Correlations between experienced fatigue, physiological fatigue and motor unit function in patients with demyelinating peripheral nerve disorders

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Fatigue is a common complaint in patients with neurological disorders. Fatigue is well documented in disorders of the central nervous system such as multiple sclerosis and post-stroke. Although acknowledged, fatigue in disorders of the peripheral nervous system is less studied and underlying pathophysiology is not understood. It has been hypothesised that fatigue may relate to progressive loss of peripheral motor units or disorganised peripheral motor unit firing. This study aimed to explore experienced fatigue in chronic demyelinating disorders of the peripheral nerves and relationship with quality of life. The study also aimed to investigate peripheral motor unit function using a newly-developed electrophysiology technique, explore how this relates to self-reported experience of fatigue and development of muscle fatigue during exertion.

Fatigue in patients with chronic demyelinating disorders of the peripheral nervous system appears to be negatively correlated with quality of life. Patients with both acquired and hereditary chronic demyelinating peripheral nerve disorders have reduced number of motor units assessed using MUNIX technique compared to control subjects. However, no clear relationship is found between number of functioning peripheral motor units and fatigue levels experienced by patients. Depression and reduced grip strength were significant predictors of higher experienced fatigue levels in patients with chronic inflammatory demyelinating polyneuropathy. This suggests fatigue in this patient group is likely to be multifactorial, with physical and psychological contributors. Significant changes in MUNIX values were found following intravenous immunoglobulin therapy in patients with chronic inflammatory demyelinating polyneuropathy, highlighting a potential role as a monitoring tool for treatment response.

Key words (5):

- Fatigue
- Electrophysiology
- Polyradiculoneuropathy, Chronic Inflammatory Demyelinating
- Charcot Marie Tooth disease
- MUNIX

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List of accepted and submitted papers

Lawley A, Seri S, Rajabally YA. Motor unit number index (MUNIX) in chronic inflammatory demyelinating polyneuropathy: a potential role in monitoring response to intravenous immunoglobulins. *Clin Neurophysiol*. 2019;130(10):1743-49. doi: 10.1016/j.clinph.2019.06.231

Lawley A, Abbas A, Seri S, Rajabally YA. Electrophysiology studies in relation to fatigue in patients with chronic inflammatory demyelinating polyneuropathy. (Manuscript submitted to Clinical Neurophysiology December 2019)

Lawley A, Abbas A, Seri S, Rajabally YA. Clinical associations of fatigue in chronic inflammatory demyelinating polyneuropathy. (Manuscript submitted to Muscle and Nerve December 2019)

Contents

Abbreviations

ADM = abductor digitii minimi

AH = abductor hallucis

ALS = amyotrophic lateral sclerosis

APB = abductor pollicis brevis

CIDP = chronic inflammatory demyelinating polyneuropathy

CIS = checklist of individual strength

CMAP = compound muscle action potential

CMT = Charcot-Marie Tooth disease

CMT1A = Charcot-Marie Tooth disease type 1A

DML = distal motor latency

EDB = extensor digitorum brevis

EFNS = European Federation of Neurological Societies

EMG = electromyography

FIS = fatigue impact scale

FSS = fatigue severity scale

GBS = Guillain-Barre syndrome

HADS = hospital anxiety and depression scale

ICC = intraclass correlation coefficient

ICMUC = ideal case motor unit count

INCAT = Inflammatory Neuropathy Cause and Treatment group

IVIg = intravenous immunoglobulins

MRC = medical research group

MGUS = monoclonal gammopathy of undetermined significance

MUNE = motor unit number estimation

MUNIX = motor unit number index

MUSIX = motor unit size index

NBS = norm-based score

NCS = nerve conduction study

ONLS = overall neuropathy limitations scale

PMP22 = peripheral myelin protein 22

PNS = Peripheral Nerve Society

QoL = quality of life

R-FSS = Rasch-built fatigue severity scale

RMS = root mean square

R-ODS = Rasch-built overall disability scale

SEMG = surface electromyography

SF-36 = medical outcomes study 36-item short form health survey

SIP = surface interference pattern

SNAP = sensory nerve action potential

TA = tibialis anterior

List of Tables and Figures

1. Background

Fatigue is a recognised feature of a wide-range of neurological disorders[1]. The experience of fatigue usually refers to the physical or mental experience of a lack of energy or lack of motivation, although perhaps unsurprisingly given its inherently subjective nature, no universally agreed definition of fatigue exists in the scientific literature[2]. Approaches to the study of fatigue also vary. Exploration of an individual's experience of fatigue typically utilises self-report psychometric scales to assess either fatigue levels or the impact of fatigue on daily functioning[3]. Research from the physiological scientific literature typically focuses on development of muscle fatigue during activity or exertion, expressing fatigue as a decline in the maximal force that may be generated by a muscle or muscle group over time[4]. How these separate aspects of fatigue relate, if at all, is far from clear.

The available evidence in neurological disorders mainly involves studies exploring fatigue in the context of disorders of the central nervous system, including multiple sclerosis[5], stroke[6] and Parkinson's disease[7]. It is only recently that fatigue has received attention in disorders of the peripheral nervous system. This is perhaps surprising given that fatigue has been a long-recognised sequelae of disorders such as Guillain-Barre syndrome (GBS), at least anecdotally, and has been reported subjectively by patients as one of the most disabling features of their disorder[8].

GBS is the most common acute paralysing neuropathy affecting roughly 100,000 people annually worldwide, with distinct subtypes including forms where loss of nerve myelin is the predominant pathophysiological feature and forms where axonal degeneration predominates[9,10]. GBS is characterised by immune-mediated inflammation of nerve roots and peripheral nerves; a history of infection by viral or bacterial or agents such as *Campylobacter jejuni,* or of immune-activating event, such as influenza A vaccination is present in some but not all cases[11]. GBS is typically a monophasic illness with peak clinical deficit seen two to four weeks following onset and around one quarter of patients requiring ventilatory support[9,12]. During the acute phase of the illness, immunomodulatory treatments such as intravenous immunoglobulin therapy (IVIg) and plasma exchange are proven to reduce disease progression and speed recovery [13,14], with a recovery period which may last for several months or years. A first-year mortality rate of 4.4% and persistent severe motor sequalae in 13.9% of patients at one year or later are reported[15]. In addition, some reports suggest only 33% of patients feel subjectively fully recovered at one year[16], with 37.8% needing to change employment during this time[17].

Nearly fifty years after GBS was first described by Guillain, Barre and Strohl[18], a chronic form of corticosteroid responsive polyneuropathy was described by James Austin[19], later termed "chronic inflammatory demyelinating polyneuropathy (CIDP)". CIDP is a chronically-progressive or relapsing-

remitting condition. Clinical presentation typically involves weakness of proximal or distal muscles, which is usually symmetrical, loss of sensation and neuropathic-type pain[20]. Clinical presentation may be heterogeneous, however, with less commonly reported presentations including asymmetrical or intermittent weakness, ataxia or muscle fatigue[21]. CIDP may present at any age, with cases reported from infancy to the ninth decade and median age of onset in the sixth decade[22,23]. CIDP has a prevalence of up to 9 per 100,000 of the population[23] and unlike the monophasic course of GBS, CIDP is a chronically active disease. It has been suggested by some authors that these conditions form part of a spectrum[24]. There is substantial evidence supporting CIDP as an immune-mediated process from animal models, pathological studies and observed response to immunomodulatory therapies[25]. The classical pathological hallmark of CIDP is multifocal and segmental demyelination starting in nodal regions, with autopsy studies demonstrating preferential involvement of proximal segments of motor nerves[25]. Despite evidence supporting an immune-mediated aetiology, no specific triggering infective agent or immune event have been identified in CIDP to date[20] and autoantibodies are identified in only a minority of patients[26].

IVIg is an effective treatment in CIDP, improving disability and preventing disease relapse[27]. In relapsing CIDP response to IVIg may be biphasic, with an initial rapid response seen within days, followed by a "wearing-off effect"[28,29] requiring repeat infusions to maintain therapeutic effect in around 65% of patients[30]. Doses should be given at the maximal interval required to maintain a stable clinical response[31], which may vary from 2 to 6 weeks[32–35], possibly reflecting interindividual-variability in pharmacokinetics of IVIg[33,36,37]. Current trials examining the role of immunosuppressant or immunomodulatory treatments in GBS and CIDP tend to focus on outcome measures assessing motor or sensory function, grip strength, standard electrophysiological parameters such as compound motor action potential amplitude, or disability scores[13,38,39]. There is growing awareness of residual deficits in daily and social activities and reduced quality of life outcomes, which are only partly explained by impairments assessed using these methods[40,41]. Fatigue has been identified as a potentially disabling symptom in immune-mediated neuropathies, which may impact quality of life outcomes[42]. However, assessment of fatigue is not currently routinely included as an outcome measure in studies exploring effect of treatments for immune-mediated neuropathies.

GBS and CIDP represent the acquired end of the spectrum of demyelinating disorders of the peripheral nervous system. Of the hereditary demyelinating disorder of the peripheral nervous system, Charcot-Marie-Tooth disease (CMT) type 1 is the most prevalent, affecting around 36 per 100,000 of the population[43]. The most common subtype, CMT1A, results from duplication of the peripheral myelin protein 22 (PMP22) gene located on the short arm of chromosome 17[43,44]. In keeping with a hereditary condition, CMT1A typically presents in the first two decades of life[45].

However, patients may not present with symptoms until the fifth decade of life or later[43], reflecting a variable rate of disease progression, which has been reported amongst families[46] and even identical twins[47]. Typical symptoms include distal muscle weakness, distal muscle atrophy, foot deformity with pes cavus and clawing of toes, loss of fine motor skills, sensory loss, balance disturbance due to sensory proprioceptive loss and tremor[43,48]. Deficits in mobility, muscle weakness, fatigue, pain and body image have been demonstrated to negatively impact quality of life in patients with CMT[49]. The classic pathological hallmark found on nerve biopsy is diffuse "onion-bulb" formation, where fragments of Schwann cells appear too thin for the diameter of the axon they surround, reflecting chronic demyelination-remyelination[50].

There is no treatment available for CMT, although several promising compounds are being studied in animal and cellular models[51]. Outcome measures in clinical trials include the CMT neuropathy score, the CMT paediatric scale or measures of fine motor skills such as the nine-hole peg test[45,52,53]. The aforementioned scales contain sum scores derived from clinical assessments of motor and sensory function and electrophysiological studies of sensory and motor nerve function. At present, no clinical trials have used measures of fatigue as an outcome measure.

Given the recognised impact of fatigue on patient quality of life, some authors have called for improved recognition of the importance of fatigue and inclusion of fatigue as an outcome measure in clinical trials for acquired and hereditary demyelinating polyneuropathies[42]. However, there are several potential obstacles, including difficulties in defining and assessing fatigue, a lack of understanding of the underlying pathophysiology of fatigue in this patient group and a lack of biomarkers for fatigue. Lack of understanding of underlying pathophysiology presents a particular problem to the study of fatigue in patients with peripheral nerve disorders. No correlation is found between fatigue levels and functional recovery in GBS[54] and fatigue does not appear to correlate with clinical markers of disease severity in CIDP[55,56]. In fact, in a minority of patients with CIDP fatigue may be the main presenting symptom[57]. Standard electrophysiological markers used to assess nerve dysfunction also show no correlation with severity of experienced fatigue in patients with GBS[58], although to date no similar explorations have been made in patients with CIDP or CMT. It has been hypothesised that fatigue may relate to more complex alterations in peripheral motor unit function, not readily assessable by standard electrophysiology studies[58,59]. Motor unit function index (MUNIX), is a recently developed technique[60,61], which allows parameters related to the number of motor units (MUNIX) and size of motor units (MUSIX) innervating a muscle to be obtained. It has the advantage of being non-inferior to and technically easier to perform than currently available motor unit number estimation (MUNE) techniques[62], and is therefore easier to incorporate into routine clinical practice. Application of this technique to patients with peripheral nerve disorders may allow

improved understanding of the relationship between fatigue and peripheral nerve dysfunction. In order to understand these issues in more detail, a systematic review of the literature is conducted.

2. Literature review: A systematic review of fatigue in demyelinating disorders of the peripheral nervous system

A systematic review of the literature was undertaken, including only English-language, primary research studies published in peer-reviewed journals. Randomised and non-randomised controlled trials, cohort studies, cross-sectional studies and case series with reference to fatigue in demyelinating disorders of the peripheral nervous system were selected. All age groups were included. Single case reports were excluded due to lack of generalisability.

Ovid MEDLINE (1946 to February 2018) and Pubmed databases were searched on 4th February 2018, using the search terms 'fatigue and neuropathy', 'fatigue and Charcot', 'fatigue and Guillain', 'fatigue and polyneuropathy', 'fatigue and CIDP' and 'fatigue and neurophysiology', as well as their derivations, as keywords or text words. Reference lists from identified articles were manually screened.

All relevant studies were reviewed and analysed using a data collection form adapted from the Cochrane Handbook for Systematic Reviews of Interventions[63] (see Appendix 1). The following objectives were identified:

- To address how fatigue is defined in patients with demyelinating polyneuropathies
- To understand methods of assessing fatigue in this patient population
- To determine the severity of fatigue and how this may impact on quality of life measures
- To explore the underlying pathophysiology of fatigue in this patient population
- To determine how treatment may impact fatigue for these patients
- To identify weaknesses and gaps in the existing literature

2.1 Findings

2.1.1 Search results

The search returned 123 articles, reduced to 47 following initial screening and application of inclusion criteria. Following review of the articles, 32 were included. A further 7 articles were identified on screening of reference lists, giving a total of 39 articles included in this review. These are summarised in Table 1. In addition, 2 relevant case reports were identified[21,64]. Despite not meeting inclusion criteria and therefore not appraised, these provide useful illustration of broader themes. Review of the grey literature identified 1 journal letter relating to fatigue in CIDP[65] and 1 personal account of experience of fatigue following GBS[8], which are discussed and referenced where relevant.

Authors	Year	Study design	*N*	Demographics	Fatigue assessment	Key findings
Studies relating to GBS and CIDP						
Wessely, S and Powell, R[66]	1989	Cross-sectional observational study	Mixed patient population; 3 GBS	NR	Self-report questionnaire (authors own design)	• In a small number of patients with neuromuscular disorders, fatigue was only reported in the presence of co-existent psychiatric illness
Merkies, ISJ *et al*[55]	1999	Cross-sectional observational study	83 GBS, 22 CIDP, 8 MGUS	Age range 14-84 years; 54 female	Fatigue severity scale	• Fatigue is a major symptom for patients with immune-mediated polyneuropathy • Fatigue levels did not correlate with motor or sensory function, age or time since diagnosis, and severe fatigue could exist in the absence of weakness or sensory deficit • Fatigue levels higher in female than male patients • Fatigue severity scale has good internal consistency, reliability and validity in this population
Merkies, ISJ *et al*[67]	2003	Cross-sectional observational study	83 GBS, 22 CIDP, 8 MGUS	Age range 14-84 years; 54 female	Fatigue severity scale	• Associations between measures of impairment, disability and handicap demonstrated in patients with immune-mediated polyneuropathy • Fatigue did not significantly contribute in regression models for handicap or disability
Garssen, MPJ *et al*[68]	2004	Case-control study	16 GBS, 4 CIDP	Age range 22-66 years; 14 female	Fatigue severity scale *and* Fatigue impact scale	• A graded-exercise programme improved self-reported fatigue, functional outcome and quality of life in in patients with immune-mediated polyneuropathy
Boukhris, S *et al*[57]	2005	Case series	11 CIDP	Age range 39-77 years; 0 female	Fatigue severity scale	• Fatigue may be the main presenting symptoms of CIDP • Anecdotal reports of fatigue improving with immunomodulatory treatments in some patients
Garssen, MPJ *et al*[58]	2006	Cross-sectional observational study	13 GBS, 3 CIDP	Age range 26-68 years; 10 female	Fatigue severity scale	• Fatigue experienced post-GBS not related to residual nerve dysfunction assessed by conventional NCS
Garssen, MPJ *et al*[69]	2006	Cross-sectional observational study	13 GBS, 2 CIDP	Age range 26-68 years; 9 female	Fatigue severity scale	• Fatigue experienced post-GBS did not appear related to changes in conduction velocity distribution in the median nerve, a measure of persisting peripheral nerve demyelination/suboptimal remyelination
Garssen, MPJ *et al*[54]	2006	Prospective survey	100 GBS or variant	Mean age 44.9 years; 47 female	Fatigue severity scale	• Fatigue experienced post-GBS not related to disease severity at nadir, antecedent infection, impairment of motor or sensory function or time to follow-up

Garssen, MPJ *et al*[70]	2006	Randomised, placebo-controlled, double blind crossover trial	74 GBS	Median age 47.5 or 52 years in different groups; 40 female	Fatigue severity scale *and* Fatigue impact scale	• Amantadine was not superior to placebo in treating fatigue post-GBS • A significant placebo effect was observed, attributed to increased medical attention
Garssen, MPJ *et al*[71]	2007	Cross-sectional observational study	9 GBS	Age range 48-67 years; gender NR	Fatigue severity scale *and* abbreviated fatigue questionnaire. Muscle fatigue measured from biceps brachii	• Central factors (i.e. proximal to neuromuscular junction) are involved in development of muscle fatigue in GBS • Muscle fibre hypertrophy may develop post-GBS as a compensatory mechanism for loss of motor units • Self-reported fatigue levels did not correlate with assessments of muscle fatigue
Bussmann, JB *et al*[72]	2007	Secondary analysis of data from Garssen *et al* 2004	16 GBS, 4 CIDP	Age range 22-66 years; 14 female	Fatigue severity scale	• Changes in fatigue seen following a graded-exercise programme are not related to changes in physical fitness • Correlation between changes following an exercise programme in fatigue and physical domains of the SF-36
Kuitwaard, K *et al*[56]	2009	Cross-sectional survey	245 GBS, 76 CIDP	Age range 7-94 years; gender NR	Fatigue severity scale	• High levels of severe fatigue lower quality-of-life scores than reference values in patients with immune-mediated polyneuropathies • Patients with severe fatigue more severely affected on the GBS-severity scale, but no correlation between fatigue level and time since diagnosis
Davidson, I *et al*[73]	2009	Cross-sectional survey	742 GBS	Age IQR 56-74 years; 378 female	Fatigue severity scale	• High levels of severe fatigue reported post-GBS. No correlation between fatigue level and time since diagnosis, age or gender • No difference in fatigue levels observed between patients who did and did not receive physiotherapy
Rekand, T *et al*[74]	2009	Cross-sectional survey	Mixed patient population; 58 GBS	Mean age 54.6 years; 9 female	Fatigue severity scale	• Fatigue, pain and muscle weakness are common sequalae to GBS, contributing to lower physical quality of life scores in these patients • Fatigue levels did not correlate with a measure of emotional affectivity
Westblad, ME *et al*[75]	2009	Cross-sectional observational study	21 CIDP	Age range 18-82 years; 6 female	Fatigue severity scale	• Significant number of Swedish patients with CIDP experience severe fatigue, although lower level than reports from other countries • Patients score lower on physical domains of the SF-36

						compared to reference values
van Nes, SI *et al*[76]	2009	Cross-sectional observational study	83 GBS, 22 CIDP, 8 MGUS	Age range 14-84 years; 54 female	Fatigue severity scale *and* short-form fatigue scale	• The fatigue severity scale does not meet expectations of the Rasch model due to difficulty of differentiating between multiple response options and one item being biased for patients who could walk independently • A new Rasch-built fatigue severity scale was developed and good reliability and validity demonstrated
Davidson, I *et al*[77]	2010	Secondary analysis of data from Davidson *et al* 2009	237 GBS	Median age 62 years; 118 female	Fatigue severity scale	• Good outcome post-GBS should be defined as an F-score (functional scale in GBS) of 0, as patients with scores of 1 or more had significantly higher anxiety, depression, fatigue and poorer physical functioning
Forsberg, A *et al*[78]	2012	Prospective cohort study	29 GBS	Mean age 49 years; 13 female	Fatigue severity scale	• Patients with poorer functional outcomes up to 10 years after GBS had higher fatigue levels than those with better functional outcomes
Drory, VE *et al*[79]	2012	Retrospective observational study	24 GBS	Mean age 57 years; 9 female	Fatigue severity scale	• Severe fatigue may persist for over 20 years post-GBS • Fatigue levels correlate with disease severity at time of hospital admission but not age, gender or time since diagnosis
Drenthen, J *et al*[59]	2013	Cross-sectional observational study	39 GBS	Mean age 58 years; 19 female	Fatigue severity scale	• Severe fatigue post-GBS correlates with more pronounced axonal loss, represented by lower MUNE values and SNAPs • No correlation between fatigue levels and standard NCS values, age and muscle weakness
Studies relating to CMT						
Lindeman E *et al*[80]	1999	Cross-sectional observational study	Mixed patient population; 29 CMT (23 demyCMT, 6 axoCMT)	Mean age 37 years; 15 female	Muscle fatigue measured from quadriceps muscle	• Maximum voluntary contraction, median frequency spectrum of SEMG and the ratio between torque and RMS of SEMG recorded from quadriceps muscles was significantly lower in CMT patients compared to controls • No significant difference found between endurance of quadriceps contraction or rate of decline in median spectral frequency seen between patients and controls with high inter-subject variation
Lindeman E *et al*[81]	1999	Randomised clinical trial	29 CMT	Mean age 37 years; 15 female	Muscle fatigue measured from quadriceps muscle	• A resistance strength training programme improved maximal force generation but not muscle fatigue in CMT • An increase in RMS of the SEMG signal seen early during the programme, interpreted as evidence for change in “neural mechanisms”

Videler, AJ *et al*[82]	2002	Prospective cross-sectional observational study	20 CMT (types I and II)	Age range 18-70 years; 11 female	Muscle fatigue measured using grip and pinch strength	• Hand and pinch grip strength were significantly lower in CMT patients than controls but the rate of strength decline during an intermittent fatiguing task did not differ between the two groups • The fatiguing protocol used had a poor reproducibility (ICC 0.62)
Kalkman, JS *et al*[83]	2005	Prospective cross-sectional observational study	Mixed patient population; 137 CMT	Age range 19-63 years; 81 female	Checklist of individual strength	• Severe fatigue experienced by majority of patients • Severely fatigued patients had lower scores in all domains of a quality of life assessment • Age did not contribute to level of fatigue
Carter, GT *et al*[84]	2006	Case series	4 CMT	Age range 33-65 years; 2 female	No formal assessment	• Anecdotal reports of improvement in subjective fatigue in a small case series
Kalkman, JS *et al*[85]	2007	Prospective cross-sectional observational study	73 CMT1	Age range 20-58 years; 43 female	Checklist of individual strength	• Lifetime psychiatric disorders reported in 32% of patients, most commonly depression and phobia • Fatigue levels same in patients with and without co-morbid psychiatric conditions
Kalkman, JS *et al*[86]	2007	Prospective cohort study	73 CMT1	Age range 20-58 years; 43 female	Checklist of individual strength	• Level of physical activity most strongly predictive of experienced fatigue in CMT patients. Frequent sleep disturbance, pain, muscle strength and neuropsychological impairment (assessment of concentration) also contributed to experienced fatigue
Schillings, ML *et al*[87]	2007	Case-control cross-sectional observational study	73 CMT1	Age range 20-58 years; 43 female	Checklist of individual strength *and* abbreviated fatigue questionnaire	• CMT patients have higher experienced fatigue levels than age-matched controls • In assessing muscle fatigue, patients have higher central activation failure than controls • Weak negative correlation between experienced and physiological fatigue found in patients
Kalkman, JS *et al*[88]	2008	Prospective cross-sectional observational study	73 CMT1	Age range 20-58 years; 43 female	Checklist of individual strength	• Experienced fatigue and peripheral fatigue appear to be unrelated in CMT patients, with central activation failure during a fatiguing task only weakly related to experienced fatigue
El Mhandi, L *et al*[89]	2008	Cohort study	8 CMT (4 CMT1A, 4 CMT2)	Age range 20-44 years; 0 female	Visual analogue scale for fatigue. Muscle fatigue measured from quadriceps muscle	• Interval training improved self-reported fatigue but not muscle force production or indices of muscle fatigue

