that serial nerve conduction studies (NCS) have played an important role in our improved understanding of GBS. In this article, we describe a case that was initially diagnosed as AIDP in whom repeated NCS revealed acute motor conduction block neuropathy (AMCBN). This prompted us to look at serial NCS of a further five patients who presented within a period of a year with GBS, FS or FS/GBS overlap.

2. Materials and methods

2.1. Patients

Eight patients with GBS (n=4), FS (n=2) or FS/GBS overlap (n=2) presented to University Malaya Medical Centre between April 2010 and February 2011. The diagnosis of GBS was made based on a history of progressive weakness within a period of four weeks affecting more than one limb associated with hyporeflexia or areflexia [10]. FS

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was diagnosed based on the clinical presentation of ophthalmoplegia, ataxia and hyporeflexia/areflexia without limb weakness [11]; whereas, FS/GBS overlap was diagnosed when there was associated significant limb weakness [8]. Six of the eight patients consented to having serial NCS performed on them. The study was approved by the hospital Medical Ethics Research Committee.

2.2. Nerve conduction studies

NCS were performed using the Medelec™ Synergy EMG machine. At least two limbs were assessed; four motor nerves and three sensory nerves as well as F wave latencies. Nerve stimulation and recorded compound motor action potentials (CMAPs) were as follows: median nerve was stimulated at the wrist and elbow, recording over abductor pollicis brevis muscle; ulnar nerve was stimulated at the wrist, below elbow and above elbow, recording over abductor digiti minimi muscle; tibial nerve was stimulated at the ankle and popliteal fossa, recording over the abductor hallucis muscle [12]. Sensory studies of the median and ulnar nerves were performed by using the orthodromic method of stimulating the index finger and little finger respectively and the sensory nerve action potentials (SNAPs) recorded over the wrist crease. The radial and sural nerves however, were recorded using the antidromic method. The radial nerve was stimulated at the forearm and recorded over the anatomical snuffbox whereas sural nerve was stimulated at the calf and recorded below the lateral malleolus. Reference values were derived from NCS performed on normal patients at our laboratory. The electrodiagnosis of AIDP or AMAN was made based on the electrodiagnostic criteria set by Ho et al. [13].

2.3. Enzyme-linked immunosorbent assay

Sera samples were obtained at acute progressive phase of the illness, and measured for IgG and IgM antibodies to GM1, GM1b, GD1a, GalNAc–GD1a, GD1b, GT1a and GQ1b, as described elsewhere [14]. In brief, serum samples diluted to 1:500 were placed in separate microtiter plate wells. The mean value for triplicate reference wells without antigen was subtracted from the mean value for triplicate wells of each sample, and the optical density assessed. An optical density of more than 0.5 was judged to be positive. Using the strict cut-off value, sera from patients with acute transverse myelitis (n = 9), acute disseminated encephalomyelitis (n = 46) and multiple sclerosis (n = 44) were negative for those anti-ganglioside antibodies.

3. Results

3.1. Clinical features

Of the six patients, four fulfilled the clinical criteria for GBS, one for FS and one for FS overlapped by GBS (FS/GBS overlap). We describe two interesting cases; one of GBS and one of FS/GBS.

3.1.1. Patient 4

A 25-year-old Malaysian Indian male presented with a 2-day history of progressive bilateral upper and lower limb weakness. A week before his presentation, he described having a sore throat. He denied any sensory symptoms. He was admitted on Day 3 of his illness when he was unable to mobilize independently. His upper limb power was MRC grade 4 for shoulder abduction and MRC grade 2 in the first dorsal interossei and abductor pollicis brevis. In the lower limbs, power was MRC grade 3 for hip flexion, 3 for knee flexion and 2 for ankle dorsiflexion bilaterally. His tendon reflexes were depressed. His sensory examination was normal. CSF analysis on the day of admission showed albumino-cytological dissociation with protein of 1.15 g/L (normal, less than 0.45 g/L) and no leucocytes. He was treated with intravenous immunoglobulin. On Day 7, he was able to walk independently although some of his muscles were still weak. The MRC grades of his

limb muscles were as follows bilaterally: shoulder abduction 5, abductor pollicis brevis 3, first dorsal interossei 3, hip flexion 4+, knee flexion 4 and dorsiflexion 3. On further review of his muscle power on Day 20, these were all normal apart from a slight weakness of his left abductor pollicis brevis and first dorsal interossei to 4.

3.1.2. Patient 6

A 61-year-old Malay female presented with a week's history of numbness in her hands and feet, unsteadiness and visual blurring. She described a history of a dry cough occurring a week before the onset of her neurological symptoms. She denied having any diarrhoea. On Day 3, her neurological symptoms had progressed and she was no longer able to mobilize and was confined to her bed. On Day 7, she was alert. There was complete ophthalmoplegia and her pupils were dilated at 5 mm and unreactive to light. Facial muscle power was intact. Her upper limb power was reduced to MRC grade 4 in shoulder abduction and 3 in abductor pollicis brevis and first dorsal interossei. In the lower limbs, her hip flexion was reduced to 4 and the rest of her muscle power was intact. Her tendon reflexes were absent throughout and plantar responses were flexor bilaterally. There was also reduced pinprick up to the elbows in the upper limbs and midthighs in the lower limbs. Proprioception was intact in the lower limbs but reduced in the upper limbs up till the wrists. She was markedly ataxic with evidence of truncal ataxia (she could not sit unsupported) as well as upper and lower limb ataxia. CSF analysis on the day of admission showed a raised protein of 0.78 g/L with no leucocytes. She was treated with immunoglobulin. By Day 17, she was able to sit unsupported and her muscle power had recovered to MRC grade 5 apart from the right APB which was grade 4. There was also now vertical and horizontal eye movement although lateral abduction was still weak. Her pupils were also responding to light.

3.2. Nerve conduction studies

NCS were performed in all six patients, and the results are shown in Table 1. Three of the four GBS patients (Patients 1, 2 and 3) fulfilled the electrophysiological criteria for AIDP. Their subsequent NCS showed prolongation of distal motor latencies (DMLs) within the first 21 days of their illness.

In Patient 4, the first NCS done on Day 5 fulfilled the criteria of AIDP based on the presence of demyelinating features of prolonged DMLs in two or more nerves. There was also evidence of conduction block in both the median and ulnar nerves. Sensory studies were within normal limits. A repeat NCS done on Day 20 showed complete recovery of the DMLs to within normal limits and also recovery of the conduction block in some nerves. The F waves also reappeared although delayed in some nerves. By Day 55, the NCS was back to within normal limits. The representative waveforms are shown in Fig. 1A.

Patient 5 had FS and the first study performed on Day 3 of her illness showed abnormal SNAPs with reduced amplitudes but these recovered to within normal limits at the second NCS on Day 128. Patient 6 had FS/GBS overlap and her initial NCS showed no demyelinating features but the motor CMAPs were reduced in amplitude with preserved conduction velocities along with absent SNAPs. A second NCS a week later showed improvement in the CMAP amplitudes although the SNAPs remained absent. On Day 31, the sensory potentials reappeared in some nerves and the motor CMAPs were within normal limits. The SNAPs were present in all nerves at Day 90. The representative waveforms are shown in Fig. 1B.

3.3. Anti-ganglioside antibodies

Sera from five patients (Patients 1, 2, 3, 4 and 6) were available for anti-ganglioside testing. Patient 4 had IgG antibodies against GM1, GD1a, GalNAc–GD1a and GD1b in serum obtained on Day 5. Each

Table 1
Nerve conduction study results.

			Patient 1			Patient 2		Patient 3				Patient 4			Patient 5		Patient 6			
Clinical diagnosis			GBS			GBS		GBS				GBS			FS		FS/GBS			
Neurophysiological diagnosis			AIDP			AIDP		AIDP				AMCBN			FS		FS/AMAN			
Age (years), gender			36, male			39, female		34, male				25, male			71, female		61, female			
Ethnic group			Chinese			Chinese		Malay				Indian			Chinese		Malay			
Motor studies			Day 3	Day 10	Day 103	Day 5	Day 70	Day 7	Day 14	Day 21	Day 70	Day 5	Day 20	Day 55	Day 3	Day 128	Day 10	Day 17	Day 31	Day 90
Median	DML (ms)		**16.1**	**Absent**	**6.8**	**19.5**	**7.7**	**5.8**	**6.8**	**7.3**	4.5	**5.2**	**2.9**	2.9	3.3	3.2	4.3	3.8	4.1	3.6
	CMAP (mV)	Wrist	0.5		11	2.6	7.4	1.6	4.2	5.5	12.0	**8**	**11.3**	**12.2**	12.4	9.9	**4.9**	**5.9**	**7.2**	**8.2**
		Elbow	0.4		11	2.1	7.5	0.9	3.2	4.1	11.5	**3.9**	**7.1**	**11.4**	10.4	7.7	**4.2**	**5**	**6.5**	**7.9**
	CV (m/s)		42		50	49	46	49	50	44	52	51	54	54	64	61	53	54	47	51
	F wave (ms)		Absent		35.3	Absent	34.2	Absent	Absent	Absent	26.2	**38.1**	31.3	30.5	24.0	23.7	27.6	26.5	27.6	25.4
Ulnar	DML (ms)		3.4	Absent	3.5	**4.9**	**5.1**	3.7	4.2	5.3	3.1	**4.2**	**2.4**	**2.4**	2.0	2.0	2.4	2.2	2.5	2.1
	CMAP (mV)	Wrist	3.8		5.5	2.2	5.7	1.9	4.0	4.2	7.5	**2.3**	**8.5**	**10.2**	6.4	5.2	**6.1**	**8.3**	7.7	8.1
		BE	2.6		4.4	1.6	5.0	0.7	1.9	1.7	7.1	**1.4**	**6.2**	**9.4**	6.0	4.0	**6.4**	**8.1**	7.4	7.7
		AE	nd		4.7	nd	4.1	0.5	1.0	1.3	7.0	**0.2**	**3.7**	**8.8**	6.2	4.5	**6.2**	**5.8**	7.3	7.7
	CV (m/s)	Wrist-BE	55		46	44	55	40	26	34	47	48	64	58	64	64	57	55	48	54
		BE-AE	nd		37	nd	37	40	27	21	31	**18**	**42**	**55**	60	57	50	59	49	50
	F wave (ms)		Absent		35.9	Absent	33.4	Absent	Absent	Absent	34.5	Absent	32.9	26.0	25.8	24.3	25.7	25.5	25.7	24.7
Tibial	DML (ms)		**6.3**	**8.7**	5.1	Absent	6.0	**6.8**	**9.1**	**10.8**	6.4	**8.8**	**3.8**	**3.8**	3.8	3.2	6.7	5.7	4.9	4.8
	CMAP (mV)	Ankle	7.3	1.3	5.9		2.9	0.8	1.1	1.5	3.5	**0.7**	**2.3**	4.5	7.2	7.6	11.7	10.6	12.9	12.5
		PF	4.6	0.5	3.8		2.3	0.2	0.5	0.7	1.7	**0.5**	**0.3**	**0.4**	6.0	6.9	8.1	9.5	11.1	11.9
	CV (m/s)		42	32	42		39	38	41	33	44	40	44	44	45	55	37	41	44	44
	F wave (ms)		Absent	Absent	64.4		49.7	Absent	Absent	Absent	Absent	Absent	63.2	57.1	48.2	41.2	54.1	52.9	53.2	52.3
Sensory studies																				
Median	SNAP (µV)		Absent	Absent	Absent	Absent	Absent	1.3	2.0	0.5	4.1	20.8	12.4	19	**3.7**	**11.0**	**Absent**	**Absent**	**Absent**	6.8
Ulnar	SNAP (µV)		Absent	Absent	2.4	Absent	Absent	Absent	Absent	Absent	4.8	15.9	10.2	6.3	**3.3**	**14.1**	**Absent**	**Absent**	**Absent**	3.5
Radial[a]	SNAP (µV)		17.2	Absent	17.1	Absent	11.6	22.3	20.4	24.3	nd	45.8	nd	nd	nd	35.7	**Absent**	**Absent**	14.4	**41.8**
Sural[a]	SNAP (µV)		8.9	1.1	10.2	4.2	11.3	7.3	3.0	Absent	9.1	11	20.2	18	14.4	21.6	**Absent**	**Absent**	2.5	**5.9**

DML = distal motor latency; CMAP = compound muscle action potential; CV = conduction velocity; SNAP = sensory nerve action potential; BE = below elbow; AE = above elbow; PF = popliteal fossa; nd = not done. Serial studies were performed on the same side.
The abnormal findings are underlined and the significant changes discussed in the article are highlighted in bold.

[a] Antidromic study.

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antibody titer decreased on Day 20 and a further reduction was seen on Day 55. Anti-ganglioside antibodies were not detected in the other patients.

4. Discussion

Understanding the pathophysiology of GBS and FS provides a means of improving the treatment and prognosis of these conditions. NCS are useful as they offer insight into the underlying pathogenesis especially when pathological studies are often not justified. In 1990, Asbury and Cornblath [10] discussed the need for sequential NCS when the initial study failed to reveal the diagnosis. However, this was in recognition of the normal or minimally abnormal studies that were seen in the early phase of illness in up to 14% of GBS patients. More recently, the rationale for serial NCS has changed as it has become

increasingly recognized that a single NCS does not necessarily provide the correct electrodiagnostic diagnosis [7,15,16]. Several studies have shown early NCS features that were compatible with demyelination such as conduction block, prolonged F waves and prolonged DMLs can rapidly resolve on subsequent NCS to uncover forms of axonal neuropathy [7,17].

In the current study, we performed serial NCS on six patients with GBS, FS or FS/GBS overlap. Three patients with AIDP (Patients 1,2 and 3) showed typical features of gradual progression in their DMLs [18]. One patient (Patient 4) had features of "demyelination" such as conduction block and prolonged DML on the initial NCS. Although the sensory amplitudes were variable, they were always within normal limits. The patient went on to recover within a week after receiving immunoglobulin and subsequent NCS two weeks later showed resolution of conduction block and distal motor latencies returned
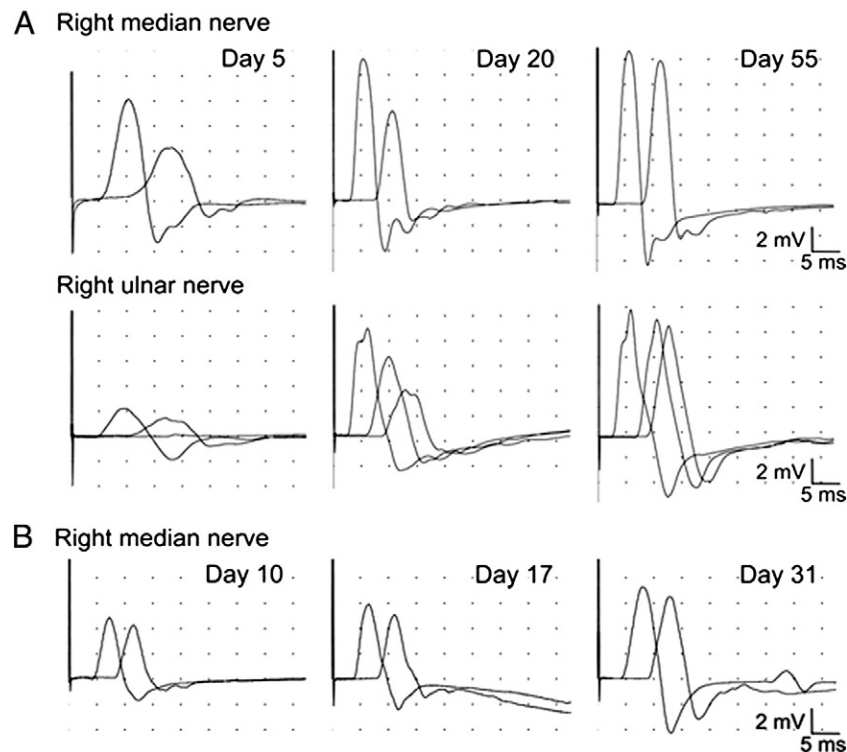
Fig. 1. (A) Serial motor conduction studies of the right median and ulnar nerves in Patient 4. Day 5 shows reduced CMAP amplitudes for both median and ulnar nerves. In particular, proximal stimulation of the right ulnar above the elbow shows conduction block. Day 20 shows improvement in CMAP amplitude and distal motor latencies for both median and ulnar nerves. The study was within normal limits at Day 55. (B) Serial motor conduction studies of the right median nerve in Patient 8. The median CMAP amplitudes were reduced on Day 10 but showed gradual improvement in the subsequent studies on Days 17 and 31.

to within the normal ranges. The rapid resolution of his motor studies along with normal sensory findings would be in keeping with the AMCBN subtype of AMAN which is associated with IgG antibodies against GM1, GD1a, GalNAc–GD1a and GD1b in the acute sera of this patient [6,7]. Our current observations are in keeping with some of the previously reported studies of serial NCS in GBS patients where repeated NCS have led to a significant change to the initial electrodiagnostic criteria. Hiraga et al. [15] described patients with demyelinating features of absent F waves and prolonged DMLs who were initially diagnosed with AIDP. These features resolved at the second test in keeping with reversible conduction failure and they argued that a repeat NCS at 3–6 weeks would be required before making an electrodiagnostic conclusion in GBS. Similarly, Uncini et al. [16] also made a change to their classification to an axonal subtype in 24% of patients. In contrast, Hadden et al. [19] who performed two NCS in their GBS patients did not report a significant change in the overall classification. However, at the time, the possibility of reversible conduction failure in axonal neuropathy was not a recognized phenomenon.

