The role of auto-antibodies and paraproteinemia in polyneuropathy

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The role of auto-antibodies and paraproteinemia in polyneuropathy

De rol van auto-antilichamen en paraproteinemie bij polyneuropathie

(met een samenvatting in het Nederlands)

Proefschrift

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Contents

1

Introduction and outline of thesis

Polyneuropathy is a clinical syndrome characterized by often symmetrical, predominantly distal sensory deficits and muscle weakness. Polyneuropathies are further subdivided according to the primary site of damage, either the nerve axons (axonal) or the myelin sheath (demyelinating). There are many causes of polyneuropathy, including diabetes, infection, metabolic disturbances, deficiencies and inflammation (Table 1.1). Polyneuropathy associated with monoclonal gammopathy is a subgroup of neuropathies that may be amenable to treatment.

Monoclonal gammopathy is the presence in blood (or urine) of abnormal quantities of identical proteins, either immunoglobulins or parts thereof. These immunoglobulins are usually produced by monoclonal expansions of plasma cells. Monoclonal gammopathies can be a symptom of hematological malignancies, but may also be found in patients without malignancy (monoclonal gammopathy of unknown, or undetermined, significance, MGUS)

Both polyneuropathy and monoclonal gammopathy are relatively common occurrences, and the incidence of both increases with age. (Niermeijer 2010, Kyle 2011) That they may occur in the same, often elderly, patient is therefore not in itself remarkable or reason to suspect a causative relation. The great majority of monoclonal gammopathies consist of antibodies of the IgG class. The relatively rare IgM MGUS is strongly associated with the presence of a primarily demyelinating polyneuropathy characterized by very specific clinical and electrophysiological characteristics. (Rajabally 2011, Franssen 2006) Typically, patients have a symmetrical polyneuropathy that is slowly progressive over years. Distal weakness may be present, but sensory deficits predominate. Functional impairments are common and are above all caused by sensory ataxia. Nerve conduction studies show a demyelinating, length dependent polyneuropathy, with typical prolongation of the distal motor latency (DML).

Table 1.1 Causes of polyneuropathy

Category	Example
Paraproteinaemic	Anti-MAG neuropathy
Metabolic	Diabetic neuropathy
Ischemic	Vasculitis
Infection	Leprosy
Non-infectious inflammation	Guillain-Barré syndrome
Toxic	Chemotherapy induced peripheral neuropathy
Deficiency	Vitamin B12 deficiency
Paraneoplastic	Neuropathy associated with anti-Hu antibodies
Genetic	Charcot-Mary-Tooth disease
Idiopathic	Chronic idiopathic axonal polyneuropathy (CIAP)

The suspicion that monoclonal IgM antibodies in patients with MGUS might be specific for antigens expressed in nerves was confirmed by several groundbreaking studies in the 1980s, which identified anti-myelin associated glycoprotein (MAG) as a target in approximately half of the patients with polyneuropathy associated with monoclonal gammopathy. (Latov 1981, Braun 1982) The pathogenic potential of these monoclonal IgM anti-MAG antibodies was also suggested by the presence of IgM deposits in nerve biopsies from these patients. Their presence correlated with myelin damage and complement depositions. (Hays 1988, Ritz 1999) These studies suggested a pathogenic mechanism of monoclonal antibodies reacting with nerve sheath components, followed by complement activation and deposition and myelin damage – at least for patients with anti-MAG antibodies. It remained unclear whether polyneuropathy associated with IgM monoclonal gammopathy without anti-MAG antibodies was caused by a similar mechanism.

Anti-MAG neuropathy may lead to significant functional deficits (Niermeijer 2010) and the identification of an immune-mediated etiology suggested that removal of the pathogenic antibodies might be an effective treatment strategy. This could be done either directly, for example by plasmapheresis, or by chemotherapy aimed at the monoclonally expanded B-cells producing them, or attenuation of their downstream effects, for example inhibition of complement activation. Results from natural history studies showing that polyneuropathy associated with monoclonal gammopathy may cause significant disability in a subgroup of patients underlined the need for treatment strategies. Unfortunately, efficacy of several treatment modalities for anti-MAG neuropathy and other IgM gammopathy associated neuropathies (IgM-PNP) was disappointing, with a recurring pattern of initial enthusiasm after the publication of suggestive uncontrolled studies and case-reports followed by controlled trials that failed to confirm a positive effect on the disease course. Intravenous immunoglobulins, fludarabine, cyclophosphamide-prednisone were tried but all lacked efficacy. (Haas 1988, Ellie 1996, Nobile-Orazio 2000, Gorson 2001, Ochsenhendler 1995, Comi 2002, Mariette 1997, 2000, Notermans 1996, Niermeijer 2006, 2007)

More recently investigators have concentrated on the possibility of improving the disease course of anti-MAG neuropathy by treatment with rituximab, a chimeric monoclonal antibody against CD20, a phosphoprotein expressed on all developmental stadia of the B cell. (Weiner 2010) Whether the pattern of eventual disappointment after initial hope holds for rituximab is still debated. For many individual patients and in small series clear improvement of the neuropathy has been described. (Levine 1999, Pestronk 2003, Benedetti 2007, Niermeijer 2009) Although two randomized trials failed to reach their pre-defined

primary endpoint, both also described a subset of patients with improvement of functional disability scores after rituximab treatment. (Dalakas 2009, Leger 2013)

More detailed knowledge of the pathophysiology of polyneuropathy associated with monoclonal gammopathy would probably help the development of treatment strategies. Determining antibody specificity in anti-MAG negative patients would help to firmly establish a causal relationship between monoclonal gammopathy and polyneuropathy. Furthermore, elucidating the causes of heterogeneity in severity of anti-MAG neuropathy could help to develop treatment strategies. Differences in severity cannot be explained by anti-MAG antibody titers. One possible explanation might be innate differences in immune activity, such as the complement system, as has been shown to be the case for other (acute) immune-mediated neuropathies. (Geleijns 2006)

The proportion of patients with IgM-PNP who lack anti-MAG antibodies has shrunk from 50 to approximately 20-30% of patients after the introduction of more sensitive (ELISA) tests. (Kuijf 2009) In some of these patients IgM antibodies against gangliosides, specific glycolipids that are nerve constituents, have been found (Figure 1.1). IgM antibodies against

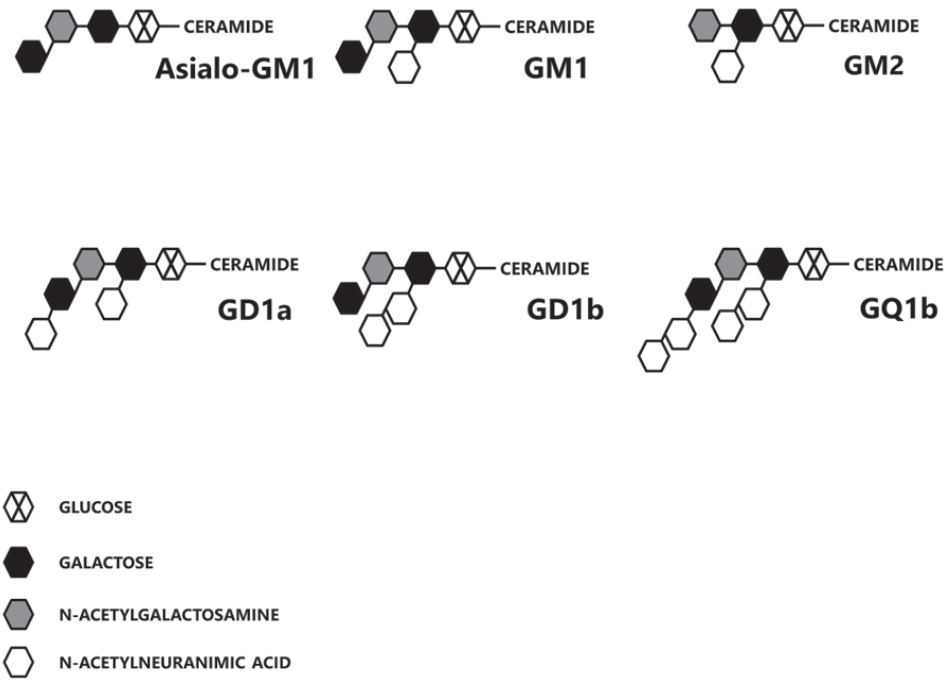

Figure 1.1 Schematic structure of relevant gangliosides.
Based on: Willison, Yuki. Peripheral neuropathies and anti-glycolipid antibodies. Brain 2002.

several gangliosides (GM1, GM2, GD1a, GD1b, GQ1b and against asialo-GM1) have been described. Anti-ganglioside antibodies (IgM, IgA, IgG) are also found in subtypes of Guillain-Barré syndrome (GBS), an acute immune-mediated neuropathy. (Kaida 2010) The pathogenic properties of these anti-ganglioside antibodies in GBS have been convincingly shown (Kuwabara 2013) and have led to the hypothesis that IgM anti-ganglioside antibodies in IgM-PNP have pathogenic properties as well. Anti-GQ1b antibodies in IgM-PNP are associated with chronic ataxic polyneuropathy, often in combination with ophthalmoplegia and cold agglutinins (CANOMAD). (Willison 2001) For other anti-ganglioside antibodies, the relation with the clinical manifestations of the neuropathy are less clear. Nothing approaching the detailed clinical and electrophysiological descriptions of anti-MAG neuropathy exists in IgM-PNP with anti-ganglioside antibodies. It is not known whether the IgM anti-ganglioside antibodies display pathogenic properties, suggestive of a causative relation. Since gangliosides are expressed on neural surfaces within larger groups of glycoplipoproteins, complexes of two or more different gangliosides may form new relevant epitopes. (Kusunoki 2011) The relevance of these ganglioside complexes has been shown in patients with GBS.