Ramdharry, GM *et al*[90]	2009	Case-control cohort study	18 CMT	Mean age 37 years; 8 female	Fatigue severity scale. Muscle fatigue measured from quadriceps muscle	• Self-reported fatigue levels were higher in patients and negatively correlated with physical endurance • In CMT patients, hip flexors compensate for distal weakness whilst walking, and hip flexor fatigue greatly reduces walking distance
Minis, MAH *et al*[91]	2010	Secondary analysis of data Kalkman 2005	135 CMT1	Age range 18-68 years	Checklist of individual strength	• No difference in was observed in fatigue scores in CMT patients in employment or not in employment
Boentert, M *et al*[92]	2010	Cross-sectional survey	227 CMT	Age range 18-78 years; 130 female	Multidimensional fatigue inventory-20	• CMT patients experience higher fatigue levels than controls • Fatigue level correlated inversely with physical functioning domain of a quality of life assessment • Fatigue level did not correlate with poor sleep, daytime sleepiness or patient age
Jageresma, E *et al*[93]	2012	Cross-sectional observational study	55 CMT1A	Age range 12-18 years; 30 female	Checklist of individual strength	• Higher rates of severe fatigue in children with CMT compared to healthy controls, correlating negatively with quality of life scores
Menotti, F *et al*[94]	2012	Case-control cross-sectional observational study	8 CMT1A	Mean age 36 years, 5 female	Muscle fatigue measured from biceps and quadriceps muscles	• Patients had weaker knee extensors than controls and impaired neuromuscular recovery after a fatiguing task • No difference between patients and controls in biceps
Ramdharry, GM *et al*[95]	2012	Qualitative interview-based study	25 CMT	Age NR; 17 female	Qualitative study of fatigue	• Qualitative exploration of fatigue in a cohort of CMT patients, exploring 4 key areas: definition, key triggers, impact and management
Menotti, F *et al*[96]	2014	Case-control cross-sectional observational study	6 CMT1A	Mean age 40 years; 3 female	Fatigue severity scale	• Movement-related cortical potentials were higher in amplitude over the prefrontal cortex in a fatiguing task in patients, interpreted as demonstrating higher cognitive effort and awareness of movement complexity
Bachasson, D *et al*[97]	2014	Case-control cross-sectional observational study	8 CMT1A	Mean age 41 years; 5 female	Fatigue severity scale	• Assessment of feasibility of using femoral nerve magnetic stimulation to differentiate between peripheral and central muscle fatigue • In CMT patients, this protocol was unable to achieve supramaximal stimulation
Anens, E *et al*[98]	2015	Case-control cross-sectional observational study	44 CMT	Median age 59.5 years; 20 female	Fatigue severity scale	• Fatigue severity correlated negatively with self-reported physical activity level • Fatigue, poor balance, weakness and pain were identified as common barriers to physical activity

Table 1 Summary of the articles identified in the systematic literature search, including details of study design, patient demographics and key findings (GBS=Guillain Barre Syndrome; CIDP=chronic inflammatory demyelinating polyneuropathy; CMT=Charcot-Marie-Tooth disease; MGUS=monoclonal gammopathy of undetermined significance; NR=not reported; QoL=quality of life; NCS=nerve conduction studies, EMG=electromyography, MUNE=motor unit number estimation, SNAPs=sensory nerve action potentials, RMS=root mean square, SEMG=surface electromyography, ICC=intraclass correlation coefficient)

2.1.2 Definition of fatigue

Fatigue is a subjective experience, which may encompass physical exhaustion, lack of motivation, difficulty concentrating and muscle weakness. Whilst several studies acknowledge the complex, vague and subjective nature of fatigue[66,74,79,80], surprisingly few offer a formal definition. Westblad et al[75] and Kalkman et al[83] define fatigue as "a sense of physical tiredness and lack of energy, distinct from sadness and weakness". Highlighting difficulties in defining the perception of fatigue, Ramdharry et al[95] undertook a detailed qualitative study exploring the experience of fatigue in 25 patients with CMT. Interesting themes to emerge included most participants distinguishing between local muscle fatigue resulting from activity and refreshed by rest, and an overall sensation of energy depletion not necessarily refreshed by rest. Participants also stressed the abnormality of this sensation and how it differs from feelings of tiredness, suggesting fatigue and tiredness are separate phenomenological entities. Only basic demographics are provided, with details of comorbid conditions and medications an important omission, making it difficult to determine generalisability of these observations. Similar explorations of the subjective experience of fatigue have not been undertaken in patients with acquired forms of demyelinating polyneuropathy, although a personal account published by Gregory[8] details a strikingly similar experience.

Several authors[71,87,94,97] distinguish between the subjective experience of fatigue, which may have both physical and psychological components, and physiological fatigue. Definitions of physiological fatigue are quoted from two review articles[99,100], both generally agreeing that physiological fatigue is a reduction in muscle strength induced by sustained exertion. Physiological fatigue may result from failure to maintain muscle contraction at the level of the muscle or neuromuscular junction, termed peripheral fatigue[87,99,100]. Alternatively, suboptimal activation of the muscle by the nervous system is referred to as central activation failure, with its development during exercise termed central fatigue[71,87,99,100]. Somewhat paradoxically, fatigue resulting from a disorder of the peripheral nerves constitutes central fatigue using this definition.

Definitions of the subjective experience of fatigue, henceforth referred to as 'experienced fatigue', and muscle fatigue induced by prolonged exertion, henceforth referred to as 'physiological fatigue', are drawn from extensive research in the psychological and physiological sciences, respectively. These are not derived from direct research involving patients with disorders of the peripheral nerves. Therefore, the usefulness of this distinction in conceptualising fatigue in this population and ultimately exploring pathophysiology needs to be addressed.

2.1.3 Assessment of fatigue

Experienced fatigue is most commonly assessed using self-report scales. Dittner and colleagues[3] identify 30 fatigue assessment scales frequently used across a range of conditions, although over 250 are reported in the literature[101]. The first major study[55] to assess experienced fatigue in patients with immune-mediated neuropathy was published in 1999 using the Fatigue Severity Scale (FSS). This 9-item, 7-point Likert scale assesses impact and functional outcomes related to fatigue and was initially validated in patients with systemic lupus erythematous and multiple sclerosis[102]. Merkies and colleagues[55] demonstrate good internal consistency, test-retest reliability and validity in patients with immune-mediated polyneuropathy when correlated with the vitality domain of the SF-36®health survey. One hundred thirteen age- and sex-matched healthy controls also completed the survey. All subsequent studies have used the FSS in this patient group.

Given the influence of this study, several important limitations of the study need to be addressed. Patients were recruited from a central databank, but diagnostic criteria are not defined. No assessment was made of comorbid physical or mental health conditions that may also cause fatigue. The authors somewhat arbitrarily define severe fatigue as mean score above the 95th centile in normal controls. Several future studies adopted this definition in defining patients as 'severely' and 'non-severely' fatigued[56,59,68,75,79]. Arbitrarily dividing fatigued patients into binary categories may reduce ability to detect subtle trends when studying correlations of fatigue in these patients. Finally, the FSS is based on classical test theory. Assessment of sum scores assumes equal relevance and weighting of each item, when in fact the relevance of each item to a patient's level of ability is not assessed. To overcome this important limitation, van Nes and colleagues used modern Rasch technology to develop a 7-item, 4-point linearly weighted scale for use in patients with immune-mediated neuropathies (R-FSS), demonstrating good test-retest reliability and validity[76].

The first study to explore experienced fatigue in patients with hereditary polyneuropathy utilised the Checklist of Individual Strength (CIS)[83]. The CIS is a multidimensional 20-item, 7-point Likert scale, providing subscores for 'subjective experience of fatigue', 'concentration', 'motivation' and 'physical activity'[103]. This scale was developed for use in patients with chronic fatigue syndrome, and has been demonstrated to differentiate between patients with chronic fatigue syndrome, multiple sclerosis and controls[104]. Good internal consistency and split-half reliability are demonstrated, although test-retest reliability has not been formally assessed[3]. Other scales employed less frequently include the fatigue impact scale[68,70], the abbreviated fatigue questionnaire-9[71,87] and the multidimensional fatigue inventory-20[92].

Several studies have explored physiological fatigue in patients with CMT[80–82,87–90,94,97], with only one pilot study assessing physiological fatigue in patients with immune-mediated neuropathies[71]. Although choice of muscle group and testing protocol is highly variable between studies, all protocols essentially involve measurement of the deterioration in force-generating capacity of a muscle over time. Both continuous and intermittent muscle contraction have been used, with physiological fatigue expressed as the force-generating capacity of the muscle at the end of the task as a percentage of the initial maximal force. Only Videler and colleagues[82] have assessed the test-retest reliability of fatiguing studies, finding a modest intraclass correlation coefficient of 0.62. Superimposed tetanic electrical muscle stimulation has also been employed during the fatiguing contraction to determine 'central activation failure'[71,87], based on the principles of the 'twitch interpolation technique'[105]. However, this technique has been criticised by some authors as painful and insensitive to small changes in muscle fatigue[97].

The relationship between physiological fatigue and experienced fatigue is unclear. Garssen and colleagues[71] found no significant correlation between experienced fatigue, assessed using the FSS and the abbreviated fatigue questionnaire-9, and any parameter used to assess physiological fatigue. Schillings *et al*[87] report a weak negative correlation between physiological fatigue and the abbreviated fatigue questionnaire-9. Ramdharry[90] found experienced fatigue correlated with walking endurance but not force-generating capacity of hip flexor muscles. Kalkman[88] surmises that 'experienced' and 'physiological' represent separate and unrelated aspects of fatigue.

These findings suggest assessment of physiological fatigue is unhelpful in understanding patients' subjective experience of fatigue. However, important limitations need to be acknowledged. All studies exploring correlation between physiological and experienced fatigue assess maximal force generating capacity of proximal muscle groups (biceps brachii or hip flexors). From a methodological point of view this makes sense as force generation can easily be assessed in isolation. Biceps brachii can also easily be electrically stimulated as part of the twitch interpolation technique to assess for central fatigue. However, most peripheral neuropathies are 'length-dependent', with more severe involvement of the most distal nerve fibres. In addition, patients often report fatigue induced by low-intensity daily activity[8,95]. Techniques assessing development of muscle fatigue in distal muscles or during "real-life" tasks may be more helpful in understanding the relationship between physiological and experienced fatigue in this patient group.

2.1.4 Severity of fatigue and impact on quality of life measures

In acquired demyelinating polyneuropathy, severe fatigue is usually defined as a mean score of 5 or greater on the FSS, based on Merkies *et al*'s[55] influential study. In total, 8 studies report prevalence of severe experienced fatigue, with values ranging from 38%[59,74,75] up to 80%[55]. Patients also frequently rate fatigue as one of their most disabling symptoms[55,56]. Merkies *et al*[55] found significantly higher levels of fatigue in their GBS group compared to CIDP, whilst Kuitwaard and colleagues[56] found a higher rate of severe fatigue in CIDP patients.

Quantification of experienced fatigue in CMT patients is more variable. Significantly higher fatigue levels are reported in comparison to healthy controls using the FSS[90], the abbreviated fatigue-questionnaire 9[87], the multidimensional fatigue index-20[92] and the CIS[83,93]. Using CIS scores, Kalkman[83] and Jagersma[93] report severe fatigue in 64% of adult patients and 24% of paediatric patients with CMT, respectively. Using a different scale, Boentert and colleagues[92] report prevalence of severe fatigue of 43% in adult patients.

The use of different fatigue scales, which explore different aspects of the subjective experience of fatigue[3], no doubt underlies some of the variation in the CMT population. However, variation is also seen between studies using the same scales. These differences may reflect variation based on sampling of patients, although other important differences in data collection need to be considered. Fatigue is not specific to peripheral neuropathies and may result from other physical or psychological disorders. When assessing CMT patients with co-morbid health conditions, Boentert and colleagues found severe experienced fatigue in 74%, compared to 43% of the entire cohort[92]. Only 3 of the 13 studies in this review that report prevalence of severe fatigue screened for and excluded co-morbid conditions which may contribute to fatigue. One further study assessed for medication which may cause fatigue only. Of the studies with no clearly defined exclusion criteria, 6 conducted a postal or telephone survey, making it difficult to perform detailed clinical assessment. This methodology also creates a potential sampling bias, with more severely affected patients more motivated to respond. High non-responder rates of between 27 and 42% are reported in these studies[56,73,74,83,93]. There is also variation in how self-reported scales were completed, with some studies sending surveys to patients to be completed[56], some providing written instructions[55] and others requiring examiners to read surveys to patients[75]. The later methodology has the advantage of allowing patients to clarify misunderstandings. All of these factors may contribute to the variation in reporting of severe experienced fatigue.

Severity of fatigue can be assessed indirectly by exploring impact of fatigue on quality of life. A single study[70] has assessed impact of fatigue in this patient group using the Fatigue Impact Scale, demonstrating that results closely correlated with FSS scores. Five studies have shown a significant inverse correlation between FSS scores and subsections of the SF-36®health survey[55,72,83,90,92]. The SF-36®health survey assesses self-reported quality of life and health perceptions and includes the subscales physical functioning, physical role function, emotional role function, bodily pain, general health, vitality, social functioning and mental health[106]. These scores can be converted to physical and mental component subscores. Studies in patients with both acquired and hereditary demyelinating polyneuropathies consistently find an inverse correlation between fatigue levels and either the physical functioning and physical role function domains of the SF-36®health survey or the physical domain subscale[55,72,83,90,92]. Relationship between fatigue levels and other domains, including the mental domain subscale, is inconsistent.

Given the observed inverse correlations between fatigue severity and self-reported quality of life, it is perhaps surprising that the only study to evaluate links between impairment, disability and handicap, as defined by the World Health Organisation, found fatigue was the only impairment measure assessed with no significant contribution to level of disability or handicap in univariate or multivariate regression modelling[67]. The tools used to assess disability and handicap in this study focus predominantly on functional abilities, including arm or leg strength and ability to perform certain tasks, respectively. It is possible these parameters may be more affected by impairments in strength or sensation, whereas fatigue is more important in patient's perception of their abilities.

2.1.5 Pathophysiology of fatigue

Hereditary and acquired demyelinating disorders of the peripheral nervous system share pathophysiological similarities although are fundamentally different disorders. It should not be assumed that the mechanisms underlying fatigue in these disorders are identical. The literature for both disorders will be considered with similarities and differences analysed.

An early study exploring fatigue in neuromuscular disorders suggested this resulted from co-existent psychiatric illness rather than a direct effect of neuromuscular dysfunction[66]. This included a heterogenous group of neuromuscular disorders and only 3 patients with GBS. The authors also devised their own fatigue assessment and symptom attribution scales, failing to demonstrate validity or reliability of either. Subsequent work involving patients with acquired and hereditary demyelinating polyneuropathies have largely disproved this theory. Although psychiatric illness such as depression and phobias exist in CMT, the prevalence of these conditions does not differ between

severely fatigued and non-fatigued patients[85]. Experienced fatigue in patients with immune-mediated neuropathies shows no correlation with measures of emotional affectivity[74]. In addition, reduced levels of motivation in a mixed group of patients with neuromuscular disorders appears independent of depression[85]. Studies exploring the relationship between fatigue and sleep disturbance in patients with CMT find no consistent relationship[86,92]. Severe fatigue also does not appear related to employment status in this population[91]. Level of physical activity is associated with experienced fatigue in this patient group[86,98], although the relationship between these variables is unclear. It is possible fatigue is a sequelae of deconditioning resulting from lack of activity. Alternatively, high experienced fatigue levels may make patients feel less able to maintain high activity levels. These findings suggest experienced fatigue cannot be fully explained by factors related to chronic illness, such as co-morbid psychiatric illness, sleep disturbance or socio-economic factors.

Long-term electrophysiological studies find weakness and persistent neurological disability are associated with more severe axonal loss in patients with GBS[107,108]. However, numerous case reports illustrate severe experienced fatigue may exist in the absence of significant motor or sensory deficits[21,57,64]. This observation is supported by larger cross-sectional studies, which demonstrate no correlation between experienced fatigue and clinical assessments of sensory or motor function[54,55,59]. In addition, no significant correlation has been found between experienced fatigue and standard electrophysiological parameters reflecting degree of axonal loss, including amplitude and area of sensory and compound motor nerve action potentials[58,59]. This has led to the hypothesis that fatigue may be related to persistent nerve demyelination or suboptimal remyelination[69]. Links between persistent fatigue in well-recovered patients with CIDP and activity-dependent conduction block have been suggested[65]. However, no correlation between experienced fatigue and sensory or motor nerve conduction velocities has been found[58,59], including detailed studies assessing conduction velocity of smaller myelinated motor units[69].

Several authors have investigated physiological fatigue in CMT[80,82,87,90,94,96]. Although methodological differences make direct comparisons difficult, the majority of studies demonstrate reduced maximal force generating capacity of the muscle group being tested in patients compared to healthy controls[80,82,87,90,94]. However, no consistent difference between endurance of muscle contraction and deterioration in force generating capacity has been demonstrated. This includes protocols using both intermittent[82] and continuous[87] fatiguing muscle contractions. The only pilot study exploring this area in immune-mediated neuropathies found no difference between maximal force generating capacity or development of physiological fatigue in biceps brachii between 10 patients with well-

recovered GBS and 12 healthy controls[71]. This appears likely to reflect pathophysiological differences between patients with a subacute, acquired, immune-mediated neuropathy and a chronic, hereditary neuropathy.

Similar protocols have been used to explore underlying causes of physiological fatigue in patients with CMT and GBS[71,87]. Despite differences between these conditions, both studies found higher central activation failure and lower peripheral fatigue in patients compared to healthy controls. As mentioned earlier, central activation failure refers to failure of neural drive to the muscle, whereas peripheral fatigue is used to refer to fatigue developing at the level of the muscle or neuromuscular junction itself. It is hypothesised that increased central activation failure results from fewer surviving peripheral motor neurons, resulting in a proportionately higher dropout rate during the fatiguing task. Lower peripheral fatigue is observed because of submaximal muscle activation. However, if physiological fatigue results from lower number of surviving motor axons, it would be expected that maximal muscle contraction force would be lower in patients compared to controls and the decline in force of muscle contraction would be more rapid. Neither of these observations is found[71,87].

Several studies utilise surface-EMG (SEMG) to assess neural drive to muscles during fatiguing contractions in patients with CMT and acquired demyelinating polyneuropathy[71,80,81,87,94]. SEMG signals contain summated muscle-fibre action potentials. Area and power of the rectified SEMG signal can be analysed using the mean absolute value or root mean square, respectively[80,94], providing an indirect assessment of the number of motor units activating over time[109]. Median frequency of the power spectrum of the SEMG signal has been used as a measure of firing frequency[94,109]. Differences in these parameters are reported between patients and controls, although no relationship between any of the SEMG parameters and experienced or physiological fatigue has been demonstrated, although inter-subject variability is high[71,80,87,94]. It could be argued that this finding further disproves the hypothesis that physiological fatigue is directly related to number of surviving peripheral motor neurons. However, there are several limitations with this methodology. SEMG activity does not exclusively reflect neural activity and will be affected by factors such as production of lactic acid during exercise[110,111]. This limitation may be overcome by recording muscle-fibre conduction velocity, which slows as intramuscular pH falls, using specialised multi-electrode arrays[71,87,94]. However, many other factors complicate recording of SEMG activity, including tissue inhomogeneities and effects on signal volume conduction, electrode contact, size and interelectrode distance and phase cancellation of action potentials at higher firing frequencies[109,112]. These factors make the relationship between SEMG signals, force and neural activation complex.

Menotti and colleagues[96] explore central factors influencing physiological fatigue using movement-related cortical potentials. They identified higher activity in the prefrontal cortex and lower activity in the primary motor area in CMT patients than controls. Movement-related cortical evoked potential amplitude in the motor area is related to the level of muscle force and number of motor units[113,114]. The lower amplitude responses seen in CMT patients are attributed to lower number of peripheral motor units, and the authors hypothesis that higher activity in the prefrontal cortex is a compensatory mechanism. Given the role of the prefrontal cortex as a cognitive association area, the authors imply that this reflects increased cognitive effort involved in motor tasks. Compensatory mechanisms involving the prefrontal cortex may allow patients to maintain activity level whilst leading to an earlier perception of fatigue during routine motor tasks. This may account for the ability of patients with CMT to maintain muscle contraction despite fewer peripheral motor neurons, and the lack of correlation between experienced and physiological fatigue. Several other groups have advanced the hypothesis that experienced fatigue may result from the need to exert greater effort to perform normal daily activities[71,72,95].

Key to this hypothesis is a relationship between experienced fatigue and a lower number of functioning motor units. However, as previously described, no relationship between experienced fatigue and standard electrophysiological parameters of motor unit function has been demonstrated[58,69]. Following acute axonal loss in GBS or during ongoing axonal loss in CIDP or CMT, denervated muscle fibres may be incorporated into surviving motor units through sprouting of collateral motor axons. This results in a smaller number of larger motor units, disrupting the normal orderly recruitment of motor units, whilst maintaining the amplitude and area of CMAPs measured during standard electrophysiological studies. Drenthen and colleagues[59] explored the relationship between Motor Unit Number Estimation (MUNE) and experienced fatigue in a cohort of 39 patients with GBS, discovering a correlation between MUNE and experienced fatigue level and that severely fatigued patients have larger motor unit size. MUNE is a detailed technique for assessing number of functioning motor units, although has several potential limitations. It is technically challenging to perform and requires repeated electrical stimulation, which may be poorly tolerated. Perhaps more importantly, the technique assumes incremental increase in compound motor action potentials seen with gradual increases in strength of electrical stimulation is due to recruitment of single motor units, which may lead to underestimation of the number of functioning motor units. Therefore, whilst this finding offers an insight into the pathophysiology of experienced fatigue in patients with immune-mediated neuropathy, this is a single study with potential technical limitations and no similar exploration has been made in patients with chronic polyneuropathies.

2.1.6 Treatment of fatigue

There is very limited information available regarding the effect of treatment on fatigue in patients with acquired or hereditary demyelinating polyneuropathy.

A single case series reports improvement in FSS scores following treatment of 11 CIDP patients[57]. Treatments were heterogenous, however, with patients receiving intravenous immunoglobulin, corticosteroids, cyclophosphamide, or a combination of all three. This study is severely limited by the small sample size and the lack of a control group, meaning it cannot be discounted that observed improvements are due to placebo effect or natural variation in fatigue levels over time. Similarly, anecdotal evidence of improvement in fatigue using modafinil in CMT should be interpreted cautiously[84].

Inspired by the benefits of Amantadine in patients with multiple sclerosis, treatment effect of Amantadine in 80 patients with recovered GBS has been assessed[70]. This double-blind, randomised-controlled trial is the only study of its kind in this patient group using changes in fatigue level as a primary endpoint. The authors used established diagnostic criteria for GBS as part of the inclusion criteria and carefully assessed for any comorbid conditions that may cause fatigue as part of the exclusion criteria. No significant impact from Amantadine was found, although there was slight improvement in FSS and FIS scores across both groups, which the authors attributed to the effect of increased medical attention. This finding demonstrates a potential placebo effect on fatigue level, which would need to be carefully controlled for in future studies assessing effects of treatment on fatigue.

It has also been suggested that exercise can improve experienced fatigue in patients with immune-mediated demyelinating polyneuropathy[68] and CMT[89,95]. Garssen and colleagues[68] explored benefits of a graded-exercise programme in a study recruiting 16 patients with GBS and 4 patients with CIDP, all with “severe fatigue” as assessed using the FSS. Parameters including fatigue levels (assessed using the FSS and FIS), depression scores, SF-36®health survey, disability and handicap scores were measured during a 12-week supervised exercise programme. Significant improvements were seen across all assessments. El Mhandi and colleagues[89] report improvement in fatigue assessed on a visual analogue scale following a 24-week interval training exercise programme in CMT patients. Whilst highlighting a potentially promising area for further exploration, the lack of a control group in both studies means that improvements resulting from increased supervision, as seen in the Amantadine study, cannot be excluded. In Garssen’s study, the significance of improvement in co-morbid depression on fatigue is also unclear.