In human and rabbit AMAN, the earliest and mildest pathological changes consisted of lengthening of the nodes of Ranvier with distortion of the paranodal myelin, and in some instances with breakdown of the outermost myelin terminal loops [20,21]. In the spinal anterior roots of AMAN rabbits, IgG antibodies bind to nodes where GM1 is highly expressed. This autoantibody binding activates complement resulting in the formation of membrane attack complex. There is then disappearance of sodium channels, detachment of terminal myelin loops and lengthening of the nodes of Ranvier. The nodal and paranodal changes caused by autoantibody binding and complement activation are novel mechanisms by which there is motor nerve conduction failure and muscle weakness in AMAN. This cascade of pathological changes in particular the alteration of sodium channels may explain the "failed" conduction seen in AMAN. In cases

where this conduction failure is reversible, rapid electrophysiological improvement can be demonstrated on subsequent electrophysiology. It remains debatable if the "reversibility" seen in AMCBN is the natural evolution of the disease or a result of immunotherapy. In most published reports describing either AMCBN [6] or reversible conduction failure [7], patients have received some form of immunotherapy. In the latter study, one patient with reversible conduction failure refused immunotherapy as she was pregnant and spontaneous rapid resolution of conduction block was observed.

There have been conflicting reports as to the underlying pathophysiology of FS. Jamal and Ballantyne [4] reported demyelination neuropathy based on serial studies of three patients, whereas Fross and Daube [3] argued an axonal pattern based on studies of 10 patients. In the former study, the diagnosis of demyelinating neuropathy was made based on initial absent or prolonged F waves. The authors also noted that although the DML and conduction velocities were within normal limits in the initial study, an improvement in both DML and conduction velocity supported a demyelinating process. We now know that these are features that can be seen in the less extensive forms of AMAN such as AMCBN. In the latter study, Fross and Daube noted that the sensory nerves were predominantly more affected than the motor nerves in the limbs of FS patients. These findings were also observed in our FS patient (Patient 5) who had reduced SNAP amplitudes at initial NCS which recovered when the study was repeated three months later. Two of the 10 patients described by Fross and Daube had serial NCS which showed a progressive decline in the CMAP and SNAP amplitudes. These findings suggested to them an axonal pattern of disease in their FS patients.

In addition to ophthalmoplegia, ataxia and hyporeflexia, some patients also exhibit limb muscle weakness and can go on to develop ventilatory failure [8]. This group of patients is thought to have FS overlapped by GBS. In contrast to FS, immunotherapy is warranted in these patients who run the risk of developing the associated morbidity

and mortality of GBS. The fact that FS and GBS can occur in unison suggests that the two share a common pathophysiological process. Serial NCS in this group of patients can shed further light on the underlying neuropathic process. In our patient with FS/GBS overlap (Patient 6), serial NCS showed fairly rapid recovery of the initial low amplitude CMAPs. In comparison, SNAPs were absent in our patient at initial assessment but reappeared in some nerves after one month and present in all by 3 months. These neurophysiological findings would support an axonal pattern of neuropathy and a similar pathophysiology to AMAN as described earlier exists in FS and FS/AMAN.

The neurophysiology from previous reports of FS/GBS overlap cases also support an axonal pattern of neuropathy, suggesting that FS overlaps specifically with AMAN [8,9,22,23]. At the time, Katsuno et al. [22] as well as Uncini and Lugaresi [23] referred to this group of patients as FS with tetraparesis. Katsuno et al. [22] compared motor conduction findings in four FS patients with severe tetraparesis to five patients without tetraparesis as well as 14 GBS patients. They found that the tetraplegic FS patients had lower CMAP amplitudes compared to non-paretic FS but otherwise had none of the demyelinating features that were seen in 10 of their 14 GBS patients. This suggests their "tetraparetic" FS patients were more in keeping with FS overlapped by AMAN rather than AIDP. Uncini and Lugaresi [23] described reversible conduction block in the serial study of their patients but at the time argued a presynaptic neuromuscular pathology mediated by anti-GQ1b antibodies. As we have described earlier, conduction block is seen with AMCBN suggesting that their case was probably FS with AMCBN overlap. The pathophysiology is more likely to be due to reversible antibody binding at the nodal and paranodal sites rather than neuromuscular junction. Funakoshi et al. [8] reported that six FS/GBS overlap patients but was able to electrophysiologically confirm AMAN in two of them. The other four patients were noted to have absent F waves but otherwise no other demyelinating features to suggest AIDP. More recently, Rajabally et al. [9] reported a similar pattern of reversible motor and sensory conduction failure in two patients. However, the repeated neurophysiology studies in their patients were performed almost three months apart making it difficult to further comment on the rate of recovery of the electrophysiological abnormalities. In the current study, our data support a recovery within a week of immunotherapy with corresponding clinical improvement in our FS/GBS overlap patient.

Although making a clinical diagnosis of GBS and FS can be relatively straightforward, the neurophysiological diagnosis continues to be challenging. The current study adds to the growing literature emphasizing the importance of serial NCS in forming the final electrodiagnosis in patients with GBS. Serial NCS also provide invaluable insight into the possible underlying pathophysiology of GBS and FS and future studies involving larger GBS and FS patient populations are required to formulate more accurate neurophysiological criteria than currently exists.

Acknowledgements

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3.2   Publication 2

Two sets of nerve conduction studies may suffice in reaching a reliable electrodiagnosis in Guillain-Barré syndrome. Shahrizaila N, Goh KJ, Abdullah S, Kuppusamy R, Yuki N. Clinical Neurophysiology. 2013 Jul;124(7):1456-9.

3.2.1   Contribution of co-authors:

Design and concept of study	N Shahrizaila
Acquisiton of data	N Shahrizaila
Analyses of data	N Shahrizaila
Drafting of manuscript	N Shahrizaila
Revising manuscript for intellectual content	All authors
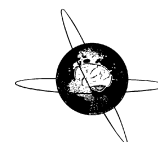
Two sets of nerve conduction studies may suffice in reaching a reliable electrodiagnosis in Guillain–Barré syndrome

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ARTICLE INFO

HIGHLIGHTS

- The existing electrodiagnostic criteria in Guillain–Barré syndrome are unreliable when applied at the initial stages of disease onset.
- Nerve conduction studies performed at two time courses, within 2 weeks and 3–8 weeks, may better reflect the final electrodiagnosis of Guillain–Barré syndrome.
- The pattern of recovery in Guillain–Barré syndrome is heterogeneous and validation of the current Erasmus Guillain–Barré syndrome outcome score in different patient populations is required.

ABSTRACT

Objective: Recent studies have advocated the use of serial nerve conduction studies (NCS) in the electrodiagnosis of Guillain–Barré syndrome (GBS). The current study aims to elucidate when and how frequent NCS can be performed to reflect the disease pathophysiology.
Methods: A prospective study of GBS patients documenting the initial and final electrodiagnoses following serial NCS performed at three time intervals: 1–2 weeks, 3–8 weeks and 8–12 weeks.
Results: Twenty-one patients were recruited over a period of 2 years. Electrodiagnosis within 2 weeks revealed 17 acute inflammatory demyelinating polyneuropathy; two acute motor axonal neuropathy and two unclassified. After 12 weeks the final diagnoses were: 12 acute inflammatory demyelinating polyneuropathy; seven acute motor axonal neuropathy and two unclassified. NCS performed within the 3–8 week period reflected the true electrodiagnosis. Patients with acute inflammatory demyelinating polyneuropathy had persistent demyelination features at the 8–12 week NCS.
Conclusion: Two sets of NCS performed within the first 2 weeks and between 3–8 weeks of disease onset is likely to suffice in elucidating the true electrodiagnosis of GBS.
Significance: These findings can be incorporated into a much-needed revision of the existing GBS electrodiagnostic criteria.

1. Introduction

Guillain–Barré syndrome (GBS) has classically been described as an acute ascending paralysis with areflexia, associated with antecedent infective illness and cerebrospinal fluid (CSF) albuminocytological dissociation (Guillain et al., 1916). GBS can be classified into demyelinating and axonal subtypes. Nerve conduction studies (NCS) play an important role in establishing these subtypes. The commonly used electrodiagnostic criteria are those by Ho et al. and Hadden et al. (Ho et al., 1995; Hadden et al., 1998),

which were derived in the mid to late 1990's. However, in recent years, it has been demonstrated that there may be flaws in these criteria especially when applied at the early stages of the disease (Uncini and Kuwabara, 2012). Early changes on NCS can mimic demyelination in certain axonal subtypes such as acute motor conduction block neuropathy (Kokubun et al., 2010; Capasso et al., 2003). Serial NCS are advocated when establishing the electrodiagnosis of GBS. However, it is less clear as to when and how frequent subsequent NCS should be performed to allow a more accurate electrodiagnosis of GBS to be made. In the current study, patients with GBS were prospectively recruited and serially assessed through clinical examination and NCS. The primary objective was to elucidate the optimum timeframes that best correlated with the final electrodiagnosis. We also compared the predicted prognostic score of each patient based on a recent validated GBS

prognostic scale, Erasmus GBS outcome score (EGOS) (van Koningsveld et al., 2007; Walgaard et al., 2011), against the actual clinical outcome in our cohort of patients.

2. Materials and methods

2.1. Patients

Patients presenting with a diagnosis of GBS to the University Malaya Medical Centre in Kuala Lumpur, Malaysia were prospectively recruited between June 2010 to November 2012. The study received ethics approval from the University of Malaya Medical Centre Medical Research Ethics Committee. A diagnosis of GBS was made based on published criteria (Asbury and Cornblath, 1990). In brief, patients were included when they presented with progressive, relatively symmetrical motor weakness, involving more than one limb, which may also be associated with facial and bulbar palsies as well as external ophthalmoplegia. Disease nadir must be reached by 4 weeks and areflexia or at least, distal hyporeflexia during the course of illness was required. Details of antecedent infections, clinical symptoms and signs, number of days to nadir, Hughes functional grade scores at nadir, EGOS at 2 weeks, modified EGOS at admission and at one week and true clinical outcome were documented. Serial NCS were performed by one of the authors (NS). NCS were performed at three time periods when permitted; within 2 weeks of illness (which is represented by NCS performed soon after admission); between 3 and 8 weeks of illness onset and between 8 and 12 weeks of illness onset.

2.2. Nerve conduction studies

Nerve conduction studies were performed as described elsewhere (Shahrizaila et al., 2011). At least 2 limbs were assessed; 4 motor nerves and 3 sensory nerves as well as F wave latencies. Reference values were derived from NCS performed on normal subjects at our laboratory. The electrodiagnosis of AIDP or AMAN was made based on the existing electrodiagnostic criteria (Ho et al., 1995; Hadden et al., 1998). AMSAN was diagnosed when there was a reduction in sensory nerve action potential amplitude by 50% of the lower limit of normal in at least 2 nerves (Feasby et al., 1993; Rees et al., 1995).

2.3. Serological analyses

Serum IgG antibodies to gangliosides GM1, GM1b, GD1a, GalNAc-GD1a, GD1b, GT1a, and GQ1b were measured by enzyme-linked immunoabsorbent assay, as described elsewhere (Yuki et al., 1997). In the present study, serum was considered positive when the optical density was 0.5 or more at a 1:500 dilution. Sera was obtained from patients at admission and before immunotherapy was instituted.

3. Results

3.1. Patient characteristics

During the current study period, 21 patients were recruited who fulfilled the criteria of GBS and 14 (67%) of these were male. The median age at presentation was 51 (range 13–90). The patients' ethnic groups were 12 (57%) Malays, 5 (24%) Chinese and 4 (19%) Indians. Most patients described an antecedent upper respiratory tract infection (48%) and one patient each presented with preceding diarrhea, varicella-zoster infection and dengue fever. The neurological signs, apart from limb weakness, included areflexia or hyporeflexia (100%), sensory disturbance (81%), facial palsy (62%), bulbar palsy (57%), ventilatory failure (29%) external ophthalmoplegia (24%) and ataxia (19%).

3.2. The electrodiagnostic classification of GBS patients

The final diagnoses of patients were as follows: 12 AIDP, 3 AMAN, 4 AMSAN and 2 patients were unclassified.

The electrodiagnostic classification of each patient at the different time periods is shown in Table 1. Based on NCS performed within the first 2 weeks, 17/21 (81%) GBS patients fulfilled the existing criteria for AIDP, 2 (9.5%) AMAN/AMSAN and 2 (9.5%) unclassified. However, based on NCS performed within 3–8 weeks, the number of AIDP cases were reduced to 12 (57%) patients, AMAN/AMSAN increased to 7 (33%) patients and two patients remained unclassified. NCS after 8 weeks did not change this classification further. All 12 AIDP patients had persistent features of demyelination to fulfill the electrodiagnostic criteria at NCS performed after 8 weeks.

Table 1
The electrodiagnostic classification based on nerve conduction studies performed at different time intervals.

Patient No.	Nerve conduction studies (weeks)			Final electrodiagnosis	IgG antibodies against
	1–2	3–8	8–12		
1	AIDP	AMAN	Not done	AMAN	GM1, GD1a, GalNAc-GD1a, GD1b
2	AIDP	AIDP	AIDP	AIDP	None
3	AIDP	AIDP	AIDP	AIDP	None
4	AIDP	AIDP	AIDP	AIDP	None
5	AIDP	AMSAN	AMSAN	AMSAN	GM1b, GalNAc-GD1a
6	AIDP	AIDP	AIDP	AIDP	None
7	AMAN	AMAN	Not done	AMAN	GM1, GD1b
8	AIDP	AIDP	AIDP	AIDP	None
9	AIDP	AMSAN	Not done	AMSAN	None
10	AIDP	AIDP	AIDP	AIDP	None
11	AIDP	AMSAN	AMSAN	AMSAN	GD1a
12	AIDP	AIDP	AIDP	AIDP	None
13	AIDP	AMAN	AMAN	AMAN	GalNAc-GD1a
14	AIDP	AIDP	AIDP	AIDP	None
15	Unclassified	Unclassified	Unclassified	Unclassified	None
16	AIDP	AIDP	AIDP	AIDP	None
17	AIDP	AIDP	AIDP	AIDP	None
18	AIDP	AIDP	AIDP	AIDP	None
19	Unclassified	Unclassified	Unclassified	Unclassified	None
20	AMSAN	Not done	Not done	AMSAN	GT1a, GQ1b
21	AIDP	AIDP	AIDP	AIDP	GD1a, GT1a, GQ1b

AIDP = acute inflammatory demyelinating polyneuropathy; AMAN = acute motor axonal neuropathy; AMSAN = acute motor and sensory axonal neuropathy.

One patient (*Patient 20*) took her own discharge and further NCS could not be performed. Her initial NCS showed features consistent with AMSAN. NCS in two patients did not meet the electrodiagnostic criteria for either AIDP or AMAN. *Patients 15* and *19* had significant abnormalities involving only the sensory nerve action potentials. However, serial NCS in *Patient 19* did reveal an improvement in the amplitude of the compound motor action potentials on the second NCS suggesting the presence of reversible conduction failure but these changes did not meet the existing GBS electrodiagnostic criteria.

