PAPER

Axon loss is an important determinant of weakness in multifocal motor neuropathy

J T H Van Asseldonk, L H Van den Berg, S Kalmijn, R M Van den Berg-Vos, C H Polman, J H J Wokke, H Franssen

J Neurol Neurosurg Psychiatry 2006;**77**:743–747. doi: 10.1136/jnnp.2005.064816

See end of article for authors' affiliations

Correspondence to:
Dr H Franssen, Department of Clinical Neurophysiology, University Medical Centre Utrecht, PO Box 85500, 3508 GA Utrecht, The Netherlands; h.franssen@neuro.azu.nl

Received 2 April 2005
Revised form received 22 May 2005
Accepted 23 May 2005

Background: Multifocal motor neuropathy (MMN) is characterised by asymmetrical weakness and muscle atrophy, in the arms more than the legs, without sensory loss. Despite a beneficial response to treatment with intravenous immunoglobulins (IVIg), weakness is slowly progressive. Histopathological studies in MMN revealed features of demyelination and axon loss. It is unknown to what extent demyelination and axon loss contribute to weakness. Unlike demyelination, axon loss has not been studied systematically in MMN.

Aims/Methods: To assess the independent determinants of weakness in MMN, 20 patients with MMN on IVIg treatment were investigated. Using a standardised examination in each patient, muscle strength was determined in 10 muscles. In the innervating nerve of each muscle, axon loss was assessed by concentric needle electromyography, and conduction block or demyelination slowing by motor nerve conduction studies. Multivariate analysis was used to assess independent determinants of weakness.

Results: Needle electromyography abnormalities compatible with axon loss were found in 61% of all muscles. Axon loss, and not conduction block or demyelination slowing, was the most significant independent determinant of weakness in corresponding muscles. Furthermore, axon loss and conduction block were independently associated with each other.

Conclusion: Axon loss occurs frequently in MMN and pathogenic mechanisms leading to axonal degeneration may play an important role in the outcome of the neurological deficit in patients with MMN. Therapeutic strategies aimed at prevention and reduction of axon loss, such as early initiation of treatment or additional (neuroprotective) agents, should be considered in the treatment of patients with MMN.

Multifocal motor neuropathy (MMN) is characterised by slowly progressive asymmetrical weakness of the arms more than the legs, without sensory loss.^{1–4} Various placebo controlled studies have shown that treatment with high dose intravenous immunoglobulins (IVIg) leads to improvement of muscle strength and disability in patients with MMN.^{5–13} Despite longterm IVIg maintenance treatment, necessary to maintain the initial improvement, follow up studies have shown decreased muscle strength over a period of years.^{14–17}

Conduction block, the electrodiagnostic hallmark of MMN, is believed to underlie the weakness in MMN. Conduction block was associated with demyelination slowing on nerve conduction studies and probably results from demyelination.¹⁸ A previous study revealed that besides conduction block and demyelination slowing, an association with weakness was found for decreased distal compound muscle action potential (CMAP) amplitude in univariate analysis.¹⁸ A decreased distal CMAP in MMN may represent distal conduction block as well as axon loss.¹⁹ The occurrence of axon loss is supported by the finding of muscle atrophy in MMN, which may be mild at first, but becomes prominent during the course of the disease in most patients.²⁰ Histopathological studies at the site of conduction block revealed demyelination and small onion bulbs indicative of poor remyelination, in addition to axonal damage and regenerative clusters indicative of axonal regeneration.^{21–22} These findings indicate that axon loss may also contribute to the weakness seen in MMN. However, it is not known to what extent axon loss occurs in MMN and to what extent it contributes to weakness.

In our present study, 20 patients with MMN underwent a standardised examination. Muscle strength was determined in 10 muscles, and in the innervating nerve of each muscle, axon loss was assessed by needle electromyography (EMG), and conduction block or demyelination slowing by motor nerve conduction studies. This enabled us to perform a multivariate analysis to assess the independent determinants of weakness in patients with MMN.

METHODS

Patients

Twenty patients with MMN were recruited on the basis of an asymmetric lower motor neurone syndrome without sensory abnormalities, motor conduction block in at least one nerve segment, normal sensory conduction, and a favourable response to high dose IVIg.²³ All patients fulfilled the criteria for definite MMN.²³ Table 1 presents the patient characteristics.

