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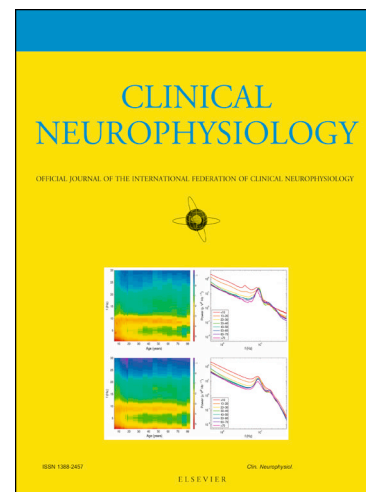
Motor unit remodelling in multifocal motor neuropathy: the importance of axonal loss

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ABSTRACT

Objective: To estimate the degree of axonal loss in patients diagnosed with multifocal motor neuropathy (MMN) using a novel assessment of motor unit numbers and size. *Methods:* Automated motor unit number estimation using a compound muscle action potential (CMAP) scan was undertaken in median nerves with conduction block. Results were compared with 30 age-matched healthy controls. *Results:* Compared with healthy controls, MMN patients had fewer motor units (MMN: 33 ± 11 vs HC: 93 ± 36 [mean $\pm$ SD]; $p<0.0001$) and larger 'size of the largest unit' (MMN: 1.2 ± 0.5mV vs HC: 0.4 ± 0.1mV; $p<0.0001$), despite having normal distal CMAP amplitudes (MMN: 7.6 ± 1.8mV vs HC: 8.7 ± 2.5mV; $p=0.24$). *Conclusions*: MMN is associated with marked axonal loss which may be masked by striking re-innervation resulting in preservation of distal CMAP amplitudes. *Significance:* Assessment of motor unit properties should be incorporated into assessment of disease progression in MMN, given that nerve conduction studies are insensitive to motor unit remodelling.

Keywords: Neurophysiology; neuromuscular disease; autoimmune diseases; multifocal motor neuropathy; axonal degeneration.

Highlights

- Axonal degeneration is an integral part of the disease process in MMN.

- Axonal loss in MMN may be masked by prominent re-innervation.

- Nerve conduction studies are not sensitive to motor unit remodelling in MMN.

1. INTRODUCTION

Progressive axonal degeneration is a prominent feature of multifocal motor neuropathy (MMN), differentiating it from the other immune-mediated neuropathies (Vlam et al., 2011). The aim of immunomodulatory treatment with intravenous immunoglobulin (IVIg) has been to reduce the rate of axonal loss as this is the most important determinant of permanent weakness and disability (Van Asseldonk et al., 2006; Vucic et al., 2004). It has been suggested that the effectiveness of IVIg may decline over time, correlating with the development of axonal degeneration (Terenghi et al., 2004).

Unfortunately, mechanisms of conduction block and axonal degeneration in MMN remain poorly understood and there remains debate as to whether MMN is primarily a demyelinating or axonal disorder (Kiernan et al., 2002). Anti-GM_1 IgM is present in approximately 50% of patients with MMN with GM_1 enriched in the nodal and paranodal regions (Vlam et al., 2011; Willison and Yuki, 2002). It has recently been suggested that disease processes targeting these regions represent a distinct group of neuropathies characterised by a continuum from conduction block to axonal degeneration, a concept which may be pivotal in understanding the pathophysiology of conditions such as MMN (Uncini and Kuwabara, 2015).

Axonal loss is typically only identified late in the disease process and only once the compound muscle action potential (CMAP) amplitude has reduced on nerve conduction studies (NCS). The present study was prompted by the frustration at the lack of an objective method to monitor treatment response and disease progression in MMN patients. As such, a novel technique was utilised to quantify the degree of axonal loss and compensatory reinnervation in MMN as a potential tool for disease monitoring.

2. METHODS

2.1. Patient cohort and selection criteria

Consecutive patients fulfilling European Federation of Neurological Societies/Peripheral Nerve Society criteria for MMN (definite or probable) were prospectively recruited between April 2015 and December 2016. Cases of 'possible' MMN were excluded (Joint Task Force of the EFNS and the PNS, 2010). Results were compared with 30 healthy control subjects who were screened based on their medical history. All participants gave written informed consent to participate in the study. The study was approved by the Sydney Local Health District Ethics Review Committee (Royal Prince Alfred Hospital).

