INVESTIGATION OF DIABETIC SENSORIMOTOR POLYNEUROPATHY THROUGH NEUROPHYSIOLOGY AND NERVE ULTRASONOGRAPHY IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

The present study explored the role of ultrasonography (US) and neurophysiology in characterizing type 2 diabetes patients according to their neuropathy severity as determined by the Toronto Clinical Scoring System (TCSS). This study also aimed to comprehensively determine the relationship between nerve US and nerve conduction study (NCS) parameters in diabetic patients. The second research question was to investigate if there are any imaging markers that could possibly differentiate diabetic patients with neurophysiological evidence of demyelination and true chronic inflammatory demyelinating polyneuropathy (CIDP) patients. The study subjects were 100 symptomatic distal symmetrical polyneuropathy (DSP) patients and 40 age-matched healthy controls. A subset of nine DSP patients with neurophysiological features of demyelination (D-DSP) and six true CIDP patients were also recruited. DSP severity was ascertained through TCSS where patients are grouped into mild (score 6-8), moderate (9-11) and severe (12-19). Nerve electrophysiology and ultrasound were performed on both lower limbs and the non-dominant upper limb in DSP subjects, and in both upper and lower limbs in true CIDP and D-DSP subjects. Nerves cross sectional area (CSA) recordings were taken at standard anatomical sites. A diagnosis of DSP and CIDP was made based on existing criteria. Statistical analyses were performed using SPSS version 22. Our findings revealed that sural nerve was inexcitable in 19.1% of mild, 40.0% of moderate and 69.0% of severe DSP groups. In contrast, CSAs were measureable in all nerves of DSP patients and were significantly larger compared to controls. Patients with severe DSP had significantly larger nerves in the ulnar, peroneal, tibial and sural, compared to mild DSP patients. By receiver operating characteristic analysis, the cut-off value for sural at 2 mm^2 was a good discriminator with area under curve (AUC) of 0.88 between the presence and absence of DSP (sensitivity 0.90 ;specificity 0.74) but performed less well in discriminating between severity of DSP

(cut-off 2.75mm^2; AUC 0.62; sensitivity 0.59; specificity 0.73). Significant correlations were demonstrated between TCSS, most neurophysiology parameters and nerve CSA of ulnar, peroneal, tibial and sural. Significant enlargement of nerves was also found in true CIDP patients compared to D-DSP patients at non-entrapment sites in the proximal regions of the upper extremities. This research found that nerve US in DSP revealed enlarged CSA and these changes worsen with increasing disease severity thus serving as a useful, reliable and practical diagnostic tool especially when neurophysiology is unrevealing. The present study also found nerve US aids in differentiation of true CIDP from D-DSP patients by differences in the nerve enlargement and electrophysiological profile between these two groups. This is important when managing these groups of patients as CIDP is treatable.

ABSTRAK

Kajian ini bertujuan untuk menerokai peranan ultrasonografi (US) dan neurofisiologi dalam mengenalpasti pesakit diabetes mellitus (DM) jenis 2 berdasarkan severiti neuropati seperti yang dikenalpasti melalui sistem skor klinikal Toronto (TCSS). Kami juga bermatlamat untuk mengkaji hubungan di antara US saraf dan parameter kajian konduksi saraf (NCS) secara komprehensif untuk menentukan sama ada US boleh digunakan untuk membezakan neuropati dengan pelbagai gred severiti.Persoalan kajian kedua adalah untuk menyiasat sama ada terdapat ciri-ciri pengimejan yang boleh membezakan pesakit 'distal symmetrical polyneuropathy'(DSP) dengan neurofisiologi menunjukkan ciri-ciri penyahmielinan (D-DSP) dengan pesakit 'Chronic Inflammatory Demyelinating Polyneuropathy'(CIDP). Subjek kajian merupakan 100 pesakit DM dengan simptom DSP dan 40 subjek sihat yang dipadankan umur sebagai kawalan. Subset 9 pesakit D-DSP dan 6 pesakit CIDP juga direkrut. Severiti neuropati berdasarkan TCSS; ringan (skor 6-8), sederhana (9-11) dan teruk (12-19). US dilakukan pada kedua dua kaki dan satu tangan tidak dominan dalam pesakit DSP,tetapi pada kedua dua belah kaki dan tangan dalam pesakit D-DSP dan CIDP. Bacaan CSA diambil pada tempat anatomi secara seragam. Diagnosis DSP dan CIDP dibuat berdasarkan kriteria yang sedia ada. Analisis statistik dibuat melalui SPSS versi 22. Kajian kami mendapati perbezaan signifikasi antara ketiadaan aksi potensi deria sural di antara pesakit neuropati ringan (19.1%), sederhana (40.0%) dan teruk (69.0%). Walaubagaimanapun, kawasan kerataan rentas (CSA) dapat dinilai dalam semua saraf pesakit DSP dan secara signifikasinya, lebih besar berbanding kumpulan orang sihat yang dijadikan kawalan. CSA saraf dalam kumpulan pesakit gred severiti teruk adalah lebih besar secara signifikasi dalam saraf ulnar , peroneal , tibial dan sural berbanding kumpulan pesakit gred severiti ringan . Analisis kawasan bawah lengkung (AUC) dijalankan untuk membezakan pesakit dengan DSP dan pesakit yang tiada DSP.Kami

mendapati bahawa saraf sural adalah yang diskriminator terbaik dengan nilai CSA 2 mm^2 yang mempunyai AUC 0.88, sensitiviti 0.90 and spesifisiti 0.74 tetapi tidak menunjukkan perbezaan teliti dalam membezakan CSA saraf pesakit DSP teruk dari DSP tidak teruk dengan nilai 2.75 mm^2; AUC 0.62; sensitiviti 0.59; spesifisiti 0.73.Hubungan secara signifikasi didapati antara TCSS,hampir kesemua parameter neurofisiologi dan CSA saraf ulnar, peroneal, tibial dan sural. Pembengkakan saraf secara signifikasi didapati dalam saraf pada bahagian proximal pada tangan dalam pesakit CIDP. Penyelidikan ini telah mendapati bahawa US saraf dalam DSP menunjukkan pembengkakan saraf dan menjadi lebih teruk dengan bertambahnya severiti neuropati. Dengan ini,US menjadi alat diagnostik yang berguna,praktikal dan boleh dipercayai khususnya apabila aksi potensi melalui neurofisiologi tidak dapat dirangsang. Kajian ini juga membantu dalam membezakan pesakit CIDP dengan D-DSP melalui definisi keratan rentas saraf dan profil neurofisiologi yang berbeza di antara dua kumpulan pesakit ini yang membantu dalam pengurusan rawatan. Kajian ini menunjukkan corak pembengkakan saraf pada bahagian proximal pada tangan dalam pesakit CIDP berbanding pesakit D-DSP melalui kajian US yang membantu mengenalpasti blok konduksi saraf yang selalunya sukar dikenalpasti melalui NCS, khususnya dalam bahagian proximal saraf yang boleh membantu pengurusan rawatan bagi pesakit yang terjejas.

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LIST OF SYMBOLS AND ABBREVIATIONS

AAN	:	American Academy of Neurology
AN	:	Autonomic neuropathy
AUC	:	Area Under Curve
CIDP	:	Chronic Inflammatory Demyelinating Polyneuropathy
CMAP	:	Compound Muscle Action Potential
CMT	:	Charcot–Marie–Tooth
CSA	:	Cross sectional area
CSF	:	Cerebrospinal Fluid
CTS	:	Carpal Tunnel Syndrome
CV	:	Conduction Velocity
dCMAP	:	Distal Compound Muscle Action Potential
D-DSP	:	DSP patients with neurophysiological features of demyelination
DM	:	Diabetes Mellitus
DML	:	Distal Motor Latency
DN	:	Diabetic neuropathy
DSP	:	Distal symmetrical polyneuropathy
EFNS	:	European Federation of Neurological Societies
IVIg	:	Intravenous immunoglobulin
mCV	:	Motor Conduction Velocity
NCS	:	Nerve Conduction Studies
NCV	:	Nerve Conduction Velocity
nNOS	:	Neuronal Nitric Oxide Synthase
NO	:	Nitric Oxide
NOS	:	Nitric Oxide Synthase

PE	:	Plasma Exchange
ROC	:	Receiver Operating Characteristic
sCV	:	Sensory Conduction Velocity
SNAP	:	Sensory Nerve Action Potential
SNAP	:	Sensory Nerve Action Potential
TCSS	:	Toronto Clinical Scoring System
UMMC	:	University Malaya Medical Centre
US	:	Ultrasonography
UNE	:	Ulnar Nerve Entrapment

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.0 Introduction

Diabetes mellitus (DM) is increasingly prevalent in both developed and developing countries. A decreased quality of life, mortality, and morbidity are major consequences from diabetes and its complications. One of the long-term complications that affect the nerves is neuropathy. There are a broad diversity of neuropathies in diabetes, affecting single (mononeuropathy), several (mononeuropathy multiplex), or many nerves (polyneuropathy). The most common presentation of diabetic neuropathy (DN) that presents with symmetrical sensorimotor symptoms is distal symmetrical polyneuropathy (DSP), with a reported prevalence of about 50% (P. J. Dyck et al, 2010). In DSP, there are indications that small fiber sensory modalities are involved and minor distal motor weakness may follow. The fundamental pathology in DSP has been shown to be of distal axonal degeneration of dying back type (distal axonopathies) (Said, Slama, & Selva, 1983) which are typically seen as 'length-dependent' or 'glove-and-stocking' neuropathies with relative preservation of dorsal root ganglion cells (Dolman, 1963; Watkins et al, 1995).

The clinical diagnosis usually relies on the patients' description of pain, where symptoms are distal, symmetrical, often associated with nocturnal exacerbations, and commonly described as prickling, sharp and burning with hyperalgesia and frequent allodynia upon examination. Validated scales and questionnaires such as Toronto Clinical Scoring System (TCSS) and McGill's Pain Questionnaire can be used to estimate the severity of the neuropathic pain (Tesfaye et al, 2010).

Conventionally, nerve conduction studies (NCS) have been widely used to diagnose DSP (England et al., 2009). For electrodiagnostic confirmation of DSP, the minimum criterion is abnormality of any attribute of nerve conduction in two separate nerves, one of which must be the sural nerve (Callaghan, Cheng, Stables, Smith, & Feldman, 2012).

Since DSP presents in a length-dependent manner, NCS in the lower limbs would be more suitable to assess DSP severity. However, NCS in the lower limbs is time consuming and in patients with severe DSP, action potential in the lower limbs often cannot be stimulated (Tsuneo Watanabe et al., 2010).

In recent years, peripheral nerve ultrasonography (US) have emerged as an additional tool in the assessment of peripheral nerve disorders demonstrating morphological changes in patients with different forms of neuropathy.

In the diagnosis of entrapment neuropathies, there is a substantial body of literature on US, however, the US changes of polyneuropathy, particularly in DM has not been fully explored. Another scope of interest is in making the distinction between DSP patients with neurophysiological evidence of demyelination (D-DSP) and true chronic inflammatory symmetrical polyneuropathy (CIDP) patients. CIDP characteristically affects the most proximal regions of the peripheral nervous system, nerve roots and major plexuses. In clinical practice, simultaneous occurrence of CIDP and DM (diabetic CIDP or CIDP-DM) is frequently seen; however, it is still unclear whether the two disorders are pathogenetically correlated (Lozeron et al, 2002; Sharma et al,2002; Stewart, McKelvey, Durcan, Carpenter, & Karpati, 1996).

In the current study, we prospectively recruited DM patients with clinical symptoms suggestive of neuropathy and assessed them for objective evidence of DSP through NCS in patients with different severities of DSP as determined by the TCSS. As measured by sural nerve morphology and NCS, the TCSS is a valid instrument to reflect the presence and severity of DSP (Bril & Perkins, 2002). To our knowledge, no studies have investigated the severity of DSP. We also aimed to determine if US could reliably discriminate between the different grades of severity of DSP. This is crucial as currently, there is no objective evaluation of assessing DSP severity, and our approach might enable appropriate specialist referral for treatment.

We also assessed the validity of ultrasound as an additional adjunct diagnostic modality in DSP. This is important as the current 'gold standard' for diagnosis and staging of DSP severity, which is NCS, is unable to assess small fiber involvement in DSP and nerves are frequently inexcitable in patients with severe disease. We hypothesized that US can perform as an equitable evaluation to determine severity of DSP. One of our objectives is also to examine the validity of the TCSS in our cohort by correlating this tool with NCS parameters. We examined a subset of D-DSP patients and compared this group to patients with true CIDP to investigate if there are any sonographic features that can differentiate these two groups of patients. It is vital to distinguish true CIDP patients from D-DSP patients due to the implications of prognosis and therapy because CIDP is treatable whereas DSP is not (Sharma et al, 2002) . To date, no specific nerve parameters have shown to specifically distinguish between CIDP and DSP.

1.1 Objective/s

1. To investigate the patterns of peripheral neuropathy in diabetic patients using clinical symptom scores, NCS and US

2. To assess the validity of ultrasound as a diagnostic modality in diabetic peripheral neuropathy

3. To correlate the findings of nerve conduction studies and ultrasound with clinical symptom scores in patients with DM

4. To investigate the utility of ultrasound as a tool to distinguish demyelinating diabetic neuropathies from true CIDP patients

CHAPTER 2: LITERATURE REVIEW

2.1 Burden of DN in the global context

With more industrialization, globalization and reformation in lifestyles worldwide, we are experiencing a shift in disease paradigm, with chronic disease such as DM becoming more widespread. DM is increasingly prevalent in both developed and developing countries. In 2010, the world prevalence of DM among adults was 6.4% affecting 285 million adults and by 2030, this number is likely to increase to 7.7 % i.e. 439 million (Shaw, Sicree, & Zimmet, 2010). The International Diabetes Federation (IDF) estimated that 371 million people worldwide were living with DM in 2012, of which about half live in South Asia, the Western Pacific, and Eastern Mediterranean regions (Abdullah, Attia, Oldmeadow, Scott, & Holliday, 2014). Asia is now the focal point of a growing diabetes epidemic, largely due to population growth and ageing in India and China. By 2030, projections indicate that more than 60% of worldwide diabetes cases will come from Asia (Shaw et al, 2010; Wild, Roglic G Fau - Green, Green A Fau - Sicree, Sicree R Fau - King, & King, 2004) with the vast majority of these being type 2 DM. A cross-sectional multicentre study performed in the United Kingdom hospital clinic population revealed the prevalence of type 2 (non-insulin-dependent) DM patients was 32.1 % (30.6–33.6 %) and diabetic peripheral neuropathy increased with age, from 5% (3.1– 6.9 %) in the 20–29 year age group to 44.2 % (41.1–47.3 %) in the 70–79 year age group (Young, Boulton, MacLeod, Williams, & Sonksen, 1993). A report from Pittsburgh epidemiology of diabetes complications study demonstrated 34% (18%, 18-29 yr old, 58% ≥30 yr old) prevalence of DN (Maser et al., 1989). In a study done in the Malaysian cohort, the prevalence of DN was found to be 54.7% (Abougalambou & Abougalambou, 2012).

The most common presentation of DN is DSP, affecting more than 90% of the patients (Tesfaye, Boulton, & Dickenson, 2013). Small and/or large nerve fibers may be affected. DSP is a major and independent risk factor for mortality (Forsblom et al, 1998) and morbidity because of foot ulceration and amputation (Abbott et al., 2002).