All patients were on IVIg maintenance treatment.²³ Serum IgM anti-GM1 ganglioside antibody tests and magnetic resonance imaging of the brachial plexus were performed as described previously.^{24–25} All participants gave informed consent to the study, which was approved by the medical ethics committee of the University Medical Centre, Utrecht, the Netherlands.

Abbreviations: CMAP, compound muscle action potential; EMG, electromyography; IVIg, intravenous immunoglobulins; MMN, multifocal motor neuropathy

Table 1 Characteristics of 20 patients with MMN

Characteristic	
Number (%) male sex	15 (75%)
Mean (SD) age at onset (years)	34.4 (8.1)
Mean (SD) years untreated	6.9 (6.6)
Mean (SD) years treated	4.5 (2.4)
Number (%) with upper limb onset	14 (70%)
Mean (SD) MRC sum score for arms	41 (6)
Number (%) with raised anti-GM1 antibodies*	5 (31%)
Number (%) with abnormal MRI brachial plexus	8 (40%)

MRC sum score arm: summation of MRC scores, which were bilaterally determined in 5 arm muscles (maximum values, 50). Abnormal MRI brachial plexus: swelling and/or increased signal intensity on magnetic resonance imaging.

*Investigated in 16 patients.

MRC, Medical Research Council; MRI, magnetic resonance imaging.

Assessment of weakness, axon loss, conduction block, and demyelination slowing

For five nerves in each arm, we determined the presence of conduction block or demyelination slowing by motor nerve conduction studies, axon loss by needle EMG of the muscle from which the CMAP was recorded for the motor nerve conduction study, and weakness by manual testing of this muscle. Two long nerves (median nerve innervating the m. abductor pollicis brevis and ulnar nerve innervating the m. abductor digiti V), two intermediate length nerves (median nerve innervating the m. flexor carpi radialis and radial nerve innervating the m. extensor carpi ulnaris), and one short nerve (musculocutaneous nerve innervating the m. biceps brachii) of both arms were tested. Each of these investigations was carried out by a different investigator who was blinded to the results of the other investigations.

Muscle strength was assessed according to the Medical Research Council scale.²⁶ Weakness was considered to be present when the Medical Research Council score for a muscle was less than five.

Concentric needle EMG was performed according to standard techniques.²⁷ For each muscle, the transversal plane was sampled by insertions made in three directions; for each direction activity was sampled in at least five sites. Axon loss was considered to be present in a nerve when needle EMG of the muscle innervated by that nerve showed spontaneous muscle fibre activity, defined according to strict criteria,²⁸ in at least one site ("denervation"), or a severely reduced pattern on maximal voluntary effort, with at least half of the motor unit potentials being polyphasic or large (>7 mV) ("reinnervation").²⁷ The number of clearly distinct sites with denervation was scored for each muscle.

Motor nerve conduction studies included the following segments—(1) median nerve to the m. abductor pollicis

brevis and ulnar nerve: lower arm, upper arm, and shoulder (axilla-Erb's point); (2) median nerve to the m. flexor carpi radialis and radial nerve: upper arm and shoulder; and (3) musculocutaneous nerve: shoulder.²⁹ The elbow segment of the ulnar nerve was not analysed. The arms were warmed in water at 37°C for at least 30 minutes before investigation, and thereafter kept warm by infrared heaters.³⁰ Nerves for which the distal CMAP amplitude was below 1 mV were not analysed because low CMAPs preclude the assessment of demyelination.³¹ Responses were only scored if supramaximal stimulation was possible (at least 20% above the strength yielding a maximal CMAP; for Erb's point at least 30%).³² In the case of conduction block, we ensured that the proximal CMAP did not increase after setting the stimulator at maximal output. If necessary, a collision technique was used to detect the effects of co-stimulation.²⁷ Conduction block was considered to be present in a nerve when in at least one segment the negative CMAP area reduction on proximal versus distal stimulation was at least 50%.³³ Demyelination slowing was considered to be present in a nerve when in at least one segment, motor conduction velocity was below 75% of the lower limit of normal, or when duration prolongation of the negative CMAP on proximal versus distal stimulation was at least 30%, indicating increased temporal dispersion.^{31 34–36}

Determinants of outcome

The following features were analysed as potential determinants of weakness as outcome measure: (1) axon loss; (2) conduction block; (3) demyelination slowing; (4) years untreated, number of years during which the patient had symptoms of MMN but received no treatment; (5) years treated, number of years during which the patient had been treated; and (6) nerve length, because long arm nerves are more frequently affected in MMN.¹⁸