It is unclear whether patients with anti-MAG neuropathy should be treated with rituximab. The possibility that a subgroup of patients may benefit from treatment indicates that we need better insight in the risks and benefits of rituximab treatment, as well as better criteria to select those patients that may respond.

Finally, we need more insight in the relevance of IgG and IgA MGUS for the development of polyneuropathy. Although there are several well-defined clinical syndromes that combine MGUS of these isotypes with polyneuropathy (e.g. POEMS), the association is less clear in the majority of patients.

Outline of this thesis

In **Part I** we examined the role of gangliosides in IgM-PNP: we investigated the presence and characteristics of antibodies against both gangliosides and ganglioside complexes in a cohort of anti-MAG negative patients (**chapter 2**). We also investigated whether the presence of specific anti-ganglioside antibodies correlated with clinical characteristics of the neuropathy (**chapter 3**).

In **Part II** we investigated whether the level of innate activity of the complement system (**chapter 4**), or general immune activation as expressed by serum cytokine patterns (**chapter 5**), correlated with disease severity.

In **Part III** we investigated treatment options for both patients with IgM-PNP or anti-MAG neuropathy. We investigated the possibility that efficacy of rituximab treatment depends on polymorphisms of leukocyte IgG receptor (Fc gamma receptor), which are the natural bridge between rituximab and the effector cells of the patient's immune system (**chapter 6**). We also described a series of patients who suffered relatively rare but relevant adverse effects during rituximab treatment (**chapter 7**).

Treatment of IgG or IgA gammopathy to improve a concomitant neuropathy is controversial. We investigated in **Part IV** the efficacy of this approach from 2 directions: we systematically reviewed the literature on treatment of these neuropathies (**chapter 8**). We also tried to establish a tentative proof of principle for the usefulness of treatment, however aggressive, of the haematological disorder in these patients to improve the neuropathy (**chapter 9**). We therefore selected patients who developed a monoclonal gammopathy and a neuropathy in the course of hematological malignancy that warranted treatment with a stem cell transplantation. This allowed us to investigate whether transplantation has an effect on the polyneuropathy.

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PART I

Antibodies against gangliosides

2

Prevalence, specificity and functionality of anti-ganglioside antibodies in neuropathy associated with IgM monoclonal gammopathy

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J Neuroimmunol. 2014 Mar 15; 268(1-2), 89-94.

Abstract

IgM antibodies against gangliosides and their complexes were studied in sera from 54 patients with polyneuropathy and IgM monoclonal gammopathy (IgM-PNP) without anti-MAG antibodies. Anti-ganglioside or anti-asialo GM1 antibodies were found in 19 (35%) patients. Five (9%) patients had antibodies against ganglioside complexes. IgM antibodies against gangliosides activated complement *in vitro*. Light chain usage was restricted to kappa or lambda in most, but not all patients.

In conclusion, anti-ganglioside antibodies in IgM-PNP are common, display pathogenic properties and do not always arise from a monoclonal B cell proliferation.

Introduction

Polyneuropathy associated with IgM monoclonal gammopathy (IgM-PNP) is characterized by distal weakness, sensory disturbances and varying degrees of sensory ataxia. Treatment with immune-modulating drugs failed to show lasting improvement of primary outcome measures. (Dalakas 2009, Niermeijer 2006, Niermeijer 2007, Comi 2002) Treatment effects in subgroups of patients could not be excluded due to the lack of statistical power, (Dalakas 2009, Niermeijer 2006, Niermeijer 2007, Comi 2002) improvement of secondary outcome measures (Niermeijer 2007) and clinical heterogeneity of included patients. (Niermeijer 2006, Comi 2002)

The specificity of serum IgM antibodies may be used to identify subgroups of patients with IgM-PNP. (Dalakas 2009, Comi 2002) IgM antibodies against myelin-associated glycoprotein (MAG) are detected in serum from approximately 50-70% of patients with IgM-PNP. IgM antibodies against gangliosides, glycolipids that are abundantly expressed in peripheral nerves, are target antigens in some patients with IgM-PNP without anti-MAG antibodies, but prevalence and antibody specificities of these antibodies have only been studied in relatively small groups of patients. (Eurelings 2001, Nobile-Orazio 2010, Larue 2011) More recently, antibodies that bind complexes of more than one ganglioside have been identified in patients with Guillain-Barré syndrome (GBS) and chronic immune-mediated neuropathies including one patient with IgM-PNP (Kaida 2006, Kaida 2007, Ogawa 2009, Kaida 2008, Kanzaki 2008, Kusunoki 2008, Kaida 2004, Hamaguchi 2007, Notturno 2009, Nobile-Orazio 2010) and could represent important additional target antigens in patients with IgM-PNP. (Comi 2002) We therefore investigated the presence of antibodies against single gangliosides and their complexes in sera from 54 patients without anti-MAG antibodies from a prospective cohort of 140 patients with polyneuropathy associated with IgM gammopathy. (Niermeijer 2010) We also investigated complement-activating properties of ganglioside-specific IgM antibodies to assess their pathogenic properties and assessed light chain use to investigate their relation with the monoclonal gammopathy.

Materials and methods

Patients

All patients participated in a cohort study between 1985 and 2007. A total of 140 patients with IgM-PNP were enrolled. Patient characteristics have been published previously.

(Niermeijer 2010) At inclusion, a complete neurological examination of strength, gait, sensory deficits and ataxia was performed. Nerve conduction studies were performed using a standardized protocol. (Franssen 2006) Research criteria of the American Academy of Neurology were used to classify polyneuropathies as demyelinating, the remainder were classified as axonal. (Ad Hoc Subcommittee of the American Academy of Neurology, 1991) The presence of anti-MAG IgM antibodies in serum was confirmed by ELISA or Western blot in 63 patients. (Kuijf 2005) These patients were excluded from the study. Serum samples were available from 54 of the remaining 77 (70%) patients. Eight of these patients were included in a previous study. (Eurelings 2001) Haematological malignancy was excluded in all patients. Patient characteristics are summarized in Table 2.1. Twelve patients (63%) had received immunomodulatory treatment prior to inclusion in this study (cyclophosphamide and prednisone, fludarabine, corticosteroids, IVIg or rituximab). Six patients improved in clinical or functional scales after treatment. Three of the 4 patients treated with IVIg improved after treatment. All patients had clinical signs of sensorimotor polyneuropathy with the exception of patients 2, 8 and 11 who had pure motor (patient 2) and pure sensory polyneuropathy (patients 8 and 11), respectively. Serum samples from patients were frozen and stored at -80°C until use. Approval of the UMC Utrecht METC (medisch-ethische

Table 2.1 Patient characteristics for all anti-MAG antibody negative patients

Patient characteristic	Anti-ganglioside IgM positive	Anti-ganglioside IgM negative	p-value
Number of patients	19 (35%)	35 (65%)	
Mean age (range)	66 (53-83)	62 (40-84)	0.26
Males	15 (79%)	24 (69%)	0.53
Presence of weakness			
Arms	10 (53%)	12 (34%)	0.25
Legs	17 (89%)	26 (74%)	0.29
Presence of sensory deficits			
Arms	9 (47%)	16 (46%)	1.00
Legs	18 (95%)	35 (100%)	0.35
Presence of ataxia	12 (63%)	22 (63%)	1.00
Presence of ophthalmoplegia	1 (5%)	0	0.35
Nerve conduction studies			
Demyelinating	15 (83%)	18 (55%)	0.06
Axonal	3 (17%)	15 (45%)	
Monoclonal gammopathy			
IgM-κ	9 (47%)	21 (60%)	0.41
IgM-λ	9 (47%)	12 (34%)	
Both IgM-κ and IgM- λ	1 (5%)	2 (6%)	

toetsingscommissie, institutional ethical standards committee for research with human subjects) was obtained for this study.