2.2 Discussion

Fatigue appears to be a frequent and significant problem experienced in both hereditary and acquired demyelinating disorders of the peripheral nervous system. Patients with higher fatigue levels appear to have lower perceptions of their physical abilities, possibly reflecting a greater awareness of the difficulties of motor tasks and physical activity. Limitations of the available literature exist, including use of heterogenous assessment methods, somewhat arbitrary classification of patients' as "severely" and "non-severely" fatigued and lack of control for comorbid conditions causing fatigue. Despite this, evidence from multiple sources across different patient populations consistently show higher self-reported fatigue levels compared to healthy control groups. In addition, fatigue appears distinct from tiredness, muscle weakness or co-morbid psychiatric conditions, suggesting it represents a distinct entity in this patient population, which is inversely correlated with quality of life.

The term "fatigue" may be used to refer to patients' subjective experience or to deterioration in muscle strength during exercise. Defining the aspect of fatigue being addressed is important, yet surprisingly few of the studies in this review offer a formal definition of fatigue. Experienced fatigue is typically assessed using psychometric, self-report scales. Selection of an appropriate scale may be difficult as numerous methods exist and many lack validity in this patient group or have limitations if based on classical test theory. The development of a new Rasch-built assessment tool developed specifically for assessment of fatigue in this patient group is an important development. The optimal method to assess physiological fatigue is even less clear. To date, no link between patient's subjective experience of fatigue and physiological fatigue as assessed by reduction in force-generating capacity of muscle over time has been established. Whether study of physiological fatigue will lead to discovery of pathophysiological mechanisms helping to understand experienced fatigue is far from clear.

The pathophysiological mechanisms underlying fatigue are unclear. There may be a psychological component to patient's experience of fatigue. A single medication trial demonstrates a placebo effect, indicating fatigue may improve in response to greater medical attention. Whilst comorbid depression may be another important factor, the available literature suggests fatigue experienced by this patient group cannot be completely attributed to comorbid psychiatric illness. Standard electrophysiological measures have failed to show a direct relationship between fatigue and loss of sensory or motor nerve axons or severity of nerve demyelination. This observation appears intuitive when patients may complain of little or no impairment of sensory or motor function but overwhelming fatigue. Interesting insights into central components of fatigue are provided by

detailed studies of physiological fatigue and movement-related cortical evoked potentials. These findings suggest fatigue may develop as a result of increased cognitive effort involved in planning and execution of everyday tasks. Based on the findings of a single study, it has been hypothesised that this occurs as a compensatory strategy for loss of peripheral motor units or disordered peripheral motor unit function. Development of more detailed neurophysiological techniques to explore motor unit function may help to understand mechanisms underlying fatigue in these patients. Initial results from a single study in patients with GBS are encouraging.

As may be expected given the limited understanding of causes of fatigue in patients with peripheral neuropathy, studies of potential treatments have focused on broad rather than targeted therapies, such as physical therapy, or speculative treatments which may offer benefit in unrelated conditions. None of the large, multi-centre studies exploring the impact of immunomodulatory treatments for GBS or CIDP have included fatigue assessments as outcome measures, instead focusing on assessments of motor or sensory function or disability scores. This may be due to a lack of understanding of the importance of fatigue for these patients, lack of robust methods for assessing fatigue, lack of biomarkers or a combination of all of these factors. Understanding the pathophysiology of fatigue becomes more important to allow targeted therapies to be developed.

2.3 Conclusion

Fatigue is a frequent problem encountered by patients with demyelinating disorders of the peripheral nervous system, whether acquired or hereditary. High experienced fatigue negatively impacts quality of life measures. Perhaps due to a combination of factors, including lack of robust assessment tools or poor understanding of underlying pathophysiological mechanisms, therapeutic trials in these conditions rarely use fatigue as an outcome measure. This review identifies several gaps and limitations in the existing literature, which offer potential avenues for investigation to improve understanding of fatigue in patients with these conditions.

3 Research aims

The primary research aim of this study is

i) To explore the relationship between experienced fatigue, physiological fatigue and peripheral nerve function in patients with acquired and hereditary demyelinating peripheral neuropathy.

Secondary research aims of this study are:

i) To investigate short-term changes in clinical and electrophysiological assessments after treatment in patients with chronic demyelinating polyneuropathy

ii) To assess short-term changes in fatigue levels and how these correlate with clinical and electrophysiological assessments after treatment in patients with chronic demyelinating polyneuropathy

This study will utilise the newly-developed Rasch-built fatigue severity scale to assess experienced fatigue. The use of a linearly-weighted scale will allow assessments of subtle trends rather than artificially dividing patients into “severely” and “non-severely” fatigued dichotomous categories. Physiological fatigue will be explored using hand-held grip dynamometry. In addition to standard electrophysiological assessments, more detailed exploration of motor unit function will be undertaken using a recently developed technique; Motor Unit Number Index (MUNIX).

The use of linearly-weighted experienced fatigue scale will also allow exploration of correlation between fatigue and comorbid depression, disability level and health-related quality of life scores, assessed using previously validated scales.

Finally, assessment can be performed before and after treatment in patients receiving regular intravenous immunoglobulin infusions as part of their standard NHS care. If there is a true correlation between these variables, it would be expected that similar changes with treatment would be observed.

It is hoped to gain insights into the pathophysiology of fatigue in demyelinating disorders of the peripheral nervous system, which may lead to identification of biomarkers that could be useful to monitor treatment response and may be useful in future therapeutic trials.

4 Methodology

4.1 Participants

All patients with CIDP and CMT1A attending specialist Neuromuscular Clinics at the Queen Elizabeth Hospital, Birmingham, were invited to participate, irrespective of treatment status. The rationale for inviting this cohort was regular attendance at follow-up appointments, aiding study recruitment. Patients were approached at routine clinic visits and provided with study information leaflets. They were subsequently contacted by telephone to arrange a study appointment.

Inclusion criteria were age 18 years and older, with CIDP meeting diagnosis of "definite" or "probable" CIDP as per EFNS/PNS Guidelines[31] and diagnosis of CMT1A confirmed by genetic studies. Exclusion criteria included co-morbid conditions contributing to fatigue; known malignancy, psychiatric diagnosis preceding onset of the neuropathy, anaemia, hypothyroidism, obstructive sleep apnoea, cardiac or pulmonary disorders. Patients with permanent pacemakers or implantable cardioverter defibrillators (ICDs) were also excluded due to theoretical risks associated with electrical stimulation during electrophysiology studies.

All patients with CIDP and CMT attended an initial appointment where clinical assessments, electrophysiological and MUNIX studies were performed. Patients with CIDP also attended a repeat appointment. For CIDP patients receiving regular IVIg therapy the initial appointment was scheduled immediately prior to a planned infusion, with a repeat assessment performed at the midpoint between infusions. For example, if a patient was receiving infusions with a 4 week interval, the repeat assessment was performed 2 weeks after the infusion. Identical clinical assessments, electrophysiological and MUNIX studies were performed at both appointments.

Healthy control subjects were recruited from hospital colleagues as part of preliminary component of this study, undergoing MUNIX and physiological fatigue studies.

4.2 Assessment methods

4.2.1 Self-report scales

Experienced fatigue was assessed using the linearly-weighted modified Rasch-built fatigue severity scale (R-FSS)[76]. In addition, the checklist of individual strength (CIS), was used to provide a multidimensional assessment of patients' experience of fatigue[83,103,104].

Disability was assessed using the linearly-weighted Rasch-built overall disability sum score (R-ODS), which was developed for use in patients with immune-mediated polyneuropathies and has good

test-retest reliability and validity[115]. A second disability scale, the widely used overall neuropathy limitations scale (ONLS) was also used[116].

The Hospital Depression and Anxiety scale (HADS)[117] was used to assess for co-morbid depression and anxiety, which may contribute to fatigue. This 14-item questionnaire has separate subscales for depression and anxiety, with a score of 0 to 7 out of a maximum of 21 for each subscale considered normal. Finally, the medical outcomes study 36-item short form health survey (SF-36) adapted into English (United Kingdom) was used to assess self-reported, health-related quality of life[106]. The survey contains subscales for 8 domains; physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role-emotional and mental health. These domains can be combined to form a physical component summary and mental component summary. Norm-based scores in each subscale range from 0-100 and allow comparison with data drawn from a general UK population. Higher scores indicate better functioning, with an average score of 50 for each subscale.

4.2.2 Clinical assessments

Muscle strength was assessed using Medical Research Council (MRC) grading[118] and maximal grip strength using a hand-held Jamar grip dynamometer[119]. MRC grading (range 0-5) of shoulder abduction, elbow flexion, wrist extension, finger abduction, hip flexion, knee flexion, ankle dorsiflexion and hallucis dorsiflexion was assessed bilaterally, giving a maximal score possible of 80. Lower scores correspond to greater degree of abnormality. 10-metre timed walking test was used to assess "focal disability"[120].

Sensory function was assessed using the modified INCAT sensory sum score, incorporating revised assessment of two-point discrimination assessment[121,122]. This sensory sum score assesses fine touch, pin-prick, vibration and proprioception (range 0-4) in upper and lower limbs and two-point discrimination (range 0-1) in upper limbs only, giving a maximum score of 33, with higher scores corresponding to greater degree of abnormality. Sensory function was also assessed using a Rydell-Seiffer tuning fork[123], calculating a sum score for vibration thresholds at the interphalangeal joint of hallucis, the medial malleolus, the patella, the distal interphalangeal joint of the index finger, the ulnar styloid and the medial epicondyle of the humerus. A maximal score of 48 is possible with lower scores corresponding to greater degree of abnormality.

4.2.3 Electrophysiological assessment

Nerve conduction studies were performed according to standard protocols[124] using a Dantec™ Keypoint® Focus machine. All studies were performed unilaterally on the dominant side. Skin surface

temperature was checked and raised to above 32°C in the hands and 30°C in the feet prior to testing. Antidromic sensory NCS of sural and superficial radial nerves and orthodromic sensory NCS of median and ulnar nerves were performed measuring amplitude and sensory conduction velocity. Median nerve motor NCS were performed recording from abductor pollicis brevis (APB) and stimulating at the wrist, elbow and axilla. Ulnar nerve motor NCS were performed recording from the abductor digiti minimi (ADM) and stimulating at the wrist, below the elbow, above the elbow and axilla. Tibial nerve motor NCS were performed recording from the abductor hallucis (AH) and stimulating posterior to the medial malleolus and popliteal fossa. Peroneal nerve motor NCS were performed recording from the extensor digitorum brevis (EDB) and stimulating at the ankle, below and above the fibular head. For each motor NCS, distal motor latency (DML), onset-to-peak amplitude, negative peak area, negative peak duration, minimum F-wave latency and F-wave persistence were evaluated. Average values for each parameter were calculated by summation then division by the number of nerves from which values could be recorded. Motor conduction block is classified according to published guidelines[125].

MUNIX and MUSIX sum scores were recorded from 3 muscles, again on the dominant side; APB, ADM and tibialis anterior (TA). An active electrode was placed over the muscle belly and a reference electrode was placed over the proximal thumb interphalangeal joint (for ABP), the fifth metacarpal-phalangeal joint (for ADM) and the distal tibia (for TA). Published descriptions of the technique were followed[60,61]. Firstly, a supramaximal CMAP was recorded from the muscle being studied (see Figure 1A). Amplitude, area and power were calculated from the negative peak of the CMAP[126].

Secondly, surface interference patterns (SIP) were recorded using identical electrode positioning, asking the patient to perform 10 isometric muscle contractions ranging from 10-100% of maximal force whilst the examiner applied resistance (see Figure 1B). SIP was recorded in 300ms epochs for each contraction, using filter settings of 3-3000Hz[127,128]. SIP area and power were calculated from each epoch.

Finally, area and power of the CMAP and all SIP epochs were transferred to a mathematical formula developed by Nandedkar and colleagues[60,61]. Ideal case motor unit count (ICMUC) was calculated from the following formula:

$$ICMUC = (Cp \times Sr)/(Cr \times Sp)$$

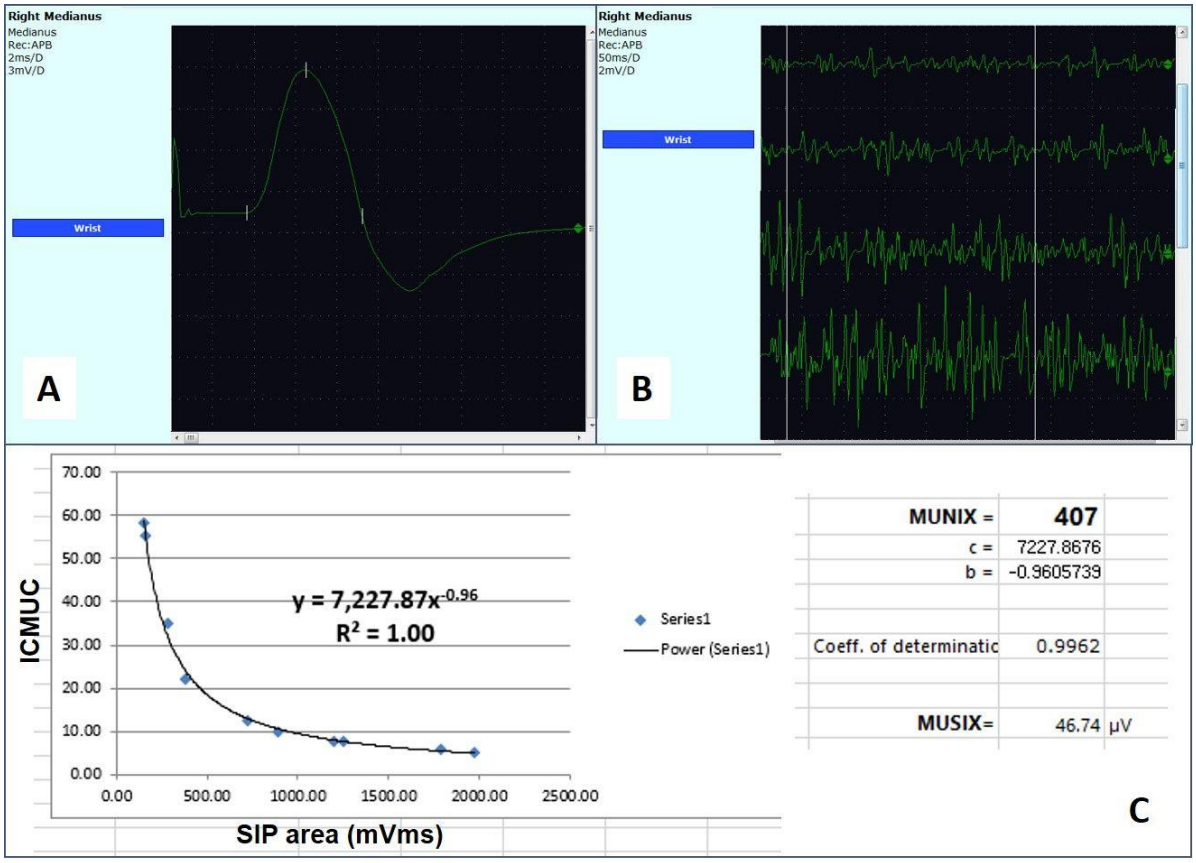


Figure 1: A) Compound muscle action potential recorded from abductor pollicis brevis stimulating the median nerve at the wrist. B) Surface interference patterns recorded from abductor pollicis brevis at increasing force levels. C) Plot of ideal case motor unit count against surface interference pattern area, demonstrating calculation of MUNIX and MUSIX values. Data acquired using a Dantec™ Keypoint® Focus system.

Cp and *Cr* refer to the power and area of the CMAP, respectively, and *Sp* and *Sr* refer to the power and area of the SIP. The parameter calculated is referred to as "ideal case" as the mathematical model makes the assumptions that all individual motor unit potentials have identical area and do not overlap during muscle contraction. For each SIP epoch, ICMUC is plotted on a graph against SIP area (see Figure 1C). SIP area is used as an indirect correlate of force. Henneman's size principle states that at low levels of isometric muscle contraction, smaller motor units are recruited, with larger motor units recruited as the force of muscle contraction increases[129]. Therefore, area of the SIP increases as force of muscle contraction increases.

Regression modelling was performed using the equation:

$$ICMUC = A\,(Sr)^{\alpha}$$

MUNIX was arbitrarily defined as the ICMUC when *Sr*=20, corresponding to a very low level of force:

$$MUNIX = A\,(20)^{\alpha}$$

Finally, MUSIX was calculated by:

MUSIX = CMAP amplitude (µV) /MUNIX

4.2.4 Physiological fatigue

Assessment of physiological fatigue was performed using protocols adapted in provisional trials in healthy controls. Force of grip strength during a maximal muscle contraction is assessed using a Vernier® grip dynamometer sampling at 10Hz. Data is collected in LoggerLite® software before being transferred to Microsoft Excel for further analysis. Patients are sat comfortably in a chair with the elbow flexed at 90 degrees and the forearm resting on a pillow. Three brief maximal contractions are performed initially to assess consistency of response. Patients are then instructed to perform maximal grip and attempt to maintain this for 60 seconds, stopping in the event of any discomfort. Constant visual feedback is provided regarding grip strength. Average force is calculated for each 1 second epoch, with physiological fatigue defined as the force of grip strength at the end of the assessment as a percentage of the initial value (See Figure 2).

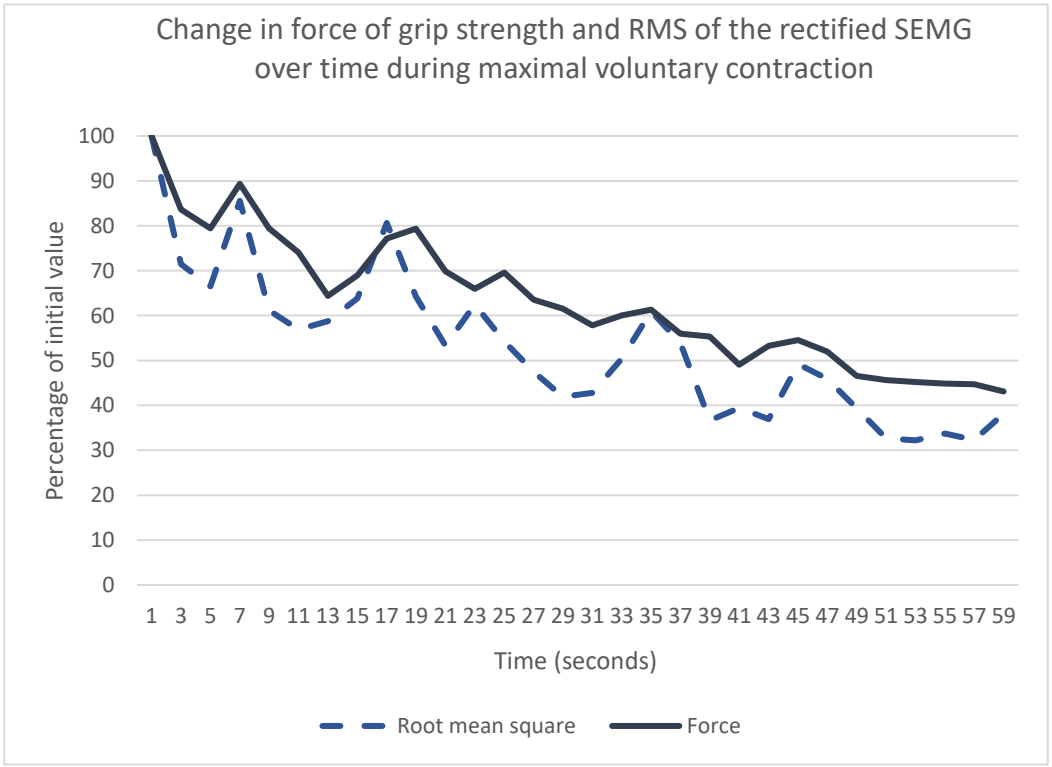


Figure 2: Graph demonstrating decline in force of grip strength (solid blue line) and root mean square (RMS) of the rectified surface EMG signal (dashed line) over time during a fatiguing contraction (both values are expressed as % of the initial value). Recordings taken from a 32-year-old male with no history of neurological disease. In this example physiological fatigue is calculated as 54.0% with a corresponding decline in RMS of 61.9%

SEMG was recorded simultaneously using an active recording electrode placed over forearm flexor muscles (flexor digitorum superficialis) and a reference electrode over the lateral aspect of the wrist. One second epochs of SEMG activity are recorded every 2 seconds using Dantec™ Keypoint® software, calculating average root mean square of the rectified SEMG signal for each epoch (See Figure 2).

Finally, after a rest period of at least 10 minutes, patients were asked to maintain grip strength corresponding to roughly 30% of maximal grip strength. The rationale for this procedure was to assess SEMG activity during maximal and submaximal contractions. It was hypothesised that patients with fewer functioning motor units would require a greater proportion of the available pool to maintain a submaximal contraction, and as a result root mean square of the SEMG signal would be similar during maximal and submaximal contractions in patients with lower MUNIX values. RMS of the SEMG signal during submaximal contraction is expressed as a percentage of RMS at the beginning of maximal contraction.

4.3 Statistical analysis

4.3.1 Sample size calculation

In contrast to previous studies exploring fatigue in this patient population, this study utilises a linearly-weighted scale for assessment of fatigue. Sample size calculation therefore relies on ability to detect a significant correlation between two variables under investigation. In our primary outcome measure, this is the correlation between experienced fatigue and MUNIX values. No previous studies have investigated the correlation between these variables, making *a priori* determination of a correlation coefficient difficult. However, Delmont and colleagues[130] have demonstrated strong correlation between MUNIX values and two disability scales in patients with CIDP (Spearman's Rank correlation coefficients of 0.70 and 0.71).

Under the assumption we expect to see similar correlation coefficients, we can use the technique described by Bland[131] to determine the required sample size. Using an α value of 0.05 and a power (β) of 90%, a minimum sample size of 17 would be required to determine whether the correlation between these two variables differs from zero.

4.3.2 Descriptive statistics

Distribution of all variables was assessed using the one-sample Kolmogorov-Smirnov test. Parametric data is presented as mean values and standard deviation. Nonparametric data is presented as median values and interquartile ranges.

4.3.3 Intraclass correlation coefficient

Intraclass correlation coefficient (ICC) was used to assess test-retest reliability of MUNIX in healthy controls using a 2-way, mixed effects model looking for absolute agreement. ICC values nearer 1.0 indicate greater similarity between results, with ICC greater than 0.9 indicating excellent reliability[132]. ICC values of 0.75 to 0.9 were taken to indicate good reliability and values of 0.5 to 0.75 were taken to indicate moderate reliability[133].

4.3.4 Association analysis

For parametric variables, differences between the three groups were assessed one-way ANOVA with post-hoc Bonferroni correction for multiple comparisons. Differences between CIDP patients receiving treatment and not receiving treatment were assessed using independent, two-tailed student t-test. For non-parametric variables, differences between the three groups were assessed using the independent-samples Kruskal-Wallis test with post-hoc Bonferroni correction for multiple comparisons. Changes in variables across repeat appointments were assessed using paired, two-tailed student t-test for parametric variables and Wilcoxon signed ranks test for nonparametric variables.

4.3.5 Correlation and regression analysis

Association between individual numerical variables was assessed using correlation analysis. Pearson correlation coefficient was used to assess statistical association between variables with normal distribution. Correlation analysis in nonparametric data was performed using Spearman's Rank correlation. For both analyses, an alpha value of <0.05 is deemed significant.

Multivariate stepwise linear regression analysis was used in the CIDP patient group, with probability of F to enter of <0.05 and to remove of <0.1. Only variables showing significant correlation in the univariate analysis were included in the regression model. Dependent variables are identified in the relevant sections.

All statistical analysis was performed using IBM SPSS® software version 25 (SPSS Inc., Chicago, IL).

5 Ethical considerations

Control data were collected from healthy volunteers (hospital staff) as part of a provisional component of this study. Ethical approval was granted from the Queen Elizabeth Hospital research department.

Ethical approval for the main component of the study involving recruitment and assessment of patients from an NHS hospital has been granted from the NHS Health Research Authority (IRAS project ID: 206150, REC reference 17/LO/0798, Protocol number 162-2016-YR). Local ethical approvals are in place from the University Hospitals Birmingham Research and Development Governance Office (project reference RRK 5804) and Aston University, who are acting as the sponsor for this study (AHRIC ref no: 162-2016-YR).