2.2. Assessment Tools

Muscle strength was assessed using Medical Research Council (MRC) grading by a single investigator (N.G.) in 15 muscle groups bilaterally (shoulder abduction, elbow flexion, elbow extension, wrist flexion, wrist extension, finger extension, finger flexion, first dorsal interosseus, abductor digiti minimi, thumb abduction, hip flexion, knee flexion, knee extension, ankle dorsiflexion and ankle plantarflexion). The MRC grades were summed to calculate an expanded MRC sum-score (maximum score 150). Serum from each patient was tested for anti-GM1 IgM antibodies as previously described (Yuki et al., 1997).

2.3. Nerve conduction studies

All patients underwent NCS at the time of recruitment by a single neurologist/neurophysiologist (N.G.) to ensure criteria for MMN were fulfilled and to identify median nerves with conduction block within the forearm segment. All patients had motor NCS of the median nerves bilaterally with stimulation at the wrist and elbow and recording over abductor pollicis brevis. In addition, patients underwent ulnar, common

peroneal and tibial motor studies and median, ulnar and radial sensory studies unilaterally.

Conduction block was defined as negative peak CMAP area reduction of at least 30% on

stimulation of wrist versus elbow with duration increase of 30% or less, or CMAP area

reduction of 50% when CMAP duration increase was greater than 30% (Joint Task Force of

the EFNS and the PNS, 2010).

2.4. Assessment of motor unit properties using a novel CMAP scan

CMAP scans (Bostock, 2016) were recorded using the TRONDNF protocol within

QTRACW software (© Institute of Neurology, University College London, UK). All

recordings were undertaken by stimulating the median nerve at the wrist with a 0.2 ms wide

stimulus using an isolated constant current stimulator (DS5; Digitimer, Welwyn Garden City,

UK). The active recording electrode was placed over the motor point of abductor pollicis

brevis and reference electrode over the proximal phalanx. The stimulus strength was

manually increased until the supramaximal CMAP was reached. Following this, stimuli were

delivered twice per second with each stimulus intensity being 99.8% of the preceding

stimulus until no response was recordable. 20 pre- and post-scan sweeps were recorded to

assess variability of supramaximal (*MScan Peak*) and baseline responses respectively with

each scan taking approximately 5 minutes in total.

The *MScanFit* program contained within the *QTRACW* software was used to derive a motor

unit number estimate (*MUNE*), and *Size of the largest unit* (in *mV* and as a percentage of the

MScan Peak). The program uses a mathematical model to simulate the recorded scan. The

modelled scan is then 'fitted' to the recorded scan by making sequential adjustments until the

discrepancy between the two scans is minimised as previously described (Bostock, 2016).

Median nerves with forearm conduction block were selected for the current study as this is a frequent site of involvement in typical MMN and the forearm segment is not a typical site of entrapment neuropathy. Median nerves with distal motor latency prolongation on NCS (>4ms) were excluded due to the possibility of entrapment neuropathy at the wrist. In patients with bilateral median nerve forearm CB, only results from one side were used in the analysis.

2.5. Statistics

An independent samples t-test was used to compare age, *MScan Peak* and *MUNE* between MMN patients and healthy controls. Results of the *Size of the largest unit* were not normally distributed in MMN patients. Hence, the Mann-Whitney U test was used. Pearson correlation coefficient was used to investigate the relationship between *MUNE*, *MScan Peak* and age followed by linear regression analysis. Significance was defined by a p-value of <0.05. Results are presented as mean ± standard deviation. Statistical analysis and graph construction was performed using Graph Pad Prism 7 and IBM SPSS Statistics (Version 22).

3. RESULTS

A total of 12 patients fulfilling EFNS/PNS criteria for definite or probable MMN were recruited. All MMN patients were established and controlled on maintenance IVIg therapy. Ten patients were identified with median nerve motor conduction block in the forearm. Two patients had high thresholds limiting supramaximal stimulation and hence accurate motor unit number estimation could not be calculated. The remaining eight patients underwent median nerve CMAP scans on the ipsilateral side of conduction block. Clinical and laboratory features of the patients were typical of MMN (Table 1). Results were compared with recordings from 30 healthy controls [13:17 (M:F)] who were of similar age (MMN: 54.6 ±

13.6; HC: 55.1 ± 16.5; p = ns). 63% of MMN patients were positive for anti-GM_1 IgM. The mean IVIg dose was 0.9 ± 0.4 grams/kg/4-weeks.