2.2 Pathophysiology of DN

The pathophysiological mechanisms of DN are not yet fully established, although pain is one of the main symptoms. It is generally accepted that the toxic effects of hyperglycemia plays a significant role in the development of this complication, but several other hypotheses have been proposed (Dobretsov, Hastings, Romanovsky, Stimers, & Zhang, 2003; Oyibo, Prasad, Jackson, Jude, & Boulton, 2002). Both metabolic and vascular factors are involved in the pathophysiology of DN. Hyperlipidemia, hypertension, cigarette smoking, consumption of alcohol, and obesity are other comorbid factors associated with DN. Hyperglycemia plays a prominent role in the pathogenesis which results in the following (Kaur, Pandhi, & Dutta, 2011) (**Fig 2.1**):

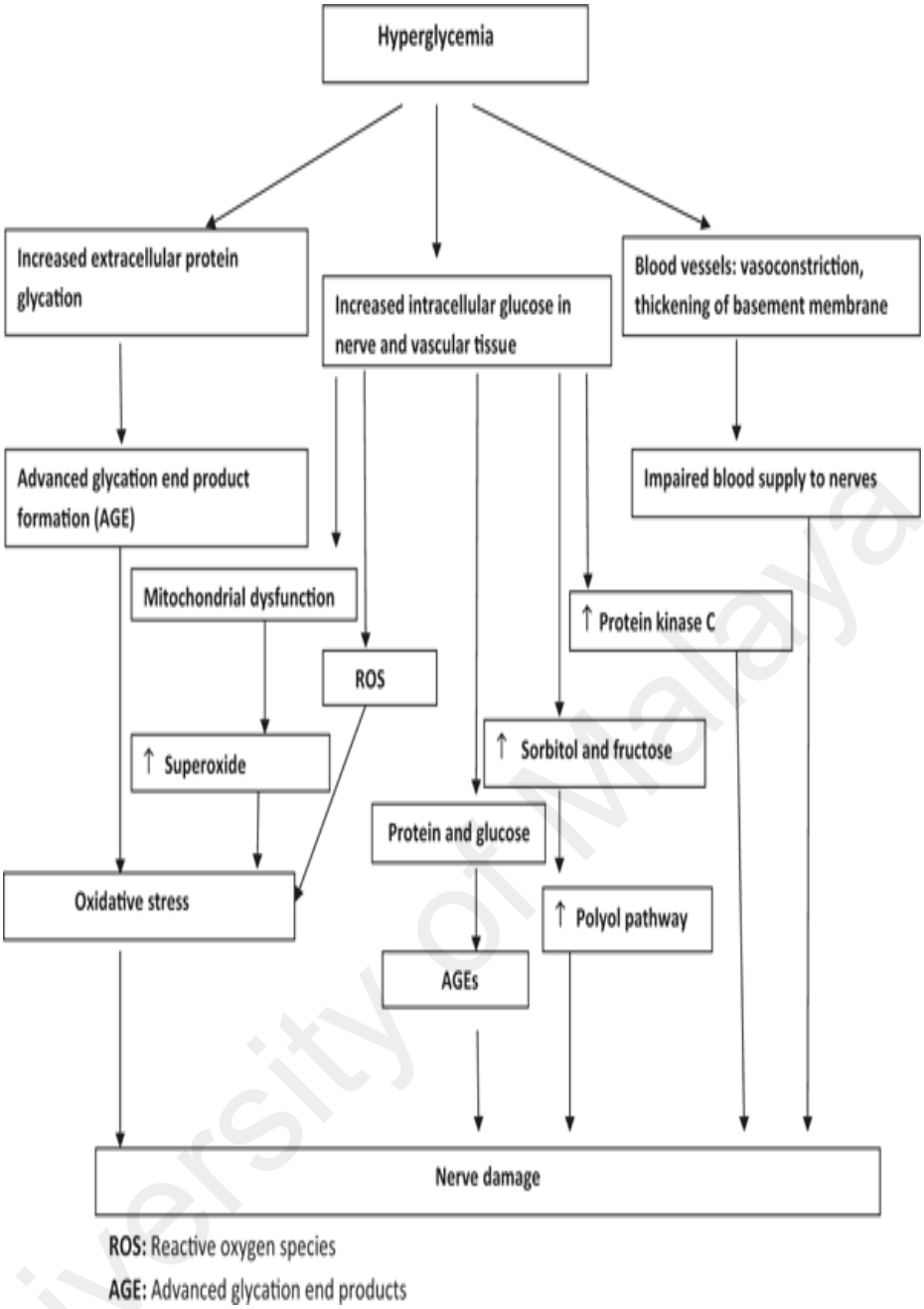


Figure 2.1 Pathophysiology of DN

(Kaur et al, 2011)

2.2.1 Formation of advanced glycation end products

DM results in an increase in oxidation products. Increased flux through one or more glucose metabolism pathways leads to excess intracellular glucose production which leads to incorporation of glucose into proteins nonenzymatically by an unregulated glycation reaction (Head, 2006). Haemoglobin, plasma albumin, lipoproteins, fibrin and collagen are among the different proteins that gets glycated. All these glycated end products are responsible for causing the tissue damage.

2.2.2 Oxidative stress

Production of reactive oxygen species or defective scavenging of free radicals provides major evidence that points to increased oxidative stress in DN. Excess glucose undergoes auto-oxidation and this leads to formation of reactive oxygen species. By obstructing the nitric oxide (NO) production by the endothelium and thereby leading to ischemia of nerves, these oxygen free radicals cause damage of the nerves. A study analyzed markers of oxidative stress in 189 people with diabetes and 85 controls (Ziegler, Sohr, & Nourooz-Zadeh, 2004). Subjects with DN exhibited significant rise of all oxidative stress markers, as well as significant decreases in the protective antioxidant vitamins C and E. In another study to examine effects of pro-oxidants, rats that were exposed to two pro-oxidant interventions show diminished nerve conduction velocity (NCV), nerve growth factor in the sciatic nerve, and neuropeptides compared to diabetic rats that were not exposed to additional oxidative stress (Hounsom, Corder, Patel, & Tomlinson, 2001).

2.2.3 Accumulation of polyols

Glucose is capable of diffusing passively without insulin into certain type of cells, including nerve cells. Once inside the cell, glucose undergoes conversion to sorbitols and other polyols by the enzyme aldose reductase. Due to polyols inability to passively diffuse out of cells, polyols concentrate within cells such as neurons, thus creating a concentration gradient that allows excess sodium and water to flow (Raccah et al, 1998). With polyol accumulation, free carnitine and myo-inositol content in the caudal nerves of diabetic rats were remarkably decreased. (Nakamura et al, 1998).

2.2.4 Deficiency of NO

The pathogenesis of DN has also been associated with vascular factors. NO plays a vital role in controlling (Na+ /K+)-ATPase activity (Gupta et al, 2002), a reduction of which has been implicated in the pathogenesis of DN (Stevens et al, 1994). Experimental analysis showed hyperglycemia results in an excess of endothelial superoxide radicals that result in decreased stimulation of NO on (Na+ /K+)-ATPase activity; this effect is inhibited by L-arginine (Gupta et al, 2002). Nerve blood flow is reduced in experimental DN, and many studies have shown it may be mediated by variation in NO metabolism. One such study investigated nerve blood flow and nitric oxide synthase (NOS) activity in the microvasculature serving peripheral nerves in diabetic rats (Kihara & Low, 1995). A significant decrease of nerve blood flow was observed compared to controls due to hyperglycemia. An *in vivo* study also demonstrated disruptions in neuronal nitric oxide synthase (nNOS) in experimental diabetes. Reduced nNOS expression was associated with a higher degree of neuropathic pain (Sasaki, Yasuda, Maeda, & Kikkawa, 1998).

Experimental analysis has further demonstrated that hyperglycemia results in an excess of endothelial superoxide radicals that result in diminished stimulation of NO on (Na+ /K+)-ATPase activity (Gupta et al, 2002). However, no relationship between altered NO activity and the development of sensory peripheral neuropathy was found in another study (Thomsen, Rubin, & Lauritzen, 2002).

The interrelationships between the various pathogenetic features of DN are not clearly understood. An animal study aimed to clarify a possible connection between aldose reductase activity (enhanced polyol pathway activity) and decreased NO activity. The study demonstrated NO to be an important mediator of nerve (Na+ /K+)-ATPase and aldose reductase activity on NCV. The study concluded that hyperglycemia increases the activity of aldose reductase, subsequently reducing NO synthase activity via cofactor competition (Stevens et al., 1994).

2.3 Classifications and characteristics of DN

Multifarious neurological complications in DM are seen, affecting different parts of the nervous system, and may manifest in various clinical presentations. There are many classification of DN (Boulton, Malik, Arezzo, & Sosenko, 2004). One such classification is shown in **Table 2.1** (Thomas, 1997). The most common form of DN is DSP, representing 70% of DN (Zochodne, 2007). DSP is the focus of this thesis and will be discussed in further detail. Other forms of DN are also described to a lesser extent.

Table 2.1 Classification of DN

Neuropathy class	Neuropathy type
Generalized/symmetrical polyneuropathies	Sensory-motor (chronic) Acute sensory Autonomic
Focal and multifocal neuropathies	Proximal motor (amytrophy) Focal limb Cranial Thoracolumbar radiculoneuropathy
Rapidly reversible neuropathy	Hyperglycemic
Superimposed CIDP	

(Thomas, 1997)

2.3.1 DSP

A consensus statement on the definition of DN was "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes (Boulton, Gries, & Jervell, 1998). Other metabolic neuropathies and causes of neuropathy such as hereditary and inflammatory should be excluded.

DSP is primarily sensory in nature (Papanas et al, 2007). In DSP, there are indications that small fiber sensory modalities are involved and minor distal motor weakness may follow.

Accumulation of sorbitol due to increased flux in the polyol pathway secondary to hyperglycemia is the major metabolic abnormality of peripheral nerves in DSP (Chalk, Benstead, & Moore, 2007). In human DM patients, axonal proteins have been shown to be abnormally glycated (Brownlee, Cerami, & Vlassara, 1988).

Since DSP is a distal axonopathy of the dying back type, there is a possible interference with the operation of growth factors by the diabetic state resulting in nerve cells being unable to maintain their distal axons. Failure of axonal regeneration is an important aspect of DSP (Thomas, 1994).

DSP is characterized by burning or aching pain, numbness, paraesthesia, and hyperalgesia in both feet and lower limbs (symmetrical). These symptoms begin in the feet and spread proximally in a length-dependent fashion, eventually involving distal hands, in what is referred to as 'stocking-and-glove' distribution. Sensory symptoms appear to be more prominent than motor involvement. The array of symptoms associated with DSP has many downstream effects that can affect patients' quality of life, both physically and mentally. DSP-associated numbness frequently results in balance difficulties, which can lead to falls (Callaghan et al, 2012). Additionally, patients with severe DSP are at risk of ulcerations and lower-extremity amputations, with 15% developing an ulcer during the course of their disease. Despite the availability of many successful therapies, however, less than half of patients are treated for pain. Currently, the only treatments available to patients with DSP are improved glucose control and pain management (Bril, Hirose, Tomioka, & Buchanan, 2009).

2.3.2 Autonomic neuropathy (AN)

Diabetic AN can involve the complete autonomic nervous system. It is manifested by impairment of one or more organ systems (e.g., cardiovascular, gastrointestinal [GI] genitourinary, sudomotor, or ocular) (Vinik, Maser, Mitchell, & Freeman, 2003). General symptoms include dizziness (orthostatic hypertension), resting tachycardia, oedema, bladder dysfunction, and erectile dysfunction. An increased mortality risk is seen among DM patients with AN compared to DM patients without AN, chiefly due to renal failure, sudden death and cardiovascular events (Vinik et al, 2003).

Autoimmunity is thought to play an important role in the pathogenesis of AN (Granberg, Ejskjaer, Peakman, & Sundkvist, 2005; Sundkvist, Lind,Bergstrom, Lilja, & Rabinowe, 1991; Vinik et al., 2003). Autoantibodies against adrenal medulla, sympathetic ganglia, and the vagal nerve have been identified and associated to future development of cardiac and peripheral AN (Granberg et al., 2005). Treatment is mainly symptomatic, but glycaemic control seems to improve AN (Vinik et al, 2003).

2.3.3 Focal and multifocal neuropathies

Focal/asymmetrical diabetic neuropathies may involve a single nerve (mononeuropathy), or few different nerves (mononeuropathy multiplex). Nerve entrapments, usually involving the ulnar, median, and peroneal nerves are one of the common causes of some focal neuropathies (Boulton et al, 2004; Boulton et al, 2005). DM patients have an increased susceptibility to nerve compression (Dahlin, Stenberg, Luthman, & Thomsen, 2008) and about one third of them have nerve entrapments (Boulton et al, 2005).

Carpal tunnel syndrome (CTS) is the most common nerve entrapment i.e. compression of the median nerve at the wrist (Boulton et al, 2004). Focal neuropathies such as mononeuritis multiplex has an acute onset, associated with pain and heal spontaneously, between 6-8 weeks. They are caused by vascular obstruction typically in the cranial nerves III, VI, and VII, ulnar, median, and peroneal nerves. Other focal/multifocal neuropathies in DM may have an ischemic basis and often exist with sudden onset of severe pain. Microvascular nerve infarct results in cranial neuropathies, typically involving the third, fourth, sixth, and seventh cranial nerves (Boulton et al, 2004; Boulton et al, 2005).

Cranial neuropathies are rare and usually present in older individuals with a long duration of DM (Boulton et al., 2004). Mostly, cranial neuropathies resolve spontaneously over several months but can recur in 25% of patients (Boulton et al, 2004). In the proximal lower limb motor neuropathy (amyotrophy), nerve infarcts have also been indicated but there is evidence that focal inflammatory lesions (including vasculitic) may be related (Boulton et al, 2004; Thomas, 1997).

2.4 Diagnosis of DSP

Although DSP can be diagnosed by experienced clinicians with a clinical examination, there are still inconsistencies in the diagnostic criteria that exists in the literature. The American Academy of Neurology (AAN) in conjunction with the American Association of Electrodiagnostic Medicine and the American Academy of Physical Medicine and Rehabilitation reported a case definition of DSP to systematize and facilitate clinical research and epidemiologic studies (England et al, 2005). The combination of neuropathic symptoms, signs, and electrodiagnostic findings accordingly gives the most accurate diagnosis of DSP.

2.4.1 Clinical examination

The clinical examination consists of a detailed inspection of peripheral sensation, tendon reflexes, and muscle strength. Neuropathic symptoms with distal sensory loss, absent tendon reflexes, and abnormal nerve conduction studies are distinctly suggestive of DSP (England et al, 2005).

2.4.4.1 Clinical Scoring System

The clinical diagnosis usually relies on the patients' decription of pain, where symptoms are distal, symmetrical, often associated with nocturnal exacerbations, and commonly described as prickling, sharp and burning with hyperalgesia and frequent allodynia upon examination. Validated scales and questionnaires such as TCSS and McGill's Pain Questionnaire are used to estimate the severity of the neuropathic pain (Tesfaye et al, 2010). In recent years, different clinical scoring systems were developed to document the presence and severity of DSP quantitatively (Dyck, 1988; Perkins, Olaleye, Zinman, & Bril, 2001). One study has indicated that the Michigan Neuropathy Screening Instrument (MNSI) is a good screening tool for diabetic neuropathy and that the Michigan Diabetic Neuropathy Score (MDNS) coupled with NCS gives a simple means to confirm this diagnosis (Feldman et al, 1994). However, none of these methods was validated against morphological criteria for DSP. In another study, the TCSS was implemented for a simple screening for DSP to classify patients into severity categories and correlated well with NCS findings and complications in subjects with DSP in that study (Perkins et al, 2001) . Consequently, TCSS was considered as a simple method for evaluation of DSP. However, further justification in patients with documented DSP was required.

A study has assessed the correlation of TCSS with the presence and severity of DSP as ascertained by electrophysiological criteria and the additional morphological gold standard of myelinated fiber density on sural nerve biopsy in an independent group of DSP patients. The study demonstrated that the clinical neuropathy as characterized by the TCSS is associated with the morphological severity of DSP and hence suggests that the TCSS may prove to be useful in documenting and monitoring DSP in the clinic and in clinical research trials (Bril & Perkins, 2002).