As part of the ethical application, patient information sheets, consent forms and GP letters were drafted. These are included in Appendices 2, 3 and 4, respectively.

A minor amendment to the study protocol was made in June 2018 to prolong the period of recruitment and allow patients identified on screening of clinic lists to be contacted directly by the clinical team, with the aim of improving recruitment to the study.

The major ethical considerations of this study are:

- Safety of research participants

The techniques being employed in this research study are very low risk. Nerve conduction studies utilise small electrical pulses in the range of 1-100mA to stimulate peripheral nerves and are widely used in clinical practice without complication[134]. Prior to undertaking the study, a thorough risk assessment was undertaken. There is a theoretical risk of leakage currents from electrical equipment used to perform nerve conduction studies. In order to minimise this risk all equipment underwent PAT testing prior to study commencement and ground electrodes are used throughout. Patients are disconnected from stimulators when equipment is turned on or off as theoretically the risk of current leakage is greater at these times. Patients with permanent cardiac pacemakers and implantable defibrillators may be at increased risk during electrical nerve stimulation as the pacemaker/defibrillator leads provide a lower resistance pathway for electrical current to reach the heart. Patients with these devices were excluded from the study.

- Informed consent

All potential participants are approached through the existing clinical team and provided with written information regarding the study. They are given a period of time to consider the information before being contacted again to decide whether they wish to participate. It is re-emphasised at this point that the study is optional and should they choose to decline or withdraw at a later date it will not affect their existing clinical care. With the exception of transient fatigue, no potential risks are envisaged. On their first study visit, participants are given the opportunity to ask questions and are taken through the consent form. Informed consent is taken by one of the study investigators (AL), who has training in taking informed consent as part of medical training and Good Clinical Practice qualification.

- Confidentiality

Potential participants are identified through screening of clinic lists at the Queen Elizabeth Hospital, Birmingham. A database of potentials participants is stored electronically in a password protected folder on an NHS computer system, which only the study investigators have access to. Each patient is given a unique identification code, used on all research data subsequently collected at Aston University. All research data is stored securely in a locked filing cabinet at Aston Brain Centre.

- Plan for action in adverse events

As mentioned previously, this study employs low-risk methods for assessment. However, unforeseen events such as trips, falls and intercurrent illness need to be considered. Standard operating procedures are in place at Aston University for these potential adverse events. Participants are seen only on days when at least two staff members with intermediate life support training are available. Standard operating procedures for notification of medical staff are in place should a participant become unwell during their study visit.

6 Results

6.1 Demographic data

Twenty healthy control subjects were recruited from hospital staff. Ten were female and age range was 29–80y; mean age 44.4y (13.9).

Forty-three patients with CIDP were identified. Of these 9 met exclusion criteria and a further 8 declined invitation to participate. Twenty-six patients with CIDP were included (5 female; age range 49–79y; mean age 62.5y (9.5)). Average time between diagnosis of CIDP and enrolment was 61 months. All patients were clinically stable; 15 undergoing regular IVIg therapy at 3 to 6 weekly intervals; 1 receiving subcutaneous immunoglobulins and 9 receiving physiotherapy input only.

Thirty-five patients with a genetically-confirmed diagnosis of CMT1A were identified. Of these 6 met exclusion criteria and 7 patients could not be contacted. One patient was unable to provide informed consent and 6 patients declined invitation to participate. Fifteen patients with CMT1A were included (11 female; age range 21-70y; mean age 47y (15.3)). Further clinical details of recruited patients are provided in Tables 2 and 3.

CIDP patients attended repeat appointments. All 15 patients receiving IVIg therapy were seen 2 to 3 days prior to a planned infusion and had a repeat appointment on average 15 days after the infusion. In addition, 5 patients with CIDP not receiving regular IVIg therapy also attended a repeat appointment on average 43 days later. A flowchart detailing patient recruitment is shown in Figure 3.

Patient ID	Age (years)	Sex	Months since diagnosis	Current treatment	Attended repeat appointment?	Co-morbid medical conditions	Current medication
CIDPF001	79	F	36	IVIg	Yes	Hypertension	Indapamide
CIDPM002	49	M	21	IVIg	Yes	Depression	Folic acid, Lansoprazole, Sertraline
CIDPM003	68	M	24	IVIg	Yes	Mild thrombocytopenia	Nil
CIDPF005	74	F	7	IVIg	Yes		Nil
CIDPM006	61	M	19	IVIg	Yes	Hypertension, Chronic leg ulcer	Lansoprazole, Gabapentin, Atenolol, Dipyridamole
CIDPM007	75	M	36	IVIg	Yes	IgG paraproteinaemia	Propranolol, Alendronate, Gabapentin
CIDPM008	53	M	15	IVIg	Yes	Hypertension, low level IgG paraproteinaemia	Bendroflumethiazide, Doxazosin, Lercanidipine, Propranolol
CIDPM009	74	M	14	IVIg	Yes	Hypertension, GORD, prostatitis	Lansoprazole, Indapamide
CIDPM010	70	M	43	IVIg	Yes	Hypertension, Depression	Citalopram, Alendronate, Adcal D3, Ramipril
CIDPM011	61	M	192	Physio		Hypertension	Nil
CIDPM012	58	M	12	Physio	Yes	Asthma	Inhalers, Pregabalin
CIDPF013	52	F	99	IVIg	Yes		Lansoprazole, Adcal D3, Alendronate
CIDPM014	60	M	19	IVIg	Yes	Type II Diabetes Mellitus, Hypertension, CKD stage III	Linagliptin, Amlodipine, Doxazosin, Bisoprolol, Furosemide, Vitamin D
CIDPF015	58	F	19	IVIg	Yes	Raynaud's syndrome, hypercholesterolemia	Aspirin, Alendronate, Atorvastatin, Omeprazole
CIDPM016	70	M	264	IVIg	Yes	IgM paraproteinaemia	Alendronate, Adcal D3
CIDPM017	52	M	17	Physio			Nil
CIDPM018	62	M	36	IVIg	Yes		Lansoprazole, Co-codamol
CIDPM019	68	M	15	Physio		Hypertension, Hypercholesterolemia	Perindopril, Lercanidipine
CIDPF020	53	F	72	IVIg	Yes		Nil
CIDPM021	49	M	23	Physio	Yes	Depression	Citalopram, Paracetamol, Pregabalin

CIDPM022	63	M	47	Physio	Yes	Hypertension, raised kappa light chain	Amlodipine, Atorvastatin, Ramipril
CIDPM023	56	M	20	Physio		Depression, Hypertension, GORD	Aspirin, Ranitidine, Lisinopril, Atorvastatin, Citalopram, Bisoprolol
CIDPM024	57	M	168	SCIg		Gout	Nil
CIDPM025	73	M	60	Physio	Yes	Mild emphysema, Hypercholesterolemia	Pregabalin, Simvastatin, Bendroflumethiazide
CIDPM026	51	M	36	IVIg	Yes	Monoclonal gammopathy of undetermined significance	Nil
CIDPM027	78	M	276	Physio		Asthma, Hypertension	Aspirin, Lansoprazole, Bendroflumethiazide

Table 2: Clinical details of recruited patients with chronic inflammatory demyelinating polyneuropathy. IVIg=intravenous immunoglobulins; SCIg=subcutaneous immunoglobulins; CKD=chronic kidney disease; GORD=gastro-oesophageal reflux disease

Patient ID	Age (years)	Sex	Co-morbid medical conditions	Current medication
CMTF001	63	F		Amitriptyline
CMTF002	44	F		Nortriptyline
CMTF003	43	F	Benign paroxysmal positional vertigo	Pregabalin
CMTF004	37	F		Nil
CMTF005	38	F		Sertraline
CMTM006	67	M	Hypertension, Gout	Colchicine, Doxazosin, Indapamide, Quinine Sulphate, Ramipril
CMTF007	71	F		Alendronate
CMTM008	24	M		Oxycodone, Pregabalin
CMTF009	54	F	Restless leg syndrome	Gabapentin
CMTM010	63	M	Asthma, Eczema, Type II Diabetes Mellitus (diet controlled)	Inhalers
CMTF011	43	F	GORD	Gaviscon
CMTM012	48	M		Nil
CMTF013	32	F		Mirtazapine, Lansoprazole, Co-codamol
CMTF014	57	F		Adcal D3, Amitriptyline, Lansoprazole, Solifenacin
CMTF015	21	F		Nil

Table 3: Clinical details of recruited patients with Charcot-Marie Tooth disease type IA; GORD=gastro-oesophageal reflux disease

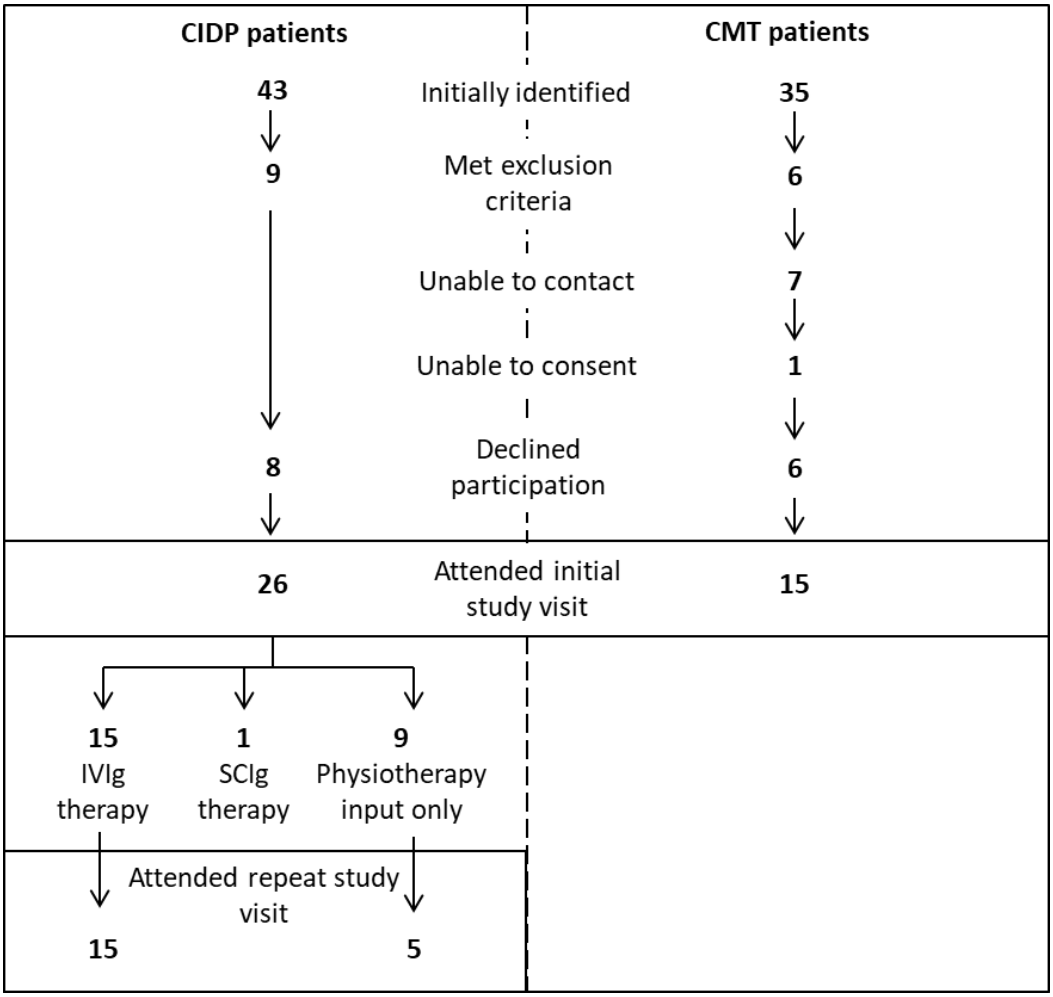


Figure 3: Flowchart detailing patient recruitment to the study. CIDP=chronic inflammatory demyelinating polyneuropathy; CMT=Charcot Marie Tooth disease; IVIg=intravenous immunoglobulins; SCIg = subcutaneous immunoglobulins

6.2 MUNIX: normative data and assessment of intra-rater reliability

MUNIX and MUSIX values showed normal distribution in healthy controls. Mean MUNIX sum scores were 516.9 (91.4) and mean MUSIX sum scores were 178.5 (32.2). Values compared to previously published reports, including from individual muscles, are shown in Table 5.

All controls underwent repeat studies at least 1 month later performed by the same investigator. ICCs are shown in Table 4. All MUNIX and MUSIX values demonstrated good reliability (ICC between 0.75 and 0.9), except for ADM MUSIX, TA MUNIX and TA MUSIX. MUNIX sum scores demonstrated higher ICC values than any of the individual muscles, and MUSIX sum scores demonstrated higher ICC values than any individual muscle except for APB.

Muscle	MUNIX ICC	95% CI	MUSIX ICC	95% CI
APB	0.78	0.52-0.91	0.80	0.56-0.92
ADM	0.75	0.48-0.89	0.58	0.22-0.81
TA	0.53	0.14-0.78	0.24	-0.18-0.60
Sum scores	**0.85**	**0.67-0.94**	**0.75**	**0.48-0.89**

Table 4: Intraclass correlation coefficients (ICC) for MUNIX and MUSIX calculated from individual muscles and sumscores calculated from all three muscles combined. APB=abductor pollicis brevis, ADM=abductor digitii minimi, TA=tibialis anterior

APB	**This study**	**Neuwirth *(2011)*[135]**	**Boekestein (2012)[136]**	**Paramanathan (2015)[137]**	**Delmont (2016)[130]**	
n=	20	66	24	20	28	
Mean age (years)	44.4	49	62	46.7	64	
MUNIX mean (SD)	195.8 (50.0)	145.7 (54.4)	121 (31)	198 (NR)	133*	
MUSIX mean (SD)	65.0 (19.8)	NR	71 (13)	60 (NR)	61.9*	
ADM	**This study**	**Nandedkar *(2010)*[126]**	**Ahn *(2010)*[138]**	**Neuwirth (2011)[135]**	**Furtula (2013)[139]**	**Delmont (2016)[130]**
n=	20	34	62	66	48	28
Mean age (years)	44.4	48	NR	49	44.4	64
MUNIX mean (SD)	176.1 (40.5)	158 (40)	142 (42)	162.9 (47)	176 (46)	134*
MUSIX mean (SD)	66.5 (14.0)	68 (13)	NR	NR	NR	68*
TA	**This study**	**Neuwirth *(2011)*[135]**	**Sandberg (2011)[140]**	**Delmont (2016)[130]**		
n=	20	66	30	28		
Mean age (years)	44.4	49	62	64		
MUNIX mean (SD)	145.1 (36.8)	132 (38.4)	103 (26)	102*		
MUSIX mean (SD)	47.0 (6.5)	--	53 (7.3)	51*		
Sum scores	**This study**	**Delmont (2016)[130]**				
n=	20	28				
Mean age (years)	44.4	64				
MUNIX mean (SD)	516.9 (91.4)	379*				
MUSIX mean (SD)	178.5 (32.2)	184*				

*Table 5: Normative data for MUNIX and MUSIX values recorded from abductor pollicis brevis (APB), abductor digitii minimi (ADM) and tibialis anterior (TA), including comparison with previously published normative values. NR=not reported. *median values presented*

6.3 MUNIX: values in patients and correlations with clinical assessments

6.3.1 MUNIX and MUSIX values

Mean MUNIX values in CIDP patients were 94.3 (59.6) for APB, 73.4 (49.0) for ADM and 46.3 (41.7) for TA. Mean MUSIX values were 92.8 (46.5) for APB, 105.8 (59.3) for ADM and 61.5 (29.2) for TA. Mean MUNIX sum score was 214.0 (124.4) and mean MUSIX sum score 251.2 (96.2). Mean MUNIX values in CMT patients were 52.3 (38.8) for APB, 54.1 (36.3) for ADM and 33.3 (37.8) for TA. Mean MUSIX values were 95.6 (36.1) for APB, 86.6 (56.7) for ADM and 40.6 (31.0) for TA. Mean MUNIX sum score was 139.7 (87.3) and mean MUSIX sum score 222.8 (88.8).

MUNIX sum scores were significantly lower in patients with both CIDP and CMT compared to controls (*p<0.001*). Although lower MUNIX values were observed in CMT patients compared to CIDP patients, this did not reach statistical significance (*p=0.095*). Mean MUSIX sum scores were higher in patients with both CMT and CIDP compared to controls. However, no statistically significant differences were observed between MUSIX sum scores in any of the groups (see Figure 4).

No difference was found between MUNIX or MUSIX sum scores in CIDP patients on treatment versus untreated patients (*p=0.343 and p=0.947, respectively*).

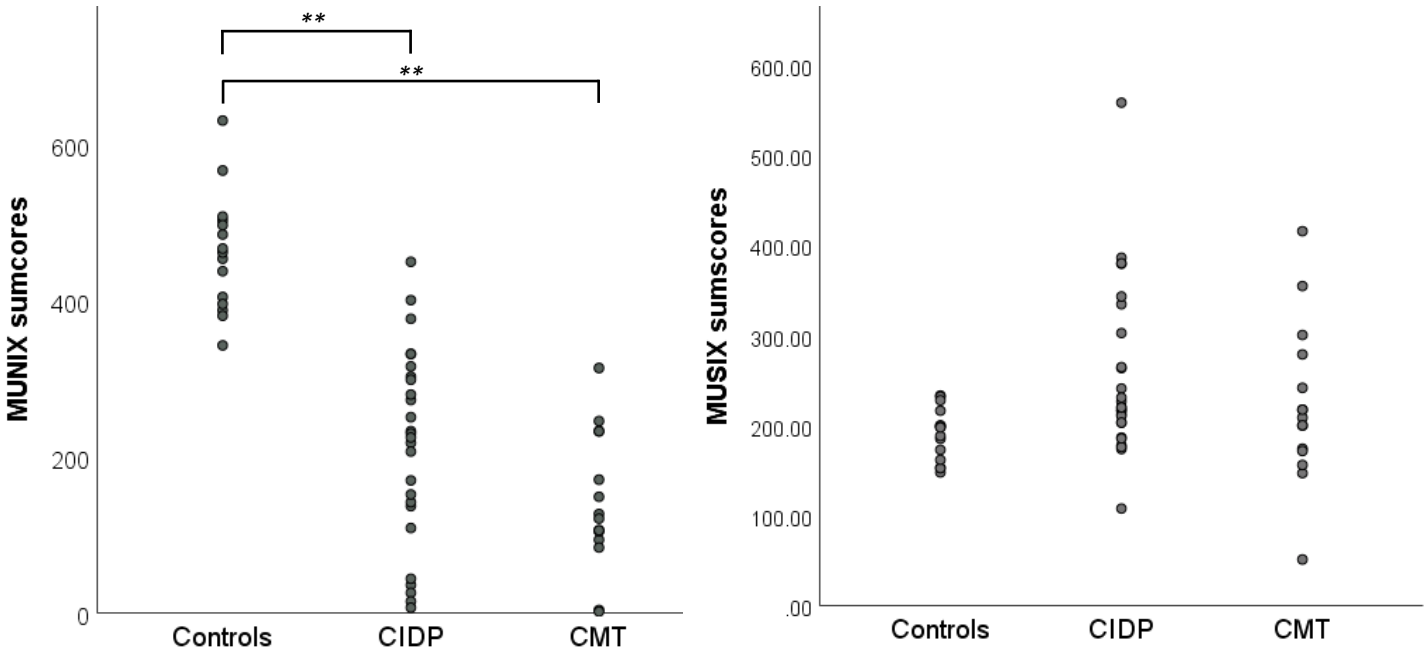


*Figure 4: Scatterplots comparing MUNIX and MUSIX sum scores in healthy control subjects, CIDP patients and CMT patients. **=p<0.001*

6.3.2 Correlations with clinical assessments and self-report scales

Median MRC muscle strength sum score in CIDP patients was 73.5 (59.3-78.0) out of 80. Mean grip strength was 26.9kg (13.4) with no significant difference observed between hands. Mean INCAT sensory sum score was 7.3 (4.6) and vibration threshold sum score 27.2 (10.2). Median 10m walk time was 10.4s (8.3-14.2). Mean R-ODS was 56.7 (16.6) and mean ONLS was 3.7 (2.1).

Vibration threshold sum scores were higher in untreated than treated CIDP patients at baseline (mean value 33.4 vs 23.9, respectively; *p=0.043*). No significant differences were seen in any of the other assessments.

Positive linear correlation, assessed using Pearson correlation coefficient, was observed between MUNIX sum scores and MRC sum score (r=0.696, *p<0.001*), hand grip strength (r=0.412, p=*0.037*), vibration threshold sum score (r=0.618, *p=0.001*) and R-ODS (r=0.480, *p=0.013*). Negative linear correlation was observed in CIDP patients between MUNIX and MUSIX sum scores (r=-0.439, *p=0.025*), INCAT sensory sum score (r=-0.598, *p=0.001*) and ONLS (r=-0.607, *p=0.001*) (see Figure 5). No significant correlation was observed between MUNIX sum scores and patient age, time since diagnosis or 10 metre timed walk test. Positive linear correlation was observed between MUSIX sum scores and patient age (r=0.419, *p=0.033*) but none of the other variables.

Median MRC muscle strength sum score in CMT patients was 68.0 (57.0-72.0) out of 80. Mean grip strength was 24.2kg (7.5) with no significant difference observed between hands. Mean INCAT sensory sum score was 9.9 (2.8), with a mean vibration threshold sum score of 23.9 (8.6). Median 10m timed walk test was 9.5s (8.8-12.1). Mean ONLS was 3.4 (1.7) and mean R-ODS score was 62.3 (16.3).

Positive linear correlation, assessed using Pearson correlation coefficient, was observed between MUNIX sum scores and MRC muscle strength sum score (r=0.816, *p<0.001*), hand grip strength (r=0.693, *p=0.004*) and vibration threshold sum score (r=0.703, *p=0.003*). Negative linear correlation was observed between MUNIX sum scores and INCAT sensory sum scores (r=-0.703, *p=0.003*) and ONLS scores (r=-0.824, *p<0.001*) (see Figure 6).

In contrast to CIDP patients, a negative correlation was also observed between MUNIX sum scores and patient age (r=-0.688, *p=0.005*) and between MUNIX sum scores and 10 metre timed walk test (r=-0.577, *p=0.024*). No significant correlation was observed between MUNIX sum scores and R-ODS scores.

No significant correlation was observed between MUSIX sum scores and any of the CMT patient demographic data or any of the clinical assessments.

In CIDP patients, only F-wave persistence showed similar correlations with the clinical assessments as those demonstrated by MUNIX, although higher correlation co-efficients were seen with MUNIX sum scores. Similar correlation with clinical assessments was not observed with any of the other electrophysiological parameters. In CMT patients, proximal CMAP amplitude and proximal CMAP area showed similar correlations with the clinical assessments as those demonstrated by MUNIX. Again, in general higher correlation co-efficients were seen with MUNIX sum scores. However, both proximal CMAP amplitude and CMAP area demonstrated positive correlation with R-ODS score, which was not observed with MUNIX sum scores. Details of electrophysiological studies and correlation analysis in patients with CIDP and CMT are included in Appendix 5 and Appendix 6.