3.3. Serological analyses

IgG anti-ganglioside antibodies were positive in 7/21 patients as follows; 1/12 AIDP patients, 3/3 AMAN patients, 3/4 AMSAN and 0/2 unclassified patients. Of the AMAN patients, *Patients 1* and *13* had features on NCS in keeping with reversible conduction failure and their serology was positive for IgG anti-GM1, -GD1a, -GalNAc-GD1a, and -GD1b, and IgG anti-GalNAc-GD1a antibodies, respectively.

3.4. Clinical outcome

With the exception of *Patient 20* who declined treatment, all GBS patients received either plasma exchange or intravenous immunoglobulin. The outcome of patients is shown in Table 2. The majority of GBS patients made good recovery and walked with at least an aid by 6 months. *Patient 7* with a diagnosis of AMAN was still wheelchair dependent at 6 months whereas *Patient 18* was only recently recruited and currently in the recovery phase of GBS and at 18 weeks, the Hughes functional grade was 4 (Hughes et al., 1978). The patterns of recovery in our group of patients were heterogeneous. The predicted outcome based on the EGOS and mE-GOS did not always match the actual outcome.

4. Discussion

The current electrodiagnostic criteria were derived from studies performed in the mid to late 1990s (Ho et al., 1995; Hadden et al.,

1998). However, several studies since have called into question the accuracy of the existing criteria as important neurophysiological changes such as reversible conduction failure in axonal GBS have been overlooked (Kokubun et al., 2010; Kuwabara et al., 1998; Capasso et al., 2003; Hiraga et al., 2005). An update of the electrodiagnostic criteria of GBS, incorporating serial NCS is required. Whilst it may be possible to perform weekly NCS in a research setting, this approach may not be feasible in reality due to limitations in resources as well as patient willingness. In a recent review, the authors recommended two sets of NCS to be performed within the first four to six weeks of disease onset (Uncini and Kuwabara, 2012). However, it is less clear if this will suffice in providing an accurate electrodiagnosis of GBS. An initial NCS is required at admission to at least confirm the presence of neuropathy in support of GBS. The timing and frequency of subsequent NCS is less certain.

In the current prospective study of GBS patients, we performed NCS at three different time intervals. We found that the electrodiagnosis of patients did not change after a second NCS was performed in the 3–8 weeks interval following the onset of disease. Similar to the findings of previous investigators (Kuwabara et al., 2004), we also found that neurophysiology performed in the first 2 weeks of hospital admission overestimated the number of AIDP patients. 17/21 (81%) GBS patients initially fulfilled the existing electrodiagnostic criteria for AIDP and only two patients were classified as AMAN. However, a second NCS performed in the 3–8 weeks interval reduced the number of AIDP cases to 12 (57%) patients whereas an additional five patients were reclassified to AMAN/AMSAN (Table 1). The neurophysiology changes of this latter group no longer demonstrated significant demyelinating features but were more in keeping with axonal features. This included rapid recovery of distal motor latencies and conduction blocks, which also led to improved conduction velocities and normalisation of F waves. In contrast, patients with AIDP showed persistent demyelinating features at NCS performed even after 8 weeks.

Our findings are in keeping with that of Kuwabara et al. who described NCS in typical AIDP patients demonstrating progressive prolongation in distal motor latencies during the 8 weeks following the onset of disease, which likely reflects the slow-conducting remyelinating fibres of distal nerve segments (Kuwabara et al.,

Table 2
Treatment and outcome.

Patient No.	Electro-diagnosis	Therapy	Hughes scale at nadir	EGOS at 2 weeks (% predicted not walking at 6 months)	mEGOS at admission(% predicted not walking at 3 months/6 months)	mEGOS at one week (%predicted not walking at 3 months/6 months)	Actual time to independent walking/function at 6 months or last review
1	AMAN	IVIG	4	1 (<7%)	4 (22/12)	0 (Negligible)	Day 1
2	AIDP	PE	5	5(25%)	6(40/28)	9 (52/34)	Week 6
3	AIDP	IVIG	5	5 (25%)	6(40/28)	8 (42/26)	Week 22
4	AIDP	PE	4	4 (7%)	6(40/28)	9 (52/34)	1 stick at 6 months
5	AMSAN	PE	4	5 (25%)	6(40/28)	2 (2/0)	Week 6
6	AIDP	IVIG	5	5.5 (40%)	7 (52/38)	10 (64/44)	Week 20
7	AMAN	IVIG	4	5 (25%)	7 (52/38)	10 (64/44)	Wheelchair at 6 months
8	AIDP	IVIG	4	4 (7%)	2 (12/6)	0 (Negligible)	Week 8
9	AMSAN	PE	4	4.5 (20%)	1 (10/4)	1 (Negligible)	Week 5
10	AIDP	IVIG	5	7 (85%)	9 (70/60)	12 (80/65)	Week 15
11	AMSAN	IVIG	4	5 (25%)	2 (12/6)	2 (2/0)	Day 17
12	AIDP	IVIG	4	4.5 (20%)	3 (18/10)	4 (12/6)	Week 11
13	AMAN	IVIG	3	3 (<7%)	0 (Negligible)	0 (Negligible)	Day 13
14	AIDP	IVIG	5	6 (55%)	2 (12/6)	11 (72/56)	Week 7
15	Unclassified	IVIG	3	2 (<7%)	0 (Negligible)	0 (Negligible)	Day 5
16	AIDP	IVIG	4	5 (25%)	8 (60/50)	11 (72/56)	Walking frame at 12 weeks
17	AIDP	PE	2	1 (<7%)	0 (Negligible)	0 (Negligible)	Walking from start
18	AIDP	IVIG	5	5.5 (40%)	3 (18/10)	10 (64/44)	Bedbound at 12 weeks
19	Unclassified	IVIG	4	4 (7%)	2 (12/6)	2 (2/0)	Week 6
20	AMSAN	None[*]	4	–	–	–	–
21	AIDP	IVIG	5	6 (55%)	4 (22/12)	11 (72/56)	Week 12

AMAN = acute motor axonal neuropathy; AMSAN = acute motor and sensory axonal neuropathy; AIDP = acute inflammatory demyelinating polyneuropathy; IVIG = intravenous immunoglobulin; PE = plasma exchange; EGOS = Erasmus GBS outcome score; mEGOS = modified EGOS.
[*] Patient 20 declined treatment and took own discharge.

2004). In a previous retrospective study, the authors found that GBS patients with positive anti-ganglioside antibody had normalization or near-normalization of their serial distal motor latencies at 5 weeks whereas patients who were antibody negative had progressive prolongation of DML up till 5 weeks (Hiraga et al., 2005). In the current prospective study, we found that NCS performed within the 3–8 week interval of illness onset was able to distinguish between axonal GBS and AIDP although we acknowledge that our patient numbers were small and further studies to validate this finding in larger and more varied population are required. Serological testing for anti-ganglioside antibodies was positive in 7 of 21 GBS patients, the majority of which had a final diagnosis of either AMAN or AMSAN.

In our study, we also looked prospectively at the predicted outcome of our GBS cases in comparison to the actual outcome based on EGOS at 2 weeks and modified EGOS at admission and at 1 week (Table 2). To our knowledge, these are the only GBS prognostic scores in the current literature and have been validated in the Dutch population (van Koningsveld et al., 2007; Walgaard et al., 2011). The numbers in our cohort were too few to conduct a validation study in our cohort. However, looking at individual cases, we found variability in the actual outcome and two patients with a similar modified EGOS score can have very different outcomes. Having a tool with which to prognosticate the recovery of patients with GBS is important especially when deciding on escalation of treatment. Modified EGOS is helpful but validation studies in GBS patient population with different patterns of GBS subtype to the Western population are required. Predicting the outcome in a heterogeneous patient population such as GBS may require other ancillary investigations such as NCS and electromyography assessments to look for ongoing axonal denervation changes.

In conclusion, our prospective study of GBS patients demonstrates that performing NCS at 2 time intervals (at admission and within 3–8 weeks of disease onset) is sufficient in making an accurate electrodiagnosis of GBS as early NCS underestimates axonal subtypes. Patients with AIDP demonstrated persistent demyelinating features even after 8 weeks. Further studies to validate these findings as well as the prognostic scale, EGOS in different patient populations are required before we can be confident of the true electrodiagnosis and predict the patient outcome in GBS.

5. Funding

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3.3   Publication 3

Sensory nerves are frequently involved in the spectrum of Fisher syndrome. Shahrizaila N, Goh KJ, Kokubun N, Tan AH, Tan CY, Yuki N. Muscle Nerve. 2014 Apr;49(4):558-63.

3.3.1   Contribution of co-authors:

Design and concept of study	N Shahrizaila
Acquisiton of data	N Shahrizaila
Analyses of data	N Shahrizaila
Drafting of manuscript	N Shahrizaila
Revising manuscript for intellectual content	All authors

SENSORY NERVES ARE FREQUENTLY INVOLVED IN THE SPECTRUM OF FISHER SYNDROME

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ABSTRACT: *Introduction:* Differing patterns of neurophysiological abnormalities have been reported in patients with Fisher syndrome. Fisher syndrome is rare, and few series have incorporated prospective serial studies to define the natural history of nerve conduction studies in Guillain–Barré syndrome. *Methods:* In an ongoing prospective study of Guillain–Barré syndrome patients, patients who presented with Fisher syndrome and its spectrum of illness were assessed through serial neurological examinations, nerve conduction studies, and serological testing of IgG against gangliosides and ganglioside complexes. *Results:* Of the 36 Guillain–Barré syndrome patients identified within 2 years, 17 had features of Fisher syndrome. Serial nerve conduction studies detected significant abnormalities in sensory nerve action potential amplitude in 94% of patients associated with 2 patterns of recovery—nondemyelinating reversible distal conduction failure and axonal regeneration. Similar changes were seen in motor nerves of 5 patients. *Conclusions:* Patients with the Fisher syndrome spectrum of illness have significant sensory involvement, which may only be evident with serial neurophysiological studies.

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Since the first description of Guillain–Barré syndrome (GBS) in 1916,[1] our understanding of the clinical patterns associated with the disease has evolved. The most recognizable of these variants is Fisher syndrome (FS), initially described in 1956.[2] It is characterized by ophthalmoplegia, ataxia, and areflexia, along with cerebrospinal fluid albuminocytological dissociation. The latter supports its link with GBS in conjunction with reports of patients with FS who have developed GBS during the course of their illness.[3]

Following the discovery of anti-GQ1b antibodies in FS patients,[4] it became evident that a clinical spectrum also exists within FS. Patients with anti-GQ1b antibodies can present with less extensive variants such as acute ophthalmoparesis or more extensive variants such as Bickerstaff brainstem encephalitis.[5] The electrodiagnostic findings in FS and its related conditions have been reported as demyelinating in some series, and as axonal in others.[6,7] In a recent study, non-demyelinating reversible conduction failure was demonstrated in 6 of 15 (40%) patients with FS and its related conditions.[8] Although well-recognized as a GBS variant, FS is rare, and few prospective studies have comprehensively investigated this patient cohort.

As part of an ongoing prospective study of GBS patients in a multi-ethnic Malaysian cohort, we investigated patients with the FS spectrum of disease. Patients were recruited prospectively, and serial examinations and neurophysiological studies were performed along with serological testing for immunoglobulin G (IgG) directed against gangliosides and ganglioside complexes. Our aim was to describe the clinical, electrophysiological, and serological patterns in this group of patients.

METHODS

Patients. Patients who were diagnosed with FS or any of its related conditions at the University Malaya Medical Centre in Kuala Lumpur, Malaysia, were recruited prospectively between June 2010 and November 2012. Thirty-six GBS patients were identified, 17 of whom had features of FS. Our study received approval from the medical research ethics committee of University Malaya Medical Centre. A diagnosis of GBS was made according to the Asbury criteria.[9] The 17 patients with features of FS were further classified as follows: typical FS when there was ataxia, ophthalmoplegia, and areflexia or hyporeflexia without altered consciousness[2]; acute ophthalmoparesis when only paresis of extraocular muscles was present without ataxia; Bickerstaff brainstem encephalitis when there was altered consciousness or hyperreflexia[10]; FS with pharyngeal–cervical–brachial weakness (FS/PCB) when there was weakness in the oropharyngeal and cervicobrachial muscles; and FS with GBS (FS/GBS) in patients who first presented with features of FS but subsequently developed limb weakness of Medical Research Council grade ≤4. Other

Table 1. Demographics, serological findings, and neurophysiological patterns among the patients studied.

Patient	Age/gender	Diagnosis	IgG antibodies	Pattern of sensory amplitude change	Pattern of motor conduction failure when present
1	61/W	FS/GBS	Negative	Progressive improvement	Non-demyelinating
2	69/W	FS	ND	Reversible	—
3*	90/W	FS/GBS	GT1a, GQ1b	Abnormal at baseline	Non-demyelinating at baseline
4*	32/M	FS	ND	Abnormal at baseline	—
5	21/W	FS	GT1a, GQ1b	Reversible	—
6	61/W	BBE	GT1a, GQ1b	Both persistent and reversible	—
7	45/M	FS	GD1b, GT1a, GQ1b, cGA1/GT1b	Progressive improvement	—
8	48/M	AO	GT1a, GQ1b, cGA1/GQ1b	Normal study	—
9	72/M	FS/GBS	GD1a, GD1b, GT1a, GT1b, GQ1b	Persistent	Demyelinating
10	66/W	FS	Negative	Persistent	Demyelinating
11	38/M	FS/PCB	GT1a	Reversible	Non-demyelinating
12	60/W	FS	Negative	Reversible	—
13	52/M	FS	Negative	Both reversible and persistent	—
14	57/W	FS	LM1,GQ1b	Progressive improvement	—
15	67/W	FS	Negative	Reversible	—
16*	64/M	FS	LM1, GD1b, GT1a, GT1b, GQ1b	Abnormal at baseline	—
17	52/W	FS	Negative	Reversible	—

M, man; W, woman; FS, Fisher syndrome; FS/GBS, FS overlapped with Guillain–Barré syndrome; BBE, Bickerstaff brainstem encephalitis; AO, acute ophthalmoparesis; FS/PCB, FS overlapped with pharyngeal–cervical–brachial weakness; c (prefix), complex; ND, not done; Abs, absent.
*Did not attend follow-up appointment.

possible mimics of a brainstem syndrome, such as brainstem stroke, myasthenia gravis, infectious encephalitis, and metabolic encephalopathy, were excluded by history, examination, and investigations that included cerebrospinal fluid analysis and brain imaging, when indicated.

Nerve Conduction Studies. Serial nerve conduction studies (NCS) were performed by 1 of the authors (N.S.). NCS were done at 3 time periods when permitted: within 2 weeks of onset of illness; 3–8 weeks after onset; and 8–12 weeks after onset,[11] as described elsewhere.[12] At least 2 limbs were assessed, including 4 motor nerves, 3 sensory nerves, and F-waves. Sensory studies of the median and ulnar nerves were performed orthodromically, whereas sural nerve studies were done antidromically. Reference values were derived from NCS performed on normal patients in our laboratory. Patients were classified as having "demyelinating" or "axonal" changes on NCS based on the existing electrodiagnostic criteria for GBS.[13,14] The criteria for abnormal sensory NCS were derived from the criteria for acute motor and sensory axonal neuropathy in which a reduction in sensory nerve action potential (SNAP) amplitude to 50% of the lower limit of normal in at least 2 nerves was considered abnormal.[15] When the initial sensory NCS were normal, any changes in the SNAP amplitude of 45% for median nerve, 49% for the ulnar nerve, or 60% for the sural nerve would be considered abnormal.[16] Motor conduction block was classified according to the American Association of Electrodiagnostic Medicine criteria as follows: (a)

definite—presence of at least 50%, or 60% in the tibial nerve, reduction of proximal vs. distal compound muscle action potential (CMAP) amplitude in the nerves of the upper and lower limbs, respectively, with minimal temporal dispersion (<30% increased CMAP duration); or (b) probable—presence of either at least 40% or 50% reduction of proximal vs. distal CMAP amplitude in the nerves of the upper and lower limbs, respectively, with minimal temporal dispersion (see above), or at least 50% or 60% reduction of proximal vs. distal CMAP amplitude in the nerves of the upper and lower limbs, respectively, with moderate temporal dispersion (31–60% increased CMAP duration).[17] Reversible distal conduction failure was defined as a decrease in distal CMAP amplitude that resolved without abnormal temporal dispersion (duration increase >30%), or other demyelinating features.[18]

Serological Analysis. Patient sera were collected on admission and prior to any immunotherapy. Serum IgG to asialo-GM1 (GA1) and gangliosides LM1, GM1, GM1b, GD1a, GalNAc-GD1a, GD1b, GT1a, GT1b, and GQ1b were measured by enzyme-linked immunosorbent assay, as described elsewhere.[19] Serum was considered positive when the optical density was ≥0.5 at a 1:500 dilution. IgG antibodies against ganglioside complexes were tested with a mixture of individual antigens at 5 pmol/well each. For example, ganglioside complex GM1/GD1a was tested with a mixture of GM1 and GD1a (each at 5 pmol/well) as antigen. Anti-GM1/GD1a complex antibodies were considered positive when the optical density was more than

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Table 2. Summary of neurophysiological patterns based on serial studies in different Fisher syndrome variants.