2.5 Neurophysiological examination

NCS are the most widely accepted objective evaluation for the diagnosis of DSP (Bae & Kim, 2007; Kim, Kwon, Lee, & Sunwoo, 2000) and CIDP (Dyck et al, 1975). NCS are non-invasive, standardized technique that provides an objective and sensitive measure of the functional status of sensory and motor nerves. An electrical impulse, an action potential, is evoked by stimulating the nerve and conducted along a motor or sensory axon. The distribution of abnormality (focal, multifocal, or diffuse), and whether the pathophysiology is predominantly a segmental demyelination or axonal degeneration can be determined using NCS. The axonal degeneration and progressive loss of nerve fibers are the most important features in DSP. These axonal changes are identified by reduced motor and sensory action potential amplitudes, with normal or slightly reduced conduction velocities secondary to loss of the largest and fastest-conducting axons (Callaghan et al, 2012). In CIDP, the diagnosis is based on NCS evidence of conduction block and temporal dispersion (Stewart et al, 1996).

A study has suggested that the residual latency (RL), terminal latency index (TLI) and modified F ratio (MFR) which indicates distal conduction slowing, may be a useful guide to identify subclinical DN. The study also demonstrated that electrophysiological changes that are unclear in routine NCS are present before the clinical manifestation (Bae & Kim, 2007). Another study has evaluated the reproducibility of NCS. The study demonstrated that the median and tibial F-wave latencies produce the most reproducible measures for NCS, serving as one of the best measures in multicentre drug trials for DN (Kohara et al, 2000). Another study aimed at evaluating the relationship of abnormal parameters in commonly tested peripheral nerves and clinical findings in DN through NCS and found the amplitude of sensory nerve action potential to be a vital parameter in detection of early DN (K. W. Lee, Hwang, & Kim, 1999).

Motor nerve conduction was studied along the entire course of nerves from the spinal cord to the muscle in diabetic and normal controls. The study found a diffuse pattern of motor conduction abnormalities in DN over the total length of the nerve, being extreme in the distal than proximal segment. Additionally, both proximal and distal segments were more often affected in the lower than in the upper extremities (Kimura, Yamada, & Stevland, 1979). NCS in the lower limbs would be more suitable to assess DSP severity as it presents in a length-dependent fashion. However, in patients with severe disease, action potential in the lower limbs cannot be stimulated. A study reported that the sensory nerve conduction velocity was not measurable in many patients, and instead they looked at distal motor latency (DML) and motor conduction velocities (mCV) to evaluate DSP (Mizumoto, Hashizume, Senda, Nagoshi, & Inoue, 2003). At times, these too cannot be recorded due to the small foot muscle wasting.

The sensory nerve action potential amplitude (SNAP) and compound muscle action potential (CMAP) amplitude is reduced due to axonal loss. Reduction of the nerve conduction velocity and increased dispersion are caused by demyelination. Motor and sensory neuropathies can be diagnosed since both afferent and efferent nerves can be tested. When polyneuropathy is suspected, the ulnar and median nerves in the arm and the peroneal, posterior tibial, and sural nerves in the leg are commonly tested. For electrodiagnostic confirmation of DSP, the minimum criterion is an abnormality of any attribute of nerve conduction in two separate nerves, one of which must be the sural nerve (England et al, 2005). To test whether physicians can validly and reproducibly diagnose DSP, one study demonstrated that there was significant agreement between 75% group diagnosis and confirmed nerve conduction abnormality. When compared to nerve conduction score, individual physicians' clinical diagnoses were greatly variable and mostly inaccurate, often overestimating DSP (Peter J Dyck et al, 2010).

2.6 US

While NCS remain fundamental to confirm the diagnosis of DSP and CIDP, the test is time consuming and uncomfortable for patients. Sonographic examinations can potentially be an alternative to assess peripheral nerves with less discomfort and there have been studies that have proven its clinical use in the evaluation of disorders of the peripheral nervous systems. (Goedee et al, 2013). In recent years, the role of US of peripheral nerves has been investigated. In the diagnosis of entrapment neuropathies, there is a substantial body of literature on nerve ultrasound, however, the US changes of polyneuropathy, especially in DM has not been thoroughly explored.

Nerve US when performed along with NCS aids in visualizing nerve morphology, not only in mononeuropathies but also in peripheral neuropathies. Most US studies have concentrated on entrapment neuropathies. In DN, ultrasound studies have been less comprehensive, typically only looking at distal lower limb nerves.

Currently, US has proven its usefulness for the diagnosis of compressive neuropathy in CTS, and there is evidence that substantiates its usefulness in ulnar neuropathy at the elbow (Suk, Walker, & Cartwright, 2013). In the diagnosis of peripheral nerve damage in entrapment syndromes, nerve tumors, and focal nerve lesions, the use of peripheral nerve US has been demonstrated clearly and has gained vast interest in recent times (Grimm, Heiling, Schumacher, Witte, & Axer, 2014). In one study, larger nerve cross sectional area (CSA) was observed in patients with common fibular neuropathy than controls (Visser et al, 2013). An increased CSA was also observed on magnetic resonance imaging and on US in immune-mediated and demyelinating hereditary peripheral neuropathies with more prominent changes in hereditary neuropathy (Grimm et al, 2014).

Peripheral nerve polyneuropathy US data, especially axonal forms, so far have been based chiefly on small patient numbers or single case studies (Goedee et al, 2013).

An enlargement of distal parts of the tibial nerves in patients with DM were observed in one study (D. Lee & Dauphinee, 2005). There have been reports on enlargement of affected nerves attributed to the process of attempted remyelination in patients with Charcot-Marie-Tooth disease (CMT), multifocal motor neuropathy (MMN) and CIDP (Beekman et al, 2005; Cartwright et al, 2009; Heinemeyer & Reimers, 1999; Martinoli et al, 2002; Taniguchi et al, 2000) .

A sonographic distinction was seen through a larger nerve area and fascicular diameter in the CMT 1A disease compared to patients with the other types of disease (including CMT2 and CMTX) and the control subjects (Martinoli et al, 2002).

Another study studied the peripheral nerves of individuals with CMT type 1B and found that patients with CMT 1B have significantly larger median and vagus nerves than healthy controls, but no difference was observed in cranial nerve size between those with versus those without cranial neuropathies (Cartwright et al, 2009). An increased nerve CSA has also been demonstrated in vasculitic neuropathy, amyloidosis, neurofibromatosis and POEMS syndrome (AD, Skare, Sakuma, & Barros, 2015; Bohm, 2009; Ito, Kijima, Watanabe, Sakuta, & Nishiyama, 2007; Lucchetta, Pazzaglia, Granata, Briani, & Padua, 2011). One study has evaluated the usefulness of US to detect abnormalities in tibial vasculitic neuropathy at the medial ankle and they found the affected nerve area was significantly larger than in controls (Ito et al, 2007).

High-frequency sonography was found helpful in one study in the diagnosis of vasculitic neuropathy in their two cases of mononeuritis multiplex and two cases of DSP and was able to detect focal morphologic lesions which could not be identified electrophysiologically due to the axonopathy (Bohm, 2009). Another study aimed to establish the value of US in the diagnosis of CTS and found the measurement of median nerve area by US performs well and could be used as first choice for the investigation of patients with CTS (AD et al, 2015).

In DN, US studies have been less comprehensive, typically only looking at distal lower limb nerves. One study aimed to determine the sonographic characteristics of lower extremity nerves in DN and correlate them with electrodiagnostic findings. The results showed measurements of lower extremity nerves in DN do not differ from controls or correlate with electrodiagnostic findings.

They concluded that further innovative US techniques might be necessary to detect differences (Hobson-Webb, Massey, & Juel, 2013). Another study determined the morphological changes of sural nerves in patients with type 2 DM using US and found that 22-MHz US may be a worthy tool for assessing diabetic cutaneous nerve neuropathy (Liu, Zhu, Wei, Bao, & Hu, 2012).

Riazi et.al demonstrated a larger nerve CSA in posterior tibial nerve in DM patients compared to control subjects and this large study of DM patients concluded US is a promising point-of-care screening tool for DM patients with DSP (Riazi et al, 2012).

2.7 CIDP

CIDP is an immune-mediated disorder. It characteristically affects the most proximal regions of the peripheral nervous system, nerve roots, and major plexuses. Simultaneous occurrence of CIDP and DM (diabetic CIDP or CIDP-DM) is frequently seen in clinical practice; however, it is still unclear whether the two disorders are pathogenetically correlated (Chio et al, 2009;Lozeron et al, 2002; Sharma et al, 2002). Making the distinction between chronic symmetric sensorimotor DPN and CIDP can be challenging. CIDP may be diagnosed in a DM patient when motor symptoms are predominant, however, it is more difficult to diagnose in DM patients with neurophysiological features of demyelination without clinical motor weakness (Ayyar & Sharma, 2004).

Stewart et al described seven DM patients with distal greater than proximal, symmetric neuropathy (polyneuropathy) that had more features in keeping with CIDP rather than a length-dependent pattern of neuropathy typically described in DN (Stewart et al, 1996). Krendel described six insulin dependent diabetics with a demyelinating neuropathy indistinguishable from CIDP, all of whom improved with varying types of immunotherapy (Krendel, Costigan, & Hopkins, 1995). To date no specific nerve parameters have been shown to specifically distinguish between CIDP and DSP. Proposals for diagnostic tools that can help clinicians to determine the probability of a patient with diabetes having CIDP exist (Lotan, Hellman, & Steiner, 2015). By listing several clinical, electrophysiological, and laboratory parameters that, when combined, were able to powerfully discriminate an immune-mediated neuropathy in patients with diabetes mellitus. Four levels of probability for a patient with diabetes to have CIDP were defined by summing the points assigned to each of these parameters.

The results demonstrated that this diagnostic tool enables the identification of diabetic patients with overlapping CIDP (Lotan et al, 2015). This is of great importance as unlike DSP, CIDP is a treatable condition.

2.7.1 Clinical presentation and diagnosis

CIDP typically arises between the ages of 30 and 60 years and is characterized by the occurrence of progressive (more than two months), symmetric proximal and distal muscle weakness. The condition also demonstrates impaired sensation, absent or reduced tendon reflexes, an elevated cerebrospinal fluid protein level, demyelinating NCS, and indications of demyelination in nerve-biopsy experiments (Barohn, Kissel, Warmolts, & Mendell, 1989).

2.8 Disease treatment and management

2.8.1 DSP treatment and management

Poor glycaemic control has been implicated as a pathogenetic mechanism in the etiology of DSP. An increase in blood glucose flux has been reported to cause pain in DSP (Oyibo et al., 2002). Currently, strategies that has been implemented for management of DSP are based on (i) improving glucose control ; (ii) symptomatic control of DSP and (iii) treatment centered on pathogenetic mechanisms (Tesfaye, 2011).

2.8.1.1 Pharmacological treatment of DSP

Some pharmacological therapies have proven to be effective in management of DSP. Tricyclic compounds have been used as first-line therapy for many years and its efficacy has been supported by several randomized clinical trials (Finnerup, Otto, McQuay, Jensen, & Sindrup, 2005;Max et al,1992; Tesfaye, 2007).

However, the uses of tricyclic drugs are limited due to its side effects including anticholinergic effects such as dry mouth and dizziness. Usually, titration of the dose of tricyclic drugs is recommended to avoid side effects (Tesfaye, 2007). Selective serotonin-reuptake inhibitors (SNRI), such as duloxetine and venlafaxine relieves pain by increasing synaptic accessibility of 5-hydroxytryptamine and noradrenalin in the descending pathways that inhibit pain impulses (Tesfaye, 2011). Anticonvulsants such as gabapentin and pregabalin have been used in the management of neuropathic pain for many years. There have been few clinical trials involving pregabalin in DSP, and these demonstrated clear efficacy in management of DSP (Freeman, Durso-Decruz, & Emir, 2008). Topical treatment, such as topical lidocaine in the form of a 5% patch is potentially effective in management of pain associated with DSP (Bril et al., 2011).

The most often used antioxidant is α-lipoic acid, which is the pathogenetically oriented treatment for DSP. It has been shown that only α-lipoic acid administered intravenously over 3 weeks (600 mg i.v. per day) is effective in improving several neuropathic symptoms and nerve function in patients with DSP (Ziegler, Nowak, Kempler, Vargha, & Low, 2004).

2.8.1.2 Non pharmacological treatment of DSP

Alternative therapies, such as acupuncture (Abuaisha, Costanzi, & Boulton, 1998), low intensity laser therapy (Zinman et al., 2004) and transcutaneous electrical stimulation (Somers & Somers, 1999) have been used due to lack of response and unwanted side effects of conventional pharmacological treatments. These might be useful as add-on therapy at any stage of DSP.

2.8.2 CIDP disease treatment and management

The first line therapy in CIDP are steroids since the first report of their use (Austin, 1958). In one study of an unblinded randomized controlled trial with 28 subjects, prednisone was superior to no treatment (Dyck et al., 1982). It has been clearly demonstrated that that intravenously administered immune globulin (IVIg) plays a role in immunomodulation and has anti-inflammatory effects (Gelfand, 2012). In a meta-analysis of four double blind randomized control trials, IVIg showed a significant improvement was seen in disability lasting 2-6 weeks in 235 subjects (Eftimov, Winer, Vermeulen, de Haan, & van Schaik, 2013). The treatment of IVIg needs to be repeated at intervals and doses needs to be determined on individual basis (Kuitwaard & van Doorn, 2009).

Plasma exchange (PE) aims to remove circulating autoantibodies, cytokines, immune complexes, and immune cells (Lehmann, Hartung, Hetzel, Stüve, & Kieseier, 2006) to achieve fast immunosuppression. Conventionally, PE is used in acute forms of dysimmune peripheral neuropathies such as Guillain-Barré syndrome (GBS), but also patients with chronic disease such as CIDP may respond to PE in the short term, usually for 2–4 weeks (Lehmann & Hartung, 2011). Immunosuppressive drugs such as azathioprine, methotrexate , cyclosporin A and rituximab may be considered when the response to steroids, IVIg or PE is inadequate. Treatment option will rely on several variables such as initial disease severity, age, general health status, and potential contraindications.

CHAPTER 3: METHODOLOGY

3.1 Subjects and study design

The study design is a prospective cohort study. DM patients were recruited prospectively by direct approach at the outpatient clinic at University Malaya Medical Centre (UMMC), Kuala Lumpur, Malaysia. Patients with Type 2 DM irrespective of treatment type were included in the study. Exclusion criteria included all patients with previous history or concurrent history of significant exposure to potential neurotoxins (chronic alcohol consumption, environmental toxin, heavy metals such as lead, mercury and arsenic, chemotherapy drugs) and previous or concurrent neurological disease, compression or trauma to the peripheral nerves involving the lower limbs were excluded. This was based on medical history. Existing patients with CIDP from the neurology clinic at UMMC were also recruited.

The diagnosis of DSP was determined by applying the TCSS questionnaire (see next section). Age and gender matched control subjects were recruited from relatives and colleagues. Diabetic control was ascertained by HbA1C values. The values of HbA1C were determined by the National Glycohemoglobin Standardization Program (NGSP) method. Demographic data such as height and weight were collected at the time of the study. Other relevant information acquired includes HbA1C values and DM disease duration. Written consent was obtained from all the patients participating in this research. Patients with longstanding chronic renal failure were also excluded. Ethical approval was obtained from the UMMC Medical Research Ethical Committee.

3.2 DSP Clinical Screening

Patients underwent a series of examination through questionnaire and clinical examination by the candidate, who had received prior training. Clinical assessment of DSP was determined by the TCSS and AAN's estimated likelihood of DSP for case definitions that include symptoms, signs, and nerve conduction studies for assessment of diabetic neuropathy (Bril & Perkins, 2002; England et al., 2005). TCSS symptom scores include lower extremity pain, numbness, tingling, weakness, walking imbalance and upper extremity symptoms. Normal was drafted as 0 point, abnormal as 1 point, and a total of 6 points can be obtained in this section. Reflex scores, including the bilateral knee reflex and ankle reflex, were 0 point for normal, reduced 1 point and absent 2 points, providing a total of 8 points in this section. Sensory score, including light touch in the right great toe, joint position sense, vibration sense, pinprick, temperature sensation, were normal- 0 point, abnormal- 1 point, giving a total of 5 points in this section.