ELISA for IgM antibodies against gangliosides and ganglioside complexes (GSC)

The presence of IgM antibodies against the gangliosides GM1, GM2, GD1a, GD1b, GQ1b and against asialo-GM1 was tested using the standardized enzyme-linked immunosorbent assay (ELISA) of The Inflammatory Neuropathy and Treatment (INCAT) group. (Kuijf 2005) Briefly, ELISA plates (Nunc Thermo Fisher, Denmark) were coated with gangliosides GM1, GM2, GD1a, GD1b, GQ1b or asialo-GM1 at a final concentration of 150 picogram/ml in ethanol. To detect antibodies against ganglioside complexes, wells were coated with combinations of 2 gangliosides in a 1:1 molar ratio. Serum samples were incubated overnight. Specific IgM antibodies were detected using rabbit anti-human-IgM antiserum. After development, optical densities were measured at 490 nm. Cut-off values of serum samples were defined as a difference in optical density between ganglioside coated wells and control wells of ≥0.55 for asialo GM1 coated wells, ≥0.15 for GQ1b coated wells and ≥0.30 for wells coated with other gangliosides. (Kuijf 2005) Positive serum samples were tested in series of two-fold dilutions ranging from 1:100 to 1:51200, and the titer was defined as the highest dilution yielding the pre-defined cut-off values. Sera were considered to contain anti-GSC (ganglioside complex) antibodies if the optical density in wells coated with a combination of gangliosides was at least 0.20 higher than the highest OD of the wells coated with the individual gangliosides. (Kuijf 2007)

Light chain usage of IgM antibodies against single gangliosides and GSC

Light chain usage of the anti-ganglioside and anti-GSC antibodies was assessed using goat anti-human IgG kappa or lambda antiserum (Sigma medical, Israel) and expressed in microgram/ml concentrations. Purified immunoglobulins from myeloma plasma with known kappa or lambda usage and monoclonal anti-GM1 IgM antibodies were used as controls (Sigma Medical, Israel). Ratios of kappa and lambda were expressed as the ratio of concentrations of kappa and lambda light chains per patient and per anti-ganglioside antibody at a 1:100 serum dilution. We defined a κ/λ greater than 10 or smaller than 0.1 as restricted light chain usage of either kappa or lambda chains.

Complement activation by IgM antibodies against gangliosides and ganglioside complexes

Complement activation was measured following a previously published protocol. (Piepers et al., 2010) Briefly, 100 µl ethanol containing 2.5 µg of each ganglioside was added to ELISA plates and left to evaporate. Wells coated with purified IgM served as a positive control. Wells saturated with PBS 1% BSA served as a control for non-specific binding. Serum aliquots from patients and controls were heat-inactivated (to abrogate intrinsic complement activity) and incubated overnight. PBS 1% pooled healthy donor serum (or PBS in control wells) was added as a complement source and incubated at 37°C. Complement was detected by rabbit anti-human-complement factor 3, with goat anti-rabbit IgG-HRP secondary antibodies. Plates were developed using ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid). OD values at least ≥3 SD higher than the healthy donor negative controls were considered to reflect complement activation. (van Sorge 2007)

Results

IgM antibodies against gangliosides and ganglioside complexes

IgM antibodies against single gangliosides were detected in serum from 15 out of 54 (28%) patients. In the sera of 4 additional patients anti-asialo GM1 antibodies were detected (Figure 2.1). In 2 of the anti-ganglioside antibody-positive sera we found antibody activity against GM2-GD1b or GM2-GD1a ganglioside complexes but not against their constituent gangliosides. In 3 sera we measured OD for one or more ganglioside complexes or complexes

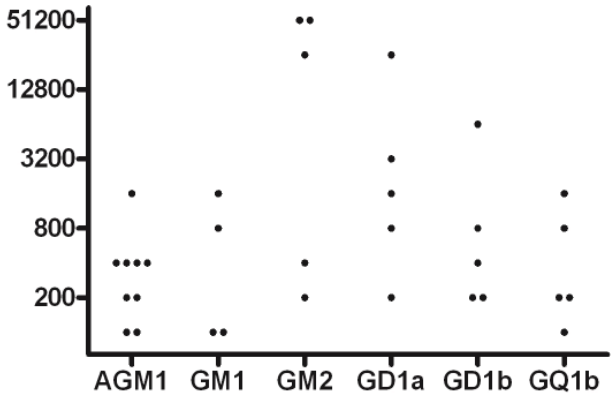

Figure 2.1 IgM antibody titers against gangliosides and against asialo-GM1, for all patients combined.

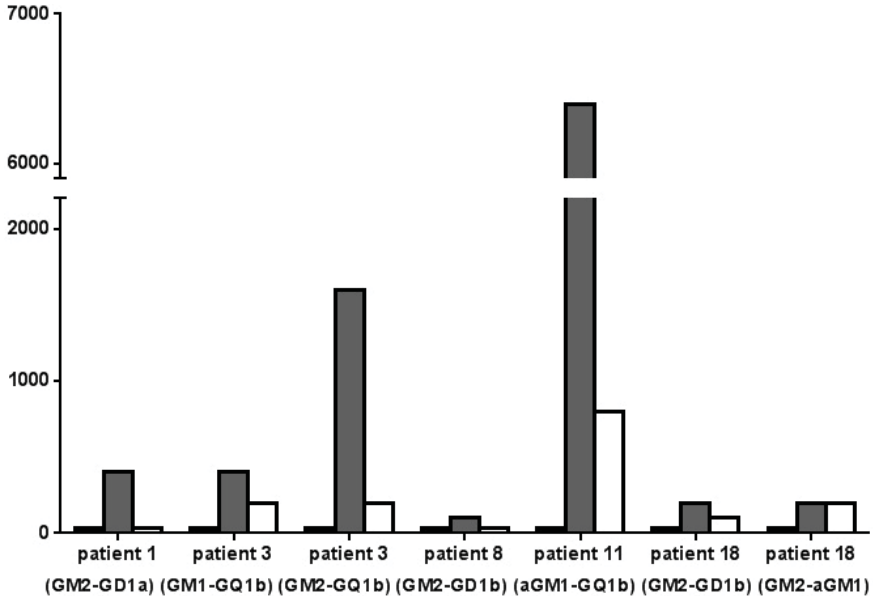

Figure 2.2 Antibody titers against GSCs (middle bars) and their constituent gangliosides (flanking bars) per patient.

of gangliosides and asialo-GM1 (GD1b-GM2; GM2-asialo-GM1; GQ1b-asialo-GM1; GQ1b-GM1; GQ1b-GM2) at least 0.2 higher than the highest of the optical densities for the gangliosides making up the complexes (Figure 2.2). We did not find anti-GSC antibodies in any of the sera without antibodies against a single ganglioside or asialo-GM1.

Light chain usage of ganglioside-specific IgM antibodies

Table 2.2 summarizes light chain usage of ganglioside-specific antibodies. Antibodies against gangliosides were restricted in light chain usage concordant with the M protein light chain (all IgM-kappa) in 7 patients. In 3 patients antibodies against gangliosides or GSCs were not restricted towards kappa or lambda light chain usage (1 had IgM-kappa M protein, 2 had IgM-lambda). In 3 patients M proteins were IgM lambda and anti-ganglioside or GSC antibodies were restricted towards kappa light chain usage. In 2 patients who had an M protein with kappa light chains, antibodies against GSC (patient 3) or asialo-GM1 and GSC (patient 8) were not restricted in light chain usage (Table 2.2). Two patients with an M protein with lambda light chains had antibodies against GSC with kappa light chains (patient 1) and antibodies against GQ1b with both kappa and lambda light chains (patient 11).

Table 2.2 Clinical and antibody characteristics of anti-ganglioside antibody positive patients

Pt.	Age	Sex	Neuropathy	NCS	M-protein	Anti-gangl. antibodies (titer)		κ/λ
1	76	M	sensorimotor	D	IgM-λ	GM1	(100)	-
						GD1b	(400)	κ
						aGM1	(100)	mixed
						GM2-GD1a	(400)	κ
2	78	M	pure motor	D	IgM-λ	GM2	(25600)	κ
3	55	M	sensorimotor	D	IgM-κ	GD1b	(6400)	κ
						GD1a	(200)	-
						GQ1b	(100)	κ
						aGM1	(100)	κ
						GM1-GQ1b	(400)	mixed
						GM2-GQ1b	(1600)	mixed
4	63	M	sensorimotor	D	IgM-κ	GM2	(200)	κ
						GD1a	(1600)	κ
5	61	M	sensorimotor	D	IgM-κ	GQ1b	(200)	κ
6	84	M	sensorimotor	D	IgM-λ	GM2	(51200)	-
						GD1a	(3200)	-
						GD1b	(200)	-
						GQ1b	(800)	-
7	53	M	sensorimotor	D	IgM-κ	GD1a	(25600)	κ
8	80	M	pure sensory	D	IgM-κ	aGM1	(100)	mixed
						GQ1b	(200)	κ
						GM2-GD1b	(100)	mixed
9	78	F	sensorimotor	D	IgM-κ	aGM1	(1600)	mixed
10	71	M	sensorimotor	D	IgM-λ	aGM1	(400)	mixed
11	59	F	pure sensory	A	IgM-λ	GQ1b	(200)	mixed
						GD1b	(100)	λ
						aGM1-GQ1b	(6400)	κ
12	65	M	sensorimotor	D	IgM-κ	aGM1	(400)	κ
13	66	M	sensorimotor	A	IgM-λ	GD1a	(200)	κ
						aGM1	(200)	κ
14	68	M	sensorimotor	D	IgM λ	aGM1	(400)	mixed
15	83	M	sensorimotor	-	IgM-λ	GM1	(100)	-
16	61	M	sensorimotor	D	IgM-λ	GM2	(100)	κ
17	62	F	sensorimotor	D	IgM-κ	GM2	(51200)	κ
					and λ	GD1a	(800)	κ
						GD1b	(800)	κ
						GQ1b	(100)	-
18	74	F	sensorimotor	D	IgM-κ	GM1	(800)	κ
						GD1b	(100)	-
						aGM1	(200)	κ
						GM2-aGM1	(200)	κ
						GM2-GD1b	(200)	κ
19	56	M	sensorimotor	D	IgM-κ	aGM1	(400)	κ

Sex: (M=male, F=female). Neuropathy: clinical classification of neuropathy. NCS: nerve conduction studies (D=demyelinating, A=axonal loss). M protein: monoclonal gammopathy and light chain usage. Anti-ganglioside antibodies: anti-ganglioside (complexes: GSC) antibodies and titer (in brackets). κ/λ: light chain usage of anti-ganglioside antibodies. Ganglioside complexes are in italics. light chain usage for anti-ganglioside antibodies marked '-' could not be established.