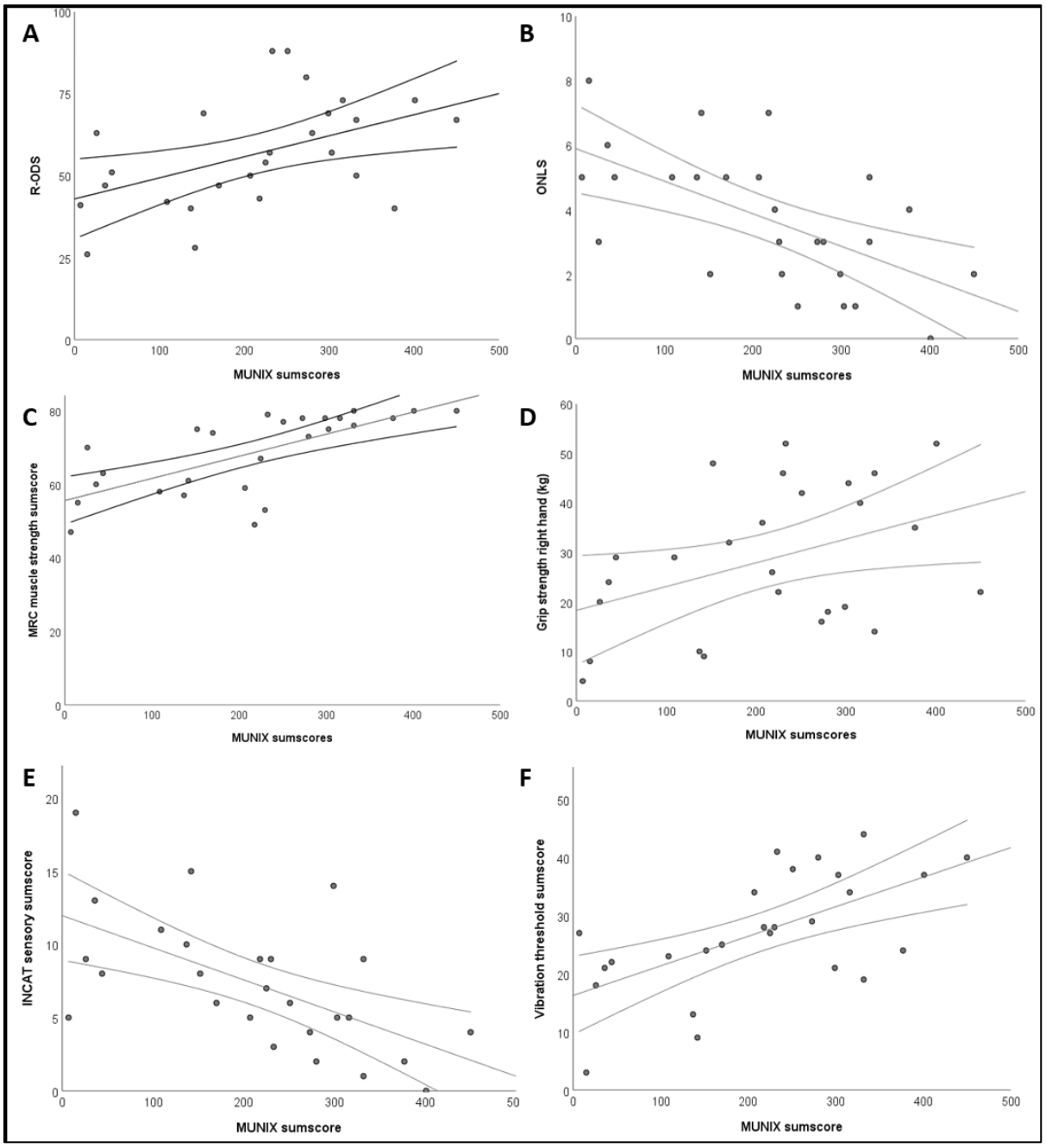


Figure 5: CIDP patients. Correlation between MUNIX sumscores and (a) Rasch-built overall disability score, R^2=0.23, Pearson correlation coefficient 0.48 (p=0.013), (b) Overall neuropathy limitations scale, R^2=0.37, Pearson correlation coefficient -0.61 (p=0.001), (c) MRC muscle strength sumscores, R^2=0.49, Pearson correlation coefficient 0.70 (p<0.001), (d) Grip strength assessed using a Jamar-grip dynamometer, R^2=0.17, Pearson correlation coefficient 0.41 (p=0.037), (e) INCAT sensory sumscore, R^2=0.36, Pearson correlation coefficient 0.60 (p=0.001), (f) Vibration threshold sumscore assessed using a Rydell-Seiffer tuning fork, R^2=0.38, Pearson correlation coefficient 0.62 (p=0.001). Graph shows best-fit line and 95% confidence band of best-fit line.

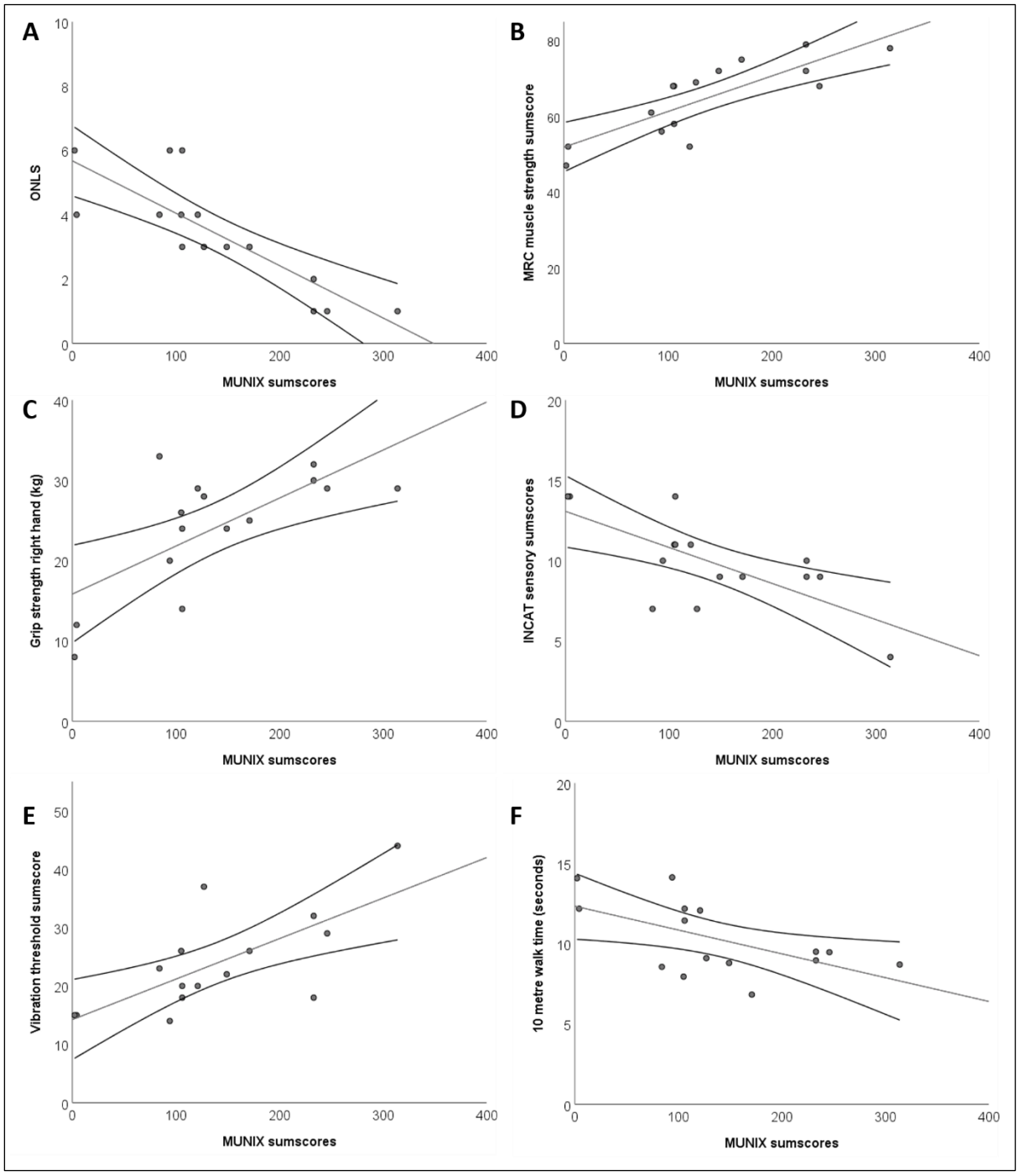


Figure 6: CMT patients. Correlation between MUNIX sumscores and (a) Overall neuropathy limitations scale, R^2=0.68, Pearson correlation coefficient -0.824 (p<0.001), (b) MRC muscle strength sumscores, R^2=0.67, Pearson correlation coefficient 0.816 (p<0.001), (c) Grip strength assessed using a Jamar-grip dynamometer, R^2=0.48, Pearson correlation coefficient 0.693 (p=0.004), (d) INCAT sensory sumscore, R^2=0.49, Pearson correlation coefficient -0.703 (p=0.003), (e) Vibration threshold sumscore assessed using a Rydell-Seiffer tuning fork, R^2=0.50, Pearson correlation coefficient 0.703 (p=0.003), (f) Timed 10 metre walk test, R^2=0.33, Pearson correlation coefficient -0.577 (p=0.024) Graph shows best-fit line and 95% confidence band of best-fit line

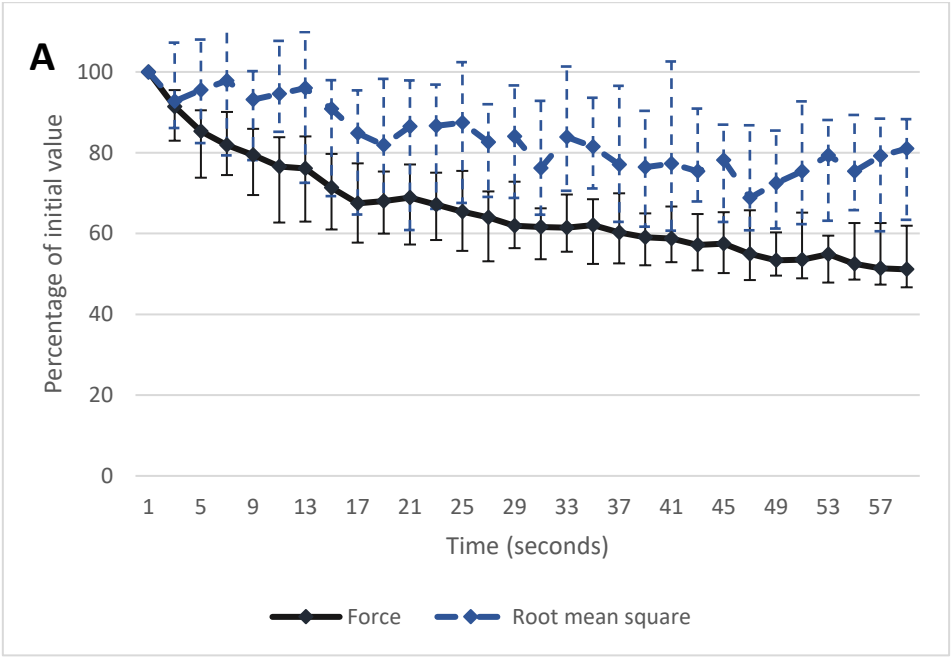
6.4 Assessment of physiological fatigue utilising hand-grip dynamometry

Assessment of physiological fatigue was performed using grip strength in the dominant hand. Maximal grip strength at baseline was significantly higher in controls compared to CIDP and CMT patients (38.9kg (12.8) *vs* 24.2kg (7.5), *p=0.007* and 38.9kg (12.8) *vs* 28.6kg (14.5), *p=0.043*, respectively).

Fifteen controls, all CIDP patients and 11 CMT patients completed the physiological fatigue assessments. Decline in force during 1-minute of maximal grip strength was greater in controls (median value 56.4% (41.2-64.6)) compared to CMT patients (39.4% (29.5-49.4), *p=0.039*). Median decline in force in CIDP patients was 49.3% (42.2-53.8), demonstrating no statistically significant difference compared to CMT patients (*p=0.425*) or controls (*p=0.473*).

A greater decline in RMS of the SEMG signal during the 1-minute grip strength test was observed in controls compared to both CMT and CIDP patients. Median decline in RMS in controls was 56.3% (30.8-68.3) compared to 19.0% (11.7-36.6) in CIDP patients (*p=0.01*0) and 30.4% (-2.4-36.3) in CMT patients (*p=0.040*). No difference was observed between CIDP and CMT patients (*p=1*.000). Decline in force of grip strength and RMS of the SEMG signal are illustrated in Figure 7.

RMS of the SEMG signal was compared during maximal grip strength and whilst maintaining a grip strength at 30% of the maximum grip strength. In control subjects, median RMS of the SEMG signal was 28.2% (20.9-34.1) during this assessment. Higher values were seen in both CIDP patients (46.5% (31.2-58.4), *p=0.006*) and CMT patients (42.7% (35.7-51.1), *p=0.073*), albeit not reaching statistical significance in CMT patients. No significant difference was observed between CMT and CIDP patients (*p=1.000*).


A
Percentage of initial value
100
80
60
40
20
0
1 5 9 13 17 21 25 29 33 37 41 45 49 53 57
Time (seconds)
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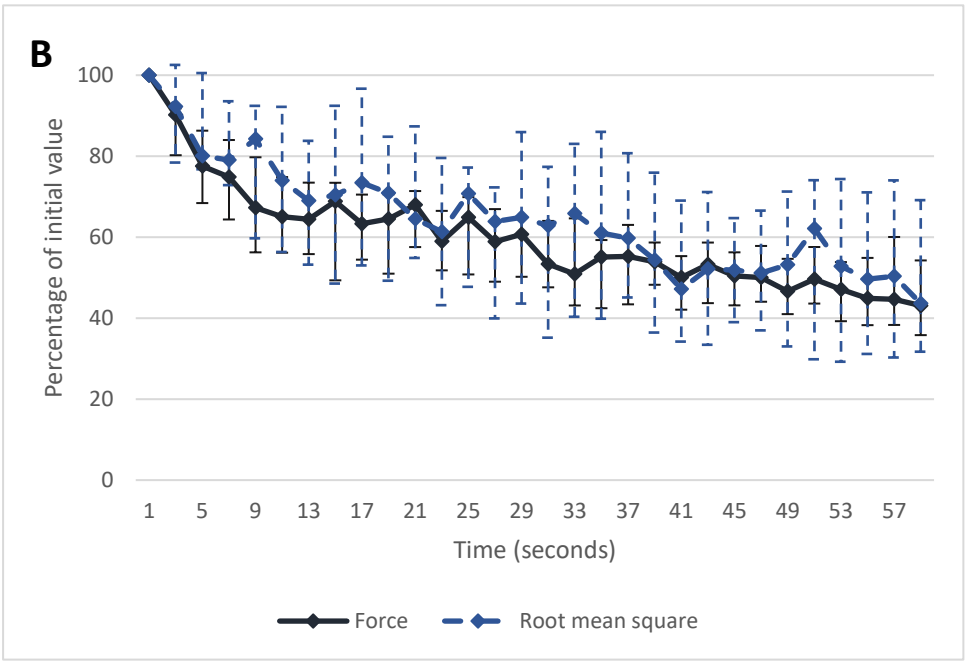
Root mean square


B
Percentage of initial value
100
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1 5 9 13 17 21 25 29 33 37 41 45 49 53 57
Time (seconds)
Force
Root mean square

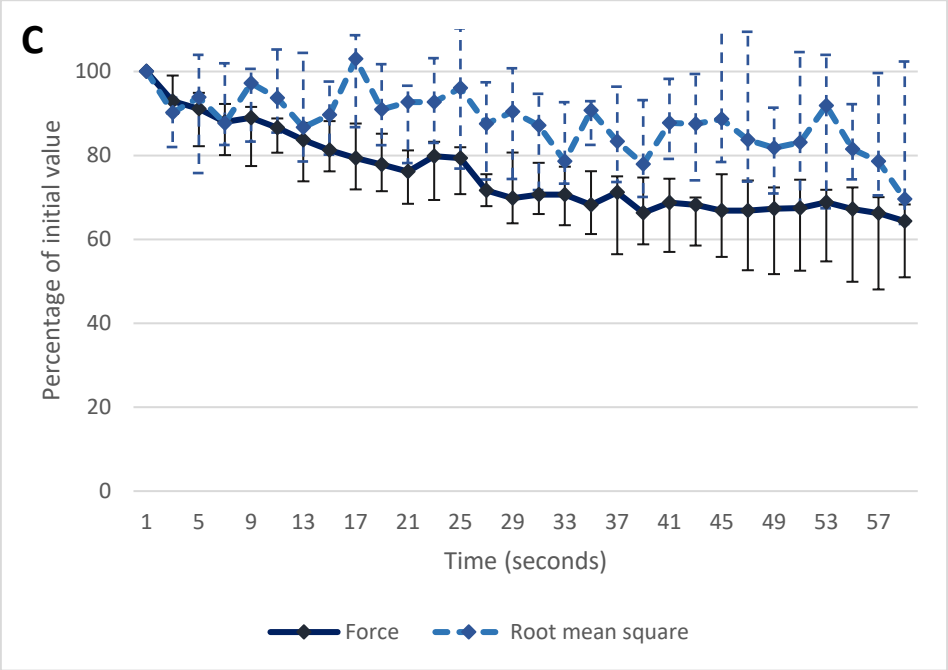

Figure 7: Graphs demonstrating decline in force of grip strength during 1 minute of maximal voluntary contraction (solid line) and corresponding decline in root mean square of the rectified surface EMG signal recorded from forearm flexors (dashed line), in A) controls (n=15), B) CIDP patients (n=26) and C) CMT patients (n=11). Graphs show median values expressed as a percentage of the initial median value. Error bars show first and third quartiles.

6.4.1 Physiological fatigue and MUNIX

Relationship between physiological fatigue and peripheral motor unit number, as assessed using MUNIX values, were assessed using Spearman's Rank correlation analysis. As described in section 6.2.3, MUNIX sum scores showed positive linear correlation with maximal grip strength at baseline. However, in CIDP patients, MUNIX sum scores showed no significant linear correlation with decline in grip strength (r=-0.045, *p=0.827*), decline in RMS of the rectified SEMG signal (r=0.138, *p=0.500*) or RMS of the rectified SEMG signal during submaximal contraction (r=-0.341, *p=0.088*). In CMT patients, MUNIX sum scores also showed no significant linear correlation with decline in grip strength (r=-0.236, *p=0.484*) or decline in RMS of the rectified SEMG signal (r=0.19, *p=0.958*). There was negative linear correlation between MUNIX sum scores in CMT patients and ratio of RMS during maximal compared to submaximal contraction (r=-0.664, *p=0.026*). MUSIX sum scores showed no significant linear correlation with any of the parameters used to assess physiological fatigue in either patient group.

6.5 Assessment of experienced fatigue and health-related quality of life

Experienced fatigue was assessed using both the Rasch-built Fatigue Severity Scale (R-FSS) and the Checklist of Individual Strength (CIS). Maximum scores are 21 for the R-FSS and 140 for the CIS, with

higher scores indicating higher level of self-reported experienced fatigue. Subdomains of the CIS (maximum scores for each subdomain in brackets) include subjective feeling of fatigue (56), concentration (35), motivation (28) and physical activity (21). Median R-FSS score in CIDP patients was 17 (13.5-19) and median CIS score was 77.5 (61-98.8). Median scores in CIS subdomains in CIDP patients were; subjective feeling of fatigue 40 (33-47.3), concentration 12.5 (7.5-18), motivation 13.5 (9.5-16.8) and physical activity 12 (7.3-17).

Median R-FSS score in CMT patients was 13 (8.5-19) and median CIS score was 88 (59.5-108). Median scores in each CIS subdomain in CMT patients were; subjective feeling of fatigue 44 (26-50.5), concentration 24 (11.5-26), motivation 16 (7.5-19) and physical activity 12 (5.5-15).

Health-related quality of life was assessed using the medical outcomes study 36-item short form health survey (SF-36). Norm-based scores in each subscale range from 0-100 and allow comparison with data drawn from a general UK population, with an average score of 50 for each subscale (see Figure 8). In contrast to the experienced fatigue scores, higher scores indicate better functioning or less pain. In CIDP patients, median physical components sum score was 34.8 (28.4-44.9) and median mental components sum score was 57.2 (48.1-59.3). In CMT patients, median physical components sum score was 35.0 (32.3-43.9) and median mental components sum score was 42.8 (39.8-55.4). Data from all assessments, including more detail of SF-36 subscales, are provided in Table 6.

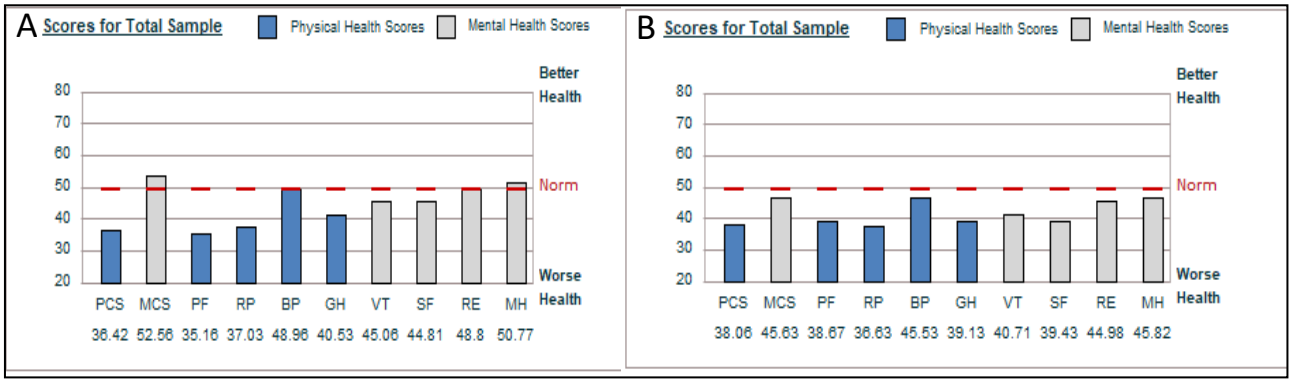

Figure 8: Charts showing norm-based scores for each subscale of the medical outcomes study 36-item, short form health survey (SF-36) in A) CIDP patients and B) CMT patients. PCS= physical components summary, MCS=mental components summary, PF=physical functioning, RP=role physical, BP=bodily pain, GH=general health, VT=vitality, SF=social functioning, RE=role emotional, MH=mental health

	CIDP	CMT	*p-value*
R-FSS (/21)	17 (13.5-19)	13 (8.5-19)	*0.398*
CIS overall (/130)	77.5 (61-98.8)	88 (59.5-108)	*0.495*
CIS-subjective fatigue (/56)	*40 (33-47.3)*	*44 (26-50.5)*	*0.512*
CIS-concentration (/35)	*12.5 (7.5-18)*	*24 (11.5-26)*	*0.968*
CIS-motivation (/28)	*13.5 (9.5-16.8)*	*16 (7.5-19)*	*0.355*
CIS-physical activity (/21)	*12 (7.3-17)*	*12 (5.5-15)*	*0.305*
SF-36			
Physical functioning NBS	*32.7 (27.4-43.7)*	*36.5 (28.8-47.5)*	*0.305*
Role physical NBS	*32.5 (30.2-45.9)*	*38.1 (30.8-41.4)*	*0.944*
Bodily pain NBS	*49.1 (38.3-60.4)*	*46.7 (39.2-50.3)*	*0.347*
General health NBS	*38.9 (30.8-52.6)*	*39.6 (30.8-43.7)*	*0.790*
Vitality NBS	*46.7 (38.5-51.9)*	*37.7 (32.5-48.1)*	*0.200*
Social functioning NBS	*47.3 (38.5-52.3)*	*37.3 (32.3-46.1)*	*0.130*
Role emotional NBS	*52.7 (42.2-56.2)*	*49.2 (35.3-55.3)*	*0.254*
Mental health NBS	*53.5 (48.3-56.1)*	*45.6 (40.4-52.8)*	*0.098*
Physical components sumscore NBS	34.8 (28.4-44.9)	35.0 (32.3-43.9)	*0.585*
Mental components sumscore NBS	57.2 (48.1-59.3)	42.8 (39.8-55.4)	***0.033***

Table 6: Results from Rasch-built Fatigue Severity Scale (R-FSS), Checklist of Individual Strength (CIS) and the medical outcomes study 36-item short form health survey (SF-36) in patients with CIDP and CMT. Data expressed as median values (interquartile range). Differences between groups compared with Mann-Whitney U Test.

Co-morbid depression and anxiety were assessed using the Hospital Anxiety and Depression Scale (HADS). Six of the 26 CIDP patients scored in the range indicating high likelihood of depression (11 or more), with 2 of these also scoring in range indicating high likelihood of an anxiety disorder. 2 of the 15 CMT patients scored in the range indicating high likelihood of both depression and anxiety disorders. A further 3 CIDP patients and 4 CMT patients scored in the borderline range for depression (score of 8 to 10).