The total possible score in TCSS taking into account the symptom score, reflex score, and sensory score was 19 points. A score of six or greater was considered abnormal, suggesting the presence of DSP. The diabetic patients were grouped into three groups of DSP according to severity: mild, moderate and severe according to TCSS scores. The AAN estimates of the likelihood of DSP were also assessed and these include symptoms, signs and NCS (England et al, 2005).

3.3 Diagnosis of CIDP and D-DSP patients

We identified eight patients that presented with a progressive symmetrical or asymmetrical polyradiculoneuropathy where the clinical course is relapsing or remitting and progressing for more than two months. Two patients with CIDP were excluded due to a concurrent history of DM . The diagnosis of CIDP was made as specified by the European Federation of Neurological Societies/Peripheral Nerve Society's (EFNS/PNS) diagnostic criteria (Van den Bergh et al, 2010). The patients fulfilled the mandatory diagnostic criteria, with evidence of sensory and motor impairment, disease duration/progression of at least 8 weeks, hyporeflexia/areflexia upon clinical examination and NCS shows evidence of demyelination [**Table 3.1 and 3.2**] (Van den Bergh et al, 2010). Cerebrospinal fluid (CSF) analysis also showed albuminocytologic dissociation in all patients. Magnetic resonance imaging (MRI) of spinal roots, brachial plexus,and lumbosacral plexus along with nerve biopsies are additional investigations to diagnose CIDP, but not mandatory as shown in **Table 3.3** (Van den Bergh et al., 2010). The diagnostic criteria of CIDP are shown in **Table 3.4**. We employed the criteria for definite CIDP which comprises of clinical criteria 1 (a or b) and 2 with electrodiagnostic criterion 1.

DM patients with DSP were defined as having demyelination (D-DSP) out of proportion to axonal loss if amplitudes were preserved and at least two NCS parameters showed conduction slowing as suggested by the EFNS criteria for CIDP (Van den Bergh et al., 2010).The diagnosis of D-DSP patients were made based on electrophysiological criteria and the patients demonstrated no clinical weakness. Cerebrospinal fluid (CSF) analysis was not performed, as patients did not consent to this procedure.

Clinical examination with quantification of muscle strength was done in all patients according to the Medical Research Council (MRC) score, ranging from 0 (absence of contraction) to 5 (full strength), in both proximal and distal muscles of four limbs.

Table 3.1 CIDP clinical diagnosic criteria

(1) Inclusion criteria

(a) Typical CIDP

Chronically progressive, stepwise, or recurrent symmetric proximal and distal weakness and sensory dysfunction of all extremities, developing over at least 2 months; cranial nerves may be affected; and Absent or reduced tendon reflexes in all extremities

(b) Atypical CIDP (still considered CIDP but with different features) One of the following, but otherwise as in (a) (tendon reflexes may be normal in unaffected limbs):

Predominantly distal (distal acquired demyelinating symmetric, DADS) or

Asymmetric [multifocal acquired demyelinating sensory and motor neuropathy (MADSAM), Lewis-Sumner syndrome] or

Focal (e.g., involvement of the brachial or lumbosacral plexus or of one or more peripheral nerves in one upper or lower limb)

Pure motor or

Pure sensory (including chronic immune sensory polyradiculopathy affecting the central process of the primary sensory neuron)

(2) Exclusion criteria

Borrelia burgdorferi infection (Lyme disease), diphtheria, drug or toxin exposure probably to have caused the neuropathy

Hereditary demyelinating neuropathy

Prominent sphincter disturbance

Diagnosis of multifocal motor neuropathy

IgM monoclonal gammopathy with high titre antibodies to myelin-associated glycoprotein

Other causes for a demyelinating neuropathy including POEMS syndrome, osteosclerotic myeloma, diabetic and nondiabetic lumbosacral radiculoplexus neuropathy. PNS lymphoma and amyloidosis may occasionally have demyelinating features

(Van den Bergh et al, 2010)

Table 3.2 CIDP electrodiagnostic criteria

(1) Definite: at least one of the following

a) Motor distal latency prolongation ≥50% above ULN in two nerves (excluding median neuropathy at the wrist from carpal tunnel syndrome), or
b) Reduction of motor conduction velocity ≥30% below LLN in two nerves, or
c) Prolongation of F-wave latency ≥30% above ULN in two nerves (≥50% if amplitude of distal negative peak CMAP
d) Absence of F-waves in two nerves if these nerves have distal negative peak CMAP amplitudes ≥20% of LLN + ≥1 other demyelinating parameter[a] in ≥1 other nerve, or
e) Partial motor conduction block: ≥50% amplitude reduction of the proximal negative peak CMAP relative to distal, if distal negative peak CMAP ≥ 20% of LLN, in two nerves, or in one nerve + ≥1 other demyelinating parameter[a] in ≥1 other nerve, or
f) Abnormal temporal dispersion (>30% duration increase between the proximal and distal negative peak CMAP) in ≥2 nerves, or
g) Distal CMAP duration (interval between onset of the first negative peak and return to baseline of the last negative peak) increase in ≥1 nerve (median ≥ 6.6 ms, ulnar ≥ 6.7 ms, peroneal ≥ 7.6 ms, tibial ≥ 8.8 ms)[b] + ≥1 other demyelinating parameter[a] in ≥1 other nerve

(2) Probable

≥30% amplitude reduction of the proximal negative peak CMAP relative to distal, excluding the posterior tibial nerve, if distal negative peak CMAP ≥ 20% of LLN, in two nerves, or in one nerve + ≥1 other demyelinating parameter[a] in ≥1 other nerve

(3) Possible

As in (1) but in only one nerve

To apply these criteria, the median, ulnar (stimulated below the elbow), peroneal (stimulated below the fibular head), and tibial nerves on one side are tested. If criteria are not fulfilled, the same nerves are tested at the other side, and/or the ulnar and median nerves are stimulated bilaterally at the axilla and at Erb's point. Motor conduction block is not considered in the ulnar nerve across the elbow and at least 50% amplitude reduction between Erb's point and the wrist is required for probable conduction block. Temperatures should be maintained to at least 33◦ C at the palm and 30◦ C at the external malleolus (good practice points).

CMAP, compound muscle action potential; ULN, upper limit of normal values; LLN, lower limit of normal values.

[a]Any nerve meeting any of the criteria (a–g).

[b]Isose S. et al. (Isose et al., 2009)

(Van den Bergh et al, 2010)

Table 3.3 Supportive criteria for CIDP

1. Elevated CSF protein with leukocyte count

2. MRI showing gadolinium enhancement and/or hypertrophy of the cauda equina, lumbosacral or cervical nerve roots, or the brachial or lumbosacral plexuses (level C recommendation)

3. Abnormal sensory electrophysiology in at least one nerve (Good Practice Points):

a) Normal sural with abnormal median (excluding median neuropathy at the wrist from carpal tunnel syndrome) or radial sensory nerve action potential (SNAP) amplitudes; or
b) Conduction velocity

4. Objective clinical improvement following immunomodulatory treatment (level A recommendation)

5. Nerve biopsy showing unequivocal evidence of demyelination and/or remyelination by electron microscopy or teased fibre analysis (Good Practice Points)

(Van den Bergh et al, 2010)

Table 3.4 Diagnostic categories of CIDP

Definite CIDP
Clinical criteria 1 (a or b) and 2 with electrodiagnostic criterion 1; or
Probable CIDP + at least one supportive criterion; or
Possible CIDP + at least two supportive criteria
Probable CIDP
Clinical criteria 1 (a or b) and 2 with electrodiagnostic criterion 2; or
Possible CIDP + at least one supportive criterion
Possible CIDP
Clinical criteria 1 (a or b) and 2 with electrodiagnostic criterion 3
CIDP (definite, probable, possible) associated with concomitant diseases.

(**Van den Bergh et al, 2010)**

3.4 Neurophysiological examination

A single assessor (neurologist) who was blinded to the patient's DSP severity at the time of study performed NCS using a standard electro-neurophysiologic device (CareFusion Nicolet EDX Systems with Synergy Software; Synergy EDX). The neurophysiological examination was performed at the Neurology Lab, UMMC. Standard techniques of supramaximal percutaneous stimulation and surface electrode recording were applied. Nerves were considered inexcitable when unrecordable after at least three attempts made with supramaximal stimulation. Recordings were performed with temperature control (32^{o}C). All diabetic and CIDP patients had bilateral nerve conduction testing of the peroneal and tibial motor nerves and sural sensory nerves in the lower limbs using standardized protocols. Diabetic patients had nerve conduction testing of the median and ulnar motor nerves and radial sensory nerve in the non-dominant upper limb, while CIDP patients had the testing in both upper limbs. Electrodiagnostic data examined includes SNAP, CMAP amplitude, conduction block/temporal dispersion, conduction velocity, distal latency, and minimal F-wave latency in motor nerves. In the D-DSP and CIDP patients, distal CMAP duration were also examined. Reference values were derived from previously established normal ranges at our laboratory. A diagnosis of DSP was made based on existing criteria (England et al, 2009). A diagnosis of co-existing median nerve entrapment across the wrist and ulnar neuropathy at the elbow was made according to previously described criteria (Bahou & Elhadidy, 2005;England et al; 2005; Moon, Kwon, Kim, Lee, & Lee, 2014).

3.5 Sonographic examination

US was performed using a 12-MHz linear array transducer (E Logic book®, GE, USA) by a single assessor who was blinded to the severity of DSP and disease category in all recruited patients. Patients were in the supine position for imaging of the median, ulnar and radial nerve in the upper limbs and in prone position for imaging of the common peroneal, tibial and sural nerves in the lower limbs. The ultrasound transducer was placed in the transverse position. The CSA recordings of each nerve were measured at standardised anatomical sites and values from individual nerves were obtained.US was performed in both lower limbs and one non-dominant upper limb in DM patients. This was to limit the time of image acquisition. As DSP is a length dependent neuropathy, it was important to include both lower limbs. Both upper limbs and lower limbs were examined in D-DSP and true CIDP patients. CSA of the median and ulnar nerves were assessed at the standard anatomical sites at distal wrist crease, mid-forearm, elbow, and mid-arm. Superficial radial nerve was assessed at mid-forearm after the split from the main trunk of the radial nerve prior to its entrance to the supinator. In the lower limbs, the peroneal nerve was assessed at the fibular head and popliteal fossa, the tibial nerve at the medial malleolus, and the sural nerve at 10 cm above the lateral malleolus **(Figure 3.1)**. These anatomical sites were chosen based on previous studies of nerve ultrasound (Hobson-Webb et al, 2013; (Tsuneo Watanabe et al., 2010). The proximal nerves such as the cervical roots were excluded, as there were limited views on ultrasound.

The frequency was set at 12 MHz. The depth and gain were kept constant. The CSA at the relevant point of each nerve was measured by tracing inside the hyperechoic rim of the nerve using an electronic tracer. The sonographer was also blinded to the NCS results.

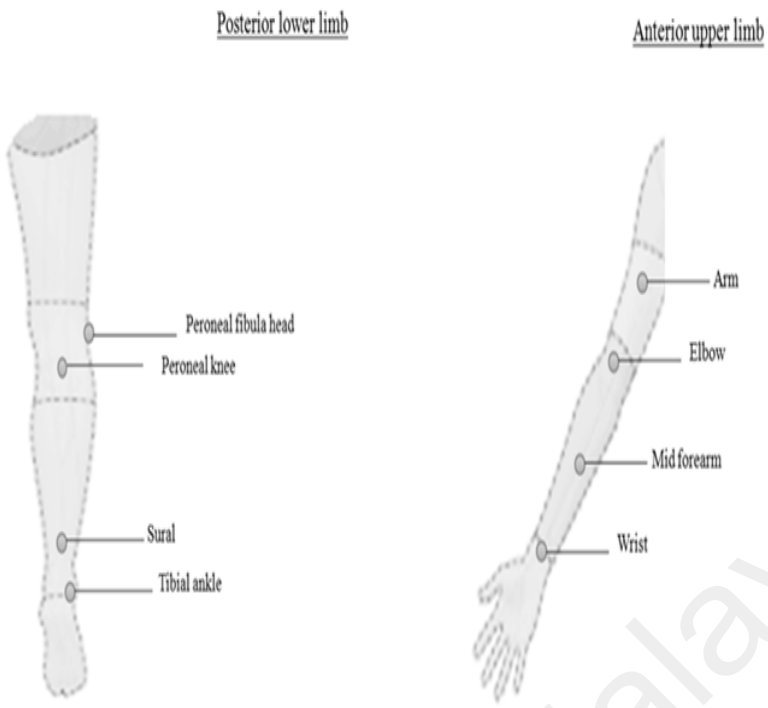


Figure 3.1: Anatomical sites of US

3.6 Statistical analyses

Statistical analyses were performed using SPSS version 22. Normality of data was tested using Kolmogrov-Smirnov normality test. Demographic data and CSAs were compared between diabetic patients and control groups using independent t-test for parametric and Mann Whitney test for non-parametric variables respectively and similarly for comparison between true CIDP patients and D-DSP patients. Comparative studies between groups were done with ANOVA for parametric variables or Kruskal-Wallis for non-parametric variables. Receiver operating characteristic (ROC) analysis was done to determine the nerve CSA cut-off values that best predict the presence of DSP as well as differentiate between severe and non-severe DSP, based on TCSS values. Correlation studies were done with Spearman's rank correlation for non-parametric variables and Pearson's for parametric variables. Statistical significance was established at $p<0.05$.

CHAPTER 4: RESULTS

4.1 Clinical Characteristics

The total number of nerves assessed was three sets of 199 lower limb nerves (one patient had a left below knee amputation) and three sets of 100 upper limb nerves. Detailed demographic data for 100 diabetic patients and healthy controls are shown in **Table 4.1.** The healthy controls are of age group between 40-70 years to match the age group of our diabetes cohort, which is in the same age range. The demographic data for true CIDP and D-DSP patients are shown in **Table 4.6**.

There were no significant differences in age, gender, height and weight between controls and diabetic patients. In the diabetic cohort, the mean disease duration, HbA1C and TCSS Score were 14.5±9.4 years, 7.9 ±1.6, and 10.5±3.5 respectively. The frequency of median nerve entrapment found in diabetes patients are 77.0%, whereas ulnar neuropathy at the elbow (UNE) is 12.0% confirmed with NCS. There were no significant differences observed in the age, height, and weight between true CIDP and D-DSP patients.