Statistical analysis

Associations between potential determinants and outcome were first evaluated with univariate logistic regression analysis. Next, multivariate logistic regression analysis was performed to determine the independent contribution of each potential determinant to outcome. Weakness, axon loss, conduction block, and demyelination slowing were analysed as dichotomous variables (present or absent), years untreated and years treated as continuous variables, and nerve length as a categorical variable (short, intermediate length, or long). To correct for intersubject and intrasubject variation, to avoid the exclusion of patients with missing data, and because several nerves within one patient were measured, multilevel analysis was performed (a random effects model for repeated measurements; software program Mln; Multilevel Model Project, London, UK) with the nerve as the unit of measurement and the patient as the subject.³⁷ Because a random part did not improve the model with weakness as the outcome variable, ordinary multivariate logistic regression analysis could be used. A p value less than 0.05 was considered to be significant.

RESULTS

Assessment of weakness, axon loss, conduction block, and demyelination slowing

Nerve conduction studies were performed in a total of 200 nerves. In 187 nerves the distal CMAP amplitude was above 1 mV, so that conduction block and demyelination slowing could be assessed. Of these 187 nerves, needle EMG was performed in 180 innervated muscles. These 180 nerves were included for analysis. Weakness was found in 50% of muscles, axon loss in 61% of nerves, conduction block in 17%, and demyelination slowing in 30% of nerves. Of the 109

Table 2 Nerves with signs of denervation or reinnervation on needle electromyography

Abnormality	N (%)
No abnormalities	71 (39)
Reinnervation only	64 (36)
Denervation at 1 site, reinnervation	19 (11)
Denervation at 2 sites, reinnervation	9 (5)
Denervation at 3 sites, reinnervation	6 (3)
Denervation at 1 site, no reinnervation	9 (5)
Denervation at 2 sites, no reinnervation	0 (0)
Denervation at ≥3 sites, no reinnervation	2 (1)

Denervation, spontaneous muscle fibre activity. Reinnervation, reduced pattern on maximal voluntary effort, with at least half of the motor unit potentials being polyphasic or large (>7 mV).

Table 3 Relation between disease duration and the percentage of nerves with weakness, axon loss, conduction block, and demyelination slowing

Disease duration (years)	No of patients	Percentage of nerves with*			
		Weakness	Axon loss	Conduction block	Demyelination slowing
0–5	4	24	54	5	3
5–10	7	44	55	12	27
10–15	6	60	65	27	42
15–20	3	86	73	27	55

*For each disease duration category, the total number of nerves with abnormalities was assessed and expressed as a percentage of the total number of nerves within that category.

muscles with abnormalities consistent with axon loss on needle EMG, abnormalities were relatively pronounced in most; 31% had signs of denervation and reinnervation, 59% had signs of reinnervation only, and 10% had signs of denervation only (table 2).

Axon loss, conduction block, or demyelination slowing were found in 67% of all investigated nerves. Of the nerves with axon loss, conduction block, or demyelination slowing, 17% had axon loss, conduction block, and demyelination slowing, 7% had axon loss and conduction block, 19% had axon loss and demyelination slowing, 2% had conduction block and demyelination slowing, 47% had axon loss only, 0% only conduction block only, and 8% demyelination slowing only. The percentage of weakened muscles, nerves with conduction block, and nerves with demyelination slowing each increased significantly with disease duration (χ^2 test for trends); axon loss was also found in patients with a short disease duration (table 3).

Determinants of weakness

Univariate analysis revealed that axon loss, conduction block, demyelination slowing, a greater number of years untreated, and a longer nerve length were each significantly positively associated with weakness (table 4). Multivariate analysis showed that axon loss was the most prominent independent determinant of weakness, followed by years untreated and nerve length (table 4). Importantly, in multivariate analysis, conduction block and demyelination slowing were no longer significantly independent determinants of weakness.

Determinants of axon loss, conduction block, and demyelination slowing

Because axon loss was the most important determinant of weakness, we subsequently analysed potential determinants of axon loss. Univariate analysis revealed that conduction block and demyelination slowing were each significantly associated with axon loss (table 5). Multivariate analysis showed that conduction block was the only independent determinant of axon loss (table 5). In this analysis the intercept was specified as a random effect to allow for individual differences.