Anti-ganglioside antibodies and clinical phenotype.

Sixteen out 19 patients with anti-ganglioside or anti-asialo-GM1 antibodies had a polyneuropathy with both sensory and motor deficits. We did not find a clear correlation of antibody specificity with clinical phenotype. Presence of antibodies against GD1b or GQ1b was not always associated with sensory ataxic neuropathy. One patient (patient 3), with anti-GQ1b, anti-GD1b, anti-GD1a and anti-asialo-GM1 antibodies, had ophthalmoplegia. However, 2 out 3 patients with only antibodies against GQ1b or GD1b using a single light chain (patients 8 and 11), had a sensory-ataxic neuropathy with complete sparing of motor function, a phenotype not found in the other 16 patients (p=0.02). Anti-GM2 antibodies were found in patient 2 with pure motor neuropathy and in other patients with sensorimotor neuropathies. In 15 out of 18 patients (83%) demyelinating characteristics were found in nerve conduction studies.

Complement activating properties of IgM anti-ganglioside antibodies

Complement activating properties of anti-ganglioside antibodies in vitro are associated with pathogenicity *in vivo*. (van Sorge 2007) Anti-ganglioside antibodies in sera from patients with IgM MGUS polyneuropathy triggered complement activation as reflected by the deposition of C3 in contrast to sera from healthy controls (Figure 2.3). Complement deposition in wells coated with irrelevant gangliosides did not differ significantly between patients and healthy controls (data not shown).

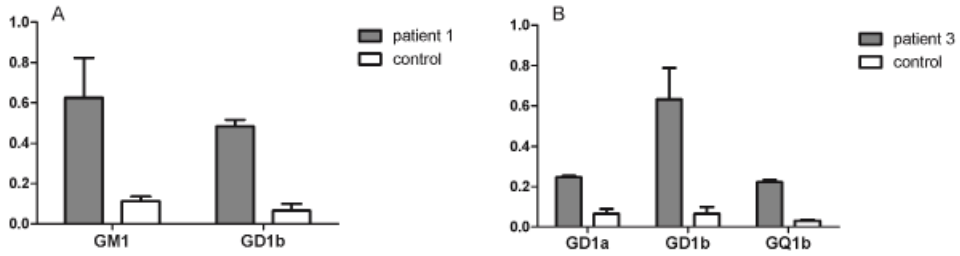

Figure 2.3 Complement activation by anti-ganglioside antibodies.
Optical densities (OD) on the vertical axis reflects deposition of complement factor 3. Results are from two representative patients A: patient 1, B: patient 3) and healthy controls. Bars represent the mean of triplicate measurement, whiskers represent 1 standard deviation.

Discussion

IgM antibodies against gangliosides were detected in sera from 28% of patients with IgM-PNP without anti-MAG antibodies. These findings show that anti-ganglioside antibodies are relatively common in patients with IgM-PNP. The complement activating properties of anti-ganglioside antibodies suggest that they may play a role in pathogenesis.

The available data seem to support that anti-ganglioside and anti-asialo-GM1 antibodies can be found in about a third of patients with IgM-PNP without anti-MAG antibodies, which translates to about 10 to 15% of all patients with polyneuropathy associated with IgM MGUS. (Kuijf 2005) We included 70% of anti-MAG negative patients in our cohort. These patients did not differ in clinical or electrophysiological characteristics from those that did not participate.

Twelve out of 19 patients (63%) with anti-ganglioside or anti-asialo GM1 antibodies had received immunomodulatory treatment prior to inclusion, a percentage similar to the 57% of patients without anti-MAG or anti-ganglioside antibodies that received immunomodulatory treatment in the same cohort. (Niermeijer 2010) An underestimation of the number of patients with anti-ganglioside IgM antibodies due to disappearance of these antibodies after immunomodulatory treatment cannot be excluded but seems unlikely, since antibodies do not completely disappear following rituximab treatment. (Pestronk 2003) Finally, a recent Italian study found anti-ganglioside antibodies in 17 out of 46 (37%) patients with IgM-PNP, a percentage that seems in line with our findings. (Mata 2011) There is convincing evidence that anti-ganglioside antibodies play a role in the pathogenesis of inflammatory neuropathies. Anti-ganglioside IgM, IgA and IgG antibodies are associated with acute axonal forms of Guillain-Barre syndrome, Miller Fisher syndrome and multifocal motor neuropathy. (Nobile-Orazio 2010, Kaida 2006, Kaida 2007, Ogawa 2009, Kusunoki 2008) Pathogenic properties, i.e. leukocyte and complement activating capacities, of anti-ganglioside antibodies in serum from patients with GBS and MMN have been documented before and are strongly associated with the onset of neuropathy in the rabbit model for GBS. (Piepers 2010, van Sorge 2003, van Sorge 2007) The complement activating properties of anti-ganglioside IgM antibodies in sera from patients with IgM-PNP therefore support their role in pathogenesis through complement-dependent and independent effects, such as activation of signalling pathways that interfere with axonal functions. (Zhang 2011) Unfortunately, animal models or cell based assays that were previously used to assess pathogenicity of anti-ganglioside antibodies have been used only to test IgG and not IgM antibodies. (Willison 2008, Yuki 2001, Bullens, 2002)

Anti-ganglioside antibody specificity correlates with the predominant clinical features, which is explained by the differential expression of gangliosides in cranial, sensory and motor nerves. (Kaida 2006, Kaida 2007, Ogawa 2009, Kaida 2008, Kanzaki 2008) We found a wide range of ganglioside specificities of IgM antibodies, including reactivity with monosialic, disialic, quatrosialic gangliosides and their complexes. Antibodies with specificities associated with severe motor variants of GBS such as GM1, GD1a and GD1a-GD1b ganglioside complexes (Kaida 2007, Kaida 2008) were relatively scarce, while specificities associated with ataxic neuropathies (GD1b and GQ1b) were relatively frequent. (Kaida 2006, (Ogawa 2009, Kanzaki 2008) In this study, presence of monoclonal antibodies against only GD1b or GQ1b was associated with a sensory-ataxic neuropathy, otherwise there was no clear correlation with antibody specificity and clinical phenotype. This may be explained by the fact that antibody reactivity with multiple gangliosides in the same serum sample was relatively frequent. Antibody cross-reactivity (e.g. GD1b and GQ1b) may explain reactivity with multiple gangliosides in some, (Willison 1994) but not all (e.g. GM2 and GD1b) patients. Proliferation of more than one B-cell clone is a more likely explanation for these findings. This hypothesis is also supported by the fact that anti-ganglioside IgM antibodies used more than one single light chain in 7 out of 19 (37%) patients. Bone marrow analysis showed plasma cell proliferation in three patients, whose biopsy materials were not available for further research, precluding analysis of clonality of B-cells in addition to IgM antibodies.

Antigenic stimulation as a result of, for example, microbial infections could explain oligoclonal proliferation of ganglioside-specific B-cells and the use of both lambda and kappa light chains. (Vanderlugt 1996) We previously reported that immunoglobulin variable (V) region use in B-cells in these patients is skewed towards V genes used for responses to bacterial infections, which further supports this hypothesis. (Eurelings 2006) Polyclonal antibody responses against multiple gangliosides may also be the result of strong antigenic stimulation after nerve damage and the release of nerve constituents. However, we feel this explanation is less likely since there was no clear correlation of the presence of anti-ganglioside IgM antibodies with disease duration or severity. Our findings suggest that the pathogenesis of IgM-PNP with anti-ganglioside and anti-MAG antibodies differ despite clinical similarities. Differences in etiology might also explain previously observed differences in efficacy of immunomodulating treatment between patients with anti-MAG and those with anti-ganglioside antibodies, who may more often respond to treatment with IVIg, similar to patients with GBS and MMN. (Mata 2011)

The specificity of IgM antibodies in patients with IgM MGUS polyneuropathy without MAG or ganglioside specific antibodies remains to be established. We found antibodies

against GSC in addition to other single anti-ganglioside antibodies in serum from only 8% of patients with IgM MGUS polyneuropathy, which is only half of the reported frequency in GBS. Glycoarray technology may be helpful to identify other antibody specificities in patients with IgM-PNP.