Table 4.1 Patient Characteristics

	Type 2 DM patients	Healthy control	P value
N	**100**	**40**	
Age(years)	59.06(8.76)	57.75(7.11)	0.402
Gender (male: female)	41:59	24:16	0.826
Height (cm)	160.1(7.47)	162.7(8.99)	0.070
Weight(kg)	71.15(16.15)	66.81(12.39)	0.119
Disease duration(years)	14.54(9.43)		
HbA1C (%)	7.87 (1.61)		
TCSS Score	10.46(3.53)		
Ethnicity, n (%)			
Indian	34(34.0)	13(32.5)	
Malay	33(33.0)	14(35.0)	
Chinese	33(33.0)	13(32.5)	
Neuropathy severity (According to TCSS), n (%)			
Mild	34(34)		
Moderate	30(30)		
Severe	36(36)		
Median nerve entrapment (%)	77		
UNE (%)	12		

4.2 Ultrasound studies

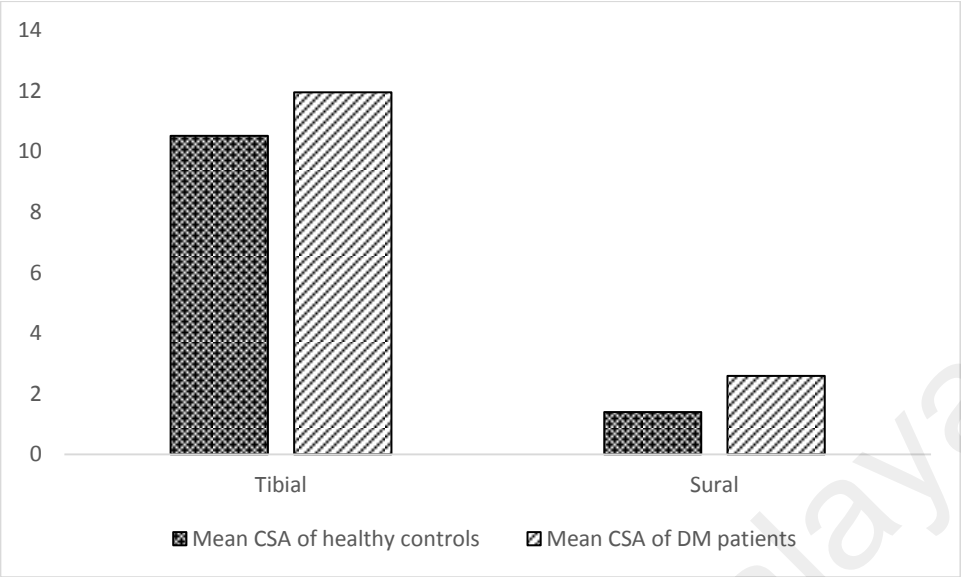
4.2.1 Comparison of nerve CSAs between DM patients and healthy group

Overall, the mean nerve CSA was larger in the diabetic patients compared to the healthy controls. There were no significant difference observed in the mean age, height and weight between the DM patients and healthy controls. The mean CSA was significantly larger in the DM patients in median nerve at wrist ($p<0.001$), in ulnar nerve at mid forearm ($p=0.016$) and elbow ($p=0.017$), in common peroneal nerve at knee ($p=0.026$), in tibial nerve at ankle ($p=0.003$),in sural nerve at ankle ($p<0.001$) and in radial nerve at midfoream ($p=0.039$) when compared to the healthy controls **[Table 4.2]**. Looking specifically at the median nerve at the wrist, we further demonstrated that DM patients had a significantly higher wrist to forearm ratio compared to the healthy controls. The wrist to forearm ratio (cutoff value ≥ 1.4) (Hobson-Webb, Massey, Juel, & Sanders, 2008) had a sensitivity of 73% and specificity of 41% ($p=0.0003$) in differentiating DM patients who were symptomatic and those who did not have CTS. As NCS were not performed on controls, asymptomatic CTS could not be definitively excluded. There were no significant differences in CSAs in the lower limbs between the left and right sides of each patient.

Table 4.2 CSA comparison between DM patients and healthy controls

	Diabetic patients	Healthy controls	P value
Age	59.06(8.76)	57.75(7.10)	0.402
Height (cm)	160.1(7.47)	162.7(8.99)	0.07
Weight (kg)	71.24(16.09)	66.81(12.39)	0.119
Nerve CSA (mm^2)			
Median			
Wrist	8.77(2.94)	6.58(1.58)	**<0.001**
Mid forearm	5.52(1.45)	5.18(0.90)	0.296
Wrist-Forearm Ratio	***1.65 (0.57)***	***1.30(0.34)***	***0.0003***
Elbow	7.63(2.15)	7.05(1.72)	0.212
Mid arm	7.92(1.91)	7.45(1.28)	0.28
Ulnar			
Wrist	4.54(1.33)	4.12(0.94)	0.122
Mid forearm	5.20(1.41)	4.60(1.06)	**0.016**
Elbow	7.15(1.91)	6.35(1.39)	**0.017**
Mid arm	6.29(1.60)	5.88(1.56)	0.121
Common peroneal			
Knee	8.53(2.06)	7.78(1.87)	**0.026**
Fibula Head	10.12(3.05)	9.52(2.25)	0.231
Tibial			
Ankle	11.96(3.01)	10.52(2.04)	**0.003**
Sural			
Ankle	2.59(0.96)	1.40(0.59)	**<0.001**
Radial			
Mid forearm	1.35(0.56)	1.15(0.36)	**0.039**

Figure 4.1: CSAs of tibial and sural nerves at the ankle in type 2 DM patients vs. healthy controls



4.2.2 Comparison of nerve CSAs between DM patients according to neuropathy severity

US measurements of DM patients with mild neuropathy were compared with the measurements of DM patients from the moderate and severe category. Patients were grouped into neuropathy severity category according to their TCSS scores. Between the three groups, ANOVA revealed that the mean CSAs in ulnar nerve at elbow (p=0.003), peroneal nerve at knee and fibula head (p=0.049; p=0.002), tibial nerve at ankle (p=0.006) and sural nerve at ankle (p=0.008) are statistically significant **[Table 4.3]**. When comparing mild and severe neuropathy group, we found that there was significant enlargement at peroneal nerve at knee and fibula head (p=0.039; p=0.002), tibial nerve at ankle (p=0.021) and sural nerve at ankle (p=0.009). Only tibial nerve at ankle demonstrated a significant enlargement (p=0.019) when we compared moderate and severe neuropathy DM patients. No significant enlargement found in any particular nerve when we compared mild and moderate neuropathy DM patients. It is clear that that there is not much difference in the mild vs. moderate neuropathy group compared to the mild vs. severe neuropathy and moderate vs. severe neuropathy group of diabetic patients.

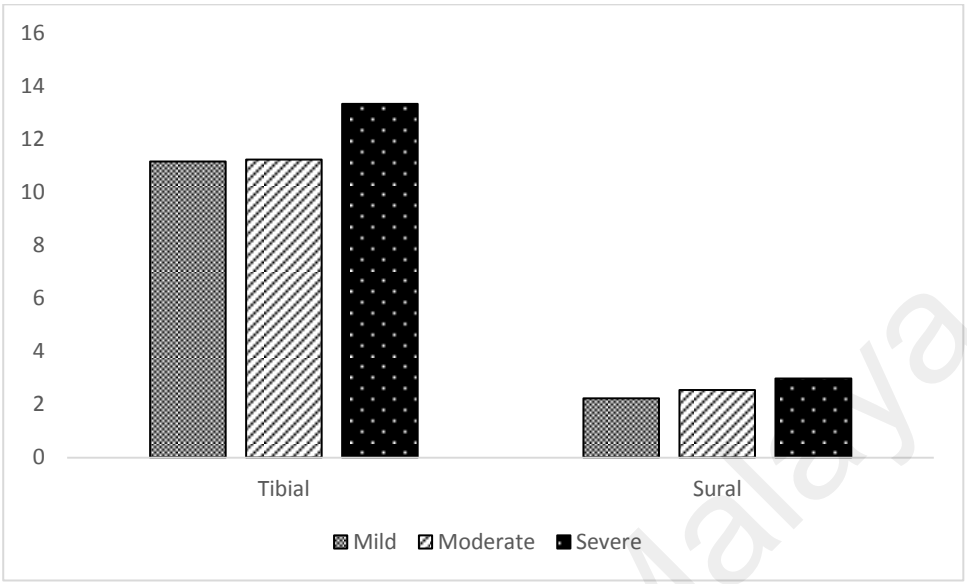
Table 4.3: CSA comparison of diabetic patients with different severities

	Mild N=34	Moderate N=30	Severe N=36	P value (between 3 groups)	P value (mild vs. severe)	P value (moderate vs. severe)	P value (mild vs. moderate)
Age	59.09(7.89)	59.17(9.39)	58.94 (9.24)	0.995	0.997	0.994	0.999
Height (cm)	161.8(8.72)	158.3(6.62)	159.8 (6.71)	0.722	0.562	0.646	0.155
Weight (kg)	69.88(18.4)	70.77(12.53)	72.93(16.68)	0.242	0.713	0.852	0.974
HbA1C (%)	7.94(1.58)	7.77(1.64)	7.89(1.66)	0.911	0.992	0.948	0.907
Disease duration (years)	12.34(8.25)	13.97(7.36)	17.17(11.47)	0.203	0.083	0.352	0.762
Nerve CSA (mm^2)							
Median							
Wrist	8.50(3.04)	9.33(2.60)	8.56(3.13)	0.207	0.936	0.614	0.561
Mid forearm	5.38(1.42)	5.50(1.61)	5.67(1.37)	0.707	0.778	0.959	0.986
Elbow	7.35(1.92)	7.20(1.85)	8.25(2.47)	0.174	0.419	0.296	0.962
Mid arm	7.65(1.70)	7.73(1.53)	8.33(2.32)	0.678	0.203	0.303	0.983

Table 4.3, continued

Ulnar							
Wrist	4.26(0.99)	4.58(1.73)	4.80(1.20)	0.557	0.471	0.876	0.248
Mid forearm	4.88(1.51)	5.03(1.22)	5.64(1.40)	0.053	0.061	0.152	0.906
Elbow	6.44(1.38)	6.93(1.96)	8.00(2.03)	**0.003**	**0.001**	0.363	0.525
Mid arm	6.12(1.67)	6.17(1.32)	6.56(1.75)	0.455	0.587	0.684	0.992
Common peroneal							
Knee	7.96(2.46)	8.45(1.28)	9.18(2.06)	**0.049**	**0.039**	0.363	0.151
Fibula Head	8.75(2.51)	10.22(2.28)	11.40(3.59)	**0.002**	**0.002**	0.138	0.152
Tibial							
Ankle	11.18(2.24)	11.25(2.22)	13.35(3.77)	**0.006**	**0.021**	**0.019**	0.995
Sural							
Ankle	2.24(0.62)	2.55(0.72)	2.99(1.26)	**0.008**	**0.009**	0.113	0.443
Radial							
Mid forearm	1.26(0.45)	1.37(0.49)	1.42(0.69)	0.648	0.296	0.742	0.443

Figure 4.2: Distribution of mean nerve CSAs at the tibial and sural nerves at ankle of DM patients with different neuropathy severities



4.2.3 ROC analysis of the nerve CSAs

We analyzed the area under the curve (AUC) that best discriminate nerve CSAs of DM patients with severe DSP (TCSS ≥ 12) from non-severe DSP (TCSS<12) which is shown in **Table 4.4.** The majority of cut-off CSA values were extremely poor discriminators (AUC < 60%). The tibial CSA at 11.75 mm^2 and sural nerve CSA at 2.75 mm^2 nerves had an AUC value of 69% and 62% respectively. Based on these values, the tibial nerve could discriminate between DSP severity at 65.1% sensitivity and 65.0 specificity whereas sural nerve at 59.0% sensitivity and 73.0% specificity. The AUC in the ROC analysis **[Table 4.5]** was determined to differentiate between patients with DSP (TCSS≥6) and without DSP (TCSS≤5). We found that the sural nerves performed the best and a cut-off value of 2 mm^2 had an AUC of 88%, sensitivity of 90% and specificity of 74%.

Table 4.4: ROC analysis of severe and non-severe DSP patients

Nerve	Site	AUC (95% C.I)	Cut-off value (mm^2)	Sensitivity (%)	Specificity (%)	P value
Median	Wrist	48.1	10.5	21.2	84.4	NS
	Mid Forearm	55.3	6.5	18.2	81.2	NS
	Elbow	58.6	9.5	21.2	92.2	NS
	Arm	53.1	9.5	24.2	89.1	NS
Ulnar	Wrist	54.9	5.5	27.3	81.2	NS
	Mid Forearm	64.9	6.5	24.2	89.1	**0.016**
	Elbow	68.6	8.5	33.3	85.9	**0.003**
	Arm	58.9	7.5	27.3	81.2	NS
Peroneal	Knee	**64.1**	10.25	21.2	87.5	**0.023**
	Fibula head	**67.1**	11.75	33.3	87.5	**0.006**
Tibial	Ankle	**69.0**	11.75	65.1	65.0	**0.004**
Radial	Mid forearm	52.3	1.5	33.3	68.8	NS
Sural	Ankle	**62.0**	2.75	59.0	73.0	**0.017**

Table 4.5: ROC analysis of DSP and non-DSP subjects

Nerve	Site	AUC (95% C.I)	Cut-off value (mm^2)	Sensitivity (%)	Specificity (%)	P value
Median	Wrist	**73.3**	9.5	33.0	75.0	**<0.001**
	Mid Forearm	54.6	5.5	40.0	72.5	0.360
	Elbow	55.8	7.5	45.0	57.5	0.281
	Arm	56.1	8.5	35.0	82.5	0.261
Ulnar	Wrist	58.4	5.5	22.0	87.5	0.120
	Mid Forearm	**62.3**	5.5	36.0	85.0	**0.023**
	Elbow	62.5	6.5	62.0	57.5	**0.021**
	Arm	57.7	6.5	44.0	72.5	0.153
Peroneal	Knee	**60.4**	9.5	29.3	85.0	**0.037**
	Fibula head	55.7	10.5	41.4	70.0	0.258
Tibial	Ankle	**63.4**	12.5	34.7	85.0	**0.008**
Radial	Mid forearm	59.6	1.5	34.0	84.6	0.079
Sural	Ankle	**88.0**	2.0	90.0	74.0	**0.001**

4.2.4 Comparison of CSAs between CIDP patients and D-DSP patients

Demographic data such as age, height, weight and mean CSAs of D-DSP and true CIDP patients are presented in **Table 4.6.** There were no significant differences in the age, height and weight between D-DSP and true CIDP subjects. At the time of assessment, the true CIDP patients were in remission. Significant enlargement in true CIDP patients were found in the median nerve at elbow (p=0.038) and mid arm (p=0.001), ulnar nerve at wrist (p=0.015), mid forearm (p=0.012) and mid arm (p=0.013), and radial nerve at mid forearm (p=0.022). The lower limb demonstrated no significant difference in the CSAs although a general enlargement is seen in the peroneal nerve at knee and tibial nerve at ankle in true CIDP subjects compared to D-DSP patients. Interestingly, sural nerve CSA was almost similar in both.