Positive linear correlation, assessed using Spearman's rank correlation, was observed between R-FSS and overall CIS scores in both CIDP (r=0.685, *p=<0.*001) and CMT patients (r=0.897, *p=<0.001*). Of the CIS subdomains, R-FSS showed positive linear correlation with subjective feeling of fatigue (r=0.793,

*p<0.*001), motivation (r=0.423, *p=0.*031) and physical activity (r=0.679, *p<0.*001) in CIDP patients and with subjective feeling of fatigue (r=0.761, *p=0.002*), motivation (r=0.932, *p<0.001*) and physical activity (r=0.839, *p<0.001*) in CMT patients.

6.5.1 Experienced fatigue and MUNIX

No significant linear correlation was observed between peripheral motor unit function, assessed using MUNIX and MUSIX values, and experienced fatigue, assessed using R-FSS and overall CIS scores (see Tables 7 and 8).

Physiological fatigue, defined as reduction in force of grip strength during 1 minute of maximal contraction, showed positive correlation with R-FSS (r=0.439, *p=0.025*) and overall CIS scores (r=0.486, *p=0.012*) in CIDP patients but not in CMT patients.

In CIDP patients, overall CIS scores showed significant correlations with clinical assessments of motor and sensory function, including MRC muscle strength sum scores (r=-0.413, *p=0.036*), grip strength (r=-0.544, *p=0.004*), INCAT sensory sum scores (r=0.507, *p=0.008*) and vibration thresholds (r=-0.497, *p=0.010*). R-FSS scores correlated with grip strength (r=-0.476, *p=0.014*) and vibration thresholds (r=-0.504, *p=0.009*) but not MRC muscle strength sum scores or INCAT sensory sum scores.

In CMT patients, both R-FSS and overall CIS scores correlated with grip strength (R-FSS r=-0.534, *p=0.041*; CIS r=-0.518, *p=0.048*). No significant linear correlation was observed with other clinical assessments of motor or sensory function.

R-FSS and overall CIS scores also showed significant linear correlation with overall disease severity scores in CIDP patients, assessed using R-ODS (R-FSS r=-0.588, *p=0.003*; CIS r=-0.641, *p<0.001*) and ONLS (R-FSS r=0.426, *p=0.030*; CIS r=0.543, *p=0.004*). In CMT patients, experienced fatigue scores correlated with R-ODS (R-FSS r=-0.583, *p=0.029*; CIS r=-0.699, *p=0.009*) but not ONLS scores. R-FSS and overall CIS scores correlated with both depression (R-FSS r= 0.536, *p=0.005;* CIS r=0.704, *p<0.*001) and anxiety (R-FSS r=0.428, *p=0.029*; CIS r=0.553, *p=0.003*) scores in CIDP patients, but not in CMT patients.

Finally, significant linear correlation was observed between R-FSS and overall CIS scores with both physical components sum scores (R-FSS r=-0.425, *p=0.030*; CIS r=-0.518, *p=0.007*) and mental components sum scores (R-FSS r=-0.457, *p=0.019*; CIS r=-0.691, *p=0.001*) of SF-36 in CIDP patients. In CMT patients, experienced fatigue scores correlated with physical components sum scores of SF-36 (R-FSS r=-0.757, *p=0.002*; CIS r=-0.804, *p=0.001*) but not mental components sum scores. In the study of Merkies *et al*[55], validity of the FSS was demonstrated by correlation with the vitality domain

of the SF-36. Both the R-FSS and CIS showed significant correlation with this subscale in CIDP and CMT patients. Full details of correlation analysis, including correlations between experienced fatigue scores and other relevant data are provided in Tables 7 and 8.

Multivariate stepwise linear regression modelling was performed in the CIDP patient group, including potential explanatory variables that demonstrated significant linear correlation in the univariate analysis. Separate modelling was performed using R-FSS and overall CIS score as the outcome variable. Included variables were clinical markers of motor function (MRC muscle strength sum scores and grip strength), clinical markers of sensory function (INCAT sensory sum score **or** vibration thresholds) HADS depression score, HADS anxiety score and physiological fatigue (decline in grip strength during a 1-minute fatiguing contraction). INCAT sensory sum score and vibration thresholds both assess sensory function and demonstrate significant collinearity. Therefore, only the method demonstrating strongest correlation with the outcome variable in the univariate analysis was included.

Using R-FSS score as the outcome variable the model identified 2 variables significantly contributing to experienced fatigue; HADS depression score (B=0.659 (95% CI 0.15-1.16), *p=0.013*) and Grip strength (B=-0.155 (95% CI -0.28--0.03), *p=0.018*). R^2 was 0.445 and f-score was 9.24 (*p=0.001*), indicating the relationship between the explanatory variables and outcome variable was significant. Using overall CIS score as the outcome variable the model identified the same 2 explanatory variables; HADS depression score (B=4.550 (95% CI 2.66-6.44), *p<0.001*) and Grip strength (B=-0.724 (95% CI -1.19--0.26), *p=0.004*). R^2 was 0.667 and f-score was 23.04 (*p<0.001*). The following regression equations were calculated:

R-FSS=15.45+0.659(HADS depression score)-0.155(Grip strength)

Overall CIS=70.52+4.55(HADS depression score)-0.724(Grip strength)

CIDP patients

	R-FSS			Overall CIS score		
	Correlation co-efficient	Significance (2-tailed)	95% CI	Correlation co-efficient	Significance (2-tailed)	95% CI
Age	-0.163	*0.425*	*0.38-0.86*	-0.077	*0.710*	*-0.47-0.36*
Time since diagnosis	0.186	*0.363*	*-0.19-0.56*	-0.077	*0.708*	*-0.44-0.36*
MUNIX	-0.330	*0.100*	*-0.66-0.14*	-0.319	*0.113*	*-0.64-0.11*
MUSIX	0.073	*0.721*	*-0.38-0.54*	0.004	*0.984*	*-0.40-0.47*
Physiological fatigue	0.439	***0.025***	*0.14-0.64*	0.486	***0.012***	*0.14-0.69*
MRC sumscores	-0.238	*0.242*	*-0.65-0.19*	-0.413	***0.036***	*-0.72--0.22*
Grip strength	-0.476	***0.014***	*-0.77--0.06*	-0.544	***0.004***	*-0.81--0.19*
INCAT sensory sumscore	0.339	*0.090*	*-0.10-0.68*	0.507	***0.008***	*0.17-0.74*
Vibration thresholds	-0.504	***0.009***	*-0.78--0.11*	-0.497	***0.010***	*-0.75--0.20*
R-ODS	-0.558	***0.003***	*-0.81--0.17*	-0.641	***<0.001***	*-0.81--0.35*
ONLS	0.426	***0.030***	*-0.01-0.73*	0.534	***0.004***	*0.15-0.81*
HADS depression	0.536	***0.005***	*0.08-0.82*	0.704	***<0.001***	*0.40-0.87*
HADS anxiety	0.428	***0.029***	*0.05-0.72*	0.553	***0.003***	*0.22-0.79*
SF-36						
Physical functioning NBS	-0.487	***0.012***	*-0.77--0.06*	-0.643	***<0.001***	*-0.86--0.29*
Role physical NBS	-0.380	*0.055*	*-0.72-0.07*	-0.592	***0.001***	*-0.82--0.24*
Bodily pain NBS	-0.289	*0.152*	*-0.62-0.16*	-0.259	*0.202*	*-0.62-0.17*
General health NBS	-0.454	***0.020***	*-0.73--0.04*	-0.590	***0.002***	*-0.84--0.20*
Vitality NBS	-0.596	***0.001***	*-0.82--0.26*	-0.881	***<0.001***	*-0.97--0.69*
Social functioning NBS	-0.708	***<0.001***	*-0.89--0.38*	-0.679	***<0.001***	*-0.83--0.42*
Role emotional NBS	-0.488	***0.011***	*-0.75--0.14*	-0.647	***<0.001***	*-0.82--0.34*
Mental health NBS	-0.208	*0.307*	*-0.59-0.25*	-0.521	***0.006***	*-0.80--0.13*
Physical components sumscore NBS	-0.425	***0.030***	*-0.71--0.01*	-0.518	***0.007***	*-0.79--0.01*
Mental components sumscore NBS	-0.457	***0.019***	*-0.75--0.04*	-0.619	***0.001***	*-0.85--0.25*

Table 7: Linear correlation analysis of experienced fatigue assessments in patients with CIDP. Experienced fatigue assessed using both the Rasch-built fatigue severity scale and the Checklist of Individual Strength. Significant correlations are highlighted in bold.

CMT patients

	R-FSS			Overall CIS score		
	Correlation co-efficient	Significance (2-tailed)	95% CI	Correlation co-efficient	Significance (2-tailed)	95% CI
Age	0.352	*0.198*	*-0.02-0.22*	0.344	*0.209*	*-0.25-0.57*
MUNIX	-0.105	*0.721*	*-0.62-0.47*	-0.115	*0.696*	*-0.63-0.43*
MUSIX	-0.473	*0.088*	*-0.81-0.12*	-0.474	*0.087*	*-0.85-0.16*
Physiological fatigue	0.331	*0.320*	*-0.41-0.81*	0.091	*0.790*	*-0.63-0.67*
MRC sumscores	-0.181	*0.519*	*-0.61-0.39*	-0.103	*0.714*	*-0.61-0.46*
Grip strength	-0.534	***0.041***	*-0.83--0.03*	-0.518	***0.048***	*-0.82--0.02*
INCAT sensory sumscore	-0.338	*0.218*	*-0.18-0.78*	0.434	*0.106*	*-0.12-0.89*
Vibration thresholds	-0.241	*0.387*	*-0.75-0.33*	-0.156	*0.578*	*-0.75-0.37*
R-ODS	-0.583	***0.029***	*-0.88-0.07*	-0.669	***0.009***	*-0.89--0.13*
ONLS	0.221	*0.447*	*-0.37-0.72*	0.221	*0.448*	*-0.30-0.67*
HADS depression	0.223	*0.444*	*-0.42-0.83*	0.257	*0.376*	*-0.39-0.87*
HADS anxiety	-0.138	*0.638*	*-0.65-0.45*	-0.139	*0.636*	*-0.69-0.50*
SF-36						
Physical functioning NBS	-0.729	***0.003***	*-0.92--0.20*	-0.709	***0.004***	*-0.93--0.22*
Role physical NBS	-0.761	***0.002***	*-0.93--0.34*	-0.799	***0.001***	*-0.95--0.45*
Bodily pain NBS	-0.141	*0.630*	*-0.67-0.51*	0.069	*0.814*	*-0.50-0.59*
General health NBS	-0.380	*0.180*	*-0.73-0.30*	-0.471	*0.089*	*-0.82-0.12*
Vitality NBS	-0.861	***<0.001***	*-0.97-0.57*	-0.889	***<0.001***	*-0.98--0.61*
Social functioning NBS	-0.336	*0.241*	*-0.80-0.29*	-0.459	*0.099*	*-0.92-0.14*
Role emotional NBS	-0.164	*0.576*	*-0.75-0.47*	-0.012	*0.967*	*-0.57-0.60*
Mental health NBS	-0.037	*0.900*	*-0.66-0.58*	0.052	*0.860*	*-0.52-0.58*
Physical components sumscore NBS	-0.757	***0.002***	*-0.94--0.30*	-0.804	***0.001***	*-0.96--0.42*
Mental components sumscore NBS	-0.136	*0.643*	*-0.70-0.47*	-0.018	*0.952*	*-0.57-0.55*

Table 8: Linear correlation analysis of experienced fatigue assessments in patients with CMT. Experienced fatigue assessed using both the Rasch-built fatigue severity scale and the Checklist of Individual Strength. Significant correlations are highlighted in bold.

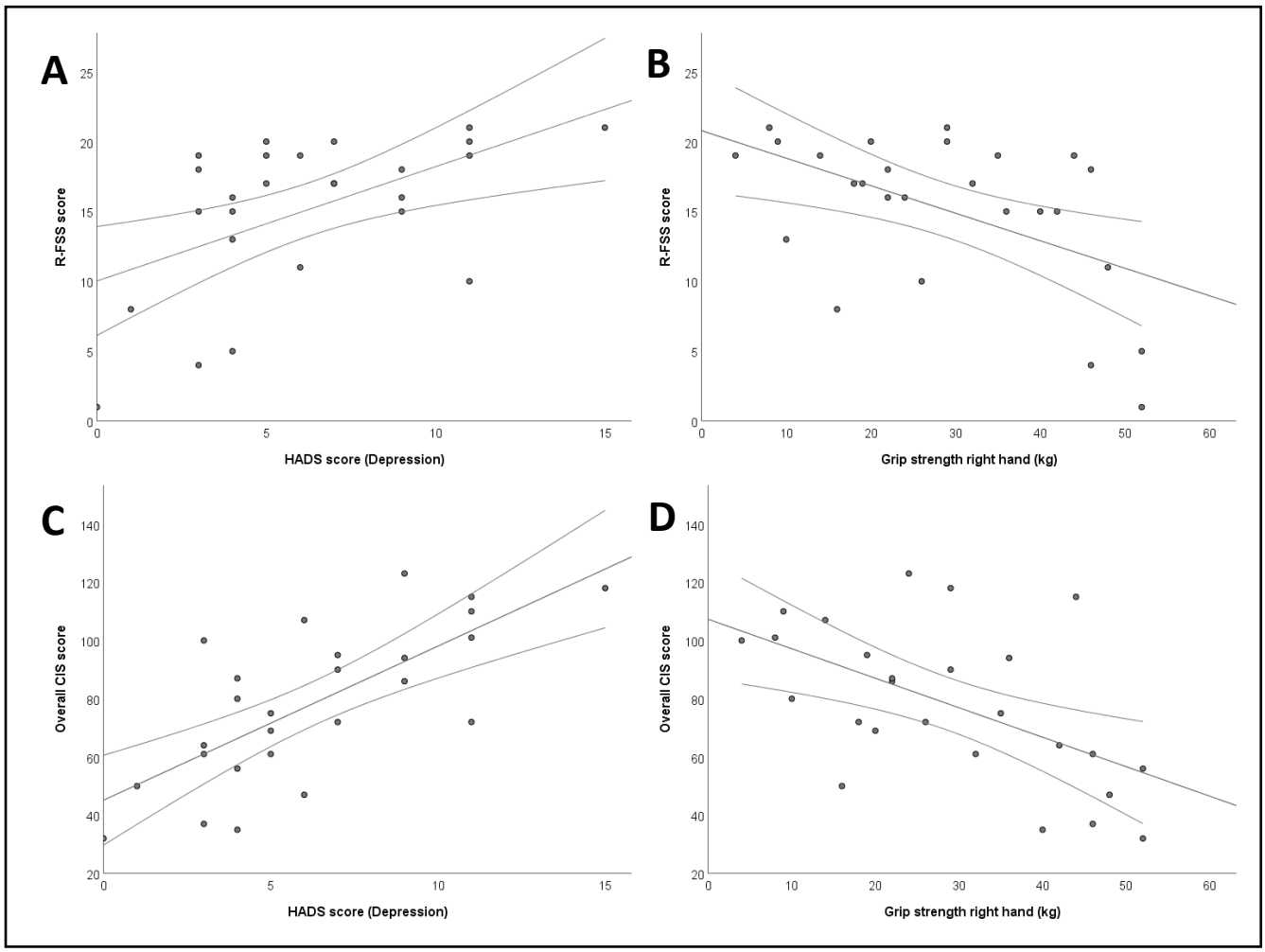

Figure 9: CIDP patients. Correlation between variables included in the multivariate linear regression models. A) R-FSS score and HADS depression scores, R^2=0.29, Spearman Rank correlation coefficient 0.54 (p=0.005). B) R-FSS score and grip strength assessed using a Jamar-grip dynamometer, R^2=0.27, Spearman Rank correlation coefficient -0.48 (p=0.014). C) Overall CIS score and HADS depression scores, R^2=0.52, Spearman Rank correlation coefficient 0.70 (p<0.001). D) Overall CIS score and grip strength assessed using a Jamar-grip dynamometer, R^2=0.31, Spearman Rank correlation coefficient -0.54 (p=0.004). Graph shows best-fit line and 95% confidence band of best-fit line.

6.6 Assessment following intravenous immunoglobulin treatment in chronic inflammatory demyelinating polyneuropathy

Repeat assessments were performed in 20 CIDP patients; 15 receiving regular maintenance IVIg and 5 receiving physiotherapy only. Patients receiving IVIg therapy were seen immediately before an infusion and on average 15 days after the same IVIg infusion. Significant improvements were seen in MRC muscle strength sum scores (mean value 67.5 to 69.4, *p=0.033*) and 10m walk time (mean value 13.2s to 11.4s, *p=0.044*). There was a trend towards improvement in R-ODS, which didn't reach statistical significance (mean value 53.3 to 55.2, *p=0.085*). In comparison, a small improvement in 10m walk time was found in 5 untreated patients participating in ongoing physiotherapy between appointments (mean value 9.7s to 9.3s, *p=0.017*), but no improvement was observed in other clinical assessments in this group.

Significant improvements were seen in MUNIX sum scores (mean value 188.3 to 226.4, *p=0.001*) but not MUSIX sum scores (mean value 266.5 to 253.5, *p=0.312*) following IVIg therapy. There was no significant change in MUNIX sum scores on repeat assessment in untreated patients (see Figure 10).

In addition to MUNIX sum scores, small but significant improvement was seen following IVIg therapy in DML (median value 6.4 to 6.1, *p=0.022*), amplitude of the distal-evoked CMAP (median value 5.0 to 5.7, *p=0.035*), duration of the proximal-evoked CMAP (median value 8.7 to 7.5, *p=0.046*) and persistence of the F-wave (median value 25 to 33.8, *p=0.014*). No significant changes were seen in any of the other electrophysiological parameters (Full details provide in Table 9).

No changes were seen in CIS scores, including subscales over repeat appointments in both patient groups. A small but statistically significant reduction in R-FSS scores were seen in CIDP patients receiving IVIg therapy (median value 17 to 15, *p=0.037*).

	CIDP patients IVIg group (*n=15*)			CIDP patients no IVIg group (*n=5*)		
	Baseline	Follow-up	*p-value*	Baseline	Follow-up	*p-value*
MRC	**67.5 (10.0)**	**69.4 (10.3)**	***0.033***	71.0 (9.4)	71.4 (10.0)	*0.648*
Grip strength (kg)	23.7 (12.7)	24.8 (11.0)	*0.332*	28.4 (10.0)	29.4 (13.1)	*0.616*
INCAT	8.3 (4.9)	7.9 (5.5)	*0.661*	5.4 (1.5)	5.8 (1.3)	*0.621*
Vibration	23.9 (10.9)	25.0 (11.1)	*0.503*	33.4 (6.8)	30.6 (7.7)	*0.300*
10m walk time (s)	**13.2 (6.8)**	**11.4 (4.2)**	***0.044***	**9.7 (2.1)**	**9.3 (1.9)**	***0.017***
R-ODS	53.2 (19.6)	55.2 (21.8)	*0.085*	59.6 (10.1)	58.4 (10.5)	*0.208*
ONLS	4.5 (2.0)	4.3 (2.1)	*0.051*	2.8 (2.1)	2.8 (2.1)	-

R-FSS	17 (14-19.5)*	16 (11.5-17.5)*	*0.054*	16 (15-19)*	16 (16-17)*	*1.000*
CIS overall	75 (66.5-104)*	79 (63.5-94)*	*0.232*	90 (87-94)*	95 (82-100)*	*1.000*
CIS subjective	*40 (33-45)**	*39 (35-49)**	*0.906*	*40 (38-52)**	*40 (39-45)**	*0.285*
CIS concentration	*14 (8-19.5)**	*14 (8-17.5)**	*0.502*	*16 (15-24)**	*22 (14-22)**	*0.593*
CIS motivation	*15 (11-16.5)**	*13 (8.5-15)**	*0.061*	*14 (13-20)**	*20 (14-21)**	*0.593*
CIS physical activity	*16 (8.5-19)**	*12 (9.5-16.5)**	*0.161*	*9 (8-16)**	*14 (8-14)**	*1.000*
MUNIX sumscore	**188.3 (110.5)**	**226.4 (132)**	***0.001***	264 (150.4)	256.6 (168.7)	*0.684*
MUSIX sumscore	266.5 (84.7)	253.5 (99.9)	*0.312*	271 (161.8)	270.7 (136.7)	*0.952*
Physiological fatigue (%)	49.7 (44.3-53.2)*	41.9 (31.4-50.0)*	*0.233*	51.1 (32.4-62.2)*	46.5 (38.4-62.2)*	*0.715*
Decline in RMS (%)	17.2 (-9.9-33.1)*	2.75 (-9.2-19.3)*	*0.363*	37.0 (20.2-39.9)*	24.7 (8.9-37.0)*	*0.686*
RMS in submaximal contraction (%)	48.4 (41.6-60.4)*	42.3 (37.1-48.2)*	*0.221*	31.0 (28.0-47.5)*	44.7 (31.1-47.5)*	*0.285*
Motor nerve conduction studies						
DML (ms)	**6.4 (5.2-6.9)***	**6.1 (4.9-7.2)***	***0.022***	4.6 (3.7-6.1)*	4.8 (4.4-5.7)*	*0.500*
dCMAP amplitude (mV)	**5.0 (3.1-5.6)***	**5.7 (3.7-6.2)***	***0.035***	5.5 (4.3-5.6)*	5.6 (4.8-5.8)*	*0.138*
dCMAP area (mV*ms)	13.8 (7.1)	14.2 (6.2)	*0.903*	13.8 (4.5)	13.9 (4.1)	*0.802*
dCMAP duration (ms)	7.5 (6.7-10.0)*	7.7 (6.5-10.2)*	*0.552*	7.6 (6.9-7.8)*	6.1 (5.3-8.4)*	*0.345*
pCMAP amplitude (mV)	4.6 (1.4-5.2)*	4.3 (1.6-5.6)*	*0.485*	5.5 (4.7-5.6)*	5.8 (5.5-5.9)*	*0.080*
pCMAP area (mV*ms)	13.9 (9.9)	13.8 (8.8)	*0.984*	15.1 (7.2)	15.8 (6.2)	*0.583*
pCMAP duration (ms)	**8.7 (7.1-13.3)***	**7.5 (6.4-8.8)***	***0.046***	6.7 (6.1-7.0)*	6.0 (5.6-6.4)*	*0.080*
MNCV (m/s)	36.1 (25.5-41.6)*	37.8 (27.3-40.2)*	*0.117*	30.2 (29.8-32.2)*	31.7 (28.6-35.9)*	*0.686*
F-wave latency (ms)	47.5 (10.3)	44.3 (8.3)	*0.079*	36.4 (7.0)	32.3 (1.8)	*0.242*
F-wave persistence (%)	**45 (35.8-60.0)***	**70.0 (55.0-81.3)***	***0.021***	25 (20-42.5)*	35 (0-47.5)*	*0.786*
Sensory nerve conduction studies						
SNAP amplitude (μV)	3.2 (0.8-4.5)*	3.3 (1.0-6.0)*	*0.062*	3.7 (2.5-4.3)*	1.5 (1.1-2.7)*	*0.066*
SNCV (m/s)	30.0 (9.8-37.6)*	30.5 (12.9-40.7)*	*0.272*	49.6 (32.4-53.4)*	34.7 (19.7-47.5)*	*0.063*

*Table 9: Repeat assessments performed in CIDP patients receiving IVIg therapy and CIDP patients not receiving IVIg therapy. *Data presented as median values (IQR). Otherwise data presented as mean values (SD). Differences assessed using paired two-tailed student t-test or Wilcoxon Signed Ranks test. Significant differences highlighted in bold.*

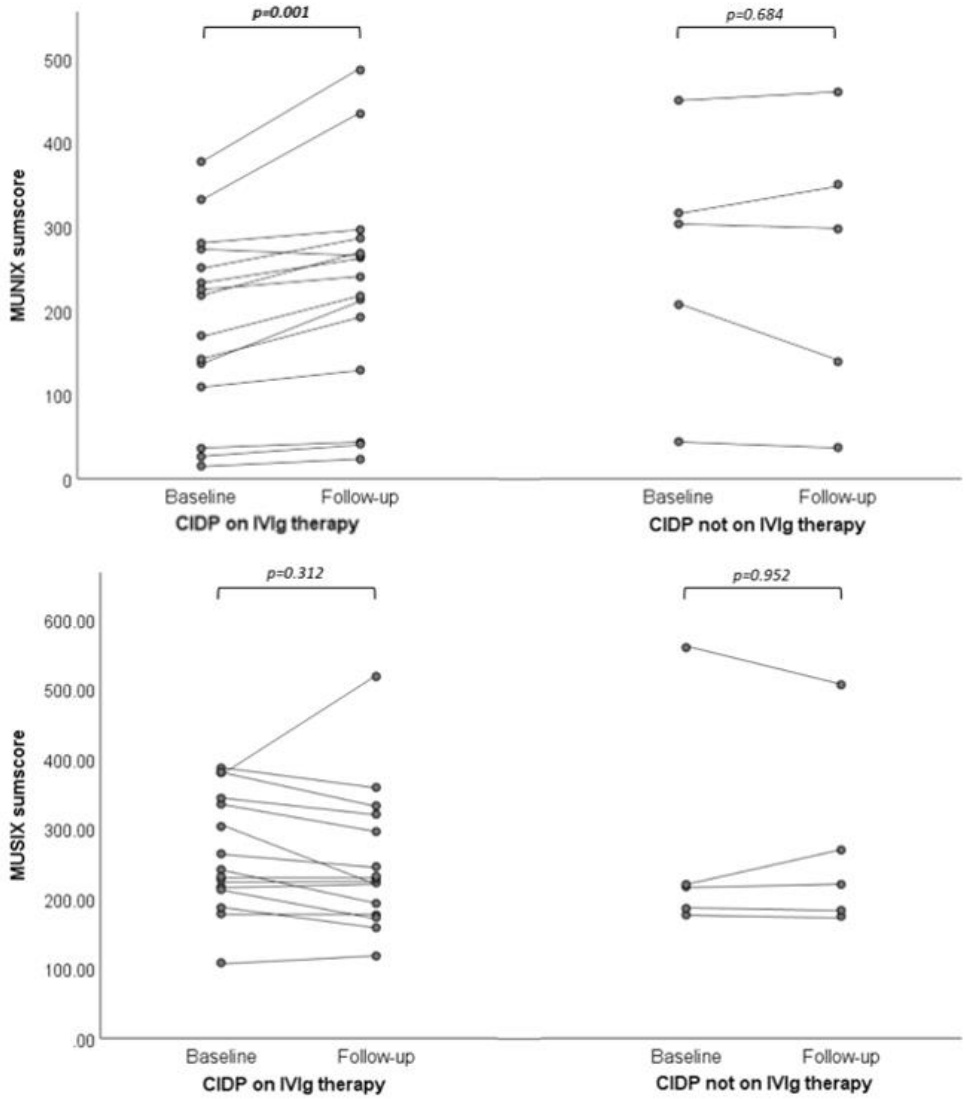

Figure 10: Scatterplots demonstrating changes in MUNIX and MUSIX sumscores between repeat appointments in CIDP patients receiving regular IVIg infusions (n=15) and patients not receiving active treatment (n=5). Changes in values within patient groups assessed using paired, 2-tail student t-test.