DISCUSSION

The aim of our study was to assess the independent determinants of weakness in MMN. Previous studies revealed associations with weakness in univariate analysis for nerve length, years treated and years untreated, and for conduction block, demyelination slowing, and decreased distal CMAP amplitudes on motor nerve conduction studies.^{18–38} Evidence of axon loss on the basis of needle EMG findings, decreased CMAPs, and pathological studies has been reported previously in MMN.^{15–17, 18–22, 38} However, systematic studies assessing the extent of axon loss by needle EMG or the contribution of axon loss to weakness were not performed. In MMN, conduction block and demyelination slowing are probably the result of demyelination, whereas a decreased distal CMAP may be caused by axon loss and distal conduction block.¹⁹ Needle EMG was shown to be more sensitive in detecting axon loss than the assessment of distal CMAP amplitude.³⁹ For these reasons, in our study, demyelination was estimated by nerve conduction studies and axon loss was assessed by needle EMG instead of by distal CMAPs. To identify independent determinants of weakness in multivariate analysis, the potential determinants were obtained in a standardised and large set of muscles and their innervating nerves.

In our present study, axon loss—scored according to strict criteria for denervation and reinnervation on needle EMG—occurred frequently in MMN. Using these criteria, other studies found spontaneous muscle fibre activity (denervation) in only 2% of limb muscles in older normal subjects.^{28–40} Despite the use of strict criteria, 61% of all muscles in our patients showed needle EMG abnormalities that were also relatively pronounced in most muscles. The frequent finding of EMG abnormalities in patients with short disease duration indicates that axon loss is an early feature of MMN.

A prominent finding of our study was that axon loss, and not conduction block, was the most significant independent determinant of weakness in corresponding muscles. Furthermore, axon loss and conduction block were independently associated with each other. However, the number of years treated with IVIg was not significantly associated with weakness. This finding was not unexpected because longterm follow up of patients on IVIg maintenance treatment showed changes consistent with improvement and worsening in

Table 4 Logistic regression analysis for the determinants of weakness

Determinant	Univariate	p Value	Multivariate	p Value
Axon loss	5.7 (2.9 to 11.1)	<0.001	4.4 (2.0 to 9.7)	<0.001
Conduction block	7.1 (2.6 to 19.4)	<0.001	2.1 (0.7 to 6.6)	NS
Demyelination slowing	6.6 (3.1 to 14.0)	<0.001	2.0 (0.8 to 4.8)	NS
Years untreated	1.1 (1.1 to 1.2)	<0.001	1.1 (1.0 to 1.2)	<0.01
Years treated	1.0 (0.9 to 1.2)	NS	1.1 (0.9 to 1.3)	NS
Nerve length	2.1 (1.4 to 3.1)	<0.001	1.9 (1.1 to 3.2)	<0.05

Data are expressed as odds ratio (95% confidence interval).
NS, not significant.

Table 5 Logistic regression analysis for the determinants of axon loss

Determinant	Univariate	p Value	Multivariate	p Value
Conduction block	12.5 (2.9 to 54.3)	0.01	8.0 (1.8 to 36.3)	0.01
Demyelinative slowing	3.6 (1.7 to 7.5)	0.01	2.0 (0.8 to 4.8)	NS
Years untreated	1.0 (1.0 to 1.1)	NS	1.0 (1.0 to 1.1)	NS
Years treated	1.1 (1.0 to 1.2)	NS	1.1 (0.9 to 1.2)	NS
Nerve length	1.5 (1.0 to 2.2)	NS	1.2 (0.8 to 1.9)	NS

Data are expressed as odds ratio (95% confidence interval).
NS, not significant.

similar numbers of nerves.^{15–17} In contrast, improvement of conduction block on nerve conduction studies was found after initial IVIg treatment.^{17–41} These findings and those of our present study indicate that conduction block may be a stronger determinant of weakness in untreated patients than in treated patients with MMN. Furthermore, the extent of conduction block may have been underestimated in our present study because transcutaneous electrical stimulation of the cervical roots, which was shown to reveal proximal conduction block in MMN, was not performed.⁴²