	Typical FS [n = 9 (65%)]	AO [n = 1 (7%)]	BBE [n = 1 (7%)]	FS/GBS [n = 2 (14%)]	FS/PCB [n = 1 (7%)]
Slow improvement/persistent changes in SNAP amplitude	5*	0	1*	2	0
Reversible changes in SNAP amplitude	6*	0	1*	0	1
Motor conduction failure without demyelination	0	0	0	1	1
Motor conduction failure with demyelination	1	0	0	1	0

FS, Fisher syndrome; AO, acute ophthalmoplegia; BBE, Bickerstaff brainstem encephalitis; FS/GBS, FS overlapped by Guillain–Barré syndrome; FS/PCB, FS overlapped by pharyngeal–cervical–brachial variant; SNAP, sensory nerve action potential.
*Presence of more than 1 neurophysiological pattern affecting different nerves in 1 individual.

half the sum of antibodies against individual GM1 and GD1a. Other ganglioside complexes similarly tested included combination of 2 of the 9 gangliosides (LM1, GM1, GM1b, GD1a, GalNAc-GD1a, GD1b, GT1a, GT1b, and GQ1b) and GA1.

RESULTS

Patient Characteristics. Thirty-six patients were diagnosed as having GBS, and 17 (47%) of them had a diagnosis that was within the spectrum of FS. Patient demographics are shown in Table 1. The FS variants were as follows: 11 typical FS; 3 FS/GBS; and 1 each of FS/PCB, acute ophthalmoparesis, and Bickerstaff brainstem encephalitis. The majority of patients with FS phenotypes presented with an antecedent illness of an upper respiratory tract infection (76%), whereas only 1 patient (patient 17) presented with preceding diarrhea with negative stool culture for *Campylobacter jejuni*. The frequencies of clinical characteristics were as follows: external ophthalmoplegia (100%); areflexia (94%); ataxia (94%); ptosis (88%); mydriasis (47%); sensory disturbance in a "glove-and-stocking" distribution (47%); facial palsy (41%); and bulbar palsy (36%). Lumbar puncture was performed at admission on all but 2 patients (patients 14 and 17). Cerebrospinal fluid analysis showed albuminocytological dissociation in 4 of 15 (patients 1, 3, 10, and 13), whereas the other patients had normal results. Magnetic resonance imaging of the brain was performed in 10 patients, none of whom had significant abnormalities.

Electrodiagnostic Features. NCS revealed significant reduction in SNAPs in all but 1 patient (94%). Table 1 depicts the corresponding neurophysiological patterns seen in each patient. Serial sensory values are shown in Table S1 (see Supplementary Material available online). The various neurophysiological patterns seen with the different variants of FS are summarized in Table 2. In 3 patients (patients 3, 4, and 16), serial studies were not performed, as patients failed to attend follow-up studies. However, all 3 had abnormal SNAP amplitudes at baseline. In patients 2, 11, 12, 15, and 17, the SNAP amplitudes more than doubled on the second set of NCS (performed within 4–5 weeks) in keeping with reversible conduction failure. In the remaining patients with SNAP abnormalities, the pattern of recovery was slowly progressive, such as that seen in axonal regeneration.

Abnormal motor studies were seen in patients 1, 3, 9, 10, and 11. Patient 1 (FS/GBS) and patient 11 (FS/PCB) demonstrated non-demyelinating reversible conduction failure. In patient 1, this was evident in the median nerve, where the CMAP amplitude recovered from 4.9 to 7.2 mV (stimulating at the wrist) and 4.2 to 6.5 mV (stimulating at the elbow) within a span of 2 weeks without demyelinating features. In patient 11, a similar pattern of rapid recovery was also seen in the ulnar nerve (5.9 to 10.3 mV at the wrist, 6.3 to 9.8 mV at the elbow). Patient 3 consented to only 1 NCS, and in this patient axonal changes were evident on the first NCS performed after admission. In contrast, 2 patients demonstrated demyelinating features on NCS (patients 9 and 10). Patient 9 had a diagnosis of FS/GBS overlap, and demyelinating findings were seen mainly in the median nerves. Patient 10 had typical FS with marked facial weakness, and NCS revealed multiple entrapment neuropathies, producing prolonged distal latencies, slowed conduction velocities, and delayed late responses. She also had underlying type 2 diabetes mellitus, and we could not exclude the possibility that these findings were part of pre-existing disease rather than the FS. The 1 patient with normal neurophysiological studies had a diagnosis of acute ophthalmoparesis. The remaining 12 patients had motor NCS that were within normal limits and without significant sequential changes.

Serological Analysis. Serological analysis was performed in 15 of 17 patients. Due to sampling error, serological testing could not be performed in 2 patients with typical FS. Of the 15 patients, IgG against a single ganglioside was positive in 9 of 15 (60%) patients (Table 1). Two patients

(patients 7 and 8) were also positive for IgG against ganglioside complexes. Positive serology was seen in 4 FS patients, including 2 (patients 14 and 16) who were positive for IgG anti-LM1 antibodies as well as IgG anti-GQ1b antibodies. The other clinical subtypes with positive results showed 2 of 3 with FS/GBS overlap, 1 with acute ophthalmoparesis, 1 with Bickerstaff brainstem encephalitis, and 1 with FS/PCB overlap.

Clinical Outcome. Patients at the severe end of the FS spectrum (FS/PCB and FS/GBS) received either plasma exchange or intravenous immunoglobulin, except for 1 FS/GBS overlap patient who declined treatment. The 1 Bickerstaff brainstem encephalitis patient improved before treatment was instituted. In view of the good spontaneous recovery seen in patients with typical FS and acute ophthalmoparesis, these patients were given the choice of no treatment or immunotherapy, which they were counseled could hasten their recovery.

The outcomes of patients with FS-related conditions are shown in Table S2 (see Supplementary Material available online). All 14 patients who returned for follow-up had made a complete recovery by week 12. The only exception was patient 10 who had persistent facial diplegia, which showed complete recovery at the 10-month review. We noted a remarkable improvement in the 1 patient with FS/PCB (patient 11) who received plasma exchange. At admission, he was severely disabled with marked truncal ataxia and bulbar weakness causing persistent drooling. After the third plasma exchange, he was able to walk independently and consume a normal diet. By the time he was discharged after completing the fifth exchange, there were no residual neurological deficits.

DISCUSSION

In this prospective study, we identified involvement of sensory nerve fibers in all but 1 patient (94%) who presented with FS or 1 of its variants. This frequency is higher than that reported previously in 1 other comparable prospective study.[8] However, the methodology and criteria used in our study were different. In our study, orthodromic assessment of both median and ulnar nerves was performed, whereas the antidromic method was used elsewhere. In 14 of 17 patients, serial NCS were performed. In these patients, not only were significant changes detected on subsequent NCS, but the majority of patients also presented with significantly reduced SNAP amplitudes in at least 2 nerves. The sensory changes were detected in patients regardless of whether there were sensory symptoms or signs. Although SNAPs are affected by age, we investigated longitudinal changes, and thus age bias was unlikely in our

analyses. Some SNAP amplitudes were normal for age but increased on subsequent studies, which suggests that the initial values were abnormal.

Previous studies of sensory conduction in patients with acute motor axonal neuropathy and FS patients have shown 2 patterns of recovery.[8,16] Sensory conduction can recover rapidly, similar to the recovery seen in reversible motor conduction failure, or it can improve slowly, as seen in axonal regeneration. We found similar patterns of recovery (Table 2). In the majority of patients, reversible changes in SNAP amplitude were detected. The pattern of rapid reversibility, occurring within weeks, without demyelinating features, suggests reversible distal conduction failure associated with dysfunction at the paranodal and nodal axolemma. In other patients, slowly progressive improvement or persistent changes in SNAP amplitude are likely to represent axonal degeneration. Based on the patterns of sensory abnormality demonstrated in this cohort, it is likely that there are pathological target antigens present in sensory nerves. In previous studies, anti-GD1b antibodies have been associated closely with sensory ataxia.[20–22] Complement-mediated nodal disruption was also observed predominantly in sensory nerves in a rabbit model of acute ataxic neuropathy associated with IgG anti-GD1b antibodies and with injection of IgG monoclonal anti-GD1b antibody.[23] In our study, only 3 patients were positive for anti-GD1b antibodies. IgG anti-GQ1b antibodies were most prevalent (7 of 15). Other unidentified antigens may play a part in the pathophysiology of the sensory neuropathy within the FS spectrum of illness.

Motor NCS were abnormal in all 3 FS/GBS patients, 1 FS/PCB patient, and 1 typical FS patient. Previous studies have identified non-demyelinating features in FS patients.[8,24] We found similar changes in 3 of our 5 patients with abnormal motor conduction. Demyelinating features were found in 2 patients (patients 9 and 10). In patient 9, the changes were limited to the median nerves, whereas patient 10 demonstrated changes more in keeping with multiple entrapment neuropathies. The latter changes are likely to be related to increased susceptibility from underlying type 2 diabetes mellitus, rather than true FS pathophysiology.

We also performed comprehensive analyses of IgG against gangliosides and ganglioside complexes (Table 1). Serological testing for anti-ganglioside antibodies was positive in 9 of 15 (60%) samples. More than 50% of the typical FS (5 of 9) patients were seronegative for the anti-GQ1b antibodies. Previous studies have suggested that the test for this antibody had sensitivities ranging from 83% ($n = 466$) to 95% ($n = 19$) in

2 separate Japanese cohorts and 100% in a British cohort ($n = 9$).[25–27] The laboratory protocol for anti-ganglioside antibody testing in our study was similar to that described in one Japanese study.[26] This raises the possibility that other pathogenic antigens may have been responsible in the development of FS in our patients. Interestingly, 2 patients with typical FS had IgG anti-LM1 antibodies. A recent study suggested that LM1 and LM1 complexes were possible antigens in the demyelinating immune-mediated neuropathies of acute and chronic inflammatory demyelinating polyneuropathy.[28] Our findings do not support this possibility, as both patients had typical FS features with ophthalmoplegia and ataxia. NCS of both patients also revealed only axonal sensory changes with no evidence of demyelination. Further studies to investigate the presence of anti-LM1 antibodies in a larger FS cohort are required to further clarify the significance of this antigen.

Some studies have suggested that there are antibodies against ganglioside complexes in FS patients who are otherwise seronegative.[29–32] In 1 study, the investigators detected antibodies against the GM1/GT1a complex in 3 of 24 seronegative FS patients.[31] In another study, FS patients demonstrated stronger reactivities with GA1 complexes than with single gangliosides.[32] The latter group also demonstrated ganglioside complexes that were associated with specific clinical features.[29,30] Patients with antibodies against GQ1b/GM1 complexes appeared to not have sensory disturbances,[29] whereas ganglioside complexes that contained GQ1b or GT1a were associated with ophthalmoplegia.[30] We found that further testing of IgG against ganglioside complexes did not increase our diagnostic yield in the seronegative patients. We detected antibodies against ganglioside complexes in 3 typical FS patients who also had IgG against single gangliosides. Patient 7 also had IgG against GT1b/GA1 complex, which has not been described previously.

Interestingly, in comparison to other reports, we noted a higher percentage of patients (47%) who presented with the FS spectrum of disease within our multi-ethnic GBS patient population. Previous studies suggested that FS is more frequent in Asian populations but the ranges varied between 19% in a Taiwanese cohort and 25% in a Japanese cohort.[3,33] Other comparable studies in terms of numbers and geographical location include a 25% FS frequency in a Singaporean GBS cohort ($n = 31$),[34] in contrast to 8% in a Thai GBS cohort.[35] The reasons for the higher frequency in our cohort are uncertain but may involve environmental and host factors that merit further investigation.

The outcome in our patient group was good, including those with FS/GBS overlap as well as FS/PCB overlap. This is similar to other reports.[8,26,36] By 12-week review, most patients made complete recovery. Patients with typical FS recovered fully regardless of whether they received immunotherapy.

In conclusion, this prospective study suggests that sensory abnormalities are common in FS and can occur in patients who are otherwise asymptomatic, but they may be evident only with serial neurophysiological studies. We detected a higher incidence of FS than had been reported previously in other Asian populations. Testing for antiganglioside complexes did not improve the yield of seropositivity in this cohort. The outcome was good, as most patients recovered fully within 12 weeks.

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3.4   Publication 4

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3.4.1   Contribution of co-authors:

Design and concept of study	N Yuki
Acquisiton of data	Créange A, N Shahrizaila
Analyses of data	All authors
Drafting of manuscript	Créange A, N Shahrizaila, H Salhi, N Yuki
Revising manuscript for intellectual content	All authors

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As the corresponding author of the article named above, I consent to Nortina Shahrizaila including the published article above as part of her collection of published works to be submitted for "PhD by prior publication" at University of Malaya, Kuala Lumpur

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RESEARCH REPORT

Association of antibodies to ganglioside complexes and conduction blocks in axonal Guillain-Barré syndrome presenting as acute motor conduction block neuropathy

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Abstract A close relationship between acute motor conduction block neuropathy and antibodies against the complex of GM1 and GalNAc-GD1a has been reported. This study investigates the hypothesis that conduction block at the early phase of axonal Guillain-Barré syndrome (GBS) is also associated with such ganglioside complexes. Sera were obtained from seven French patients with initial evidence of isolated conduction blocks that resolved or progressed to acute motor axonal neuropathy. Serum IgG to asialo-GM1 and gangliosides of LM1, GM1, GM1b, GD1a, GalNAc-GD1a, GD1b, GT1a, GT1b, and GQ1b as well as their complexes were measured. Five of seven patients progressed within the first month of disease to AMAN. One patient had IgG antibodies against the complex of asialo-GM1 and each of the other ganglioside antigens. Another patient carried IgG antibodies against GM1 complex with GM1b, GD1a, and GT1a as well as asialo-GM1 complex with GD1a and GT1a. None had IgG antibodies against GM1/GalNAc-GD1a complex. Six patients had IgG against single antigens GM1, GD1a, GalNAc-GD1a, GD1b, and asialo-GM1. In three patients, a reduced reaction against GM1/GalNAc-GD1a complex was observed. The presence of conduction block in axonal GBS is not always associated with anti-GM1/GalNAc-GD1a complex antibodies.

Key words: acute motor axonal neuropathy, acute motor conduction block neuropathy, anti-ganglioside antibody, conduction block, Guillain-Barré syndrome

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Introduction

Guillain-Barré syndrome (GBS) can be broadly classified into acute inflammatory demyelinating polyneuropathy and acute motor axonal neuropathy (AMAN). Within the spectrum of AMAN lie "acute motor conduction block neuropathy (AMCBN)" *(Uncini and Yuki, 2009)*, characterized by an acute onset of pure motor clinical deficit associated with good prognosis, "conduction block followed by axonal

degeneration" and "classical axonal degeneration (without early conduction block)" *(Kokubun et al., 2010)*. Patients with AMCBN have been associated with an antecedent *Campylobacter jejuni* infection and the presence of IgG anti-GM1 or -GD1a antibodies *(Capasso et al., 2003)*.