Our findings show that anti-ganglioside antibodies may play a role in the pathogenesis of monoclonal gammopathy associated neuropathy, but that the pathogenesis of neuropathy with IgM anti-ganglioside antibodies and anti-MAG antibodies may differ. Differences in pathogenesis of the anti-MAG and anti-ganglioside variants of IgM-PNP suggest that optimal treatment strategies of these disorders are likely to differ as well.

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3

Clinical phenotype of patients with neuropathy associated with monoclonal gammopathy: a comparative study and a review of the literature

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Abstract

Objectives To investigate if the clinical and electrophysiological phenotype of patients with polyneuropathy associated with IgM monoclonal gammopathy (IgM-PNP) is related to the presence of antibodies against gangliosides or myelin-associated glycoprotein (MAG).

Methods We compared clinical and nerve conduction study (NCS) characteristics of 11 IgM-PNP patients with antibodies against asialo-GM1 or gangliosides (GM1, GD1a, GD1b, GM2 or GQ1b) to 11 consecutive IgM-PNP patients with anti-MAG neuropathy and to 9 IgM-PNP patients without antibodies against either MAG or gangliosides.

Results Patients with anti-ganglioside antibodies could not be differentiated from those with anti-MAG antibodies based on clinical characteristics. However, within the group of anti-ganglioside antibody positive patients, antibodies against GD1b and GQ1b were associated with a purely sensory neuropathy (p=0.002), while asymmetric weakness with symmetric sensory loss was associated with anti-asialo-GM1 antibodies.

Conclusions Polyneuropathy associated with IgM monoclonal gammopathy and anti-ganglioside antibodies clinically resembles anti-MAG neuropathy. Pure sensory neuropathy and marked asymmetry may suggest the presence of anti-ganglioside rather than anti-MAG antibodies.

Introduction

Five to ten percent of patients with otherwise unexplained polyneuropathy have an IgM monoclonal gammopathy. Approximately 40-70% of these patients have antibodies against myelin associated glycoprotein (MAG). (Kuijf 2009) Anti-MAG neuropathy is a predominant sensory neuropathy with sensory ataxia, relatively mild motor deficits and nerve conduction (NCS) features compatible with length dependent demyelination. (Steck 2006) A second, smaller subgroup (15%) of patients with IgM-PNP is characterized by the presence of antibodies against asialo-GM1 or gangliosides. (Eurelings 2001) The clinical characteristics of IgM-PNP patients with anti-ganglioside antibodies have been less well described and it is not known whether response to treatment with IVIg or rituximab differs in patients with IgM-PNP with anti-MAG or anti-ganglioside antibodies. (Dalakas 2009, Attarian 2006, Leger 2013) In patients with Guillain-Barré syndrome (GBS), the specificity of anti-ganglioside antibodies is associated with the type of neurological deficits. For example, antibodies against GM1 or GD1a are associated with pure and severe motor neuropathy, while antibodies against GD1b or GQ1b are associated with ataxia. (Susuki 2001, Willison 2001, Willison 2001a) We therefore hypothesized that patients with IgM-PNP and antibodies against asialo-GM1 or gangliosides may have a distinct clinical phenotype and performed a comparative study of clinical and electrophysiological characteristics of patients with IgM-PNP with and without anti-ganglioside antibodies.

Patients and methods

Patients

Approval for this study was obtained from the ethical standards committee of the UMC Utrecht. We identified 23 patients with IgM-PNP and antibody reactivity against asialo-GM1 or gangliosides (GM1, GM2, GD1a, GD1b, or GQ1b) in a prospective cohort of 169 patients that was followed at our outpatient clinic between 1991 and 2012. (Niermeijer 2010) Eleven patients were included in this study: three patients had anti-asialo-GM1 antibodies, one had antibodies against GM1, one had antibodies against GQ1b, three patients had antibody reactivity against 2 gangliosides (one against GD1b and GQ1b, one against GM2 and GD1a, and one against GM1 and GQ1b), one patient had antibodies against three gangliosides (GM1, GD1b and asialo-GM1), and two patients had antibodies against 4 gangliosides:(one against GD1a, GD1b, GQ1b and asialo-GM1 and one against GM2, GD1a, GD1b and

GQ1b). Two patients had antibodies against both MAG as well as against asialo-GM1. The remaining 10 patients could not be included in this study for a number of reasons (death: 2; lost to follow-up: 2; concomitant disease that would influence assessment of functional impairment: 4; dementia making informed consent impossible: 2).

Twenty consecutive patients without antibodies against gangliosides were included for comparison with the anti-ganglioside positive patients. Anti-MAG antibodies were detected in 11 of these patients by commercially available enzyme-linked immunosorbent assay (ELISA) (Bühlmann Laboratories, Switzerland) with a cut-off point of 1500 Bühlmann titer units (BTU). (Kuijf, 2009) Anti-MAG titers ranged from 13,055 BTU to 349,783 BTU, with a median of 70,000 BTU. Patient characteristics are summarized in Table 3.1.

All patients were seen by the same physician (ACJS) and were interviewed using a standardized questionnaire to assess functional impairment and disease history. A full neurological examination was performed. Severity of neurological deficits and functional impairment was documented using the INCAT overall disability sum score (ODSS), medical research council (MRC) sum score, INCAT sensory sum score assessing pinprick and vibration sensations and the kinetic and balance sections of the international consilium on ataxia rating scale (ICARS). (Trouillas 1997, Merkies 2003) In addition, walking ability of patients with anti-MAG or anti-ganglioside antibodies was quantified by the shuttle walking test. (Erdmann 2005)

NCS were performed on one side by one investigator (HF). (Franssen 2006) Motor nerve conduction with stimulation up to the axilla or popliteal fossa was performed in the median, ulnar, peroneal and tibial nerves, the median nerve being investigated to hand and forearm muscles. Analysis included distal compound muscle action potential (CMAP) amplitude, distal motor latency (DML), motor conduction velocity and CMAP-area drop in each segment, and terminal latency index (TLI). Distal sensory nerve conduction was performed in the median, ulnar, radial, lateral cutaneous nerve of the forearm, and sural nerves; analysis comprised sensory nerve action potential amplitudes (SNAPs). NCS were classified as demyelinating if at least two nerves had features of demyelination and as axonal if at least two nerves had features of axonal loss in the absence of features of demyelination. One patient declined electrophysiological examination.

Bone marrow biopsies were performed on clinical indication at the discretion of the treating haematologist.

Review of previously published cases

We reviewed previously published descriptions and case series of patients with neuropathy associated with IgM monoclonal gammopathy and anti-ganglioside antibodies. We used gammopathy, gangliosides, polyneuropathy and relevant descriptive diagnoses (i.e. CANOMAD, sensory ataxic neuropathy) as search terms in Pubmed. Only papers in English in which both the clinical phenotype and performed auxiliary investigations were described in sufficient detail per patient were included.

Statistics

Mann-Whitney U test was used to assess the differences in patient characteristics, clinical and functional scales and NCS findings between anti-ganglioside and anti-MAG antibody positive patients and between anti-ganglioside positive patients and those with IgM-PNP without known antibodies. To correct for multiple testing, we used the Bonferroni correction for p-values, considering the p-value of 0.0125 significant for differences in motor NCS. The Fisher's exact-test was used to assess differences in categorical variables between the same groups regarding the presence or absence of demyelinating NCS and the presence of monoclonal cells in bone marrow biopsies. The Fisher exact test was also used to test the association between anti-ganglioside antibodies and specific clinical phenotypes, comparing patients with specific anti-ganglioside antibodies to all other patients in this study.

Results

Clinical phenotypes

Patient characteristics are summarized in Tables 3.1 and 3.2. Patients 1 and 9 were described in an earlier study. (Eurelings 2001) All patients had a clinical phenotype of a sensorimotor polyneuropathy characterized by sensory disturbances, ankle dorsiflexor weakness and gait ataxia with the exception of two patients with antibodies against GQ1b or GD1b who presented with a distinct clinical syndrome of moderately severe sensory-ataxic neuropathy with complete sparing of motor function (p=0.002, Fisher exact test). Two other patients with antibodies against asialo-GM1 but not against gangliosides had marked asymmetric weakness for which no other cause could be identified, not found in patients without anti-asialo GM1 antibodies (p=0.01, Fisher exact test). One of these patients had weakness in his left leg only, and the other predominantly in the right arm, with symmetric mild sensory

Table 3.1 Patient characteristics, clinical scores and nerve conduction studies

	Anti-ganglioside antibodies (N=11)	Anti-MAG antibodies (N=11)	Antibody specificity unknown (ASU) (N=9)
Age at diagnosis	54.5 (44-81)	60 (39-74)	56.5 (48-74)
Sex, % male	64%	100%	67%
Duration of disease in years	3 (0-15)	1 (0-5)	6 (0-12)
Presence of monoclonal cells in bone marrow biopsy	2 (20%)	1 (13%)	0
MRC motor sum score(0-140)	132 (106-140)	133 (138-120)	134 (96-138)
INCAT sensory sum score	19 (12-29)	22.5 (9-29)	12 (0-33)
ICARS ataxia score	27 (15-44)	28.5 (10-41)	27 (15-50)
ODSS	4.0 (2-6)	4.5 (2-6)	5.0 (2-7)
Modified Rankin score	2.0 (2-3)	2.0 (2-3)	2.0 (2-4)
Shuttle walking test (no. shuttles)	18 (1-91)	21.5 (10-121)	-
AAN demyelination criteria	60%	91%	78%
DML median nerve	5.9 (3.9-10.1)	8.2 (6.3-15.1)	6.7 (4.5-9)
MCV lower arm median nerve	44 (26-59)	42.5 (33-58)	47 (24-59
Minimal F-M interval median nerve	30.9 (24-43)	35.7 (25-43)	31 (28-54)
TLI median nerve	0.28 (0.18-0.40)	0.24 (0.17-0.30)	0.29 (0.14-0.54)
SNAP median nerve	9 (0-27)	0 (0-7)	0 (0-27)
SNAP sural nerve	4 (0-8)	0 (0-6)	0 (0-7)

Data are expressed as median (range) (age, disease duration, motor, sensory and ataxia sum scores), percentage of male patients or patients meeting demyelination criteria, total number of shuttles reached (shuttle walking test) and as median and range (DML (distal motor latency), MCV (motor conduction velocity), SNAP (sensory nerve action potential) and TLIs (terminal latency indices) for the median and sural nerves).