Our results indicate that mechanisms leading to axonal degeneration may play the most important role in the outcome of the neurological deficit in patients with MMN on IVIg maintenance treatment. This is consistent with the observation that muscle strength in patients with MMN improves after a course of IVIg treatment, but rarely fully recovers to normal values.^{15–17} IVIg treatment may have an effect on immune mediated demyelination or partially reversible damage to axons, but not on irreversible axonal degeneration. Previous studies showed that patients with MMN may present with low distal CMAP amplitudes.^{15–16–18–20} In a study of patients with MMN who had never received IVIg treatment, a longer disease duration was shown to be associated with more weakness, more segments with conduction block, and more nerves with low distal CMAP amplitudes.³⁸ Longterm follow up studies of patients on IVIg maintenance treatment showed a mild decrease in muscle strength and decrease in distal CMAP amplitudes.^{15–16–43} It is unknown whether these decreased distal CMAPs represented axon loss or distal conduction block. Differentiation between these two by nerve conduction studies is difficult because distal conduction block cannot always be assessed. Follow up studies using nerve conduction studies in combination with needle EMG may be helpful in providing further insight into the progression of axon loss and demyelination over time.

Our finding that conduction block and axon loss were independent determinants of each other indicates that, within individual nerves, loss of axons and conduction block in the surviving axons often occur together. These findings may indicate a common disease mechanism that leads to conduction block in some axons and degeneration of others. A common disease mechanism is supported by studies showing that the antibodies to the ganglioside GM1, found in 20–80% of patients with MMN, bind to epitopes of the nodal axolemma and paranodal myelin, possibly leading to axon loss and conduction block, and to epitopes of spinal cord motor neurones, possibly leading to axon loss.^{23–44–47}

Alternatively, the finding that conduction block and axon loss were independent determinants of each other may indicate that an axon that is affected by a process resulting in conduction block will eventually degenerate. Conduction block was found to be randomly distributed in arm nerves and, consequently, longer nerves had more segments with conduction block; in addition, distal CMAPs were more often decreased in longer nerves, possibly indicating length dependent axonal degeneration as a result of the higher number of conduction blocks in these nerves.¹⁸ In a small

proportion of nerves, CMAPs evoked distal to a segment with conduction block were shown to decrease on follow up.^{15–17} Finally, excitability measurements have revealed axonal hyperpolarisation adjacent to sites with conduction block, thought to be secondary to intra-axonal sodium accumulation at the site with conduction block caused by reduced sodium/potassium pump activity.⁴⁸ The sodium accumulation could in turn lead to intra-axonal calcium accumulation as a result of passive reversal of the sodium/calcium exchanger and, consequently, to axonal degeneration.⁴⁹ This mechanism is supported by an animal study of inflammatory demyelination showing that sodium channel or sodium/calcium exchanger blockers prevented axonal degeneration.⁵⁰

Our present study supports findings in demyelinating disorders of the central nervous system. In multiple sclerosis, imaging studies revealed that axon loss is associated with disability,⁵¹ and is ongoing during treatment with β interferons.⁵² In chronic relapsing experimental allergic encephalomyelitis, early stage disability was determined by a combination of inflammation, demyelination, and acute axonal injury, whereas non-remitting late stage disability was mainly determined by axon loss. Furthermore, demyelination in the absence of axon loss did not necessarily cause permanent disability.⁵³ A multivariate analysis of the independent association between features of axon loss or demyelination and functional deficit may be cumbersome because, in contrast to the electrophysiological studies in the peripheral nervous system, assessment of these determinants is difficult for a specific tract or set of nerve fibres in the central nervous system.

Our results indicate that axon loss occurs frequently in MMN and that pathogenic mechanisms leading to axonal degeneration may play an important role in the outcome of the neurological deficit in patients with MMN. Therapeutic strategies aimed at prevention and reduction of axon loss, such as early initiation of treatment or additional neuroprotective or immunosuppressive agents, should be considered in the treatment of patients with MMN.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Prinses Beatrix Fonds.

Authors' affiliations

J T H V Asseldonk, H Franssen, Department of Clinical Neurophysiology, Rudolf Magnus Institute of Neuroscience, University Medical Centre Utrecht, 3508 GA Utrecht, The Netherlands
L H V den Berg, R M V den Berg-Vos, J H J Wokke, Department of Neurology, Rudolf Magnus Institute of Neuroscience
S Kalmijn, University Medical Centre Utrecht, Julius Centre for Health Sciences and Primary Care, 3508 GA Utrecht, The Netherlands
C H Polman, Vrije Universiteit Medical Centre, Department of Neurology, 1007 MB Amsterdam, The Netherlands

Competing interests: None declared.

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