More recently, there have been reports of GBS patients who developed antibodies against complexes made up of two different gangliosides, also referred to as ganglioside complexes (GSCs). The conformational structure formed by these complexes reveals an epitope that would normally not be recognized in a single ganglioside. In one Japanese study, reversible conduction block was reportedly associated with anti-GM1/GalNAc-GD1a antibodies, suggesting this GSC is a target antigen in early conduction blocks seen in AMCBN and AMAN *(Kaida et al., 2008; Ogawa et al., 2013)*. In this study, we investigated the presence of anti-GSC antibodies in patients with AMCBN and conduction block followed by axonal degeneration in a French cohort.

Patients and Methods

Patients

Patients presenting between 2000 and 2006 to Henri Mondor Hospital (Créteil) were selected when the following inclusion criteria were fulfilled: presence of a pure motor neuropathy; acute disease onset and disease onset of less than one 1 month; and isolated conduction blocks on initial neurophysiological examination, without other demyelinating neurophysiological features. Clinical data included precipitating factors, topography of the motor deficit, time course evolution, severity of motor deficit, and response to treatment. Sera were collected before initiation of intravenous immunoglobulin treatment. This retrospective study received ethical standards committee approval, and patients were informed of the collection of their anonymous data for research according to French standards.

Electrophysiological studies

All patients had one initial examination within the first 8 days of motor deficit. Between 2 and 5 subsequent neurophysiology examinations were performed in all patients from day 2 to day 480. According to previously published criteria *(Olney et al., 2003)*, conduction blocks were defined as definite, probable, or possible. The electrodiagnosis of GBS was made according to Hadden's criteria *(Hadden et al., 1998)*. Patients with (1) two or more definite partial conduction blocks outside common entrapment sites as an isolated or predominant abnormality at the time of first nerve conduction study (NCS), (2) normal sensory NCS in a minimum of three nerves, and (3) normal sensory nerve

conduction velocity across the same segments with demonstrated conduction block, were included.

Serological analyses

Serum IgG to asialo-GM1 (GA1) and nine gangliosides (LM1, GM1, GM1b, GD1a, GalNAc-GD1a, GD1b, GT1a, GT1b, and GQ1b) were measured by enzyme-linked immunosorbent assay (ELISA) *(Funakoshi et al., 2009)*. IgG antibodies against GSC were tested with a mixture of individual antigens at 5 pmol per well each. Anti-GSC antibodies were considered positive when the optical density was greater than 0.5 of the sum of antibodies against individual antigens.

Results

Seven of 87 GBS patients fulfilled the inclusion criteria. All patients apart from Patient 2 described antecedent diarrhea. All patients received a course of intravenous immunoglobulin, and complete clinical recovery was observed between 4 and 13 months (Table 1). All patients had evidence of three or more definite conduction blocks in two or more limbs, either as an isolated or predominant abnormality at the time of first NCS. Detailed data for Patient 3 are described elsewhere *(Boerio-Gueguen et al., 2010)*. A second study was performed within 1 month in all but one patient (Patient 6 did not receive a second NCS before day 250). On the basis of existing electrodiagnostic criteria *(Hadden et al., 1998)*, all patients could have initially been classified as demyelinating form of GBS. However, in Patients 2, 4, 5, 6, and 7, subsequent NCSs showed, after more than 1 month in Patient 6, decreased compound muscle action potential (CMAP) amplitudes in nerves that had originally demonstrated features of conduction block. This evolution is in keeping with axonal degeneration following the initial conduction block in the intermediate segment. Therefore, a diagnosis of AMAN was probable in these patients. In Patient 3 (and to a lesser degree Patient 1), conduction block resolved on subsequent studies without occurrence of any other demyelinating features or CMAP amplitude reduction, in keeping with a diagnosis of AMCBN. Patients 1 and 6, with persistent conduction blocks at day 250, had effort related fatigue without motor deficit in the affected nerves, not necessitating further immunotherapy.

IgG antibodies against single antigen were detected in all patients except Patient 5 who was serologically negative. The findings are depicted in Table 2. There were reactivities against the single antigens, GM1, GD1a, GalNAc-GD1a, GD1b, and GA1. Patient 1 also developed antibodies against GA1 complex with each of the other antigens. Patient

Table 1. Nerve conduction study results.

Patients	1					2		3		
Age (years), gender	31/Female					35/Male		28/Female		
Motor deficit	S/D/4L					S/P/D/4L/F		S/P/D/4L		
Motor studies (day)	2	45	75	250	480	7	14	2	9	30
Right median nerve										
CMAP (mV) wrist	10	9	10	11	15	6	*2.7*	12	21	
CMAP area P/D reduction (%)	*40**	*35**	*40**	<10	<10	*60**	<10	<10	*30**	
DML (ms)	2.8	2.9	3	2.4	2.8	3.5	3.1	2.5	2.9	
CV (limb segment) (m/s)	52	51	55	53	56	52	51	50	55	
Left median nerve										
CMAP (mV) wrist	11	11	10	9	13.5	7	6.5	11	15	14
CMAP area P/D reduction (%)	*60**	*55**	*60**	*60**	*30**	<10	<10	<10	<10	<10
DML (ms)	2.7	2.7	2.4	2.3	2.2	3	3.2	3.1	3	2.8
CV (limb segment) (m/s)	50	48	51	51	54	48	56	59	62	54
Right ulnar nerve										
CMAP (mV) wrist	9	7	9	12	16	7	7	14	11	
CMAP area P/D reduction (%)	*70**	*67**	*68**	<10	<10	<10	<10	<10	<10	
DML (ms)	2.8	2.8	2.8	2.8	2.2	2.5	2.4	3	2.9	
CV (limb segment) (m/s)	52	53	48	49	63	52	55	66	70	
Left ulnar nerve										
CMAP (mV) wrist	9	6	8.5	10	17	7	7	11	12	12
CMAP area P/D reduction (%)	*65**	*55**	*64**	<10	<10	<10	<10	*50**	*50**	<10
DML (ms)	2.8	2.6	2.8	2.9	2.5	2.8	2.2	3	2.5	2.7
CV (limb segment) (m/s)	54	52	48	56	67	59	52	56	70	70
Right peroneal nerve										
CMAP (mV) ankle	5	4	4	4	8	4.8	*0.7*	6	5.5	5
CMAP area P/D reduction (%)	*80***	*80***	*80***	*40***	<25	*81***	<25	*65***	*80***	<25
DML (ms)	4.5	4.3	4.8	3.9	4	4.3	5.5	3.2	5	3.6
CV (limb segment) (m/s)	42	42	41	42	43	*38*	45	48	46	54
Left peroneal nerve										
CMAP (mV) ankle	5	5	4.5	3	7	6.2	*1.1*	6	5	5
CMAP area P/D reduction (%)	*75***	*75***	*72***	*60***	*35***	*70***	<25	*65***	*60***	<25
DML (ms)	4.2	3.6	4.2	3.7	2.8	4.4	4.8	3	5.2	3.8
CV (limb segment) (m/s)	44	41	47	42	47	*35*	41	48	42	48
Right tibial nerve										
CMAP (mV) ankle	9	6		8.5	15	12	*3.3*	8	9	10
CMAP area P/D reduction (%)	*70***	*60***		*44***	<25	*64***	<25	<25	*70***	*40***
DML (ms)	4.4	3.8		3.8	3.3	4.7	5.9	3.8	4.9	3.5
CV (limb segment) (m/s)	48	47		42	51	*38*	43	47	*35*	42
Left tibial nerve										
CMAP (mV) ankle	10	10		11.5	19	8	*1.8*	11	8	11
CMAP area P/D reduction (%)	*70***	*73***		*48***	<25	*45***	<25	*45***	*70***	*50***
DML (ms)	4.5	4.7		4.4	3.4	4.6	6.5	4.5	4.1	3.6
CV (limb segment) (m/s)	47	51		42	45	*38*	44	46	*35*	43

Patients	4				5		6		7				
Age (years), gender	40/Female				40/Male		30/Male		50/Female				
Motor deficit	S/D/4L				S/D/4L		S/P/D/4L		S/DUL-PLL				
Motor studies (day)	5	10	21	160	3	10	8	250	2	16	50	210	360
Right median nerve													
CMAP (mV) wrist	7.5	*1*	*0.6*	*5.1*	6	*3.9*	15	*2.4*	21	*3*	*4*	12	10
CMAP area P/D reduction (%)	*40**	<10	<10	<10	*53**	*39**	*95**	*53**	*64**	<10	<10	<10	<10
DML (ms)	4	4	3.2	3.3	3.7	3.6	2.2	3.5	2.2	2.8	2.8	2.8	3.2
CV (limb segment) (m/s)	53	47	51	55	51	48	53	46	54	49	48	48	52
Left median nerve													
CMAP (mV) wrist	*3.5*	*0.6*	*0.6*	*4.6*	5.2	6.5			13	16			
CMAP area P/D reduction (%)	<10	<10	<10	<10	*55**	*37**			<10	<10			
DML (ms)	3.9	3.5	3.3	3.1	3.5	3.2			2.5	2.8			
CV (limb segment) (m/s)	53	48	53	52	54	57			58	50			
Right ulnar nerve													
CMAP (mV) wrist	10	*0.4*	*0*	*0.8*	*3.9*	*1.8*	*3*	*1.4*	15	*0.6*	*0.7*	*4*	*6*

Table 1. Continued

Patients Age (years), gender Motor deficit	4 40/Female S/D/4L				5 40/Male S/D/4L		6 30/Male S/P/D/4L		7 50/Female S/DUL-PLL				
Motor studies (day)	5	10	21	160	3	10	8	250	2	16	50	210	360
CMAP area P/D reduction (%)	*40**	<10		<10	*91**	<10	*95**	*48*	*94**	<10	<10	<10	<10
DML (ms)	2.2	2.5		2	3.2	2.8	2.5	3.4	2.2	2.6	2.4	2.8	2.5
CV (limb segment) (m/s)	65	56		52	46	53	51	*36*	66	58	52	53	56
Left ulnar nerve													
CMAP (mV) wrist	7	*0.3*	0	*1*	5.4	*2.9*			15	*2.4*			
CMAP area P/D reduction (%)	<10	<10		<10	*95**	<10			*96**	<10			
DML (ms)	1.8	2.4		2.3	3	3			2	2.8			
CV (limb segment) (m/s)	58	59		56	54	53			67	62			
Right peroneal nerve													
CMAP (mV) ankle	*2*	*0.4*	*0.3*	*0.5*	*2.1*	*2.8*	3.5	*0.8*	8	*4*	*4*	5.5	7
CMAP area P/D reduction (%)	*50***	<10	<10	<10	<25	<25	*90***	<10	<25	<25	<25	<25	<25
DML (ms)	4.3	5.7	5.2	4.8	2.7	3.6	5	5.9	4.2	3.6	3	3.9	3.1
CV (limb segment) (m/s)	41	47	45	44	49	41	47	43	55	45	43	45	49
Left peroneal nerve													
CMAP (mV) ankle	*1.8*	*0.6*	0	*0.1*	*1.8*	*2.7*	*1*	*0.9*	6.5	*3*			6
CMAP area P/D reduction (%)	*50***	<10		<10	<25	<25	<10	<10	<25	<25			<25
DML (ms)	5.7	5.6		5.2	3.7	3.3	4.8	5.5	5.2	5.5			3.4
CV (limb segment) (m/s)	49	50		47	48	42	42	40	47	45			43
Right tibial nerve													
CMAP (mV) ankle	*0.5*	*0.7*	*0.2*	*1.7*	*0.9*	*2*	*0.6*	*1.9*	6	5	9	11	11
CMAP area P/D reduction (%)	<10	<10	<10	<10	<25	<25	<10	<10	<25	<25	<25	<25	<25
DML (ms)	5.7	5.2	4.3	4.7	4.2	5.1	5.4	5.7	4	4.6	4.2	3.9	3.5
CV (limb segment) (m/s)	44	45	49	44	52	49	44	41	44	43	42	42	40
Left tibial nerve													
CMAP (mV) ankle	*0.7*	*0.4*	0	*0.1*	*0.8*	*1.7*	*0.9*	*1.2*	7	9			12
CMAP area P/D reduction (%)	<10	<10		<10	<25	<25	<10	<10	<25	<25			<25
DML (ms)	6.3	5.3		4.4	5.6	5.1	5.7	5.7	4.3	5.2			4.7
CV (limb segment) (m/s)	44	50		46	46	48	42	*38*	48	40			41

CMAP area P/D reduction (%); bold and italicized values indicate definite partial conduction block; unbold and italicized values indicate probable partial conduction block.
S, symmetric; D, distal; L, limb; P, proximal; F, face; UL, upper limb; LL, lower limb; AMCBN, acute motor conduction block neuropathy; CMAP, compound muscle action potential; DML, distal motor latency; CV, conduction velocity, *, forearm; **, leg.

2 developed IgG antibodies against complexes of GM1/GM1b, GM1/GD1a, GM1/GT1a, GA1/GD1a, and GA1/GT1a. No patients had IgG antibodies against GM1/GalNAc-GD1a complex. In Patients 1, 4, and 6, an interesting serological pattern was observed. All three patients had significantly increased reactivity against GM1 alone but demonstrated a reduced reaction against GM1/GalNAc-GD1a complex (Fig. 1).

Discussion

Recent studies have suggested that 50% of patients with anti-GM1/GalNAc-GD1a antibodies are associated with pure motor neuropathy with conduction blocks at intermediate segments *(Kaida et al., 2008; Ogawa et al., 2013)*. In this study, we investigated, early in the time course evolution of the neuropathy, at the time of neurophysiological features consistent with AMCBN diagnosis, a small group of patients with conduction blocks in AMAN and found positive antibodies against single glycolipids

and GSCs, other than GM1/GalNAc-GD1a complex. Instead, there were patients with reduced antibody titers against GM1/GalNAc-GD1a complex.

Aside from the methodology, there are several differences between this study and previous studies. In the previous studies, patients with anti-GM1/GalNAc-GD1a antibodies frequently had the presence of antecedent respiratory infections and the majority of patients had good prognoses *(Kaida et al., 2008; Ogawa et al., 2013)*. Conduction blocks were limited to forearm and leg segments of nerves. In another series, early recovery without the development of distal conduction blocks was demonstrated *(Hong et al., 2011)*. Finally, the present series is different from Kokubun *et al.* (2010) that included patients with definite AMAN associated with at least one conduction block, or patients with follow-up study of conduction blocks in the upper limb only.

In this study, most of our patients with early conduction block had antecedent diarrheal illness rather than respiratory illness. We also failed to demonstrate rapidly reversible conduction failure such as that seen
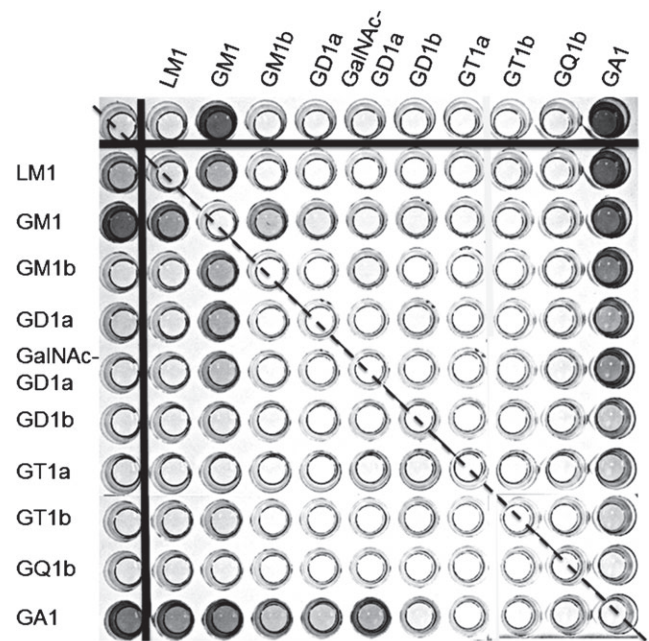
Table 2. Serological testing for IgG antibodies to single antigens and ganglioside complexes.