7. Discussion

7.1 Relationship between experienced fatigue, physiological fatigue and peripheral nerve function in patients with acquired and hereditary demyelinating peripheral neuropathy.

In this study, MUNIX technique was used as a marker for number of functioning motor units. MUNIX sum scores were significantly lower in both CMT and CIDP patients compared to healthy controls. Interestingly, whilst mean MUSIX sum scores were higher in both patient groups, there was considerable overlap with values recorded in controls, with some patients having lower MUSIX sum scores than controls. During chronic and progressive loss of motor neurones, surviving motor neurones sprout collateral terminal axon branches to re-innervate denervated muscle fibres within their motor unit territory. As a result, there is an increase in "size" of the surviving motor units. In the context of the MUNIX calculation, this means that each surviving motor unit has a greater overall contribution to the summated muscle action potential (the CMAP). Given that low MUNIX sum scores were observed in some patients without corresponding increase in MUSIX sum scores, a possible mechanism could be motor conduction block or conduction failure due to nodal/paranodal dysfunction. In both CMT and CIDP patients, the lowest MUSIX sum scores correlated with the lowest MUNIX sum scores and low amplitude CMAP potentials. It is also possible that low MUSIX sum scores could result from severe loss of motor units, with insufficient surviving motor neurones to allow reinnervation to occur. These various mechanisms likely explain the greater variation in MUSIX sum scores seen in patients with CMT and CIDP compared to controls.

Physiological fatigue was assessed using continuous grip strength measurements. CMT and CIDP patients demonstrated reduced grip strength at baseline compared to controls. In addition, CMT patients showed slower decline in force of grip strength during a fatiguing task. No difference was observed comparing decline in grip strength during 1 minute of continuous forearm muscle contraction in CIDP patients and controls.

MUNIX studies suggest patients with CMT and CIDP have fewer functioning peripheral motor neurones than healthy controls. It was therefore hypothesised that a greater proportion of available motor neurones would be activated during submaximal muscle contraction in patients compared to controls. To test this hypothesis, RMS of the rectified SEMG signal recorded from forearm flexor muscles was measured during the fatiguing muscle contraction. For the purpose of this study, SEMG was used as a surrogate marker for neural activation. There was a greater decline in this signal in controls than in both CMT and CIDP patients. The ratio of RMS of the SEMG signal during maximal and submaximal hand grip was also calculated. As predicted, the ratio was lower in both CIDP and CMT patients compared to controls (not reaching statistical significance in the CMT patient group,

although as only 11 patients completed this part of the assessment, this may be due to small sample size). Furthermore, a negative linear correlation was observed between MUNIX values and ratio of RMS of the SEMG during maximal and submaximal contraction.

In combination, these finding support the hypothesis that patients with lower number of functional motor units have altered patterns of neural activation, requiring similar neural activation to maintain a submaximal forearm muscle contraction as to maintain a maximal forearm muscle contraction. It can be hypothesised that constant activation of a greater proportion of the available motor unit pool during routine motor tasks could lead to earlier fatigue. However, there are several inconsistencies which should be addressed. Some authors have suggested there is greater relative drop-out of motor neurones in patients with peripheral neuropathy during a fatiguing task[71,87]. However, in this study, no difference was observed in development of physiological fatigue between controls and CIDP patients, and decline in power of the SEMG signal was slower in patients than controls. Whilst physiological fatigue may result from reduced neural activation, local factors will also be involved, such as production of lactic acid during exercise[110,111]. Greater peripheral fatigue in controls compared to patients with peripheral neuropathy is hypothesised to occur because patients are unable to generate maximal muscle contraction, resulting in less anaerobic respiration and lower production of lactic acid[71]. The balance between these two factors, local peripheral fatigue and neural activation, will affect development of physiological fatigue and may explain the observed inconsistencies in this study[71,82,87].

Two self-report scales are used to assess experienced fatigue in this study. A strong correlation is demonstrated between these scales in patients with CMT and CIDP. In addition, both scales show strong correlation with the vitality domain of the SF-36 questionnaire, which has been used to validate fatigue scales in previous studies in similar patient groups[55]. These findings suggest that both scales are valid methods of assessing experienced fatigue in these patient groups. It must also be acknowledged that there is some variation when scores are compared between groups. For example, median R-FSS scores were higher in CIDP patients than CMT patients, but median overall CIS scores were lower. The FSS (from which the R-FSS is adapted) assesses impact of fatigue on types of daily activity, whereas the multidimensional CIS aims to assess severity of fatigue symptoms. It is possible that patients with CMT may experience more severe symptoms of fatigue, but owing to CMT being a hereditary disorder, be less conscious of the daily impact of fatigue.

No significant correlation was observed between experienced fatigue and MUNIX or MUSIX sum scores in either patient group. In CIDP patients, a weak positive correlation was observed between experienced fatigue levels and decline in grip strength during a fatiguing contraction. However, the

only two variables to contribute to experienced fatigue in regression analysis were depression scores and maximal grip strength. Analysis using both R-FSS scores and overall CIS scores as outcome variables identified the same two explanatory variables, again increasing confidence in the validity of these assessment scales. This finding indicates experienced fatigue is likely to be multifactorial in patients with CIDP, related to markers of physical impairment and psychological factors, most importantly co-morbid depression. It is also worth noting that the regression modelling explained only 44.5% and 66.7% of the variance in R-FSS and overall CIS scores, respectively. Therefore, a significant proportion of the variance is unexplained by the explanatory variables included in this study. In CMT patients, the only explanatory variable found to correlate with experienced fatigue scores was grip strength. For this reason, and due to the smaller sample size, regression modelling could not be performed in CMT patients. In contrast to CIDP patients, depression scores showed no correlation with experienced fatigue level in CMT patients.

In recovered patients with GBS, Drenthen *et al*[59] found that only the number of functioning peripheral motor units explained residual fatigue levels, assessed using motor unit number estimation. No similar relationship was found in CIDP patients in this study. This may reflect a different method for assessing peripheral motor unit function. However, Furtula *et al*[139] have shown similar diagnostic accuracy of MUNIX and intermittent stimulation-MUNE in patients with amyotrophic lateral sclerosis, but suggest that due to lower intra-rater variability MUNIX technique is superior. An alternative explanation for the difference between these findings and those reported by Drenthen *et al* may be different pathophysiological mechanisms of fatigue in subacute and chronic peripheral neuropathies.

Menotti and colleagues[96] suggest fatigue experienced by patients with CMT is related to high activation of cognitive association areas in the prefrontal cortex during routine motor tasks. This is considered to be a compensatory mechanism for reduced activation of peripheral motor units. This study demonstrates both lower number of functional motor units in patients with CIDP and CMT and differences in neural activation during a physical fatiguing task compared to controls. However, assessment of number of functioning peripheral motor units did not correlate significantly with experienced fatigue in this study. Whilst peripheral motor unit dysfunction may play a role in experienced fatigue, this does not appear to be the strongest predictor in our study, suggesting other factors are more important.

Several studies report fatigue negatively correlates with quality of life scores in patients with CIDP and CMT[55,72,83,90,92]. Similar relationships were found in this study. Both patient groups scored lower in the physical components subscale of the SF-36 compared to a population drawn from the general

UK population. In addition, experienced fatigue levels showed strong negative correlation with the physical functioning and role physical domains of the SF-36 in both patient groups. Experienced fatigue correlated with the mental components subscale of the SF-36 and depression and anxiety scores in patients with CIDP. Similar relationships were not observed in patients with CMT, however.

7.2 MUNIX technique

MUNIX was first proposed as a method of monitoring loss of motor units in patients with amyotrophic lateral sclerosis (ALS)[126,127]. Good intra- and inter-rater reproducibility of MUNIX and MUSIX has been demonstrated in healthy controls and in ALS[138,141–145]. More recently, a multicentre study has suggested training in the technique improves reliability for the purpose of large-scale clinical trials[146].

In this study, preliminary collection of normal MUNIX data was performed in healthy controls to assess test-retest intra-rater reliability. Care was taken to ensure standardisation of the technique across repeat tests. Standard electrode positioning was used, as detailed in section 4.2.3. Signals acquired at low force levels appear to disproportionately affect MUNIX values and these epochs were carefully reviewed for artefact, with epochs where SIP area was lower than CMAP area excluded[128,147]. In addition, SIP epochs with an area of <20mV/ms or ICMUC > 100 were excluded. demonstrated good test-retest reliability, which was similar to previous reports using this technique[130,138,141–145]. Good test-retest reliability was demonstrated, with maximum variance of 21.4%, which was similar to previous reports using this technique[130,138,141–145]. This suggests that the methods used for acquiring MUNIX and MUSIX values in this study are valid for assessing longitudinal changes in patients.

Whilst similar variance was observed in sum scores calculated from 3 muscles, variance in MUNIX and MUSIX scores from TA was, however, greater than some previous reports[130]. This may result from placement of the reference electrode, with other authors utilising placement over the patella tendon[135]. Alternatively, this may reflect the method used for placement of the active (recording) electrode on repeat testing. During this study care was taken to ensure optimal electrode positioning by repositioning the active electrode for multiple stimulations until maximum amplitude CMAP was acquired. Reference electrode position was maintained according to the anatomical landmarks outlined previously. Supporting this approach, original descriptions of the MUNIX technique found that accuracy is dependent upon acquiring a maximum amplitude CMAP[128,141]. It was therefore felt important to achieve a maximal CMAP for each test. However, other authors have suggested maintaining active electrode positioning according to anatomical landmarks improves test-retest reliability (unpublished observation). This can be achieved by placing electrodes at fixed

distances from anatomical landmarks or photographing electrode placement for reference on repeat testing. It is possible that adopting this technique may improve retest reliability for large muscles such as tibialis anterior, where there is potential for greater variation in electrode placement.

Muscle selection was based on previous studies published in similar patient groups[130,137,148]. Whilst small muscles of the feet, such as extensor digitorum brevis, can be studied with MUNIX technique, this was avoided in this study. The majority of patients with chronic peripheral neuropathies demonstrate significant if not complete atrophy of distal muscles in the lower limbs, preventing accurate calculation of MUNIX values.

MUNIX and MUSIX do not provide a measure of the actual number or size of motor units, instead providing an index value accurately related to these parameters. Therefore, whilst technically not a technique for motor unit estimation, MUNIX was developed to track motor unit function over time. It has the advantage of being quicker and easier to perform and better tolerated by patients than techniques for motor unit estimation (MUNE)[127,149]. Initial studies suggest MUNIX is strongly correlated and non-inferior to incremental stimulation and high-density MUNE techniques[136,139]. It has been suggested that a very recently developed technique, MScan MUNE is more accurate at differentiating between controls and patients with ALS than MUNIX or more traditional multi-point stimulation MUNE techniques[150]. It was also observed that MUNIX showed slightly higher correlation with CMAP amplitude than other MUNE techniques, leading some authors to suggest CMAP amplitude has a greater influence on MUNIX and MUSIX than number or size of functioning motor units[151,152]. However, in this study MUNIX sum scores showed greater correlation with clinical data in both CIDP and CMT patients than distally-evoked CMAP amplitude. This finding is consistent with previous reports in CIDP[130], and indicates that despite criticism outlined above, MUNIX provides additional clinically-relevant information regarding motor unit function not available from analysis of the CMAP.

7.3 Relationship between MUNIX and MUSIX sum scores and clinical assessments in patients with CIDP and CMT

MUNIX sum scores correlated with clinical measures of motor function and disability scores in patients with CIDP. MUNIX sum scores showed a significant correlation with both grip strength and muscle strength assessed by MRC sum scores. MUNIX assessments were performed in distal muscles of upper and lower limbs. MRC sum scores incorporated clinical assessments of proximal and distal muscle strength. Despite this, significant correlations were observed between the two assessments. They also correlated with self-reported disability level, with patients with lower MUNIX sum scores experiencing higher disability levels. These findings are similar to previous reports[130]. In addition,

there was significant correlation between MUNIX sum scores and clinical measures of sensory function, which has not previously been reported. In contrast, MUSIX sum scores correlated with patient age but none of the clinical assessments.

In CMT patients, MUNIX sum scores also showed significant correlation with clinical assessments of motor function (MRC muscle strength sum scores and grip strength), 10 metre timed walk and one disability scale (ONLS). These findings are consistent with previous reports in this patient population[153]. In addition, MUNIX sum scores also showed significant correlation with clinical measures of sensory function and patient age. MUSIX sum scores did not correlate with any of the clinical assessments.

As mentioned previously, MUNIX is used to assess the number of functioning motor units. It is therefore intuitive that patients with lower MUNIX values would present with greater weakness and higher perceived level of disability. Whilst not directly assessing sensory nerve function, the correlation between MUNIX sum scores and clinical measures of sensory function in both patient groups is likely to reflect overall disease severity. Such associations have been found previously with levels of sensory dysfunction in CIDP[154].

MUNIX sum scores showed greater correlation with clinical assessments and disability scores than MUSIX sum scores. This suggests that the number of functioning motor units, rather than chronic motor unit re-innervation, contributes more to motor impairments and overall disability in these patient groups. As mentioned earlier, some authors have criticised MUNIX as an index of CMAP amplitude rather than number of functioning peripheral motor units[152]. However, in this study MUNIX sum scores are demonstrated to correlate with markers of motor and sensory function and validated disability scales. Similar correlations were not seen with distal-evoked CMAP amplitudes in either CIDP or CMT patients, indicating that MUNIX provided clinically relevant information not available from analysis of the CMAP amplitude alone. In CMT patients, proximal-evoked CMAP amplitude and area showed similar correlations with clinical assessments and disability scores. In CIDP patients, stronger correlations were observed with MUNIX sum scores than any of the other electrophysiological parameters. Interestingly, of the other electrophysiological parameters studied, only F-wave persistence showed modest correlation with both muscle strength assessments and disability scores. In CIDP, there is usually predominant involvement of nerve roots and proximal nerve segments. F-waves can be used to indirectly assess proximal nerve segments, which may explain this finding.

7.4 Short-term changes in clinical and electrophysiological assessments after treatment in patients with chronic demyelinating polyneuropathy

Short-term improvement was seen in MUNIX sum scores comparing studies performed immediately before and 2 weeks following IVIg therapy. Conversely, no significant change in MUNIX sum scores was seen on repeat testing in a small group of untreated patients. In contrast to previous studies, no significant change in averaged proximally-evoked CMAP amplitude was detected. Small improvements were seen in averaged distal motor latency, amplitude of the distally-evoked CMAP, duration of the proximally-evoked CMAP and persistence of the f-wave.

Previous authors have suggested a minimum clinically relevant change in MUNIX sum scores of 50% in CIDP[130], based on the maximum variation seen in stable patients receiving IVIg therapy. The maximum change in MUNIX sum scores observed on repeat testing in controls was 21.4% and in untreated patients (albeit in a small cohort) was 32.9%. This suggests a smaller change in MUNIX sum scores may be clinically relevant. Whilst this study was not designed to determine minimum clinically significant changes, based on our findings in healthy controls and previous reports of variance in MUNIX values, a change in MUNIX values of 25% could be considered clinically significant. A small improvement in muscle strength was also observed and mean values in self-reported disability were higher following IVIg therapy, but failed to reach statistical significance. This was despite recruitment of clinically stable patients, reflecting the well-reported "wearing off effect" of IVIg therapy observed in CIDP[28,29].

Lower MUNIX values observed in CIDP have been attributed to chronic axonal loss[137]. Higher MUSIX values were observed in some CIDP patients compared with controls, suggesting motor unit remodelling related to chronic axonal loss in some of our patient cohort. However, the improvement in MUNIX values following IVIg appears too rapid to be explained by axonal regeneration or even nerve remyelination. Similar observations have been made regarding functional improvements following IVIg therapy in CIDP[155]. Nerve excitability studies in CIDP suggest disruption of nodal sodium-channel function and resulting hyperpolarisation may interfere with nerve conduction and cause block[156–159]. Elevated thresholds on nerve excitability studies have also been demonstrated in CIDP patients with and without conduction block compared to healthy controls, possibly related to changes in the paranodal region[160]. Although autoantibodies are only identified in a minority of patients[26], it is hypothesized that IVIg competes with functionally important autoantibodies, producing rapid although reversible improvement in nodal function[155,158]. It is possible the observed improvements in MUNIX values result from functional axonal recovery due to improved nodal function after IVIg therapy. Given that motor unit size relates to motor unit remodelling in association with gradual, chronic axonal loss, it is unsurprising that no significant change in MUSIX values was observed on repeat testing over a short interval. However, a large improvement in

MUSIX sum score was observed in a single patient. This patient had one of the lowest MUNIX values in the study cohort and although it is difficult to make hypotheses based on a single observation, it is possible the increase in MUSIX reflected presence of large motor units that were "non-functioning" at the time of assessment prior to IVIg therapy.

7.5 Short-term changes in fatigue levels and how these correlate with clinical and electrophysiological assessments after treatment in patients with chronic demyelinating polyneuropathy

Short-term fluctuations were explored in CIDP patients by performing assessments immediately before an IVIg infusion and on average 2 weeks later. This methodology was designed to explore the well-reported "wearing-off effect" of treatment observed with IVIg[28,29], which requires repeat infusions at 2 to 6 week intervals[32,33,35] to maintain therapeutic benefit in around 65% of patients[30]. This study design allowed recruitment of a reasonable sample size from a single centre, which would not have been possible if recruiting newly-diagnosed and treatment naïve patients, given the low incidence of CIDP. Owing to this methodology, it should be recognised that patients receiving IVIg therapy recruited to this study would have had their dosing regimen carefully titrated to achieve clinical stability.

It is therefore perhaps unsurprising that no significant changes were observed in assessments of disability or experienced fatigue levels on repeat assessment. In addition, no significant changes were seen in any of the parameters used to assess physiological fatigue. Previous authors have suggested assessment of physiological fatigue has modest test-retest reliability[82]. Large variation was observed on repeat assessment of SEMG activity from forearm flexor muscles, even in the untreated CIDP patient group, suggesting this methodology may not be suitable to track changes in neural activation over time.

As described above, a significant improvement in MUNIX sum scores was observed, along with small but statistically significant improvements in MRC muscle strength sum scores and 10-metre walk time. This finding suggests that MUNIX sum scores may be highly sensitive to fluctuations in peripheral motor unit function in CIDP. The fact that significant changes were observed in MUNIX sum scores without corresponding changes in fatigue levels again suggests there is no simple relationship between peripheral motor unit function and experienced fatigue in this patient group.

7.6 Limitations

Several limitations of this study need to be addressed. Firstly, only 15 patients with CMT were successfully recruited, whereas a sample size calculation had suggested a sample size of 17 was

required to detect whether correlation between different variables differs from 0. In addition, only 11 CMT patients completed the physiological fatigue component of the study. Therefore, whilst significant correlation between experienced fatigue levels and grip strength was demonstrated in this patient group, it cannot be concluded that the other variables under exploration did not correlate with experienced fatigue, only that this study was unable to find a correlation.

A second potential criticism is the use of the Rasch-built fatigue severity scale and the Rasch-built overall disability scale in patients with CMT. Whilst R-ODS has been used to assess disability level in CMT patients[153], these scales have been developed for use in patient with inflammatory polyneuropathies. MUNIX sum scores demonstrated significant correlation with ONLS, which has been validated in patients with CMT, but not R-ODS in this study. R-ODS was used in both groups to create uniformity and allow direct comparison between groups. However, the use of a scale more commonly used in CMT populations, such as the CMT neuropathy score (CMTNS)[52], may have allowed more detailed exploration of correlations between peripheral motor neuron function and disease severity. The CMTNS comprises scores based on severity of clinical symptoms, signs and electrophysiological parameters. On overall score reflecting disease severity is produced, which correlates well with other markers of disability[161]. In the CMTNS, sensory signs are assessed using a Rydell-Seiffer tuning fork and pinprick sensation. These modalities were assessed in this study as part of INCAT sensory sum scores and vibration thresholds. Similarly, motor symptoms in arms and legs were assessed as part of ONLS and R-ODS disability scores and motor signs were assessed with MRC sum scores. Finally, CMTNS incorporates ulnar nerve CMAP amplitudes and radial nerve SNAP amplitudes, again both assessed in this study. Therefore, whilst not using this specific scale, all individual components were assessed in this study and correlated with fatigue scores. In addition, some authors[162] suggest that the CMTNS is not linearly-weighted and differentiates poorly between CMT patients with moderate disease severity, suggesting this scale may not be ideally suited to correlation analysis.

A third potential limitation was the methodology for assessing physiological fatigue. Specifically, advanced techniques such as multi-electrode arrays that allow calculation of muscle-fibre conduction velocity, or twitch interpolation techniques, were not employed. Therefore, whilst SEMG recording was used as a surrogate marker for neural activation, the impact of local muscle fatigue on the SEMG signal is unknown. This means that it was not possible to determine contribution of central and peripheral fatigue on overall physiological fatigue[71,87]. However, the primary objective of this study was to explore the relationship between physiological fatigue and experienced fatigue in

patients with demyelinating disorders of the peripheral nervous system. This objective is still achievable without further exploring factors contributing to physiological fatigue.