Table 3.2 Anti-ganglioside antibodies, clinical phenotype, nerve conduction studies per patient

Pt	Antibody specificity	Clinical phenotype	NCS
1	GD1a, GD1b, GQ1b, asialo-GM1	Sensorimotor	Demyelinating
2	GM2, GD1a, GD1b, GQ1b	Sensorimotor	Demyelinating
3	Asialo-GM1	Sensorimotor, asymmetric weakness in arms	Demyelinating
4	Asialo-GM1	Sensorimotor, asymmetric weakness in legs	Axonal
5	GM1	Sensorimotor	Not performed
6	GM1, GD1b, asialo-GM1	Sensorimotor	Axonal
7	Asialo-GM1	Sensorimotor	Demyelinating
8	GD1b, GQ1b	Pure sensory	Axonal
9	GM2, GD1a	Sensorimotor	Demyelinating
10	GM1, GQ1b	Sensorimotor	Demyelinating
11	GQ1b	Pure sensory	Axonal[a]

[a] NCS of patient 11 were consistent with axonal loss in sensory nerves at first presentation, but showed features of demyelination of motor nerves at repeated investigation 15 years later.

loss in the legs. The 2 patients with both anti-MAG and anti-ganglioside antibodies had a relatively mild sensorimotor neuropathy.

Clinical scores

Motor, sensory and ataxia scores, ODSS, Rankin score, shuttle walking and NCS were not significantly different between groups. Sensory function tests were more abnormal in patients with antibodies against GD1b, GQ1, or MAG than in patients with anti GM1, GD1a or asialo-GM1 antibodies, but differences did not reach statistical significance.

Nerve conduction studies

NCS in patients with anti-ganglioside antibodies did not differ convincingly from NCS in other IgM-PNP patients. Demyelinating features were observed more often in patients with anti-MAG antibodies (91%) than in patients with anti-ganglioside antibodies (60%) and with antibodies of unknown specificity (78%), but differences did not reach statistical significance. Distal demyelination of motor nerves seemed more pronounced in patients with anti-MAG neuropathy than in patients with IgM-PNP and antibodies against gangliosides as reflected by the TLI differences of the median nerve ($p=0.02$, Mann-Whitney U test) and longer DMLs ($p=0.04$). In 4 cases NCS stood out from the others: in two patients (one without anti-MAG or anti-ganglioside antibodies and one patient with anti-MAG antibodies) we found definite conduction block. One of the patients with anti-asialo GM1 antibodies and asymmetric weakness (patient 3) had markedly asymmetrical multifocal demyelination of motor nerves in the arms, and asymmetric demyelination and axonal loss in motor nerves in the legs. Sensory nerve conduction studies were abnormal in both legs. This patient did not meet diagnostic criteria for multifocal motor neuropathy (MMN). NCS of one patient (no. 11) who presented with isolated sensory-ataxic deficits showed reduced SNAPs but no abnormalities in motor nerves. Fifteen years later at follow-up sensory deficits had progressed while strength assessment remained completely normal, but NCS now also showed decreased motor conduction velocities.

Review of literature

We found 21 previously published articles with clinical descriptions of 67 patients with IgM-PNP and antibodies against asialo-GM1 or gangliosides (Table 3.3). NCS showed demyelination in 45 (67%) patients. All patients with NCS abnormalities consistent with axonal loss had anti-GD1b antibodies, 18 of these patients also had antibodies against

Table 3.3 Overview of patients with IgM monoclonal gammopathy, neuropathy and anti-ganglioside antibodies described in literature

Antibodies found	No. of pts	Clinical phenotype	Nerve conduction	References
Asialo-GM1	1	Sensorimotor	Demyelinating	Eurelings[a]
Asialo-GM1,GD1a, GD1b, GQ1b	1	CANOMAD with weakness	Demyelinating	Willison
Asialo-GM1,GM1, GD1a	1	Sensorimotor		Lehmann
Asialo-GM1,GM1, GD1b	2	1 sensorimotor, 1 motor	Demyelinating	Ilyas , Eurelings
Asialo-GM1,GM1, GD1a,GD1b	1	Motor	Demyelinating	Carpo
Asialo-GM1,GM1, GM2,GD1a, GD1b,GQ1b	3	CANOMAD, 2 of whom with weakness	Demyelinating	Willison, Löscher
Asialo-GM1,GM1, GQ1b	1	CANOMAD with weakness	Demyelinating	Sanvito
GM1	3	Motor	Demyelinating	Matà
GM1, GM2	2	Sensorimotor	Demyelinating	Ilyas
GM1,GD1b	1	Sensorimotor with cranial nerve involvement	Demyelinating	Attarian
GM1,GD1a,GD1b	1	Sensorimotor, with cranial nerve involvement	Demyelinating	Delval
GM1,GD1b,GQ1b,	1	Sensorimotor	Demyelinating	Carpo
GM1,GM2,GD1a, GD1b	1	Motor	Demyelinating	Carpo
GM1,GM2,GD1a	3	2 motor, 1 sensorimotor	Demyelinating	Oga Ortiz, Lopate
GM1, GM2,GD1b	1	Sensory	Demyelinating	Eurelings
GM2	1	Motor	Demyelinating	Eurelings
GM2, GD1a	1	Motor		Matà
GM2,GD1a,GD1b,GQ1b	1	Sensory	Demyelinating	Eurelings
GM2,GD1b,GQ1b	1	Sensorimotor, with cranial nerve involvement	Demyelinating	Carpo
GD1a	2	1 sensorimotor, 1 motor	Demyelinating	Bollensen, Eurelings
GD1a, GD1b	4	3 sensorimotor, 1 sensory	1 axonal	Lehmann, Matà, Younes-Chennoufi
GD1a,GD1b,GQ1b	9	6 CANOMAD, 5 of whom with weakness 3 sensorimotor, 2 with cranial nerve involvement	3 demyelinating, 3 axonal	Willison, Sanvito, Susuki
GD1b	1	Sensorimotor, with cranial nerve involvement	Axonal	Attarian
GD1b, GQ1b	21	4 sensorimotor, 2 of whom with cranial nerve involvement, 1 sensory with cranial nerve involvement, 15 CANOMAD, 6 of whom with weakness 2 sensory	14 axonal, 4 demyelinating, 1 neuronopathy	Willison, Matà, Kam, Susuki Delmont, Iorio, Daune, Eurelings
GQ1b	3	1 sensorimotor, 1 sensory, 1 motor	Demyelinating	Eurelings

[a] Two patients described by Eurelings et al. are described in this study as well and are not included in this table.

GD1a or GQ1b. The most frequent clinical phenotype was a purely sensory neuropathy in 18 patients (described as either "CANOMAD" or "sensory neuropathy") and antibodies against GD1b or GQ1b. Five out of 18 (28%) also had antibodies against gangliosides other than GD1b or GQ1b.

Discussion

Polyneuropathy with IgM monoclonal gammopathy is often associated with the presence of IgM antibodies against myelin-associated glycoprotein (MAG). A second, smaller subgroup of patients is characterized by the presence of antibodies against asialo-GM1 and gangliosides. (Eurelings 2001, Nobile-Orazio 2011) Most patients with a polyneuropathy associated with IgM monoclonal gammopathy have sensory disturbances, sensory ataxia and relatively mild weakness. A pure sensory or pure motor involvement or marked asymmetry of weakness suggest the presence of anti-ganglioside rather than anti-MAG antibodies. We observed a more length-dependent pattern of demyelination in anti-MAG PNP patients compared to those with anti-ganglioside antibodies, however, as we tested multiple NCS characteristics, these p-values did not meet our Bonferroni-corrected threshold and might be chance occurrences after multiple testing and not a reflection of real, statistically significant differences.