Patient number	1	2	3	4	5	6	7
IgG antibodies to							
GM1	+		+	+		+	+
GD1a	+		+				+
GalNAc-GD1a			+				
GD1b	+	+					+
GA1		+	+	+		+	+
IgG to GM1 complex with							
LM1				−		−	
GM1b		+		−		−	
GD1a		+		−		−	
GalNAc-GD1a	−			−		−	
GD1b				−		−	
GT1a		+		−		−	
GT1b				−		−	
GQ1b				−		−	
GA1				−		−	
IgG to GA1 complex with							
LM1	+			−			
GM1	+			−			
GM1b	+			−			
GD1a	+			−			
GalNAc-GD1a	+			−			
GD1b	+			−		−	
GT1a	+			−		−	
GT1b	+			−		−	
GQ1b	+			−		−	

GA1, asialo-GM1, +, positive reaction; −, the reaction was reduced when compared with reaction to a single antigen; blank, no reaction.

Figure 1. Reactivity of IgG antibodies to single glycolipid and to ganglioside complexes. Serum IgG antibodies from patient 6 react with GM1 and asialo-GM1 (GA1) as demonstrated by a darker shade (corrected averaged optical density [OD] values for GM1 = 2.2; GA1 = 2.5). In contrast, in wells that contained a combination of GM1 and all the other gangliosides, including GM1/GalNAc-GD1a, the reactions are reduced. This is visually demonstrated by the lighter shade in these wells when compared with the wells of GM1 alone. The difference between the corrected OD values of GM1 and the GM1 complexes were > 0.5. For example, the corrected OD value of GM1/GalNAc-GD1a was 0.6, which is markedly reduced compared with the corrected OD value for GM1, which was 2.2. A similar pattern of reduced reaction was also seen in the wells with ganglioside complexes GA1/GD1b, GA1/GT1a, GA1/GT1b, and GA1/GQ1b. The oblique dotted line runs through the control wells, which have no antigen added to them.

in AMCBN in six of our seven patients. All six patients had antecedent diarrhea and made slow clinical recoveries. In five patients, conduction blocks evolved into axonal degeneration typical of AMAN between days 8 and 30. In two of these patients, conduction block persisted up to day 250 of nerve studies, a feature more frequently observed in multifocal motor neuropathy but in this case, associated with an IgG anti-ganglioside immune response instead. Interestingly, only one patient (Patient 3) was seen to have reversible conduction failure that is classically seen in AMCBN. This may account for the slow recovery seen in our patient cohort. Reversible conduction failure has been associated with a better prognosis and earlier recovery. AMCBN has been described as an aborted form of AMAN with isolated conduction blocks that rapidly resolves.

None of the patients in the current cohort had positive IgG against GM1/GalNAc-GD1a complex. In this study, our findings suggest that anti-GM1/GalNAc-GD1a antibodies are not associated with conduction blocks when associated with antecedent diarrhea and subsequent progression to axonal degeneration. Instead, the serological pattern seen in the current cohort showed reactivities in all patients toward single antigens, predominantly against GM1 and GA1. Further analyses of antibodies

against GSC did not increase the yield further. The reduced reactivity or inhibitory effect against GSCs seen in some cases that were otherwise positive against single antigens raises questions as to the true significance of anti-GSCs in the pathogenesis of the development of conduction blocks in GBS patients.

In the animal model of AMAN, IgG is deposited at the nodes of Ranvier, with subsequent complement activation, lengthening of the nodal region, and detaching the paranodal myelin terminal loops. The latter feature is thought to represent paranodal demyelination, but it is important to recognize that the primary pathology is axonal rather than myelin. These changes can lead to conduction block which, when resolves rapidly, is considered reversible conduction failure, such as that seen in AMCBN *(Susuki et al., 2012)*. However, as we have demonstrated in most of our patients,

conduction block can progress instead to axonal degeneration and Wallerian-like degeneration with persistent autoimmune attack. This can occur in conjunction with other axonal insults in other nerves *(Kokubun et al., 2012)*. Similar findings with sensitization of animal models with GSC are yet to be demonstrated.

In conclusion, this study showed the association of axonal conduction block with antibodies against single anti-glycolipid and anti-GSCs but not specifically with anti-GM1/GalNAc-GD1a antibodies. While this may be attributed to the different clinical features of the current cohort of patients, further studies incorporating a range of patient presentations and patterns of conduction block is required before a more definite conclusion can be made regarding the role of antibodies against GSC in the pathogenesis of conduction blocks in the context of GBS.

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Disclosure

Author Contributions

A. C., N. S., A. S., and N. Y. contributed to drafting/revising the manuscript for content, including medical writing for content. N. Y. contributed to study concept and design. A. C., N. S., A. S., J.-P. L., and N. Y. were involved in the analysis and interpretation of data.

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3.5   Publication 5

Antibodies to single glycolipids and glycolipid complexes in Guillain-Barré syndrome subtypes. Shahrizaila N, Kokubun N, Sawai S, Umapathi T, Chan YC, Kuwabara S, Hirata K, Yuki N. Neurology. 2014 Jul 8;83(2):118-24
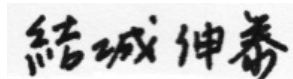
3.5.1   Contribution of co-authors:

Design and concept of study	N Shahrizaila, N Yuki
Acquisiton of data	All authors
Analyses of data	All authors
Drafting of manuscript	N Shahrizaila
Revising manuscript for intellectual content	N Shahrizaila, N Yuki

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As the corresponding author of the article named above, I consent to Nortina Shahrizaila including the published article above as part of her collection of published works to be submitted for her "PhD by prior publication" at University of Malaya, Kuala Lumpur

Signed,

結城伸番

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Antibodies to single glycolipids and glycolipid complexes in Guillain-Barré syndrome subtypes

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ABSTRACT

Objective: To comprehensively investigate the relationship between antibodies to single glycolipids and their complexes and Guillain-Barré syndrome subtypes and clinical features.

Methods: In acute sera from 199 patients with Guillain-Barré syndrome, immunoglobulin G (IgG) antibodies to glycolipids and ganglioside complexes were tested using ELISA against individual antigens from single glycolipids including gangliosides (LM1, GM1, GM1b, GD1a, GalNAc-GD1a, GD1b, GT1a, GT1b, GQ1b) and a neutral glycolipid, asialo-GM1 (GA1), and antigens from the combination of 2 different glycolipids. Based on serial nerve conduction studies, the electrodiagnoses were as follows: 69 demyelinating subtype, 85 axonal subtypes, and 45 unclassified.

Results: Significant associations were detected between acute motor axonal neuropathy subtype and IgG antibodies to GM1, GalNAc-GD1a, GA1, or LM1/GA1 complex. Reversible conduction failure was significantly associated with IgG antibodies to GM1, GalNAc-GD1a, GD1b, or complex of LM1/GA1. No significant association was demonstrated between acute inflammatory demyelinating polyneuropathy and any of the glycolipids or ganglioside complexes. Anti-ganglioside complex antibodies alone were detected in 7 patients (5 axonal subtype).

Conclusions: The current study demonstrates that antibodies to single glycolipids and ganglioside complexes are associated with acute motor axonal neuropathy or acute motor conduction block neuropathy but not acute inflammatory demyelinating polyneuropathy.

Classification of evidence: This study provides Class II evidence that antibodies to glycolipids are increased in patients with acute motor axonal neuropathy and acute motor conduction block neuropathy but not acute inflammatory demyelinating polyneuropathy. *Neurology*® 2014;83:1–7

GLOSSARY

AIDP = acute inflammatory demyelinating polyneuropathy; **AMAN** = acute motor axonal neuropathy; **AMCBN** = acute motor conduction block neuropathy; **GBS** = Guillain-Barré syndrome; **GSC** = ganglioside complex; **Ig** = immunoglobulin; **NCS** = nerve conduction study.

Guillain-Barré syndrome (GBS) is an acute immune-mediated polyneuropathy with 2 major subtypes: acute inflammatory demyelinating polyneuropathy (AIDP) and acute motor axonal neuropathy (AMAN).[1] Within the axonal subtype, there are now recognized variants evident on nerve conduction studies (NCS), which demonstrate early reversible conduction failure, referred to as acute motor conduction block neuropathy (AMCBN).[2] There is robust evidence that immunoglobulin G (IgG) anti-ganglioside antibodies are associated with the pathogenesis of AMAN, whereas the target antigens in AIDP remain elusive.[3]

In 2004, antibodies to ganglioside complexes (GSCs) were reported in patients with GBS.[4] The patients who were seronegative for antibodies to single gangliosides were found to have anti-GSC antibodies. The authors have since described further associations between anti-GSC antibodies and variants of GBS. This includes antibodies to LM1 and its complexes in AIDP,[5] to complex of GM1 and GalNAc-GD1a (GM1/GalNAc-GD1a) in AMCBN,[6] and to complexes of GD1a/GD1b and GD1b/GT1b in patients with GBS requiring artificial ventilation.[7]

Supplemental data
at Neurology.org

In the current study, we aimed to investigate the relationship between anti-GSC antibodies and specific clinical features of GBS as well as the electrodiagnostic subtypes of GBS, the latter based on serial NCS in a large cohort of patients from different geographical locations.

METHODS **Serum samples.** Acute phase sera were collected from patients with GBS presenting consecutively to 5 different centers, namely, University Malaya Medical Centre in Malaysia, National Neuroscience Institute and National University Hospital in Singapore, and Dokkyo Medical University and Chiba University in Japan. Patients from Malaysia and Singapore were prospectively recruited from 2010 to 2012. Patients recruited from the Japanese cohort were consecutively seen between 1998 and 2012. A total of 199 patients (Malaysia, 22; Singapore, 33; Japan, 144) with GBS were recruited. The clinical features in each patient, specifically, the presence of ophthalmoplegia, bulbar palsy, facial palsy, sensory impairment, and respiratory failure necessitating artificial ventilation were documented by the respective neurologists from each center.

Standard protocol approvals and patient consents. Patients' informed written consents, clinical data, and sera samples were obtained following protocol approved by the respective institution's ethics committee.

Nerve conduction studies. NCS were performed at presentation and repeated subsequently within a period of 3 to 6 weeks. The electrodiagnosis of GBS was initially defined according to existing criteria.[1] However, a final electrodiagnosis was made after the second NCS. The final electrodiagnoses were AIDP, AMAN (which included both AMCBN and acute motor and sensory axonal neuropathy subtypes), and unclassified. In a separate analysis, patients exhibiting the presence of reversible conduction failure defined by a decrease of proximal to distal compound motor action potential amplitude by 50% in intermediate nerve segments without temporal dispersion were considered to have AMCBN, a less severe form of AMAN.[8]

ELISA. Serologic analyses were performed for IgG antibodies to single glycolipids including gangliosides (LM1, GM1, GM1b, GD1a, GalNAc-GD1a, GD1b, GT1a, GT1b, and GQ1b) and a neutral glycolipid, asialo-GM1 (GA1), using ELISA.[9] Patients' sera were also assessed for IgG antibodies to GSC, which were tested with a mixture of individual glycolipids at 5 pmol/well each. Anti-glycolipid and -GSC antibodies were considered positive when the optical density was greater than 0.5 of the sum of antibodies to individual antigens. The tests were performed in quadruplicate and a mean of the optical density value was measured.

Statistical analysis. Comparative analyses of categorical outcomes were performed with the Fisher exact test or χ^2 test. A p value <0.05 was considered statistically significant.

Classification of evidence. The primary objectives of our study were to describe the relationship between antibodies against single glycolipids and glycolipid complexes and GBS subtypes. The study provides Class II evidence that antibodies to single glycolipids and glycolipid complexes are increased in AMAN and AMCBN but not AIDP.

RESULTS **Comparison between the Malaysian-Singaporean and Japanese cohorts.** The presence of ophthalmoplegia, facial palsy, bulbar weakness, sensory impairment, and need for artificial ventilation were significantly more frequent in the Malaysian-Singaporean (n = 55) than the Japanese cohort (n = 144) (table 1). Electrodiagnosis between the 2 cohorts reached no significant difference in AIDP and AMAN, but there were significantly more cases that were unclassified in the Japanese cohort. In contrast, more patients were seen to have reversible conduction failure in keeping with AMCBN in the Malaysian-Singaporean cohort. Despite the differences in the clinical patterns, there were no significant differences between seropositivity for either anti-ganglioside alone or anti-GSC alone between the cohorts.

In both cohorts, there was a significant association between the presence of antibodies to single glycolipids and AMAN as well as the absence of anti-ganglioside antibodies and AIDP (table 2). The same pattern was also observed with anti-GSC antibodies, but only in the Japanese cohort. The relationships among anti-glycolipid or -GSC antibodies, the GBS subtypes, and various clinical features were further analyzed in the entire group (tables 3 and 4).

Relationships among anti-ganglioside or -GSC antibodies, electrodiagnoses, and clinical features. The final electrodiagnoses based on serial studies for the entire group (n = 199) were as follows: AIDP = 69 patients, AMAN = 85, and unclassified = 45. The serologic analyses revealed 88 patients (44%) with positive serology. The results are shown in table 1. Analyses of IgG antibodies to individual single glycolipid and GSC revealed significant associations between AMAN and anti-GM1, -GalNAc-GD1a, -GA1, and -LM1/GA1 antibodies (table 3). Figure e-1 on the *Neurology*® Web site at Neurology.org depicts an example of seropositive findings in a patient with AMAN. AMCBN was associated with anti-GM1, -GalNAc-GD1a, and -GD1b antibodies as well as anti-LM1/GA1 antibodies. In contrast, AIDP was not significantly associated with any of the glycolipids or GSCs.

Regarding the clinical features, significant associations were detected between IgG anti-GT1a and -GQ1b antibodies and ophthalmoplegia (table 4). Patients with IgG anti-GM1, -GalNAc-GD1a, -GD1a, and -GA1 antibodies were less likely to have facial palsy, and those with IgG anti-GalNAc-GD1a antibodies were also less likely to have bulbar palsy. In addition, sensory impairment was less likely to be demonstrated in patients who had IgG anti-GM1, -GalNAc-GD1a, -GA1, -LM1/GA1, -GM1/GalNAc-GD1a, and -GM1b/GA1 antibodies. The need for artificial ventilation showed no significant association with the presence of IgG antibodies to glycolipids or GSCs.

DISCUSSION In the current study, we investigated the relationship between anti-glycolipid or -GSC

Table 1 Comparison of clinical features, electrodiagnosis, and serologic analyses

	Malaysia/Singapore (n = 55), n (%)	Japan (n = 144), n (%)	p Value	Odds ratio (95% CI)
Clinical feature				
Ophthalmoplegia	11 (20)	9 (6)	0.0039	3.7 (1.4-9.6)
Facial weakness	21 (38)	25 (17)	0.0018	2.9 (1.4-5.8)
Bulbar palsy	27 (49)	30 (21)	<0.001	3.7 (1.8-7.5)
Sensory impairment	42 (76)	67 (47)	<0.001	3.7 (1.7-8.0)
Artificial ventilation	17 (31)	18 (13)	0.014	2.4 (1.1-5.5)
Neurophysiology				
AIDP	24 (44)	45 (31)	NS	
AMAN	27 (49)	58 (40)	NS	
Unclassified	4 (7)	41 (28)	0.0013	5.0 (1.7-14.9)
AMCBN	16 (29)	16 (11)	0.0023	3.2 (1.4-7.7)
Serology positive for				
Single glycolipids	19 (35)	63 (44)	NS	
Ganglioside complexes	5 (9)	32 (22)	0.033	2.8 (1.0-7.7)
Ganglioside complexes only	0 (0)	7 (5)	NS	

Abbreviations: AIDP = acute inflammatory demyelinating polyneuropathy; AMAN = acute motor axonal neuropathy; AMCBN = acute motor conduction block neuropathy; CI = confidence interval; NS = not significant.

antibodies with electrophysiologic subtypes or specific clinical features of GBS. Patients were recruited from 2 geographical locations: Southeast Asia (represented by Malaysia and Singapore) and Japan. Although a comparison between the 2 cohorts revealed differences in the frequencies of certain clinical features, neither the electrodiagnostic classification of AIDP and AMAN nor the serologic analyses were significantly different. Analyses of the entire cohort revealed that significant associations of antibodies to certain single glycolipids and GSCs were evident in patients with an electrodiagnosis of AMAN but not AIDP. There were also specific antibodies that were significantly

associated with reversible conduction failure as well as certain clinical characteristics such as ophthalmoplegia and bulbar palsy.