3.1. Nerve Conduction Studies

Median nerve conduction data for MMN patients (summarised in Table 2) confirmed that all patients had well preserved median nerve distal CMAP amplitudes > 5mV, with a mean distal CMAP of 8.05 ± 1.9 mV (range $6.3 – 11.2$) and mean area reduction $54\% \pm 22$ (range $30 – 93\%$). The precise site of conduction block was unable to be accurately localised in the majority of patients due to the deep location of the median nerve within the forearm.

3.2. Results of CMAP Scan

Compared with healthy controls, there was no difference in the maximal response (*MScan Peak*) in the MMN group (MMN: 7.6 ± 1.8 mV vs HC: 8.7 ± 2.5 mV; p=0.24) (Figure 1A). However, MMN patients had a markedly reduced number of units having only a third of the number of units that were seen in HC subjects (MMN: 33 ± 11 vs HC: 93 ± 36; p<0.0001) (Figure 1B, Tables 2 and 3). In addition, MMN patients exhibited a strikingly larger size of the largest unit compared with healthy controls (MMN: 1.2 ± 0.5 mV vs HC: 0.4 ± 0.1 mV; p<0.0001) (Figure 1C, D) demonstrating that despite substantial axonal loss, significant reinnervation had occurred maintaining a normal distal CMAP. An example of the CMAP scan in an MMN subject and a healthy control are presented in Figure 2.

3.3. Correlations between clinical features and neurophysiology

There was a positive correlation between *MUNE* and *MScan Peak* in the HC group (R = 0.69; p < 0.0005) and a negative correlation between age and *MUNE* (R = - 0.65; p < 0.0005). These correlations were lost in the MMN group (Figure 3). No correlation was found

between *MUNE* or *Size of largest unit* and clinical features such as disease duration or expanded MRC sum-score in the MMN patients.

4. DISCUSSION

The present study highlights the marked reduction in the number of motor units innervating a muscle affected by conduction block in MMN despite preservation of the distal CMAP amplitude. The degree of axonal loss suggests that the axon may be a direct target in MMN. Furthermore, it demonstrates the striking collateral reinnervation by surviving motor units compensating for and potentially masking axon loss that has occurred.

The immune basis for MMN is supported by the association with anti-GM_1 IgM antibody and response to immunomodulatory therapy. GM_1 is enriched in the nodal and paranodal regions (Willison and Yuki, 2002) and gangliosides play an important role in the maintenance and stabilisation of the paranode and ion channel clustering (Susuki et al., 2007). Conduction block may result from a variety of processes such as lengthening of the node, detachment of myelin from the paranode, sodium channel dysfunction and/or abnormalities in axolemma polarisation (Barnett et al., 2016; Uncini and Kuwabara, 2015) and in theory, pathology at either the node and/or the paranode could lead to conduction failure and axonal degeneration. Axonal degeneration has been shown to be strongly associated with conduction block supporting the notion that the same pathophysiology may account for both processes (Vucic et al., 2007; Van Asseldonk et al., 2006) and the continuum from conduction block to axonal degeneration with disease processes affecting the nodal/paranodal regions has recently become better recognised as a distinct entity (Uncini and Kuwabara, 2015).

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Only a small number of conflicting reports have described the histopathology of motor fibres in MMN (Vlam et al., 2011). While one study found predominantly axonal pathology (Taylor et al., 2004), two others reported evidence of demyelination along with onion bulb formations (Corbo et al., 1997; Kaji et al., 1993). In two histopathology studies regenerative fibre clusters were reported in MMN (Corbo et al., 1997; Taylor et al., 2004), changes which were not seen in biopsies taken from patients with motor neuron disease (Corbo et al., 1997), highlighting that motor nerve regeneration is a prominent feature of MMN.