The clinical features of patients with anti-ganglioside antibodies are more heterogeneous than those of anti-MAG neuropathy. In Guillain-Barre syndrome a similar heterogeneity in clinical features is found in relation to variation in the specificity of anti-ganglioside antibodies. These clinical differences may be in part explained by the differential expression patterns, function or accessibility to antibody binding of gangliosides in motor and sensory nerves and roots. (Susuki 2001, Willison 2001, Willison 2001a) In our cohort the presence of anti-GD1b and GQ1b antibodies was associated with pure sensory deficits, as has been previously described in patients with Fisher syndrome, chronic sensory ataxic neuropathy and chronic sensory ataxia with ophtalmoplegia and M protein (CANOMAD). (Susuki 2001, Willison 2001, Willison 2001a) The presence of antibodies against asialo-GM1 was associated with more pronounced motor deficits and asymmetry. Although our patient cohort is too small to draw definite conclusions, these patterns are similar to the ones reported in patients with GBS. The association of antibody-specificity and clinical phenotype has been only investigated in small series of patients with IgM-PNP (Table 3.3). Although this implies the possibility of selection bias, the available studies clearly suggest an association of anti-GD1b and GQ1b antibodies with predominantly sensory or atactic neuropathies,

including CANOMAD. The available data do not support additional associations of other antibody specificities with specific clinical or nerve conduction characteristics. One patient had asymmetric weakness of the arms with multifocal demyelinating features and anti-asialo GM1 antibodies. This patient did not meet the diagnostic consensus criteria for MMN, although the clinical phenotype suggests remarkable similarities. The association of anti-asialo GM1 antibodies with asymmetry therefore needs to be corroborated in future studies.

The pathogenicity of anti-ganglioside antibodies has been extensively documented using *in vitro* and *in vivo* models for GBS. (Willison 2001) Although this is a relatively small study, the specific association of antibody specificity and neurological deficits and the analogy with GBS suggests that anti-ganglioside antibodies also play a role in the pathogenesis of monoclonal gammopathy associated neuropathies. Anecdotal reports suggest that at least some patients with IgM-PNP and anti-ganglioside antibodies may respond to IVIg, (Attarian 2006) similar to other neuropathies associated with anti-ganglioside antibodies including GBS and MMN. However, the effect of IVIg was short-lived in patients with IgM-PNP and better treatment strategies are therefore needed. (Attarian 2006) Most therapeutic trials are currently solely aimed at IgM-PNP patients with anti-MAG antibodies, of which pathogenicity is well established. (Dalakas 2009, Leger 2013) As patients with anti-ganglioside antibodies comprise approximately 15% of all patients with IgM -PNP, testing for anti-ganglioside antibodies should therefore be considered in patients with IgM monoclonal gammopathy and sensorimotor polyneuropathy without anti-MAG antibodies, as should inclusion of these patients in new therapeutic trials.

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PART II

Immune activity and polyneuropathy

4

Classical and lectin complement pathway activity in polyneuropathy associated with IgM monoclonal gammopathy

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Submitted.

Abstract

Polyneuropathy associated with IgM monoclonal gammopathy (IgM-PNP) is a slowly progressive, sensorimotor symmetrical neuropathy. IgM antibodies against myelin-associated-glycoprotein (MAG) or gangliosides are found in serum of 50-70% of patients with IgM-PNP and depositions of complement factors have been found in their nerve biopsies. It is therefore assumed that complement activation contributes to the pathogenesis of IgM-PNP.

We investigated whether differences in innate complement activity of the classical and mannose binding lectin (MBL) pathways are associated with IgM-PNP or its disease course. We measured complement activity of the classical and MBL pathways by ELISA and determined MBL serum concentrations and polymorphisms of the *MBL2* gene in 83 patients and compared these to age and sex-matched healthy controls.

We did not observe significant differences in innate complement activity, soluble MBL serum concentrations or MBL gene polymorphisms between IgM-PNP and healthy controls or between patients with progressive or more indolent disease course. It is unlikely that differences in innate complement activity contribute significantly to susceptibility to or severity of IgM-PNP.

Introduction

Polyneuropathy associated with IgM monoclonal gammopathy (IgM-PNP) is characterized by slowly progressive sensorimotor deficits, which may lead to significant disability in a subgroup of patients. (Niermeijer 2010) IgM antibodies against glycoproteins and glycolipids such as myelin associated glycoprotein (MAG) and gangliosides, which are expressed in nerves, are found in the majority of patients with IgM-PNP. It is generally assumed that binding of these antibodies to nerve constituents triggers pathogenic pathways that cause a length-dependent and most often demyelinating, sensorimotor polyneuropathy. (Latov 2014)

Complement activation has been identified as an important pathogenic mechanism in animal models of antibody-mediated neuropathies. (Halstead 2004, Jacobs 2003) The finding of complement depositions in nerve biopsies from patients with anti-MAG neuropathy suggest that antibody-complement interactions likely also contribute to the pathogenesis of IgM-PNP. (Hays 1987) The complement system consists of a cascade of plasma proteins that upon activation culminates in the deposition of the membrane attack complex (MAC), a porin that causes cellular permeability. Antibodies can activate the complement cascade via 2 pathways, i.e. the classical pathway and the mannose-binding lectin (MBL) pathway. The contribution of complement to the pathogenesis of multiple disorders has led to design and development of complement-targeted therapies. (Botto 2009, Ricklin 2007, Lambris 2007) Attenuation of complement function ameliorates nerve function in several *in vitro* and *in vivo* models of antibody-mediated inflammatory neuropathies. (Fitzpatrick 2011, Halstead 2008) Polymorphisms in complement genes partially explain inter-individual differences in innate activity of complement pathways and have been identified as risk factors for inflammatory disorders. Polymorphisms in the *MBL2* genes (Garred 2003) have been found associated with disease severity of Guillain-Barré syndrome. (Geleijns 2006) We investigated whether the innate activity of the MBL and classical complement pathways, MBL serum concentrations and MBL2 genotypes are associated with IgM-PNP or with progressive disease course.

Patients and methods

Eighty-three patients from a prospectively followed cohort with IgM-PNP, of whom 49 (59%) had anti-MAG antibodies were included in this study. (Niermeijer 2010) We used the inflammatory neuropathy cause and treatment group (INCAT) overall disability sum score (ODSS) to document disability (Merkies 2002) and the 5 point medical research council (MRC) scale to assess muscle strength of 14 muscle groups (deltoid, biceps and triceps

brachii, wrist extensors, finger flexors and extensors, first interosseous, iliopsoas, hamstrings, quadriceps femoris, anterior tibial, gastrocnemius, peroneal and extensor hallucis longus muscles) in arms and legs on both sides to calculate a MRC sumscore with a maximum of 140 points. Sensory deficits were scored using the pinprick and vibration components of the INCAT sensory sum score (ISS). (Merkies 2002) Neurological examination was performed by one of the authors (ACJS). Follow-up data 2 years after complement measurement in addition to baseline data were available of 37 patients. In this subgroup, progressive disease was defined as a gain of 1 point or more on the ODSS functional score after 2 years. Serum samples from 83 age and sex matched individuals were used as controls. Nerve conduction studies were performed for 75 patients and classified as demyelinating if at least two nerves had features of demyelination and as axonal if at least two nerves had features of axonal loss in the absence of features of demyelination.

Sera were immediately frozen and stored at -80°C until use. Presence of anti-MAG antibodies was determined by commercially available ELISA (Buehlmann Laboratories, Switzerland), with a cut-off point of 1500 BTU (Buehlmann Titer Units). The presence of IgM antibodies against the gangliosides GM1, GM2, GD1a, GD1b, GQ1b and asialo GM1 was tested using the standardized enzyme-linked immunosorbent assay (ELISA) of The Inflammatory Neuropathy and Treatment (INCAT) group (Kuijf 2005) and results have been published previously. (Stork 2014) Genomic DNA was obtained from whole blood samples of 59 patients and 71 controls using standard techniques.

Complement ELISA

The innate activity of the lectin and classical pathways of complement was determined using a previously published ELISA protocol with minor modifications. (Petersen 2001, Roos 2003) In short, ELISA plates were coated with either mannan (10 μg/ml,Sigma,St. Louis, MO, USA) for the lectin pathway or with human-IgM (3 μg/ml, Calbiochem, San Diego, CA, USA) for the classical pathway. Control wells were left uncoated. For each sample a corrected optical density (OD) was calculated (OD of coated wells minus OD of non-coated wells). Serum samples were added in triplicate. In order to block any contribution of the classical pathway to the complement activation by mannan, anti-human C1q antibodies (Sanquin, Amsterdam, The Netherlands) were added to the serum during assessment of the lectin pathway activity.(Roos 2001) To correct for day-to-day variation and variation between the plates, human pooled serum (HPS) from 10 healthy donors was included in each experiment. Lectin and classical pathway activity was expressed relative to the activity of the HPS (corrected OD sample / corrected OD HPS * 100).

MBL serum concentrations and MBL2 genotypes

Serum concentrations of the multimeric MBL protein were determined with an enzyme-linked immunosorbent assay (ELISA) following manufacturer's instructions (Sanquin, Amsterdam, The Netherlands).