In a previous comparative study between Japanese and Italian cohorts, no significant differences were found in the final GBS electrodiagnosis (also based on serial studies) and anti-ganglioside antibodies.[10] The current study also demonstrates that both GBS cohorts from Southeast Asia and Japan were not significantly different regarding the final electrodiagnoses of AIDP and AMAN or their serologic reactivities. The majority of seropositive patients had IgG antibodies to single glycolipids (with some also reacting to GSCs). In

Table 2 Antibodies to glycolipids and ganglioside complexes in Malaysian/Singaporean and Japanese populations

	Anti-glycolipid antibodies			Anti-ganglioside complex antibodies		
	Positive vs negative, n (%)	p Value	Odds ratio (95% CI)	Positive vs negative, n (%)	p Value	Odds ratio (95% CI)
Malaysian/Singaporean						
AIDP (n = 24)	1 (6) vs 23 (62)	<0.001	27.9 (3.3-233.4)	0 (0) vs 24 (48)	NS	
AMAN (n = 27)	15 (83) vs 12 (32)	<0.001	10.4 (2.2-56.6)	4 (14) vs 23 (46)	NS	
Unclassified (n = 4)	2 (11) vs 2 (6)	NS		1 (25) vs 3 (6)	NS	
Japanese						
AIDP (n = 45)	2 (3) vs 43 (53)	<0.001	34.5 (7.9-150.7)	1 (3) vs 44 (39)	<0.001	20.0 (2.6-152.2)
AMAN (n = 58)	41 (65) vs 17 (21)	<0.001	13.0 (6.0-29.0)	20 (63) vs 38 (34)	0.0042	3.2 (1.3-8.0)
Unclassified (n = 41)	20 (32) vs 21 (26)	NS		11 (34) vs 30 (27)	NS	

Abbreviations: AIDP = acute inflammatory demyelinating polyneuropathy; AMAN = acute motor axonal neuropathy; CI = confidence interval; NS = not significant.
AMAN includes acute motor conduction block and acute motor and sensory subtypes.

Table 3 Association of electrodiagnostic subtypes with antibodies to glycolipids and ganglioside complexes

IgG antibodies to	AIDP (n = 69) vs non-AIDP (n = 130)			AMAN (n = 85) vs non-AMAN (n = 114)			AMCBN (n = 32) vs non-AMCBN (n = 167)		
	No. (%)	p Value	Odds ratio (95% CI)	No. (%)	p Value	Odds ratio (95% CI)	No. (%)	p Value	Odds ratio (95% CI)
Single glycolipids									
GM1	1 (1) vs 46 (35)	<0.001	37.2 (5.0–277.0)	29 (34) vs 18 (15)	0.0031	2.8 (1.3–5.7)	12 (37) vs 35 (20)	0.044	2.3 (0.9–5.4)
GalNAc-GD1a	0 (0) vs 20 (15)	<0.001	25.7 (1.5–433.2)	16 (18) vs 4 (3)	<0.001	6.4 (1.9–23.6)	9 (28) vs 11 (6)	<0.001	5.6 (1.9–16.5)
GD1b	2 (2) vs 29 (22)	<0.001	9.6 (2.2–41.6)	17 (20) vs 14 (12)	NS		11 (34) vs 20 (11)	0.0032	3.9 (1.5–9.9)
GA1	2 (2) vs 40 (30)	<0.001	14.8 (3.4–63.7)	28 (32) vs 12 (10)	<0.001	4.2 (1.9–9.5)	10 (31) vs 30 (17)	NS	
Ganglioside complexes									
LM1/GA1	1 (1) vs 20 (15)	0.0023	12.3 (1.6–94.2)	14 (16) vs 7 (6)	0.023	3.0 (1.1–8.7)	9 (28) vs 12 (7)	0.0024	5.1 (1.7–14.7)
GM1b/GA1	0 (0) vs 10 (7)	0.030	12.1 (0.7–209.8)	7 (8) vs 3 (2)	NS		4 (12) vs 6 (3)	0.032	3.8 (1.0–14.4)
GD1a/GA1	0 (0) vs 11 (8)	0.021	13.3 (0.7–230.5)	8 (9) vs 3 (2)	0.032	3.8 (0.9–14.9)	4 (12) vs 7 (4)	NS	
GT1b/GA1	0 (0) vs 10 (7)	0.030	12.1 (0.7–209.8)	7 (8) vs 3 (2)	NS		3 (9) vs 7 (4)	NS	

Abbreviations: AIDP = acute inflammatory demyelinating polyneuropathy; AMAN = acute motor axonal neuropathy; AMCBN = acute motor conduction block neuropathy; CI = confidence interval; IgG = immunoglobulin G; NS = not significant.
Represents the single gangliosides and ganglioside complexes that showed a significant association with the electrodiagnostic subtypes.

seronegative patients, analysis of IgG antibodies to GSC identified a further 7 patients. GSCs are thought to represent new clustered epitopes that are recognized by antibodies that would normally not recognize epitopes of a single glycolipid.

LM1 is a predominant peripheral nerve ganglioside, localized in motor nerve myelin,[11,12] and thus it is possible that antibodies to LM1 and its complexes are involved in the development of AIDP. Several studies have investigated the presence of both IgG and IgM anti-LM1 antibodies in GBS, and the results have been variable. The frequencies range from 43%[13] and 23% of patients with GBS[14] to less than 10% of GBS in other series.[15–17] In a more recent study, a significant association of AIDP with antibodies to LM1 and its complexes was reported.[5] However, in the current study, we did not detect as strong an association of AIDP with IgG antibodies to LM1 and its complexes making it less likely that LM1 or its complexes are target antigens, at least in AIDP. Instead, we found that the LM1/GA1 complex was significantly associated with AMAN, reversible conduction failure or AMCBN, and the absence of sensory impairment. In previous studies, the electrodiagnosis of GBS was based on a single study. Given our current understanding that the neurophysiologic findings in GBS can rapidly change in the early stages of the disease, we believe that the diagnosis of AIDP was likely to have been overestimated. Based on our findings, pathogenic autoantibodies involved in AIDP remain elusive.

Certain electrophysiologic features, such as reversible conduction failure, have previously been associated with the presence of IgG antibodies to specific gangliosides, namely, GM1.[18–21] In the current study, we found significant associations of reversible conduction failure with IgG anti-GM1, -GalNAc-GD1a, -GD1b, and -LM1/GA1 antibodies. Reversible conduction failure was first described in 1998 and this was followed by reports of similar findings in other cohorts.[18] Some authors have referred to patients with such features as having AMCBN, associated with a better prognosis in comparison to AMAN. AMCBN is a predominantly motor neuropathy and thus it is not surprising to find associations with IgG anti-GM1 and -GalNAc-GD1a antibodies, both of which have been described in AMAN. Notably, there were significantly more patients with AMCBN in the Southeast Asian cohort compared with the Japanese cohort, and the significance of this merits further study in a larger cohort.

Previous studies have provided evidence that IgG anti-GM1 or -GD1a antibodies are pathogenic in the development of AMAN.[22] Several clinical patterns have since been described in association with certain anti-ganglioside antibodies. This includes the association of pure motor GBS with IgG anti-GM1/GalNAc-GD1a antibodies[6] and IgG antibodies to

Table 4 Relationship of clinical features with antibodies to glycolipids and ganglioside complexes

	Ophthalmoplegia			Facial weakness			Bulbar palsy			Sensory impairment		
	Yes (n = 20) vs no (n = 179), n (%)	p Value	Odds ratio (95% CI)	Yes (n = 61) vs no (n = 138), n (%)	p Value	Odds ratio (95% CI)	Yes (n = 57) vs no (n = 142), n (%)	p Value	Odds ratio (95% CI)	Yes (n = 109) vs no (n = 90), n (%)	p Value	Odds ratio (95% CI)
Single glycolipids												
GM1	2 (10) vs 45 (25)	NS		4 (6) vs 43 (31)	<0.001	6.4 (2.2-18.9)	7 (12) vs 40 (28)	0.011	0.3 (0.1-0.8)	11 (10) vs 36 (40)	<0.001	5.7 (2.7-12.1)
GalNAc-GD1a	1 (5) vs 19 (10)	NS		1 (1) vs 19 (13)	0.0091	9.5 (1.2-73.2)	1 (1) vs 19 (13)	0.018	0.1 (0.006-0.8)	4 (3) vs 16 (17)	<0.001	5.6 (1.8-17.6)
GD1b	3 (15) vs 28 (15)	NS		4 (6) vs 27 (19)	0.022	3.4 (1.1-10.3)	6 (10) vs 25 (17)	NS		12 (11) vs 19 (21)	NS	
GT1a	6 (30) vs 5 (2)	<0.001	14.9 (3.5-66.5)	5 (8) vs 6 (4)	NS		6 (10) vs 5 (3)	NS		7 (6) vs 4 (4)	NS	
GQ1b	5 (25) vs 3 (1)	<0.001	19.6 (3.6-117.2)	4 (6) vs 4 (2)	NS		5 (8) vs 3 (2)	0.042	4.5 (0.90-24.5)	6 (5) vs 2 (2)	NS	
GA1	3 (15) vs 37 (20)	NS		3 (4) vs 37 (26)	<0.001	7.0 (2.0-23.9)	7 (12) vs 33 (23)	NS		13 (11) vs 27 (30)	0.0024	3.1 (1.5-6.5)
Ganglioside complexes												
LM1/GA1	1 (5) vs 20 (11)	NS		3 (4) vs 18 (13)	NS		5 (8) vs 16 (11)	NS		7 (6) vs 14 (15)	0.044	2.6 (1.0-6.9)
GM1/GalNAc-GD1a	0 (0) vs 5 (2)	NS		0 (0) vs 5 (3)	NS		0 (0) vs 5 (3)	NS		0 (0) vs 5 (5)	NS	
GM1b/GA1	0 (0) vs 10 (5)	NS		1 (1) vs 9 (6)	NS		3 (5) vs 7 (4)	NS		1 (1) vs 8 (8)	0.0075	10.5 (1.2-85.9)

Abbreviations: CI = confidence interval; NS = not significant.
Represents the single gangliosides and ganglioside complexes that showed significant associations with the different clinical features. Artificial ventilation was found not to be significantly associated with any glycolipids or glycolipid complex.

GD1a/GD1b and GD1b/GT1b with the need for artificial ventilation.[7] We investigated the various clinical features and their associations with seropositivity in our cohort. We found that patients who lacked sensory impairment (indicating a predominant motor form of GBS) were significantly associated with IgG antibodies to GM1, GalNAc-GD1a, and GA1 as well as IgG antibodies to LM1/GA1, GM1/GalNAc-GD1a, and GM1b/GA1. In contrast, none of the patients who required mechanical ventilation had significant associations with anti-GQ1b antibodies, which has previously been reported to be predictive of mechanical ventilation.[23] Of note, in the current study, only one patient was seropositive for anti-GD1a/GD1b and none for GD1b/GT1b, both of which have also been postulated to have associations with severity of GBS.[7]

The presence of ophthalmoplegia and bulbar palsy was associated with IgG anti-GQ1b antibodies, in keeping with previous reports.[24,25] Ophthalmoplegia is a key feature of Fisher syndrome, which has a strong association with anti-GQ1b antibodies,[25] whereas bulbar palsy is typically seen in patients with the pharyngeal-cervical-brachial variant of GBS, which is associated with monospecific anti-GT1a antibodies.[26,27] In the current study, the association of bulbar palsy with anti-GT1a antibodies did not reach significance. Instead, anti-GQ1b antibodies, which are recognized to crossreact with GT1a, were significantly associated with bulbar palsy.[25] This association has been demonstrated in previous studies comparing GBS with and without bulbar palsy.[28] None of the patients in the current cohort had monospecific anti-GT1a antibodies. Contrary to previous reports, antibodies to GSCs were not significantly higher in either group of patients with ophthalmoplegia or bulbar palsy.[7] In our cohort, the presence of facial palsy was associated with a diagnosis of AIDP without significant serologic associations. Instead, the presence of IgG anti-GM1, -GalNAc-GD1a, -GD1b, and -GA1 antibodies was less likely to result in the development of facial palsy. Facial palsy in GBS has been described to occur in almost 60% of patients with GBS,[29] and there are reports that recognize the presence of "bifacial weakness and paraesthesia" as a variant of AIDP.[30,31] Our studies would support this hypothesis and that there are as yet no specific antigens that can be associated with facial palsy.

Before the current work, the majority of the literature on GSCs and the clinical characteristics associated with them has originated from a different Japanese cohort.[4] Although our findings share similarities to their cohort, there were also discrepancies such as the lack of association of antibodies to LM1 and LM1 complexes in AIDP. The most apparent reason for the differences is the different methodologies in

serologic analyses by ELISA and GBS electrodiagnosis. In comparison to previous studies, our serologic analyses utilized a reduced amount of antigen (e.g., GM1, 7.5 vs 200 ng) and a higher serum and secondary antibody dilution (1:500 and 1:2,000 vs 1:40 and 1:500, respectively). The optical density value for seropositivity also differed ($\geq$0.5 in the current study vs >0.1 in single gangliosides and >0.2 in GSCs in other studies). We believe that the methodology adopted in our study would result in more specific findings. The final electrodiagnostic criteria in the current study were based on serial studies, taking into account the existing limitations of a single study.[2] In contrast, other studies have used different criteria based on one study, which may overestimate AIDP.[32]

In a more recent study, antibodies to glycolipid complexes were assessed in sera from a Western European cohort utilizing the combinatorial glycoarray method.[33] The method differs from traditional ELISA, and discrepancies of results obtained from ELISA were noted by the authors. In the study, a large number of heterodimeric glycolipid complexes were assessed (n = 162) and the authors found an increase in seropositivity to the glycolipid complexes of patients with "demyelinating" GBS or unclassified. Similar to previous studies, the GBS electrodiagnoses were based on a single NCS. These are some of the limitations of the current study and highlight the importance of standardizing methodology of serology and electrophysiology among investigators to allow for improved and more valid comparisons of GBS patterns between cohorts. One likely platform for such work to be done could be the ongoing multicentered International GBS Outcome Study, recently initiated by the Inflammatory Neuropathy Consortium.

The current study of a large multicentered GBS population suggests that antibodies to glycolipids and GSCs are associated with classical AMAN and AMCBN but not demyelinating GBS. Future work incorporating standardized methodology, including reliable electrodiagnostic criteria for classifying GBS subtypes, is required to better clarify the true relationship between antibodies to glycolipids and GSCs and the clinical and electrophysiologic patterns.

AUTHOR CONTRIBUTIONS

Design or conceptualization of the study (N.S., N.Y.), analysis or interpretation of the data (all authors), and drafting or revising the manuscript for intellectual content (N.S., N.Y.).

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DISCLOSURE

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CHAPTER 4: CONCLUSION

It has been almost a century since the first description of GBS by French neurologists, Guillain, Barré and Strohl. (Guillain et al., 1916) Our understanding of GBS has evolved over the years but the last two decades saw significant progress made in research within this field with the discovery of antibodies against glycolipids and their relationship to antecedent infections such as *C jejuni*. (Yuki et al., 2004)

Neurophysiological studies have played a crucial role in the diagnosis of GBS, discriminating between immune-mediated insults directed towards myelin or axon. However, recent studies have found significant flaws in the existing criteria. (Hadden et al., 1998; Ho et al., 1995) that continue to be used as a reference for making the electrodiagnosis of GBS. (Uncini & Kuwabara, 2012) The main criticism is the lack of recognition of conduction blocks in axonal GBS which represent pathology at the nodo-paranodal region of peripheral nerves. (Uncini & Kuwabara, 2015) A single NCS study performed at the initial stages of the disease demonstrate features that may not necessarily be helpful at distinguishing between axonal or myelin damage. Previous studies have largely utilized single NCS to demonstrate associations between serological analyses and GBS variants. This could potentially lead to erroneous reports of serological profiling of GBS variants and their clinical features. This in turn could result in misdirection of subsequent pathological studies.