Table 4.6: Patient characteristics and CSAs of D-DSP and CIDP subjects

	D-DSP N=9	True CIDP N=6	P value
Age	57.8(1.5)	56.8(1.6)	0.555
Height (cm)	163.4(1.0)	164.5(0.9)	0.404
Weight (kg)	72.5(1.1)	67.5(1.5)	0.564
	Nerve CSA (mm^2)		
Median			
Wrist	10.0(2.9)	10.0(1.0)	0.302
Mid forearm	6.7(1.9)	7.4(2.8)	0.574
Elbow	8.5(2.7)	12.2(5.4)	**0.038**
Mid arm	8.7(2.6)	13.9(3.7)	**0.001**
Ulnar			
Wrist	4.1(0.9)	6.0(2.1)	**0.015**
Mid forearm	5.5(1.5)	7.3(1.8)	**0.012**
Elbow	7.7(1.8)	9.6(2.4)	0.078
Mid arm	7.5(1.9)	10.0(1.8)	**0.013**
Common peroneal			
Knee	9.3(2.8)	10.8(3.1)	0.412
Fibula Head	10.8(3.6)	10.5(1.8)	0.576
Tibial			
Ankle	12.9(2.8)	14.2(4.3)	0.819
Sural			
Ankle	3.4(0.9)	3.3(1.5)	0.367
Radial			
Mid forearm	1.2(0.4)	2.5(1.5)	**0.022**

Figure 4.3: Distribution of mean CSAs of D-DSP patients vs. true CIDP patients at non entrapment sites of the median nerve

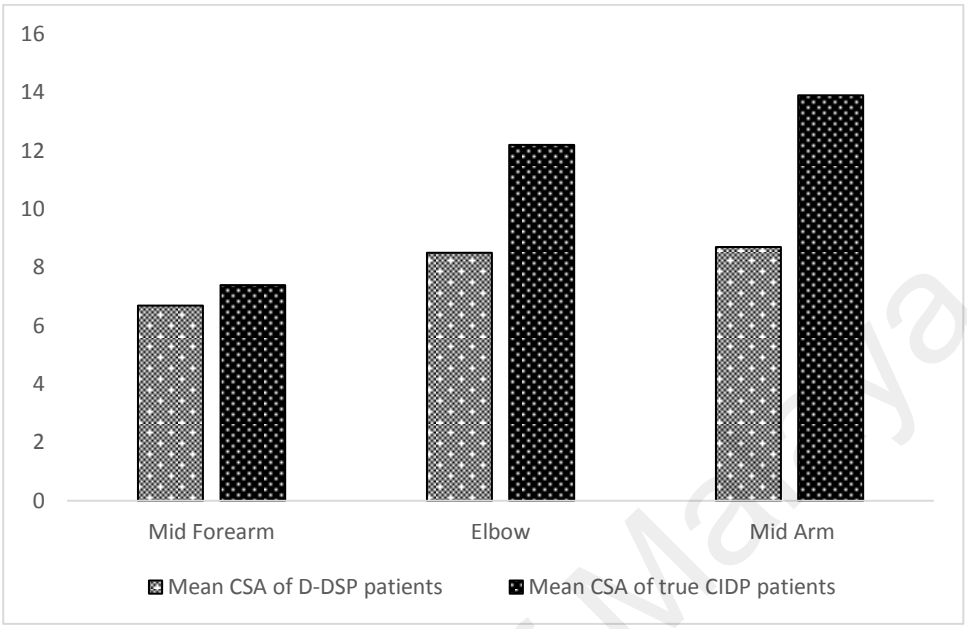
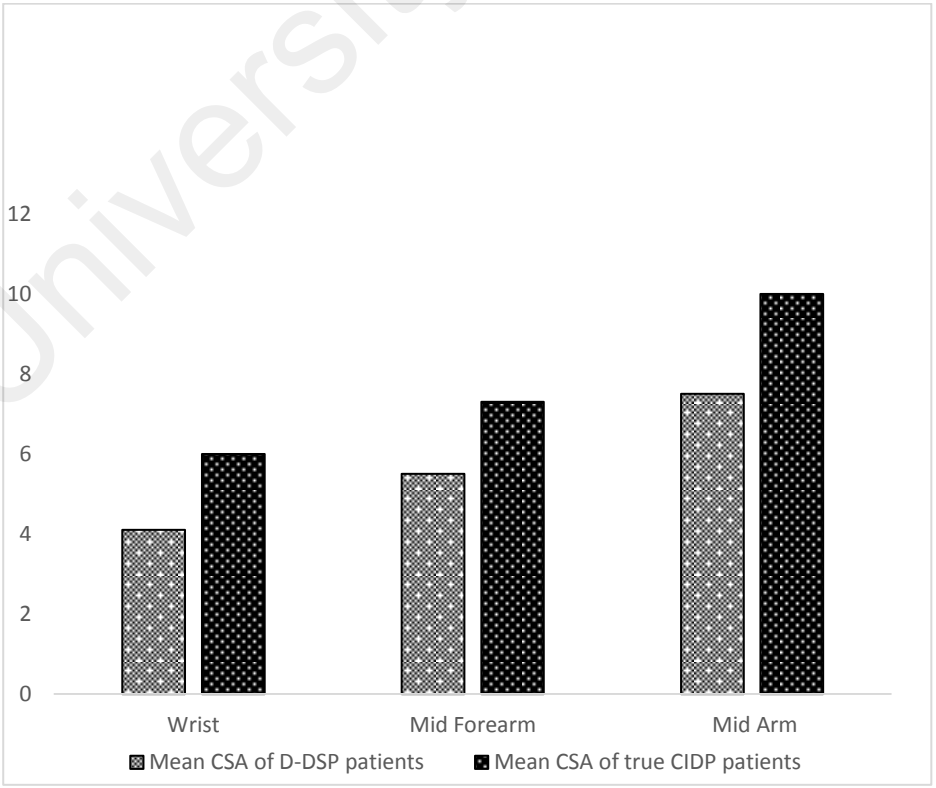


Figure 4.4: Distribution of mean CSAs of D-DSP patients vs. true CIDP patients at non entrapment sites of the ulnar nerve



4.2.5 Sonogram images of DM patients with different neuropathy severities vs. healthy controls

Figure 4.5 shows transverse sonography of the tibial and **Figure 4.6** shows transverse sonography of sural nerves of healthy controls and DM patients of different neuropathy severities. Transverse sonography of the tibial and sural nerves showed a hypoechoic structure with hyperechoic dots within it. We observed a general nerve enlargement in the DM patients when compared with healthy controls. CSA is also seen to increase with progressing severity of neuropathy in DM patients.

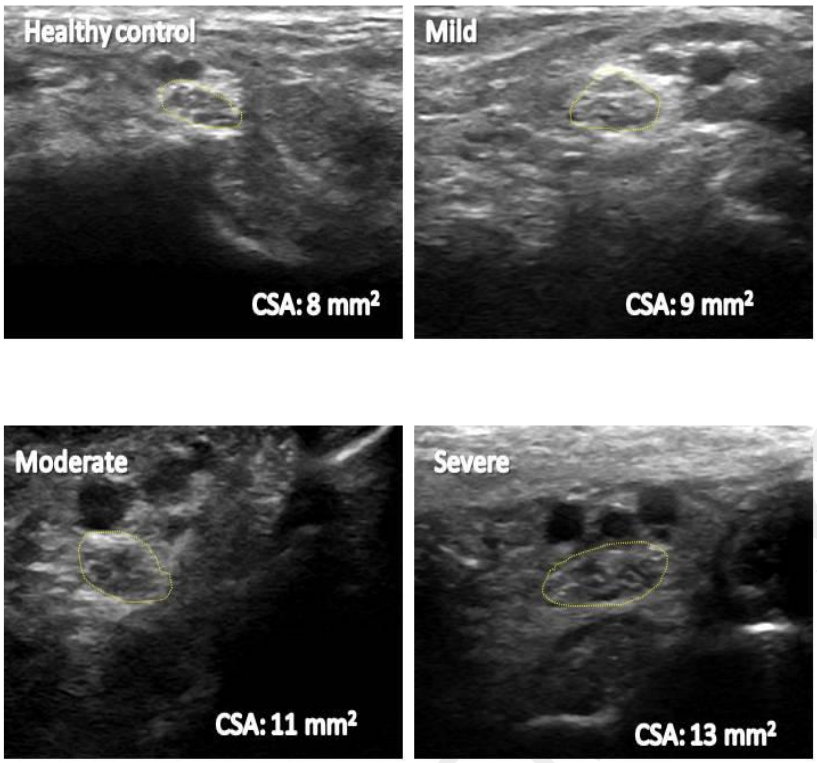


Figure 4.5: Sonogram of the tibial nerve at medial malleolus (transverse view) of healthy controls and DM patients with different neuropathy severity showing a hypoechoic structure with hyperechoic dots within.

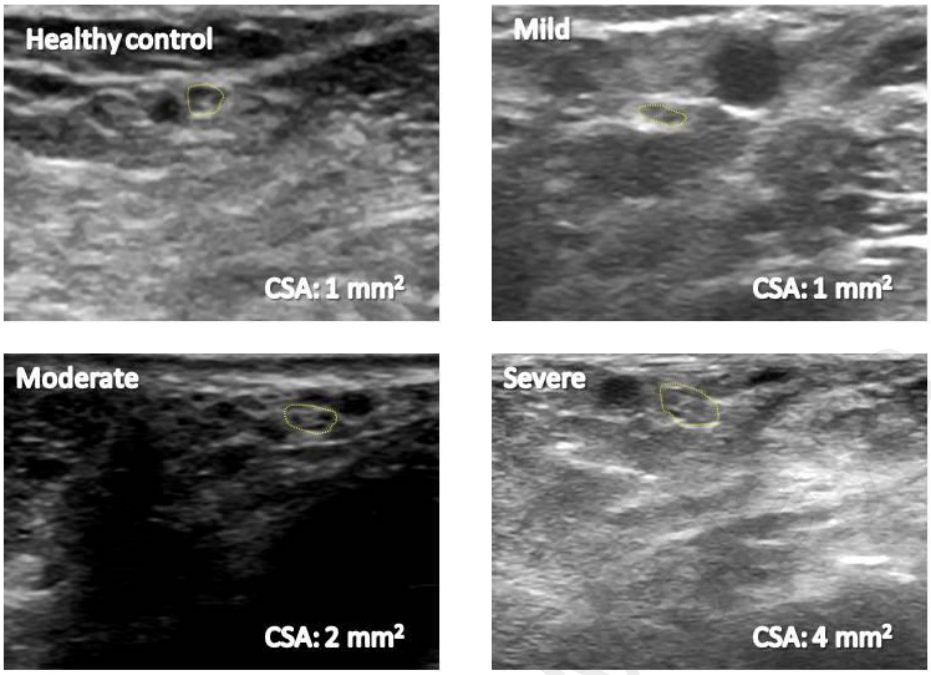


Figure 4.6: Sonogram of the sural nerve at 10 cm above the lateral malleolus (transverse view) of healthy controls and DM patients with different neuropathy severity showing a hypoechoic structure with hyperechoic dots within.

4.2.6 Sonogram images of CIDP patient and D-DSP patients

Figure 4.7 shows transverse sonography of the median mid arm of true CIDP patients versus D-DSP patients. Although both patients had enlarged CSAs, true CIDP patients had larger CSA when compared to D-DSP patients.

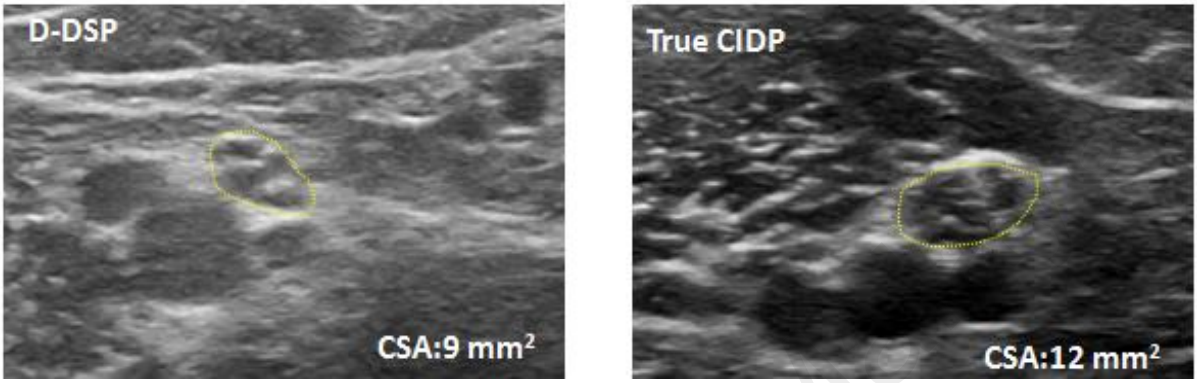


Figure 4.7: Sonogram images of the median mid arm of true CIDP patient versus D-DSP patients

4.3 Neurophysiological studies

4.3.1 Electrophysiological findings according to severity classification (based on TCSS) of DM patients

Looking specifically at the lower limb nerve conduction parameters, these were unrecordable in 43.2% sural, 13.1% peroneal and 8.0% tibial nerve potentials. There were significant differences ($p<0.001$) between absence of sural sensory potentials in the mild (19.1%), moderate (40.0%) and severe (69.0%) groups and peroneal nerve motor potentials in the mild (2.9%), moderate (10.0%) and severe (25.4%), in keeping with worsening DSP. The mean values and comparison between the DML, distal CMAP (dCMAP), mCV, SNAP and sensory conduction velocity (sCV) for the three groups of diabetic patients according to their DSP severity are presented in **Table 4.7.** The DSP patients in the severe neuropathy category (according to TCSS scores) significantly demonstrated prolonged distal latency in the ulnar nerve at wrist ($p<0.001$) and in tibial nerve at ankle ($p<0.001$).

The dCMAP in the peroneal nerve at knee ($p=0.005$) and in tibial nerve at knee ($p=0.001$) is significantly reduced when the DM patient neuropathy severity increases. The mCV is significantly slowed across the median nerve at elbow ($p<0.001$), ulnar nerve at above and below elbow ($p=0.001$,$p<0.001$), peroneal nerve at fibula head ($p<0.001$) and tibial nerve at knee ($p<0.001$) with worsening DSP severity. The SNAP amplitude was markedly reduced when the neuropathy severity increases in the ulnar nerve at wrist ($p=0.007$), radial nerve at forearm ($p=0.005$) and sural nerve at calf ($p=0.005$).We observed a significant slowing of sCV at the radial forearm ($p=0.004$) in keeping with worsening of DSP. Patients in the most severe category demonstrated significant changes in their neurophysiological parameters.

Table 4.7: Comparative studies of neurophysiology parameters between the different severity DSP groups in DM patients

Parameters	Mild N=34	Moderate N=30	Severe N=36	P value
Total lower limb nerves evaluated ,N	68	60	71	
Nerve potential inexcitable,N(%)				
Sural	13.0(19.1)	24.0(40.0)	49.0(69.0)	**<0.001**
Peroneal	2.0(2.9)	6.0(10.0)	18.0(25.4)	**<0.001**
Tibial	5.0(7.35)	2.0(3.3)	9.0(12.7)	0.143
DML(ms)				
Median	4.22(1.61)	4.22(2.26)	4.01(0.98)	0.055
Ulnar	2.34(0.36)	2.5(0.26)	2.76(0.76)	**<0.001**
Peroneal	4.13(0.89)	4.12(0.75)	4.24(0.69)	0.696
Tibial	3.96(0.83)	4.42(1.19)	5.33(1.63)	**<0.001**

Table 4.7, continued

dCMAP(mV)				
Median	7.29(3.10)	6.53(2.99)	6.31(3.14)	0.393
Ulnar	9.24(1.93)	8.20(2.08)	8.49(13.40)	0.874
Peroneal	4.82(2.90)	3.97(2.61)	3.24(2.18)	**0.005**
Tibial	8.13(3.52)	5.51(3.31)	5.17(6.85)	**0.001**
mCV(ms)				
Median	53.30(7.30)	50.91(5.65)	50.11(6.95)	**<0.001**
Ulnar Below Elbow	51.65(9.64)	53.29(8.36)	44.73(9.19)	**0.001**
Ulnar Above Elbow	56.05(6.37)	52.27(4.62)	47.16(10.31)	**<0.001**
Peroneal Knee	49.62(11.24)	47.36(12.89)	46.74(11.73)	0.565
Peroneal Fibula Head	43.23(4.73)	41.15(7.23)	38.15(5.05)	**<0.001**
Tibial	46.47(5.43)	41.69(6.67)	39.08(6.73)	**<0.001**

Table 4.7, continued

SNAP amplitude(μV)				
Median	9.97(7.28)	8.26(7.25)	6.82(5.38)	0.345
Ulnar	8.94(6.36)	6.42(5.37)	4.33(3.03)	**0.007**
Radial	32.98(24.17)	34.29(17.01)	19.05(13.10)	**0.005**
Sural	12.10(7.92)	7.61(3.95)	5.64(2.25)	**0.005**
sCV(ms)				
Radial	49.63(7.97)	49.80(6.03)	44.18(6.61)	**0.004**
Sural	43.67(6.10)	41.24(6.70)	41.08(5.37)	0.217

4.3.2 Comparison of electrophysiological findings between CIDP patients and D-DSP patients

NCS revealed significantly prolonged latency in the peroneal nerve at ankle in true CIDP patients (p=0.009). A significantly reduced CMAP amplitude was observed in true CIDP patients (p=0.011) in the ulnar nerve above elbow **[Table 4.8]**. The CIDP cohort also exhibited bigger distal to proximal ratios in CMAP amplitudes in both upper and lower extremities. Looking at the duration, we observed a prolonged duration in the median nerve at wrist (p=0.011) and elbow (p=0.001) and in the ulnar nerve below elbow (p=0.035) and in tibial nerve at knee (p=0.004) in true CIDP patients. Significant reduction in the sCV were observed in the ulnar (p=0.004) and radial nerve (p=0.020). Generally, the distal latencies and duration were prolonged; CMAP amplitude, mCV, SNAP amplitude, and sCV were reduced in true CIDP patients compared to D-DSP patients. No statistically significant differences observed in the distal to proximal CMAP ratio between true CIDP and D-DSP subjects.