Finally, control and patient groups were not age or sex-matched. Control data was collected to determine test-retest reliability of MUNIX and MUSIX sum scores. This data was also used to compare MUNIX data and physiological fatigue assessments between controls and patients. Controls were recruited on a convenience basis and were not age-matched to patients. Loss of motor units is recognised with advancing age[163], which may have influenced the differences observed between patients and controls. However, MUNIX sum scores showed no correlation with age in CIDP patients. A greater proportion of CIDP patients included in this study were male, consistent with previously published epidemiological data[23]. In contrast, only 26% of the CMT cohort were male. However, previous studies utilising MUNE techniques have found no significant difference between number of motor units in APB and ADM muscles in healthy male and female subjects[163]. In addition, biological sex did not appear to be a predictive factor of experienced fatigue level in this study. Overall, whilst there was heterogeneity in demographic data between controls and patient groups, these observations indicate that this limitation would not significantly impact the overall findings of the study.

8. Conclusion

Fatigue in patients with chronic demyelinating disorders of the peripheral nervous system appears to be negatively correlated with quality of life. Patients with both acquired and hereditary chronic demyelinating peripheral nerve disorders have reduced number of motor units assessed using MUNIX technique compared to control subjects. However, no clear relationship is found between number of functioning peripheral motor units and fatigue level experienced by patients. Depression and reduced grip strength were significant predictors of higher experienced fatigue levels in patients with chronic inflammatory demyelinating polyneuropathy. This suggests fatigue in this patient group is likely to be multifactorial, with physical and psychological contributors. However, the variables included in this study accounted for only 44.5 to 66.7% of the variation in experienced fatigue scores, suggesting that unidentified factors also contribute to experience of fatigue in this patient group. Differences in experienced fatigue levels and relationship with depression scores are observed between patients with CIDP and CMT1A, suggesting different factors are likely to contribute to fatigue in these patients.

Differences in neural activation during fatiguing forearm muscle contraction are demonstrated between controls and patients with chronic peripheral neuropathies. Patients with lower MUNIX values were found to have similar surface EMG activation patterns during maximal and submaximal

forearm muscle contraction. Based on this finding, it could be hypothesised that patients require a greater number of available motor units to be activated during routine motor tasks, which may contribute to fatigue. However, there are significant limitations with methods for assessing physiological fatigue and decline in force-generating capacity of forearm muscles over time did not correlate with experienced fatigue levels reported by patients.

Although no relationship is demonstrated with fatigue, MUNIX sum scores correlate with measures of motor function and disability levels in patients with CIDP and CMT1A. Correlations with sensory function are also demonstrated. In addition, improvement in MUNIX values are demonstrated two weeks after IVIg therapy in clinically-stable CIDP patients on long-term treatment. This new finding suggests a potential role for MUNIX sum scores as an objective marker of response to IVIg therapy. IVIg availability and cost issues are of paramount importance in CIDP treatment and the limitations of motor and disability scores as sole monitoring tools are real and concerning in long-term patients in whom placebo effects are not uncommon.

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Appendix 1: Data collection form devised for systematic literature review

First author_____
Title_____
Year of publication_____
Journal_____

Meets eligibility criteria Yes / No
If no, state which_____
Notes_____
_____
_____
_____
_____

Study design_____
Study aims_____
_____
Sample size_____
Age (mean)_____ Age (range)_____
Gender_____
Diagnostic criteria_____
Duration from diagnosis_____
Inclusion criteria_____
_____
_____
Exclusion criteria_____
_____
_____
Methods of assessment_____
_____
_____
_____

Statistical analysis
Appropriate distribution assessment made yes / no
Appropriate descriptive statistics used yes / no
Method for estimate of effect or difference_____
Method for assessment of correlation_____
Subgroup analysis_____

Definition of fatigue yes / no
Details_____
_____
_____

Method(s) of assessing fatigue yes / no
Details_____
_____
_____

Prevalence of fatigue in study population yes / no
Details_____
_____
_____

Correlations between fatigue and other assessments yes / no
Details_____
_____
_____

Insights into pathophysiology yes / no
Details_____
_____
_____

Impact of fatigue on quality of life measures yes / no
Details_____
_____
_____

Impact of treatment on fatigue yes / no
Details_____
_____
_____

Appendix 2: Patient information sheet

RESEARCH PATIENT INFORMATION SHEET

Study Title: Fatigue in Neuropathic Disorders (FIND Study)

You are being invited to take part in a research project that will be conducted at University Hospitals Birmingham NHS Trust and Aston University. Before you decide if you wish to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Please also take time to decide whether or not you wish to take part. If there is anything that is unclear or if you would like more information please do ask. Please feel free to discuss this information with others and thank you for reading.

WHO IS DOING THE RESEARCH?

This study is being conducted by a team based between the Queen Elizabeth Hospital, Birmingham and Aston University. This includes Professor Yusuf Rajabally, who is a Consultant Neurologist and Honorary Professor of Neurology at Aston University and Dr Andrew Lawley, who is a Specialist Registrar in Clinical Neurophysiology.

WHAT IS THE PURPOSE OF THE STUDY?

Fatigue is a common and often troubling symptom in nerve illnesses including Chronic Inflammatory Demyelinating Polyneuropathy (CIDP) and Charcot-Marie Tooth disease (CMT). However, it is currently unknown if fatigue might be related to loss of peripheral nerve cells in the arms and legs, or due to dysfunction of certain areas within the brain. Such information would help advance our understanding of what causes these symptoms. It could also help doctors to more accurately assess patients with these conditions who suffer from fatigue, and additionally may guide future research into different treatments for fatigue.

We are therefore conducting this study to assess if there is any relation between fatigue and either peripheral nerve loss or with dysfunction in certain brain areas. We are also interested to discover what effect your current treatment is having in relation to fatigue.

WHY HAVE I BEEN INVITED?

You are being invited to take part in a research study investigating the reason for fatigue experienced by patients with nerve disorders. You have been chosen as you suffer from CIDP, CMT or another Acquired Nerve illness.

WHAT WILL HAPPEN TO ME IF I TAKE PART?

Before being given this information sheet you will have had an initial meeting with one of the research team who will have described the outline and purpose of the study. You will be invited back for a second meeting at Aston Brain Centre within the 4 days before your regular treatment at Queen Elizabeth Hospital (either immunoglobulin or physiotherapy). At this point you will have a chance to ask any further questions you may have and if you are happy to participate we will ask you for written consent to proceed. At any subsequent appointments as part of this study, you will have the opportunity to ask questions, and at any point you may withdraw consent and stop participation. If you refuse consent or withdraw from the study, this will have no impact whatsoever on your usual clinical care.

As is common practice, your General Practitioner would be informed that you are participating in a research study and the reasons for this research study.

During your visit to Aston Brain Centre at Aston University you will have:

1. *A full examination of your neurological system, which will assess your strength, sensation, reflexes, balance, coordination, gait (as at each one of your usual visits). As part of this examination, several questionnaires will be completed to evaluate fatigue, function, mood and quality of life. This should take around 45 minutes.*

2. *A set of electrical tests (nerve conduction studies). During these tests, you may feel brief tingling sensations in your hands and legs, however these should not be painful. A new simple technique called MUNIX will also record your voluntary muscle contractions. However, you will NOT at any stage have a needle (EMG) test. This should take up to 40 minutes.*

Either on the same day, or on a separate visit within a few days, you will have the following test in the Aston Brain Centre at Aston University:

3. *A non-invasive imaging technique called functional Magnetic Resonance Imaging (fMRI). This will be performed by technicians experienced in performing this technique based at Aston University. This uses a magnetic field to produce high-quality brain images without the use of harmful radiation. During the scan, you will be asked to perform simple mental arithmetic. Once the scanning process begins there will be a loud "knocking" noise from the magnetic coils changing pulse direction. This is normal and you will be given ear plugs to keep the noise to a minimum. This should take 60 minutes. You will be given a safety button during the MRI, which can be pressed to stop the test at anytime.*

Although these tests are completely non-invasive, widely used and very safe, there are some circumstances when you should not have them, for example, if you have any metal implanted in your body. At the start of the study we will check if there any reasons that you cannot undergo a test. If you have claustrophobia, you will not undergo the MRI part of the study.

You will undergo these same steps again (1, 2 and 3), 10-15 days after immunoglobulin treatment if you have CIDP, or 3 to 5 weeks after start of your physiotherapy programme (if you have CMT or another Acquired Nerve illness), with further visitation to the Aston Brain Centre at Aston University. All travel costs will be reimbursed at a standard rate of 40p/mile.

DO I HAVE TO TAKE PART?

Your participation is entirely voluntary and the care you receive will not be affected in any way by choosing not to. If you do decide to take part in the study you will be free to withdraw at any time and for any reason. If you chose to leave the study you can also choose to have your data removed if you notify the researcher by e-mail, telephone or in person.

ARE THERE POSSIBLE BENEFITS OF TAKING PART?

You will be given detailed feedback/information regarding your levels of fatigue and quality of life, through assessments which are not a part of routine clinical care. This may help you to monitor your response to your normal treatment.

ARE THERE ANY DISADVANTAGES TO TAKING PART IN THE STUDY?

Some of the tasks you will be asked to perform are designed to cause temporary fatigue. You may therefore temporarily feel more tired than usual after the test for a short time. There are no other disadvantages to taking part in this study. The assessments used, including the physical examination, electrical nerve and MRI testing are all extremely safe and very commonly used in routine clinical care. You will not receive any new treatments as part of this study.

WILL MY TAKING PART IN THE STUDY BE KEPT CONFIDENTIAL?

Your participation in this study will be kept confidential, and all data will be handled in accordance with the Data Protection Act. During the study all paper records of your results will be stored in a secure location at Aston University and labelled with a unique patient identification code known only to the research team.

At the end of the study, the consent forms, study documentation and research data will be sent to Aston University for archiving. All data will be stored in a secure location at Aston University for a maximum of 6 years. The research data will be anonymised (i.e. no one will be able to link you to your results).

Information collected at each study visit will be used only for the purposes of the research outlined earlier in this information sheet. In the unlikely event that your MRI scan detects an unexpected finding, this will be reviewed by a Consultant who will decide if the scan needs to be looked at by a Radiologist. Any unexpected findings will be communicated to you by the research team. If you require further investigation or treatment, we may then ask your GP to refer you to an appropriate medical professional.

WHAT WILL HAPPEN TO THE RESULTS OF THE RESEARCH STUDY?

The results will be written into a study report, and may also be published in a medical journal and/or presented at a medical conference. All data included in any report will be strictly anonymous, meaning anybody reading or listening to the report would be unable to identify you. None of your personal information would be included in the study report. If you wish to obtain a summary of the research findings please leave your contact details with the researchers and these will be sent to you.

WHO HAS REVIEWED THE STUDY?

This study has been reviewed and given a favourable opinion by the NHS Health Research Authority, whose role is to ensure that all research undertaken in England protects and promotes the interests of patients.

WHAT IF THERE IS A PROBLEM?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (contact details below). If you have any questions regarding your rights as a research participant or complaints about the way the study has been conducted, please contact the Secretary of the Aston University Research Ethics Committee: [redacted] Aston University, Aston Triangle, Birmingham, B4 7ET; [redacted]: [redacted]

WHERE CAN I FIND INDEPENDENT INFORMATION ABOUT TAKING PART IN RESEARCH?

You can contact the NHS Patient Advisory Liaison Service (PALS) University Hospitals Birmingham NHS Trust if you would like advice on taking part in research. Email: PALS@uhb.nhs.uk or Telephone: [redacted]

WHAT HAPPENS NEXT?

You will be contacted by a member of the research team and invited for a further meeting to confirm if you wish to take part in this study.

FOR FURTHER INFORMATION PLEASE CONTACT:

Chief investigator	Prof Yusuf Rajabally Neurology department, Queen Elizabeth Hospital Birmingham, Mindelsohn Way, Edgbaston, Birmingham B15 2GW [redacted]
Researcher	Dr Andrew Lawley Clinical Neurophysiology department, Queen Elizabeth Hospital Birmingham, Mindelsohn Way, Edgbaston, Birmingham B15 2GW [redacted]

Thank you for taking time to read this information sheet.

Appendix 3: Study consent form

Study Number: 162-2016-YR

Participant Identification Number:

Chief Investigator: Prof Y Rajabally

CONSENT FORM

Title of Project: **Fatigue in Neuropathic Disorders (FIND) Study** Please *initial* box

1. I confirm that I have read the information sheet dated 18/05/17 (version 6) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by the study sponsor, individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I understand that the information collected about me will be used to support other research in the future, and may be shared anonymously with other researchers. ☐

5. I agree to my General Practitioner being informed of my participation in the study and informed of any unexpected finding that may need further investigation. ☐

6. I confirm that I have understood what is involved in the MRI scan. ☐

7. I agree to take part in the above study. ☐

Name of Participant	Date	Signature

Name of Person taking consent	Date	Signature

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes.

Appendix 4: GP information sheet

Queen Elizabeth Hospital Birmingham
Mindelsohn Way
Edgbaston
Birmingham
B15 2GW
[redacted]

Date

Dear Dr

Re: Your patient

Study title: Fatigue in Neuropathic Disorders (FIND Study).

Your patient has recently agreed to participate in the above study which is taking place at the Aston Brain Centre, Aston University. This is a non-interventional study and no further action is required from yourself. This letter is for information only.

Details of the study are outlined in the enclosed patient information sheet. Information collected as part of this study will be used for research purposes only. However, part of this study uses fMRI and it is possible an unexpected finding may be detected requiring further investigation or treatment. In this unlikely event, you may be contacted by one of the research team to request assistance referring the patient to an appropriate specialist or for further investigation.

If you require any further information please do not hesitate to contact me on the above number, or contact a member of our research team via e-mail [redacted]

Yours sincerely

Yusuf A. Rajabally,

Consultant Neurologist & Honorary Professor of Neurology.

Enc

Patient Information Sheet

Appendix 5: Electrophysiological studies in patients with CIDP and CMT

Motor nerve conduction studies were performed in 4 nerves unilaterally (median, ulnar, peroneal and tibial), with averaged values for each patient calculated from all the nerves from which responses could be recorded. Motor conduction velocities were calculated from distal nerve segments. Proximal-evoked potential refers to the most proximal point from which a CMAP could be elicited (excluding Erb's point due to concerns regarding submaximal nerve stimulation). Sensory nerve conduction studies were performed in 4 nerves unilaterally (sural, median, ulnar and superficial radial), with averaged values calculated in the same way. Values are presented as median values (IQR). Only parameters that could be recorded in at least 10 patients are presented (F-waves were only present in 7 of the 15 CMT patients).

	CIDP	**CMT**
DML (ms)	5.7 *(4.7-6.8)*	8.6 *(8.1-10.7)*
dCMAP amplitude (mV)	5.4 *(2.7-5.9)*	4.0 *(3.1-4.4)*
dCMAP area (mV*ms)	14.9 *(8.6-17.7)*	14.0 *(11.4-16.5)*
dCMAP duration (ms)	7.3 *(6.0-8.9)*	7.7 *(7.0-10.0)*
pCMAP amplitude (mV)	3.4 *(1.6-4.7)*	2.3 *(1.7-3.3)*
pCMAP area (mV*ms)	11.3 *(5.4-17.0)*	8.9 *(7.7-12.7)*
pCMAP duration (ms)	7.7 *(6.8-10.2)*	9.2 *(8.2-12.7)*
Motor NCV (m/s)	38.9 *(33.0-43.1)*	19.9 *(17.9-20.7)*
F-wave latency (ms)	41.0 *(39.2-49.0)*	-
F-wave persistence (%)	56.7 *(38.3-74.2)*	-
SNAP amplitude (µV)	4.7 *(3.2-7.1)*	5.6 *(2.6-5.9)*
Sensory NCV (m/s)	42.5 *(37.9-45.9)*	21.6 *(17.7-28.1*)

Appendix 6: Correlation analysis between electrophysiological parameters and clinical assessments

Tables demonstrating correlation analysis between electrophysiological parameters and clinical assessments in patients with CIDP (first table) and CMT (second table). Motor nerve conduction studies were performed in 4 nerves unilaterally (median, ulnar, peroneal and tibial), with averaged values for each patient calculated from all the nerves from which responses could be recorded. Sensory nerve conduction studies were performed in 4 nerves unilaterally (sural, median, ulnar and superficial radial), with averaged values calculated in the same way. Correlation analysis was performed using Spearman Rank correlation. Results are presented as r values (95% confidence intervals). Significant correlations (*p<0.05*) are highlighted in **bold**.

CIDP patients (*n=26*)

	MRC muscle strength	Grip strength	INCAT sensory sumscore	Vibration threshold	10m timed walk test	R-ODS	ONLS
DML	-0.261 *(-0.65-0.16)*	-0.195 *(-0.51-0.17)*	0.323 *(-0.08-0.66)*	**-0.406** ***(-0.72--0.01)***	0.140 *(-0.27-0.55)*	-0.260 *(-0.53-0.17)*	0.111 *(-0.37-0.50)*
dCMAP amplitude	0.361 *(-0.10-0.73)*	**0.456** ***(-0.01-0.75)***	**-0.490** ***(-0.75--0.10)***	**0.560** ***(0.18-0.79)***	-0.277 *(-0.66-0.18)*	0.284 *(-0.14-0.60)*	-0.277 *(-0.59-0.19)*
dCMAP area	0.346 *(-0.10-0.67)*	0.353 *(-0.14-0.72)*	**-0.446** ***(-0.69--0.06)***	**0.520** ***(0.10-0.77)***	-0.252 *(-0.62-0.24)*	0.337 *(-0.10-0.66)*	-0.303 *(-0.59-0.16)*
dCMAP duration	0.045 *(-0.39-0.45)*	0.042 *(-0.36-0.47)*	0.189 *(-0.29-0.60)*	-0.269 *(-0.65-0.21)*	-0.217 *(-0.64-0.25)*	0.031 *(-0.34-0.44)*	-0.071 *(-0.53-0.35)*
pCMAP amplitude	0.231 *(-0.24-0.64)*	0.383 *(-0.12-0.73)*	-0.334 *(-0.68-0.11)*	**0.409** ***(-0.09-0.76)***	-0.263 *(-0.66-0.26)*	0.193 *(-0.30-0.56)*	-0.276 *(-0.60-0.20)*
pCMAP area	0.239 *(-0.21-0.62)*	0.345 *(-0.16-0.73)*	-0.257 *(-0.57-0.16)*	0.330 *(-0.16-0.69)*	-0.246 *(-0.63-0.26)*	0.227 *(-0.25-0.61)*	-0.291 *(-0.60-0.17)*
pCMAP duration	0.168 *(-0.28-0.54)*	-0.029 *(-0.43-0.35)*	0.210 *(-0.22-0.58)*	-0.268 *(-0.63-0.14)*	-0.161 *(-0.55-0.26)*	0.282 *(-0.13-0.62)*	-0.218 *(-0.58-0.18)*
Motor NCV	0.099 *(-0.34-0.53)*	-0.047 *(-0.37-0.48)*	-0.150 *(-0.54-0.31)*	0.167 *(-0.28-0.56)*	-0.083 *(-0.51-0.36)*	0.518 *(-0.31-0.58)*	-0.246 *(-0.64-0.17)*
F-wave latency	0.020 *(-0.44-0.43)*	-0.262 *(-0.71-0.19)*	0.079 *(-0.39-0.50)*	0.005 *(-0.49-0.46)*	0.116 *(-0.37-0.60)*	0.179 *(-0.32-0.62)*	0.108 *(-0.41-0.59)*
F-wave persistence	**0.478** ***(-0.03-0.80)***	**0.498** ***(0.09-0.80)***	-0.346 *(-0.69-0.10)*	**0.496** ***(-0.11-0.78)***	**-0.650** ***(-0.86--0.26)***	**0.440** ***(0.06-0.68)***	**-0.638** ***(-0.84--0.27)***
SNAP amplitude	0.388 *(-0.06-0.72)*	-0.031 *(-0.54-0.41)*	-0.096 *(-0.57-0.38)*	0.102 *(-0.35-0.52)*	-0.005 *(-0.43-0.50)*	0.269 *(-0.14-0.62)*	-0.170 *(-0.58-0.31)*
Sensory NCV	**0.428** ***(-0.02-0.77)***	0.169 *(-0.29-0.58)*	-0.213 *(-0.60-0.26)*	0.252 *(-0.29-0.70)*	-0.334 *(-0.69-0.11)*	0.359 *(-0.15-0.76)*	**-0.436** ***(-0.79-0.03)***

CMT patients (*n=14, 1 patient did not tolerate NCS*)

	MRC muscle strength	Grip strength	INCAT sensory sumscore	Vibration threshold	10m timed walk test	R-ODS	ONLS
DML	-0.498 *(-0.84-0.09)*	-0.437 *(-0.80-0.09)*	0.348 *(-0.23-0.77)*	**-0.611** ***(-0.89--0.05)***	0.336 *(-0.40-0.77)*	-0.151 *(-0.67-0.36)*	**0.540** ***(-0.07-0.96)***
dCMAP amplitude	**0.627** ***(-0.07-0.90)***	**0.538** ***(-0.03-0.89)***	**-0.538** ***(-0.90-0.05)***	0.416 *(-0.28-0.86)*	-0.111 *(-0.70-0.51)*	0.501 *(-0.12-0.86)*	**-0.680** ***(-0.93-0.15)***
dCMAP area	0.400 *(-0.27-0.88)*	0.355 *(-0.30-0.86)*	-0.390 *(-0.78-0.24)*	0.102 *(-0.53-0.79)*	0.096 *(-0.59-0.73)*	0.451 *(-0.15-0.79)*	-0.434 *(-0.87-0.16)*
dCMAP duration	-0.462 *(-0.94-0.15)*	**-0.542** ***(-0.90--0.01)***	0.269 *(-0.42-0.82)*	-0.522 *(-0.88-0.05)*	0.279 *(-0.39-0.72)*	-0.021 *(-0.73-0.58)*	0.352 *(-0.26-0.86)*
pCMAP amplitude	**0.766** ***(0.32-0.96)***	**0.668** ***(0.20-0.89)***	**-0.757** ***(-0.95-0.41)***	**0.721** ***(0.27-0.95)***	**-0.581** ***(-0.93-0.10)***	**0.542** ***(0.04-0.80)***	**-0.707** ***(-0.91-0.30)***
pCMAP area	**0.768** ***(0.29-0.98)***	**0.637** ***(0.17-0.88)***	**-0.717** ***(-0.94--0.32)***	**0.613** ***(0.04-0.92)***	-0.488 *(-0.90-0.21)*	**0.560** ***(0.05-0.87)***	**-0.666** ***(-0.88--0.25)***
pCMAP duration	**-0.542** ***(-0.96-0.11)***	**-0.606** ***(-0.90--0.01)***	0.404 *(-0.19-0.84)*	**-0.562** ***(-0.86--0.02)***	0.469 *(-0.18-0.86)*	-0.189 *(-0.73-0.44)*	0.440 *(-0.10-0.81)*
Motor NCV	0.398 *(-0.27-0.93)*	**0.697** ***(0.27-0.89)***	-0.485 *(-0.90-0.12)*	**0.619** ***(0.13-0.89)***	**-0.592** ***(-0.93--0.06)***	0.100 *(-0.53-0.66)*	-0.341 *(-0.76-0.24)*
F-wave latency	-	-	-	-	-	-	-
F-wave persistence	-	-	-	-	-	-	-
SNAP amplitude	0.046 *(-0.68-0.78)*	**-0.762** ***(-0.98--0.04)***	0.349 *(-0.36-0.77)*	-0.067 *(-0.60-0.18)*	-0.310 *(-0.89-0.60)*	-0.573 *(-0.96-0.01)*	0.344 *(-0.34-0.79)*
Sensory NCV	0.287 *(-0.51-0.88)*	-0.134 *(-0.81-0.61)*	0.116 *(-0.82-0.79)*	0.177 *(-0.64-0.83)*	-0.261 *(-0.89-0.65)*	-0.134 *(-0.76-0.65)*	-0.219 *(-0.83-0.68)*

Appendix 7: Final version of manuscript submitted for publication in Clinical Neurophysiology journal

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Motor unit number index (MUNIX) in chronic inflammatory demyelinating polyneuropathy: a potential role in monitoring response to intravenous immunoglobulins

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