The current body of work presented in this thesis aimed at further clarifying the pathogenesis of GBS through studies of serial neurophysiology and their association with serological analyses. Two sets of publications have been presented, describing studies on serial NCS and studies of antibodies against glycolipids respectively. In the

first publication, (Shahrizaila et al., 2011) we investigated prospective serial nerve conduction studies in GBS and its variants in a cohort of multi-ethnic Malaysian patients. Similar to reports from the Japanese and Italian groups, (Kokubun et al., 2010; Kuwabara, Asahina, et al., 1998; Sekiguchi et al., 2012; Uncini et al., 2010) we recognized patterns of reversible conduction failure in our GBS cohort. It is now widely recognized that the latter reflect pathology at the paranodal region. In keeping with this, patients also harbor IgG against glycolipids such as GM1, GD1a and GalNAc-GD1a. These glycolipids are known to reside at the axon and abundantly the motor axons, resulting in a predominantly motor nerve involvement such as that seen in AMAN. This neurophysiological pattern of "axonal" conduction failure can only be detected when serial studies are performed. Although such studies have been previously performed, our study represented a different cohort of patients with different environmental and microbial exposures as well as different ethnic and genetic make-up. Our findings adds further to the growing literature acknowledging the need for a change in how we classify GBS based on NCS.

From a practical standpoint, performing multiple NCS may not be clinically feasible. Based on our second publication, (Shahrizaila et al., 2013) we found that rather than performing multiple studies, two sets of studies would suffice to clarify the true electrodiagnosis of GBS. Thus, we advocate studies done with 3 weeks of disease onset and a second study within 6 to 8 weeks of disease onset, as the benchmark for future proposals of GBS electrodiagnostic criteria. We also found that at 8 weeks of disease onset, patients with AIDP demonstrated persistent demyelinating features on NCS. In the same publication, we investigated the utility of the recent prognostic scale, mEGOS (Walgaard et al., 2011) at predicting disease prognosis in our cohort of patients. We found that whilst there was some merit in utilizing mEGOS especially when deciding on escalating treatment at the early stage of the disease, the scale did tend to underestimate

the potential recovery in patients. Our findings suggest that further work is required to better prognosticate patients and we believe that defining axonal degeneration through NCS and EMG are likely to be more specific. Future prognostic studies in a heterogeneous GBS population are currently underway through the Inflammatory Neuropathy Consortium-initiated International GBS Outcome Study, which the candidate is currently participating as one of the study investigator.

One of the most recognisable variants of GBS is MFS and since the discovery of IgG against GQ1b as a biomarker of MFS, further variants have been recognized representing the extent of disease involvement. In Western cohorts, the spectrum of MFS is rare. In our third publication, (Shahrizaila, Goh, et al., 2014) we found MFS variant to occur frequently in our GBS cohort, representing almost 50% of patients. This reflects the heterogeneous presentation of GBS and further highlights that factors inherent to the individual and its environment are involved in the pathogenesis of GBS. Due to the comparatively larger cohort of MFS patients, we were able to prospectively study this group of patients in greater detail. Based on serial NCS, we found that sensory nerves are preferentially involved in MFS regardless of symptoms. We also found a lower percentage of patients who were seropositive for IgG against GQ1b, compared to previous cohorts. This suggests that other target antigens are likely involved in our cohort of patients. We were also able to prospectively define the clinical features that were present in our patients and found that atypical features of ptosis, mydriasis and facial palsy were common. The latter occasionally presenting as a delay after other classic features such as ataxia and ophthalmoplegia had plateaued and started to improve. Other prior comprehensive studies of MFS have been in the Japanese cohort and similar to their studies, we found that patients have a good prognosis and all recovered to normal regardless of whether immunotherapy was initiated. Given the cost

and potential side-effects of immunotherapy, we are in a better position and more confident to not initiate immunotherapy in this group of patients.

Although some patients with axonal forms of GBS express IgG antibodies against certain glycolipids, AIDP and a proportion of axonal GBS patients remain seronegative. More recently, the Japanese group identified the presence of antibodies against complexes of gangliosides in GBS patients who were otherwise seronegative. (Kaida et al., 2004a) They went on to associate these antibodies with AIDP, conduction block and other clinical features of GBS. As previously stated, these studies have relied on single NCS which calls into question the electrodiagnosis profile of their GBS patients. The ELISA methodology employed in these studies also raised the possibility or false positives in some patients.

The second series of publications described studies of antibodies to GSCs with the aim of defining their relationship with neurophysiological and clinical characteristics of GBS. In a cohort of French patients, we found that antibodies against specific GSCs were not associated with "axonal" conduction block as had been previously described. (Creange et al., 2014) Similar findings were also found in a comprehensive multi-centre study in South-East Asian and Japanese GBS patients where there was further proof to support the lack of association between antibodies to both single and GSCs in AIDP patients. (Shahrizaila, Kokubun, et al., 2014) Our study remains the largest study to date and the only study to employ the use of serial NCS in defining GBS subtypes as well as recognizing the presence of reversible conduction failure in axonal forms of GBS. Our findings also highlighted the importance of standardizing the methodology of serological analyses in GBS and utilizing more specific methods in order to exclude false positive results. We also found that in patients who are seronegative for existing antibodies to single glycolipids, only a minority demonstrated antibodies to GSCs. One

other study by the British group have also study of IgG against GSCs, using a completely different method of glycoarray. (Rinaldi et al., 2013) The authors argue that the method allows for greater number of samples analysed simultaneously. However, they recognize that in some patients, ELISA remained the more specific diagnostic test.

The last two decades have brought greater understanding of GBS pathogenesis through neurophysiology and serological studies. However, more questions have been raised and the search for a reliable biomarker for GBS and all of its subtypes continues. The published works presented in this thesis have contributed towards a better understanding of how future studies of GBS neurophysiology should be conducted including proposals on a long overdue revision of the existing criteria. The serological work presented here has also provided a more comprehensive association study of serological analyses to GSCs. We have highlighted the need for further research to elucidate the target antigens of AIDP. It may be more meaningful for GBS researchers to work together in a collaborative manner to gain a greater perspective of the disease pathogenesis. We have witnessed the heterogeneity in its presentation amongst cohorts as well as the clinical outcome. Standardising methodology across several cohorts would be one of the ways of overcoming the current shortcomings in this area of research. It is hoped that the current ongoing international collaborative studies will address some of the questions that remain.

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APPENDIX A: LIST OF PUBLICATIONS

Original research articles represented in this thesis

1. **Shahrizaila N**, Goh KJ, Kokubun N, Abdullah S, Yuki N. Serial nerve conduction studies provide insight into the pathophysiology of Guillain-Barré and Fisher syndromes. J Neurol Sci. 2011 Oct 15;309(1-2):26-30.

2. **Shahrizaila N**, Goh KJ, Abdullah S, Kuppusamy R, Yuki N. Two sets of nerve conduction studies may suffice in reaching a reliable electrodiagnosis in Guillain-Barré syndrome. Clin Neurophysiol. 2013 Jul;124(7):1456-9.

3. **Shahrizaila N**, Goh KJ, Kokubun N, Tan AH, Tan CY, Yuki N. Sensory nerves are frequently involved in the spectrum of Fisher syndrome. Muscle Nerve. 2014 Apr;49(4):558-63.

4. Créange A, **Shahrizaila N**, Salhi H, Lefaucheur JP, Yuki N. Association of antibodies to ganglioside complexes and conduction blocks in axonal Guillain-Barré syndrome presenting as acute motor conduction block neuropathy. J Peripher Nerv Syst. 2014 Jun;19(2):115-20.

5. **Shahrizaila N**, Kokubun N, Sawai S, Umapathi T, Chan YC, Kuwabara S, Hirata K, Yuki N. Antibodies to single glycolipids and glycolipid complexes in Guillain-Barré syndrome subtypes. Neurology. 2014 Jul 8;83(2):118-24

Other published works in GBS with principal contribution from candidate during the period of thesis work

Book Chapters

1. **Shahrizaila N.**, and Yuki N. (2014) Acute Motor and Motor Sensory Neuropathy (Axonal Subtypes of Guillain-Barre Syndrome), Immunology of. In: Aminoff M.J. and Daroff R.B. (eds.) Encyclopedia of the Neurological Sciences, 2nd edition, vol. 1, pp. 49-53. Oxford: Academic Press

2. **Shahrizaila N.**, and Yuki N. (2014) Fisher's Syndrome and Bickerstaff's Encephalitis; Immunology of. In: Aminoff M.J. and Daroff R.B. (eds.) Encyclopedia of the Neurological Sciences, 2nd edition, vol. 2, pp. 308-311. Oxford:Academic Press

Review articles in ISI journals

1. **N Shahrizaila**, N Yuki. Guillain-Barre Syndrome, Fisher syndrome and Bickerstaff brainstem encephalitis: Understanding the pathogenesis. Neurology Asia 2010: 15(3):203-209 (ISI-Cited Publication)

2. **Shahrizaila N**, Yuki N.The role of immunotherapy in Guillain-Barre syndrome: understanding the mechanism of action. Expert Opinion in Pharmacotherapy. 2011 Jul;12(10):1551-60.

3. **N Shahrizaila**, N Yuki. Guillain-Barre Syndrome Animal Model: The First Proof of Molecular Mimicry in Human Autoimmune Disorder. J Biomedicine and Biotechnology. 2011;2011:829129.

4. **Shahrizaila N**, Yuki N. Antiganglioside antibodies in Guillain-Barre syndrome and its related conditions. Expert Review Neurotherapeutics. 2011 Sep;11(9):1305-13

5. **Shahrizaila N**, Yuki N. Bickerstaff brainstem encephalitis and Fisher syndrome: anti-GQ1b antibody syndrome.J Neurol Neurosurg Psy. 2013 May;84(5):576-83. doi: 10.1136/jnnp-2012-302824.

Editorials and Commentaries

1. Yuki N, **Shahrizaila N**. Keeping it simple: is there a need for the various subtyping of axonal forms of Guillain-Barre syndrome? Journal of Neurology, Neurosurgery and Psychiatry. 2011 Jun;82(6):592

2. **Shahrizaila N**, Yuki N. Peripheral neuropathies: Clinical prognostic scales in Guillain-Barre syndrome. Nature Reviews Neurology. 2011 Jun 21;7(7):362-3.

3. Yuki N, **Shahrizaila N**. How do we identify infectious agents that trigger Guillain-Barre syndrome, Fisher syndrome and Bickerstaff brainstem encephalitis? Journal of the Neurological Sciences. 2011 Mar 15;302(1-2):1-5.

Online resource

1. **Shahrizaila N** and Yuki, N. Fisher syndrome and Bickerstaff brainstem encephalitis. In : Gilman S, editor-in-chief. MedLink Neurology. San

Diego: MedLink Corporation. Available at www.medlink.com. Originally released on Dec 3,2010 and updated until Dec 3,2014

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1. Kokubun N, **Shahrizaila N**, Hirata K, Yuki N. Conduction block and axonal degeneration co-occurring in a patient with axonal Guillain- Barre syndrome. 2012 Aug 15;319(1-2):164-7

2. Kokubun N, **Shahrizaila N**, Hirata K, Yuki N. Reversible conduction failure is distinct from neurophysiological patterns of recovery in mild demyelinating Guillain-Barre syndrome. J Neurol Sci. 2013 Mar 15;326(1-2):111-4.

3. Kokubun N, **Shahrizaila N**, Koga M, Hirata K, Yuki N. The demyelination neurophysiological criteria can be misleading in Campylobacter jejuni- related Guillain-Barre syndrome. Clin Neurophysiol. 2013 Aug;124(8):1671-9.

4. Wong AH, Umapathi T, **Shahrizaila N**, Chan YC, Kokubun N, Fong MK, Chu YP, Lau PK, Yuki N.The value of comparing mortality of Guillain-Barre syndrome across different regions. 2014 Sep 15;344(1-2):60-2.

5. Sudo M, Yamaguchi Y, Sp th PJ, Matsumoto-Morita K, Ong BK, **Shahrizaila N**, Yuki N. Different IVIG Glycoforms Affect In Vitro Inhibition of Anti-Ganglioside Antibody-Mediated Complement Deposition. PLoS One. 2014 Sep 26;9(9):e107772

6. Miyaji K, Paul F, **Shahrizaila N**, Umapathi T, Yuki N. Complement regulatory proteins (CD46, 55 and 59) expressed on Schwann cells:

Immune targets in demyelinating neuropathies? J Neuroimmunol. 2014 Nov 15;276(1-2):172-4.

7. Miyaji K, **Shahrizaila N**, Umapathi T, Chan YC, Hirata K, Yuki N. Are ERM (ezrin/radixin/moesin) proteins targets for autoantibodies in demyelinating neuropathies? Hum Immunol. 2014 Nov;75(11):1089-91.

8. Fukami Y, Wong AH, Funakoshi K, Safri AY, **Shahrizaila N**, Yuki N. Anti- GQ1b antibody syndrome: anti-ganglioside complex reactivity determines clinical spectrum. Eur J Neurol. 2015 Jul 14. doi: 10.1111/ene.12769.
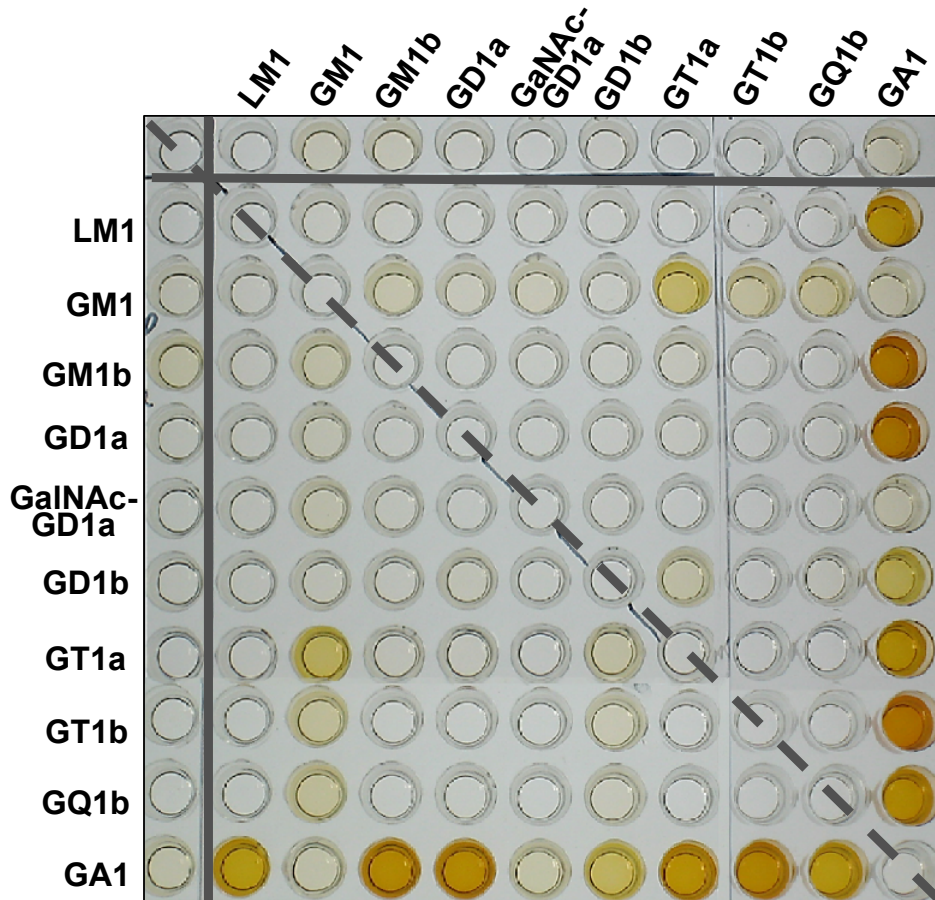
Figure. Reactivity of IgG antibodies to single glycolipid and to ganglioside complexes in a patient with acute motor axonal neuropathy. Serum IgG antibodies reacted with none of the single antigens but with ganglioside complexes of asialo-GM1 (GA1) and LM1, GM1b, GD1a, GD1b, GT1a, GT1b and GQ1b as well as complex of GM1/GT1a. This is visually demonstrated by the darker shade in these wells when compared to the other wells. The oblique dotted line runs through the control wells, which have no antigen added to them.