Table 4.8: Comparative studies of neurophysiology parameters between CIDP patient and D-DSP patients

		Reference value	Demyelinating DM patients (n=9)	True CIDP patients (n=6)	P value
Age			57.8(1.5)	56.8(1.6)	0.555
Height (cm)			163.4(1.0)	164.5(0.9)	0.404
Weight (kg)			72.5(1.1)	67.5(1.5)	0.564
DML(ms)	**Site**				
Median	Wrist	<4.4	6.6(2.2)	8.2(2.1)	0.103
Ulnar	Wrist	<3.6	3. 8(1.3)	5. 2(2.0)	0.142
Peroneal	Ankle		4.4(0.5)	9.3(1.8)	**0.009**
Tibial	Ankle	<4.9	6.7(2.2)	8.4(1.5)	0.078
CMAP (mV)					
Median	Wrist	>4.8	5.5(2.6)	3.5(2.3)	0.058
	Elbow		4.5(2.7)	2.7(1.2)	0.117
Wrist-Elbow ratio			***1.2(1.0)***	***1.3(2.0)***	0.876
Ulnar	Wrist	>4.1	6.5(3.2)	4.7(1.5)	0.057
	Below elbow		5.3(3.4)	3.4(1.1)	0.136
	Above elbow		6.1(3.5)	3.1(0.9)	**0.011**
Wrist-below elbow ratio			***1.2(0.9)***	***1.4(1.4)***	0.078
Wrist-above elbow ratio			**1.1(0.9)**	**1.5(1.7)**	0.317

Table 4.8, continued

Peroneal	Knee		3.2(2.6)	1.8(1.0)	0.786
	Fibula Head		1.8(2.1)	2.8(0.7)	0.690
	Ankle		2.4(2.5)	3.7(0.9)	0.602
Ankle-knee ratio			***0.8(0.9)***	***2.1(0.9)***	0.881
Tibial	Knee		3.1(4.5)	2.0(1.9)	0.485
	Ankle	>7.3	3.4(4.9)	3.6(2.9)	0.699
Ankle-knee ratio			***1.1(1.1)***	***1.8(1.5)***	0.927
Duration (ms)					
Median	Wrist		7.2 (0.8)	9.7(2.9)	**0.011**
	Elbow		8.4(1.9)	11.9(2.0)	**0.001**
Ulnar	Wrist		6.5(1.0)	6.6(2.5)	0.877
	Above elbow		7.5(2.2)	8.8(2.6)	0.119
	Below elbow		7.0(1.3)	9.6(3.1)	**0.035**
Peroneal	Ankle		6.5(1.5)	8.3(1.8)	0.117
	Fibula head		8.8(3.9)	9.6(2.1)	0.151
	Knee		7.6(1.1)	10.6(3.2)	0.101
Tibial	Ankle		4.7(1.2)	8.2(3.8)	0.150
	Knee		5.1(0.8)	9.6(2.5)	**0.004**

Table 4.8, continued

mCV(ms)					
Median	Elbow		33.9(7.1)	28.2(1.1)	0.227
Ulnar	Above elbow	>35	35.4(9.7)	29.5(1.3)	0.243
	Below elbow		34.1(1.0)	29.1(1.3)	0.280
Peroneal	Knee		32.2(7.2)	21.8(7.5)	0.086
	Fibula Head		33.0(3.9)	27.9(3.4)	**0.028**
Tibial	Knee	>43	29.9(2.2)	28.5(4.6)	0.337
		Sensory studies			
SNAP amplitude (µv)					
Median	Wrist	>11	4.2(2.7)	3.5(0.7)	0.764
Ulnar	Wrist	>9	3.1(1.6)	8.3(1.0)	0.433
Radial	Forearm		17.2(1.2)	11.3(8.3)	0.515
Sural	Calf	>5	14.3(0.3)	15.8(1.3)	0.699
sCV (m/s)					
Median	Wrist		34.6(4.5)	26.4(2.5)	0.053
Ulnar	Wrist		39.3(5.5)	25.8(2.5)	**0.004**
Radial	Forearm		43.3(5.1)	32.8(7.6)	**0.020**
Sural	Calf	>44	35.5(1.7)	34.4(3.6)	0.857

4.4 Correlation studies

4.4.1 Correlation studies of nerve CSAs and electrophysiology in DM patients

Correlation studies between nerve CSAs and NCS parameters revealed significant positive correlation between median DML and CSA in the median nerve at the wrist ($p<0.001$, $r=0.453$). The median dCMAP was negatively correlated with CSA in the median nerve at the wrist ($p=0.011$, $r = - 0.254$). Ulnar mCV and CSA in the ulnar nerve at the elbow were also negatively correlated ($p=0.043$, $r=- 0.206$) [**Table 4.9]**. Other parameters do not show a statistically significant correlation.

Table 4.9: Overview of correlation between nerve CSAs and neurophysiology parameters in DM patients

NCS parameter	Sonographic measurements (CSA/site)	r value	p value
DML	Median wrist	0.453	**<0.001**
dCMAP		-0.254	**0.011**
DML	Ulnar wrist	0.081	0.431
dCMAP		0.089	0.385
mCV	Ulnar elbow	-0.206	**0.043**
DML	Tibial ankle	0.132	0.220
dCMAP		-0.010	0.910
SNAP amplitude	Sural ankle	-0.147	0.285
sCV		-0.105	0.454

4.4.2 Correlation studies of nerve CSAs at different sites versus different disease markers

Table 4.10 shows correlation studies of nerve CSAs with different disease markers. TCSS scores show a significant, linear correlation with nerve CSA in the ulnar nerve at mid forearm (p=0.010, r=0.310) and at elbow (p=0.006, r=0.300), in the common peroneal nerve at fibula head (p=0.002, r=0.301), in tibial nerve at ankle (p=0.005, r=0.200) and in sural nerve at ankle (p=0.0008, r=0.335). HbA1C shows a significant, linear relationship with nerve CSA in the median nerve at wrist (p=0.001, r=0.313) only. DM disease duration correlated well with a direct, significant association between nerve CSAs in the median nerve at wrist (p=0.041, r=0.026) and in the ulnar nerve at elbow (p=0.001, r=0.321) and arm (p=0.021, r=0.231).

Table 4.10: Overview of correlation studies of nerve CSAs versus independent disease markers

	TCSS scores		HbA 1C		Disease duration	
Nerve/ sites	**r value**	**p value**	**r value**	**p value**	**r value**	**p value**
Median						
Wrist	0.810	0.421	0.313	**0.001**	0.026	**0.041**
Mid Forearm	0.800	0.410	0.115	0.323	0.007	0.944
Elbow	0.110	0.211	0.312	0.414	0.035	0.726
Arm	0.040	0.718	0.401	0.916	0.103	0.309
Ulnar						
Wrist	0.111	0.200	0.041	0.601	0.049	0.632
Mid forearm	0.310	**0.010**	0.118	0.231	0.066	0.515
Elbow	0.300	**0.006**	0.127	0.114	0.321	**0.001**
Arm	0.208	0.205	0.093	0.400	0.231	**0.021**
Common peroneal						
Knee	0.200	0.050	0.007	0.913	0.067	0.515
Fibula Head	0.301	**0.002**	0.821	0.404	0.013	0.897
Tibial						
Ankle	0.200	**0.005**	0.093	0.400	0.035	0.732
Radial	0.115	0.311	0.021	0.813	0.165	0.100
Sural	0.335	**0.008**	0.013	0.800	0.008	0.934

4.4.3 Correlation studies of NCS parameters versus different disease markers

Table 4.11 demonstrates correlation between electrophysiology parameters and different disease markers. Significant, linear association was observed between TCSS scores and distal latencies in the median nerve at wrist (p=0.011, r=0.257), in the ulnar nerve at the wrist (p<0.001, r=0.421) and in the tibial nerve at ankle (p<0.001, r=0.396). TCSS scores show an inverse, significant relationship with CMAP in the peroneal nerve at knee (p<0.001,r=-0.324) and fibula head (p<0.001,r = -0.318), and in tibial nerve at ankle (p=0.019,r=-0.173). A significant, inverse correlation were also observed between TCSS scores and mCV in peroneal nerve at fibula head (p<0.001, r=-0.289) and with SNAP amplitude in the sural nerve at ankle (p<0.001, r=-0.365). HbA1C only shows a linear, significant correlation with DML in median nerve at the wrist (p=0.003, r=0.293) and a significant, inverse association with CMAP in the median nerve at wrist (p=0.01, r=-0.249). DM disease duration correlated significantly with a linear relationship with DML in median nerve at the wrist (p=0.173, r=0.140) and a significant, inverse association with CMAP in the median nerve at wrist (p-0.007, r=-0.272), mCV in peroneal nerve at knee (p=-.032, r=-0.233) and CMAP in the tibial nerve at ankle (p=0.003,r=-0.302).

Table 4.11: Overview of correlation studies of electrophysiology parameters versus independent

			TCSS Scores		HbA1C		Disease duration	
Nerve	**Site**	**NCS parameter**	**r value**	**p value**	**r value**	**p value**	**r value**	**p value**
Median	**Wrist**	DML	0.257	**0.011**	0.293	**0.003**	0.140	**0.173**
		CMAP	-0.129	0.206	-0.249	**0.013**	-0.272	**0.007**
Ulnar	**Wrist**	DML	0.421	**<0.001**	0.072	0.481	0.147	0.153
		CMAP	-0.004	0.972	-0.084	0.437	0.136	0.887
Peroneal	**Knee**	CMAP	-0.324	**<0.001**	-0.088	0.286	-0.067	0.569
		mCV	-0.046	0.551	-0.086	0.265	-0.233	**0.032**
	Fibula Head	CMAP	-0.318	**<0.001**	-0.132	0.080	-0.082	0.449
		mCV	-0.289	**<0.001**	-0.134	0.097	-0.176	0.128
Tibial	**Ankle**	DML	0.396	**<0.001**	0.071	0.311	0.132	0.207
		CMAP	-0.173	**0.019**	-0.140	0.060	-0.302	**0.003**
Sural	**Ankle**	SNAP amplitude	-0.365	**<0.001**	0.144	0.122	-0.067	0.623
		CV	-0.121	0.201	-0.061	0.528	-0.007	0.957

4.4.4 Association between HbA1C values with neuropathy severity and DM duration

The correlation between HbA1C values with the neuropathy severity (as per TCSS) of diabetic patients failed to reach significance. However, there was a significant, linear relationship between HbA1C values and the DM duration ($p=0.013$, $r=0.248$). A significant correlation were also found between DM duration and neuropathy severity ($p=0.045$, $r=0.201$).

CHAPTER 5: DISCUSSION

5.1 Evaluation of DSP

DM is the most common cause of neuropathy. The frequency of neuropathy in patients with DM is about 30%, and up to 50% of patients will eventually develop neuropathy during the course of their disease (Callaghan et al, 2012). The diagnosis of DSP is primarily based on its characteristic symptoms. TCSS is proven to be a valid instrument to reflect the presence and severity of DSP (Bril & Perkins, 2002) . Conventionally, NCS has been widely used to diagnose DSP (Dyck, Karnes, Daube, O'Brien, & Service, 1985). However, NCS is time consuming and uncomfortable for patients. Sonographic examination can potentially be an alternative to assess peripheral nerves with less discomfort and there have been studies that have proven its clinical use in the evaluation of disorders of the peripheral nervous system (Goedee et al, 2013).

5.2 US

In the current study, US revealed that nerve CSAs varied along the length of nerves. In one study, the CSAs of multiple nerves in the upper and lower extremities of normal, healthy controls were examined (Cartwright et al, 2008). The mean area of tibial nerve at ankle observed in their study was 13.7 mm^2, which is slightly greater than the value 10.5 mm^2 that we obtained **[Table 4.2].** The participants in their study varied from other populations with regard to body composition and racial distribution and this may account for the discrepancy seen in comparison to our findings. Another study reported a mean area of the tibial nerve at the ankle, which was 7.2 mm^2 in 35 healthy individuals, which is lower than our finding. On average, nerves were smaller in the median and ulnar nerve at forearm than in the arm of the diabetic patients as well as in the healthy controls.

Nerves also appear to be larger in entrapment sites compared to their more proximal nerve segment. In asymptomatic, older subjects, where it is common for nerve to be relatively enlarged, slight nerve enlargement at the elbow or wrist relative to the more proximal site does not necessarily suggest important focal pathology (Hassan, Leep Hunderfund, Watson, Boon, & Sorenson, 2013). In DSP, nerve enlargement in the median and tibial nerves has been reported (D. Lee & Dauphinee, 2005; Watanabe et al., 2009). In our study, we found that the CSA in the median nerve at wrist and in the ulnar nerve at elbow is significantly larger than in healthy controls. This may be because the wrist is an entrapment site and most of our diabetic patients are susceptible to developing CTS i.e. median nerve entrapment [**Table 4.1]** and UNE. CTS has been documented as the frequent form of median nerve entrapment (Lo, Raskin, Lester, & Lester, 2002; Padua, Lo Monaco, Padua, Gregori, & Tonali, 1997; Pfeffer, Gelberman, Boyes, & Rydevik, 1988) and accounts for almost 90% of all entrapment neuropathies (Aroori & Spence, 2008). Entrapment of the median nerve at the level of the carpal tunnel, by the carpal bones and by the transverse carpal ligament results in CTS (Alfonso, Jann, Massa, & Torreggiani, 2010). CTS reflect a decreased function of the median nerve at that level due to increased pressure within the carpal tunnel. In DM, CTS can be considered to be caused by both chronic compression and nerve dysfunction (Comi et al, 1985). Aside from this, we found that nerves are also significantly larger in the ulnar and radial nerve at mid forearm in diabetic patients than in healthy controls. The lower extremity nerves in DSP subjects exhibit significant enlargement compared to healthy controls. This is particularly true in the peroneal nerve at knee, tibial and sural nerve at ankle **[Table 4.2].** However, the mean CSA of diabetic patients in the tibial nerve at ankle in our study (11.96 mm^2) is lower than previously reported where the threshold value was found to be 19.01 mm^2 (Riazi et al., 2012).

This might be due to the variation in the patient population where their patients are older and had longer duration of diabetes. Our findings are also in keeping with previous studies that have found sural nerves in diabetes patients to be enlarged (Liu et al., 2012; T. Watanabe et al., 2010). However, the mean CSA of sural nerve in our study of 2.59 mm^2 is higher than previously reported (1.88 mm^2) (Liu et al., 2012). The possible explanation could be their subjects had shorter duration of DM (7.5±2.6 years) compared to our subjects (14.54± 9.43).

Looking specifically at the median nerve at the wrist, we further demonstrated that the DM patients had a significantly higher wrist to forearm ratio compared to the healthy controls. The wrist to forearm ratio (cutoff value ≥ 1.4) (Hobson-Webb et al, 2008) had a sensitivity of 73% and specificity of 41% in differentiating DM patients who were symptomatic and those who were not for carpal tunnel syndrome. We found this ratio to be sensitive but not specific for clinically symptomatic median nerve entrapment in DM patients with DSP.

We also found that the CSA of all lower limb nerves examined demonstrated a significant difference between the mild and severe neuropathy group, where the severe neuropathy patient group demonstrated larger CSA than the mild neuropathy patient group **[Table 4.3].** Generally, peroneal, tibial and sural nerves showed progressively larger nerve CSAs with worsening severity. This is similar to a study that has reported a close association between morphological parameters such as nerve hyperechoic area and nerve CSA with the severity of diabetic neuropathy (Ishibashi et al, 2015).