Axonal degeneration is a key area of interest in MMN as it is the major determinant of permanent weakness and disability (Cats et al., 2010; Van Asseldonk et al., 2006). Traditionally, axon loss has been heralded by a reduction in the distal CMAP amplitude (Cats et al., 2010; Terenghi et al., 2004). The present study highlights that axonal loss may be prominent before a reduction in CMAP amplitude reinforcing the notion that nerve conduction studies may be suboptimal in monitoring disease progression and treatment response. In one study, needle electromyography (EMG) was shown to be more sensitive in detecting axonal loss than assessment of distal CMAP amplitude with EMG abnormalities demonstrated in patients with short disease duration. Furthermore, in the same study, 90% of patients had EMG signs of reinnervation (Van Asseldonk et al., 2006). The results of the present study are consistent with these findings. However, while EMG should show a reduction in motor units and increase in size of units, it is not a practical tool for long-term monitoring.

IVIg remains the mainstay of therapy for MMN with studies demonstrating its efficacy in improving muscle strength and neurophysiological parameters, although in some series this has been followed by a slow decline in CMAP amplitudes and muscle scores (Terenghi et al.,

2004; Van den Berg-Vos et al., 2002). It has been reported that the effect of IVIg often declines over time and progressive wasting and weakness may ensue despite increasing doses of IVIg (Cats et al., 2010; Terenghi et al., 2004). It has however, been demonstrated that early institution of higher doses of IVIg may prevent this decline by promoting reinnervation (Vucic et al., 2004). The general approach in clinical practice for patients established on IVIg, is to escalate treatment by increasing IVIg dosing or frequency only once a patient clinically deteriorates (Burrell et al., 2011). However, once distal CMAP reductions occur and disability ensues, it is possible that the capacity for repair has largely been exhausted and hence escalating treatment during this stage of the disease may be inadequate.

The CMAP Scan is a new technique for MUNE estimation which is practical and fast to perform, taking only approximately 5 minutes. MUNE values, particularly when small, are accurately estimated with a mean absolute error of less than 7% (Bostock, 2016). The CMAP Scan method has been shown to have good reproducibility in ALS with inter and intra-rater reproducibility exceeding that of other methods of motor unit number estimation, including multiple point stimulation MUNE (MPS) and motor unit number index (MUNIX) (Jacobsen, 2017).

MPS and MUNIX have been used to demonstrate axonal loss and reinnervation in patients with CIDP, although 25% of CIDP patients in this series had a severe reduction in CMAP amplitude (Paramanathan et al., 2015). Furthermore, multipoint incremental MUNE has shown that spinal muscular atrophy (SMA) is associated with a reduction in CMAP and MUNE and increase in measures of unit size with a lower MUNE correlating with a lower function score (Gawel et al., 2015). The lack of correlations between CMAP Scan features and clinical features in MMN patients in the current study may relate to the highly individual

and variable course of MMN and the effects of IVIg on the natural history of the disorder. Furthermore, we only studied a single muscle which may not be reflective of the overall disease process given the patchy nature of MMN, although all patients had ipsilateral median nerve involvement. The current CMAP Scan method has been most extensively studied in the APB muscle. Small muscles are best suited to the CMAP Scan to limit movement artefact and ensure patient tolerability. Ulnar-innervated muscles such as abductor digiti minimi and first dorsal interosseous are further muscles that could be studied and incorporated into a neurophysiological score which may be more sensitive for long-term monitoring.

Limitations of the study include the relatively small number of patients given the rare nature of the conditions studied. Further studies of motor unit properties in nerves not affected by conduction block may provide information about whether axonal degeneration is a direct consequence of conduction block or whether it may be a more generalised process in MMN.

5. CONCLUSIONS

MMN is associated with marked axonal degeneration to which NCS are insensitive due to the process of motor unit remodelling and reinnervation. Once a CMAP reduction has occurred, axonal loss is severe and compensatory reinnervation may no longer be adequate to compensate. Hence aims of treatment in MMN should be to address axonal loss before a significant decline in CMAP amplitude occurs. The CMAP Scan used in the present study may be a practical way to monitor disease progression and treatment response in MMN.

CONFLICTS OF INTEREST
Hugh Bostock receives from UCL a share of the royalties for sales of his Qtrac software. None of the other authors have potential conflicts of interest to be disclosed.

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Table 1. Clinical Features of MMN Patients.