AUC in ROC analysis revealed fair discriminatory cut-off CSA values for tibial and sural nerves at 11.75 mm^2 and 2.75 mm^2 respectively to differentiate between severe and less severe DSP as determined by TCSS **[Table 4.4].** Previous studies have suggested optimum threshold value that identifies DSP without specifically focusing on severity.

In one study, a cut-off value for tibial nerve of 19.1 mm^2 was suggested to identify DSP (Riazi et al, 2012). In the same study, NCS was utilized to detect the presence of neuropathy. We find that whilst NCS is useful as an objective marker of DSP, it may not be as useful in discriminating between DSP severities.

For this, TCSS has been validated as an instrument that can indicate the presence and severity of DSP (Bril & Perkins, 2002). TCSS is also a universally accessible tool that is easily implemented in DM patients. Using ROC analysis, we also found the sural nerve CSA to be a good discriminator between the presence and absence of DSP in DM patients using a cut-off value of 2.0 mm^2 **[Table 4.5] .** This is similar to another study, that reported a cut-off value of 1.685 mm^2 of the sural nerve to differentiate diabetic patients with neuropathy and diabetic patients without neuropathy (Liu et al, 2012).

To our knowledge, there has been no study that has compared the nerve sizes of true CIDP patients and DM patients with demyelinating neurophysiological characteristic. We explored the difference in CSAs of the nerves between DM patients with demyelinating neurophysiological characteristics and true CIDP patients. We found that the nerves in true CIDP patients were significantly enlarged in the upper extremities, especially at the proximal and non-entrapment sites [**Table 4.6].** This is similar to a study that exhibits bigger mean nerve CSAs especially at proximal and non-entrapment sites (Jang, Cho, Yang, Seok, & Kim, 2014). CIDP is mainly characterized pathologically by segmental demyelination. There have been previous pathological studies of CIDP that have demonstrated consistently a widespread segmental demyelination and 'onion bulb formation', which describes magnified fascicles with elevated endoneural connective tissues in which many myelinated fibers are enclosed by concentrically arranged Schwann cells (Dyck et al., 1975; Matsuda et al, 1996).

The increase in nerve size in sonographic images is likely to be due to multiple enlarged fascicles resulting from repeated demyelination and remyelination (Oguz, Oguz, Cila, & Tan, 2003). There have also been studies that have reported the diffuse nerve enlargement at multiple nerves in CIDP patients (Jang et al., 2014; Taniguchi et al., 2000). Our study further demonstrates that CSA enlargement was more prominent at sites where entrapment or compression is not common, and at proximal regions.

This may be due to the fact that DSP presents in a length dependent fashion, whereas CIDP is rather patchy or segmental. Proximal and distal weakness strongly indicates CIDP (Koski et al, 2009). Regional nerve enlargement have been reported in acquired demyelinating neuropathies (CIDP) (Zaidman, Harms, & Pestronk, 2013) which is in contrast to D-DSP, which is more likely a form of axonal neuropathy, exhibiting "demyelinating" neurophysiology. US studies in patients with CIDP have shown diffuse nerve enlargement of the median and ulnar nerves and are more common than in axonal neuropathies, and these distinction may reflect the pathologic findings associated with repeated demyelination and remyelination (Zaidman, Al-Lozi, & Pestronk, 2009).

5.3 Neurophysiological studies (NCS)

NCS was found to be useful in staging of neuropathy severity in DSP patients with significant differences found in DML in the ulnar and tibial nerves, where the severe patient group demonstrated a prolonged DML. The CMAP is also significantly reduced in the peroneal and tibial nerves examined in the severe group. The motor conduction velocities are decreased in the median nerve, ulnar nerve, peroneal nerve at fibula head and tibial nerve in the severe neuropathy patient group. As for the sensory nerves, ulnar, radial and sural nerves demonstrated a significant reduction in the sensory nerve action potential among the severe neuropathy patient group.

Only the radial nerve showed a reduced sensory conduction velocity in the severe neuropathy patient group **[Table 4.7].** It is important to note that the lower limb nerve potentials were inexcitable in 43%, 13% and 8% of total sural, peroneal and tibial nerves respectively. The unrecordable potentials were likely due to significant axonal degeneration resulting in too few remaining axons that are able to conduct an electrically quantifiable signal. Previous studies have suggested the latter to reflect more severe neuropathy with an increased risk of developing foot ulcers (Behse, Buchthal, & Carlsen, 1977; Severinsen & Andersen, 2007; Veves et al, 1991; Vinik, Bril, Litchy, Price, & Bastyr, 2005). Other possibilities for inexcitable nerves include peripheral nerve edema but this was not a significant feature in our cohort, suggesting that the nerves were truly diseased.

Comparative studies of neurophysiology parameters between true CIDP patients and D-DSP patients **[Table 4.8]** revealed that DML in the peroneal nerve and duration in the median nerve, ulnar nerve and tibial nerve were prolonged in true CIDP patients. True CIDP patients demonstrated reduced CMAP in the ulnar nerve and a slowed velocity in the peroneal nerve at fibula head. Ulnar and radial nerve in true CIDP patients exhibited decreased sensory conduction velocities. This is identical to a study that has reported similar results where they compared the NCS parameters between CIDP patients and DM patients with neuropathy (Wilson, Chawla, & Fisher, 2005). Sensory NCS abnormalities studies in CIDP have reported a high proportion of nerves with absent responses and a susceptibility for sensory nerve to exhibit somewhat lesser degree of CV slowing than is evident in motor nerves of the same patient (Krarup & Trojaborg, 1996). In other studies, evidence of demyelination on electrodiagnostic tests was found (e.g. slowed conduction velocity or prolonged DML) in sensory nerves (Oh, Joy, & Kuruoglu, 1992; Sinnreich et al., 2004).

Another study has also shown that sCV slowing is a highly specific marker for differentiating CIDP from axonal polyneuropathy (Bragg & Benatar, 2008). This is particularly true in our study where we found a significant reduction in conduction velocity in the sensory ulnar and radial nerve.

5.4 Correlation studies

Correlation analysis in the current study revealed significant associations between NCS parameters and nerve size in DSP patients **[Table 4.9].** DMLs of median nerve were positively correlated with nerve CSAs whereas CMAP amplitudes were negatively correlated with nerve CSAs in the median nerve at the wrist. Ulnar mCV was also negatively correlated with nerve CSA across the elbow. We failed to detect significant correlations in the lower limb NCS parameters and nerve CSAs. This is similar to a study that has reported the CSAs were negatively correlated with both a reduced mCV and delayed latency (Tsuneo Watanabe et al, 2010). Another study has found an increased CSA of the median nerve at wrist, which inversely correlated with conduction velocity (Watanabe et al, 2009). A study also reported that the NCV might be decreased not only due to loss of the fastest conducting axons, but also because of demyelination and acute metabolic dysregulation (Severinsen & Andersen, 2007). A secondary sodium accumulation and an increase in sorbitol may be major contributors to an increase in intracellular hydration using a ^{1}H-nuclear magnetic resonance study (Suzuki et al, 1994). The peripheral nerve in DM patients may be swollen due to increased water content related to increased aldose reductase conversion of glucose to sorbitol further causing enlarged peripheral nerves i.e. larger CSAs (D. Lee & Dauphinee, 2005).

A significant positive correlation was also seen between disease duration; nerve CSA of the median at the wrist and ulnar at the elbow, and arm **[Table 4.10].** No correlation was found between DM duration and CSA in the lower extremities. This is similar to one study that has studied lower extremity nerve CSAs and found no correlation between DM duration and nerve CSAs (Hobson-Webb et al, 2013).

We also investigated the relationship between disease duration and glycaemic control (HbA1C) with DSP severity, NCS parameters and nerve CSA. Having excluded entrapment sites, we failed to demonstrate a significant relationship between glycaemic control and DSP severity or NCS and CSA parameters **[Table 4.10 and 4.11].** This is similar to a previous study where no significant association was found between HbA1C and DSP severity (Sachedina & Toth, 2013). In one study, the authors investigated glycaemic control based on glycated haemoglobin and nerve morphology based on sural nerve biopsy (Perkins et al, 2001). The latter is an invasive method and unlikely to be a feasible measure of nerve morphology in a clinical setting. However, we did demonstrate a significant positive correlation between duration of DM with HbA1C and disease severity. This is similar to other studies that have also found disease duration to be a better predictor of DSP severity (Dyck et al., 1999; Mimi, Teng, & Chia, 2003). Our study also demonstrated significant positive correlations between TCSS and most neurophysiology parameters and with nerve CSAs **[Table 4.10 and 4.11]**. This is supported by a study that had found strong correlations between TCSS and sural nerve fiber density with electrophysiology, by both summed amplitude and summed conduction velocity values. It was further concluded that TCSS is a valid instrument to reflect the presence and severity of DSP as measured by sural nerve morphology and electrophysiology (Bril & Perkins, 2002).

5.5 Study limitations

Some of the limitations of our study include the small number of patients in the individual DSP severity groups. However, we were able to demonstrate that nerve US has a role in discriminating between DSP severities. A separate cohort of patients with asymptomatic DSP was not identified. Future studies involving a larger cohort and identification of a separate group of asymptomatic DSP patients may yield further useful information such as CSA cut-off values with better performance when discriminating between DSP severities and the presence of DSP. The use of more advanced US techniques incorporating color Doppler imaging might also provide further insight.

This study aimed to distinguish D-DSP patients from true CIDP patients, which was a pilot study with only preliminary results. However, our results show some promise as to the utility of nerve US to differentiate the two cohorts. This diagnostic value will require further validation in prospective studies involving a larger sample of patients at different clinical stages of CIDP. We also did not attempt histological evaluation, although these are not as easily available but may provide additional information and lead to evidence that is more robust.

CHAPTER 6: CONCLUSION

In the current study, we investigated the nerve morphology of DM patients through nerve US by assessing nerve CSAs in DM patients of different DSP severities as assessed by the clinical tool, TCSS. We demonstrated that US correlated well with NCS parameters in our cohort of patients, which suggests US can potentially be used to objectively evaluate for severity of DSP and can be utilized in future studies.

We found that the nerve CSA of DM patients were larger as the severity of DSP progresses. To our knowledge, there have been no previous studies that have described such a relationship utilizing nerve CSA values. Traditionally, NCS has been the gold standard for the diagnosis of DSP. However, patients with advanced DSP are likely to have inexcitable nerves thus making an objective assessment of injured nerves challenging. In these situations, we found US to be useful in assessing DSP in patients and could serve as a valid bedside evaluation method to identify and determine the severity of DSP, as currently, there is no objective practical method to determine the presence and/or severity of DSP at the bedside.

In the current study, we found this ratio to be sensitive but not specific for clinically symptomatic median nerve entrapment in DM patients with DSP. To further improve the sensitivity of US, measurement of the median nerve at different levels and using a proximal median nerve to median nerve at carpal tunnel ratio has been recommended (Pastare, Therimadasamy, Lee, & Wilder-Smith, 2009). This would be helpful in demonstrating the focality of median nerve swelling at the carpal tunnel similar to that used in NCS. Besides this, when assessment of nerve conduction abnormalities across the carpal tunnel becomes difficult in patients with DSP as median neuropathy and DSP might affect median nerve conduction, US may serve as an alternative tool to detect median neuropathy.

Both tibial and sural nerves showed progressively larger nerve CSAs with worsening severity. This suggests that both tibial and sural nerve CSAs can potentially be used as markers of disease severity in DSP. Further studies based on large multi-center cohorts of patients with broader spectrum of neuropathy including asymptomatic DSP patients may yield additional information, which may provide a more specific and sensitive cut-off values to differentiate severe and non-severe DSP patients as well as DSP and non DSP patients.

In the current study, we were also able to demonstrate significant associations between NCS parameters and nerve size in DSP patients. US can be an alternative diagnostic modality when NCS results are not confirmatory in patients suspected of DSP. NCS and US used in combination may be able to estimate DSP more accurately. Future serial studies with long-term follow up are needed to investigate correlations between changes in US findings and other parameters such as NCS and clinical characteristics. Significant positive correlations between TCSS and most neurophysiology parameters and with nerve CSAs were presented in this study. Further investigation to examine relationships between morphological changes and other clinical scoring systems in DSP patients would yield extra information on the validity of TCSS as well.

We did not detect any significant correlation between HbA1C and DSP severity or NCS and CSA parameters, but detected significant correlation between duration of DM with HbA1C and disease severity. Significant association between duration of diabetes and DM severity from our study implies that although not a modifiable risk factor, the duration of diabetes is of great significance for early identification and management of diabetic polyneuropathy. Our findings strongly suggest that nerve CSA values have a

role in determining severity of DSP, particularly when other existing objective parameters such as nerve action potentials are inexcitable.

Making the distinction between chronic DSP and CIDP can be challenging. The current study exhibits a pattern of significant nerve enlargement in non-entrapment sites in the upper extremities of true CIDP patients compared to D-DSP patients through US studies. This is of significant clinical importance, as earlier initiation of treatment is required to improve the overall prognosis and clinical outcome. Future prospective and serial studies in a larger cohort of patients are required to confirm the current findings.

In conclusion, work presented here has demonstrated the promising use of nerve US in the diagnosis of DSP including the ability to differentiate disease severity and distinguishing between DSP and CIDP.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

1. Serial peripheral nerve ultrasound in Guillain–Barré syndrome, Clinical Neurophysiology (2015)

2. Relationship between ultrasonographic nerve morphology and severity of diabetic sensorimotor polyneuropathy-Peripheral Nerve Society Meeting,Canada

APPENDIX

Appendix A

Toronto Clinical Scoring System

Date:	**Right**	**Left**
Symptom scores	Present =1 Absent=0	
Pain		
Numbness		
Tingling		
Weakness		
Ataxia		
Upper-limb symptoms		
Reflex Scores	Absent = 2 Reduced = 1 Normal = 0	
Knee reflexes		
Ankle reflexes		
Sensory test Scores	Abnormal = 1 Normal = 0	
Pinprick		
Temperature		
Light touch		
Vibration		
Position		
Total		

0-5: No neuropathy

6-8: Mild neuropathy

9-11: Moderate neuropathy

12-19: Severe neuropathy

Appendix B

Estimated likelihood of distal symmetrical polyneuropathy for case definitions that include symptoms, signs, and nerve conduction studies (recommendations for clinical research studies)-a joint report by the American Association of Electrodiagnostic Medicine, the American Academy of Neurology, and the

Neuropathic symptoms	Decreased or absent ankle reflexes*	Decreased distal sensation	Distal muscle weakness or atrophy	NCS†	Ordinal likelihood
Present	Present	Present	Present	Abnormal	++++
Absent	Present	Present	Present	Abnormal	++++
Present	Present	Present	Absent	Abnormal	++++
Present	Present	Absent	Absent	Abnormal	++++
Present	Absent	Present	Absent	Abnormal	++++
Absent	Present	Absent	Present	Abnormal	+++
Present	Absent	Absent	Absent	Abnormal	+++
Absent	Absent	Absent	Absent	Abnormal	++
Absent	Present	Absent	Absent	Abnormal	++
Present	Present	Present	Absent	Normal	++
Present‡	Absent	Present‡	Absent	Normal‡	+
Present§	Present§	Present§	Present§	Normal§	−

American Academy of Physical Medicine and Rehabilitation

Neuropathic symptoms: numbness, altered sensation, or pain in the feet. NCS, nerve conduction studies. For clinical research studies enrollment should be limited to cases above the bold horizontal line (i.e. ++++).

- *Ankle reflexes may be decreased in normal individuals >65–70 years.
- †Abnormal NCS is defined in text.
- ‡This phenotype is common in "small-fiber" sensory polyneuropathy. Determination of intraepithelial nerve fiber density in skin biopsy may be useful to confirm the diagnosis.
- §This phenotype in the presence of normal NCS is not a distal symmetrical polyneuropathy. This situation is given a negative (−) ordinal likelihood because the condition cannot be classified as a distal symmetrical polyneuropathy. It is included here to emphasize the importance of including NCS as part of the case definition for clinical research studies.