	Sex	Age	Age at onset (years)	Disease duration (years)	E-MRC SS (/150)	GM-1 IgM Antibody
Patient 1	F	70	47	23	117	No
Patient 2	M	45	39	6	141	No
Patient 3	F	53	33	17	126	Yes
Patient 4	M	71	66	5	122	Yes
Patient 5	M	35	32	3	138	No
Patient 6	F	66	43	23	139	Yes
Patient 7	M	41	31	10	143	Yes
Patient 8	F	56	50	6	142	Yes
Total	4:4 (M:F)	54.6 ± 13.6 (35 – 71)*	42.6 ± 11.8 (31 – 66)*	11.6 ± 8.2 (3 – 23)*	133 ± 10 (117 - 143)*	5/8 (62.5%)

*=mean ± SD

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Table 2. Median nerve neurophysiology findings in MMN Patients.

	Side	Distal CMAP amp (mV)	Elbow CMAP amp (mV)	CMAP area reduction (%)	Forearm CV (m/s)	Number of Units	Size of largest unit (mV)
Patient 1	Right	6.4	3.8	30	49.7	30	1.1
Patient 2	Left	7.1	1.8	58	48.4	40	1.1
Patient 3	Right	7.2	3.9	30	39.6	31	1.2
Patient 4	Left	10.4	1.9	52	53	41	1.1
Patient 5	Right	8.8	1.5	78	43.7	45	1.0
Patient 6	Left	11.2	0.6	93	13.3	38	0.9
Patient 7	Right	6.3	3.9	40	50	10	2.5
Patient 8	Right	7.0	2.7	51	58.7	25	0.7

Table 3. Summary of results from CMAP scan.

	MMN (n=8)	HC (n=30)	P-value (MMN vs HC)
MScan Peak (mV)	7.6 ± 1.8	8.7 ± 2.5	ns
MUNE	33 ± 11	93 ± 36	<0.0001
Largest Unit (mV)	1.2 ± 0.5	0.4 ± 0.1	<0.0005
Largest Unit (%age of MScan Peak)	16.2 ± 6.3.	5.3 ± 1.9	<0.0001

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Figure 1. Results of CMAP scan.

Figure legend: There was no significant difference in *MScan Peak* between the MMN and healthy control group (A); There was a marked reduction in the number of units in the MMN group compared with healthy controls (B); MMN patients had a much higher *Size of largest unit* compared with healthy controls (C and D).

HC: healthy controls

Figure 2. CMAP Scan Example.

Figure legend: Example of CMAP Scan in a subject with MMN demonstrating large units with "gaps" in the CMAP Scan (A). In contrast, the healthy control subject has a similar *MScan Peak,* but with many more units and without large "gaps" in the scan (B).

Figure 3. CMAP Scan Correlations between MScan Peak, MUNE and age.

Figure legend: Negative correlation between age and MUNE (A) and positive correlation between *MScan Peak* and MUNE (B) in healthy controls (black circles and line). These correlations were lost in the MMN group (blue circles and line)

MUNE: motor unit number estimate

ABSTRACT

Objective: To estimate the degree of axonal loss in patients diagnosed with multifocal motor neuropathy (MMN) using a novel assessment of motor unit numbers and size. *Methods:* Automated motor unit number estimation using a compound muscle action potential (CMAP) scan was undertaken in median nerves with conduction block. Results were compared with 30 age-matched healthy controls. *Results:* Compared with healthy controls, MMN patients had fewer motor units (MMN: 33 ± 11 vs HC: 93 ± 36 [mean $\pm$ SD]; $p<0.0001$) and larger 'size of the largest unit' (MMN: 1.2 ± 0.5mV vs HC: 0.4 ± 0.1mV; $p<0.0001$), despite having

normal distal CMAP amplitudes (MMN: 7.6 ± 1.8mV vs HC: 8.7 ± 2.5mV; p=0.24).

Conclusions: MMN is associated with marked axonal loss which may be masked by striking re-innervation resulting in preservation of distal CMAP amplitudes. *Significance:* Assessment of motor unit properties should be incorporated into assessment of disease progression in MMN, given that nerve conduction studies are insensitive to motor unit remodelling.

A MScan Peak

B MUNE

C Largest Unit (mV)

D Largest Unit (%)

A MUNE vs Age

HC:
$R^2 = 0.4255$
$p < 0.0001$

MMN:
$R^2 = 0.02$
$p = 0.75$

B MScan Peak vs MUNE

HC:
$R^2 = 0.473$
$p < 0.0001$

MMN:
$R^2 = 0.05$
$p = 0.59$