The X/Y promoter polymorphism (rs7096206) and the three SNPs in exon 1 (wild-type 'A' and variants 'O' rs5030737, rs1800450 and rs1800451) of the *MBL2* gene were determined using genomic DNA and a previously described denaturing gradient gel electrophoresis (DGGE) assay in a nested polymerase chain reaction (PCR) protocol. (Madsen 1995, Garred 2003) Genotypes 0/0 and XA/0 were considered MBL-deficient, and genotypes YA/0, XA/XA, XA/YA and YA/YA were considered MBL-sufficient, with the YA/YA genotype related to the highest lectin pathway activity.

Statistics

Differences in the distribution of relative complement activity and soluble MBL concentrations between IgM-PNP, anti-MAG neuropathy patients and healthy controls were assessed by Mann-Whitney-tests (SPSS v22, IBM Corporation, Armonk, New York, USA). Frequencies of MBL genotype were analysed by chi-square test. To ascertain whether a progressive disease course was correlated with higher complement activity we used the Fisher exact test to compare the distribution of the top quartile of the different complement measurements between patients with or without progressive disease (SPSS v22, IBM Corporation, Armonk, New York, USA). We used Bonferroni-corrections for multiple testing of the 4 different complement measurements. P-values <0.0125 were significant.

Results

Patient characteristics

Patient characteristics are summarized in Table 4.1.

Comparison of complement activity and sMBL concentrations between patients and healthy controls

Median intra-assay variation of complement activity was 14% for the MBL pathway (range 3-25%) and 4% (range of 0-15%) for classical pathway. There were no statistical differences

Table 4.1 Patient characteristics

	IgM-PNP	Controls
Age at inclusion (range)	64 (41-88)	62 (39-87)
Male gender (%)	58 (70%)	58 (70%)
Demyelinating neuropathy	60 (72%)	-
Anti-MAG antibodies (%)	49 (59%)	-
Anti-ganglioside antibodies (%)	14 (17%)	-

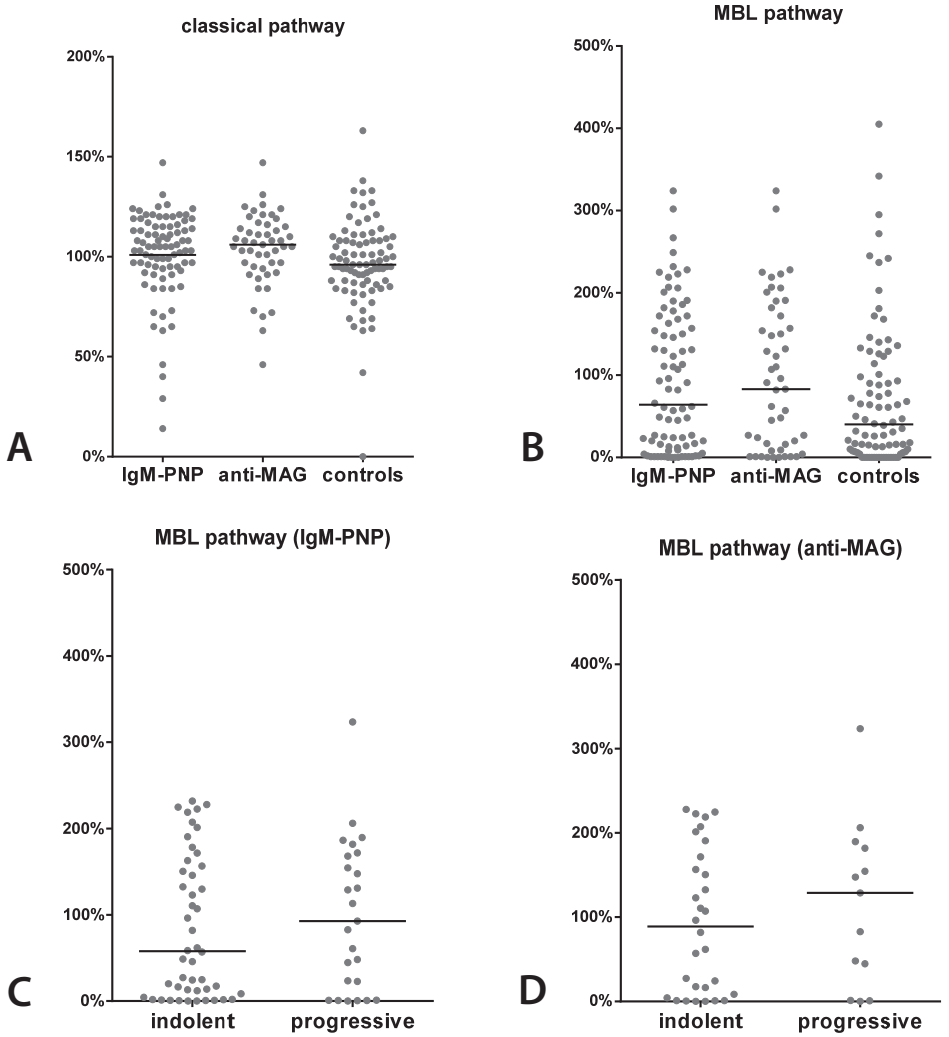

Figure 4.1A and B: complement activation via the classical and MBL pathway for all IgM-PNP patients ("IgM-PNP"), anti-MAG PNP patients ("anti-MAG") and controls, expressed as percentages of the optical densities of pooled reference sera.
Figure 4.1C and D: complement activation via the MBL pathway for patients with either a progressive or more indolent disease course of IgM-PNP and anti-MAG PNP patients.

in MBL or classical pathway activity between IgM-PNP patients and healthy controls (Table 4.1 and Figure 4.1). MBL pathway activation was higher in patients with a progressive disease course, as defined by an ODSS score of 4 or more 3 years after diagnosis, but the difference was not significant (Figure 4.1).

Innate activity of the MBL pathway, soluble MBL concentrations in serum and distribution of MBL genotypes

To test the internal consistency of our findings we compared the distributions of MBL genotype frequencies, soluble MBL and innate activity of the MBL pathway. Soluble MBL serum concentrations were significantly correlated with MBL genotypes and innate activity of the MBL pathway, as was the innate activity with MBL genotypes (p=0.00 for all tests, Mann-Whitney test). Soluble MBL serum concentrations did not differ significantly between IgM-PNP patients, anti-MAG patients and healthy controls (Table 4.2). Similarly, frequencies of MBL genotypes did not differ significantly in patients and controls, with the exception of genotypes associated with MBL deficiency (XA/0 and 0/0), which were absent in patients with anti-MAG antibodies (p=0.03, not significant after correction for multiple testing) (Table 4.3). Soluble MBL serum concentrations or MBL genotypes did not differ significantly between patients with active or more indolent disease course (data not shown).

Table 4.2 Median (and range) of complement measurements for patients and controls

	All IgM-PNP	Anti-MAG only	Controls
Classical PW activity	1.05 (0.14-1.47)	1.06 (0.46-1.47)	0.97 (0.42-1.63)
MBL PW activity	0.61 (0-8.1)	0.82 (0-3.24)	0.4 (0-4.1)
sMBL concentration	732 (0-6142)	762 (0-4509)	740 (118-4646)
Deficient MBL phenotype	5 (8%)	0	10 (14%)

Table 4.3 Frequency of MBL phenotypes for patients and controls

MBL genotype	All IgM-PNP (n=59)	Anti-MAG only (n=32)	Controls (n=71)
YA/YA	20 (34%)	12 (38%)	19 (27%)
YA/XA	10 (17%)	5 (16%)	19 (27%)
YA/0	25 (42%)	15 (47%)	23 (32%)
XA/XA	1 (2%)	0	0
XA/0	0	0	4 (6%)
0/0	3 (5%)	0	6 (8%)

Discussion

Complement depositions in nerves are a well-described feature of IgM-PNP and data from experimental studies of antibody-associated neuropathies suggest that complement-activation is an important mediator of nerve damage. (Hays 1987) The presence of IgM antibodies against specific peripheral nerve glycoproteins and glycolipids suggested that high innate activity of either the classical (after binding of IgM antibodies to nerves) or MBL pathway (after binding of MBL to sugar residues exposed on the surface of peripheral nerves upon binding of antibodies to MAG) were potential risk factors for IgM-PNP or an unfavorable disease course. High MBL levels may be unfavorable in the context of tissue damage as exemplified by disorders characterized by tissue damage such as coronary heart disease. (Busche 2009) High MBL levels were previously identified as a risk factor for Guillain-Barre syndrome (Geleijns 2006) and high innate classical pathway activity with axonal damage in patients with multifocal motor neuropathy (MMN), (Vlam 2015) two other antibody-mediated inflammatory neuropathies. However, classical and MBL complement pathway activity, sMBL concentrations and MBL2 genotypes were not associated with IgM-PNP in this study.

We cannot fully exclude the possibility that our study is underpowered, in particular to detect meaningful differences in specific subgroups of patients. The variability of the complement assay is relatively large, which could limit its sensitivity to detect biologically relevant differences. It should however be noted that although MBL pathway activity was higher in patients with anti-MAG antibodies and a progressive disease course, sMBL concentrations and MBL2 genotype frequencies were quite similar in patients with anti-MAG and rapid progression and healthy controls. The data therefore do not support a trend towards higher MBP pathway activity in these patients. The absence of genotypes that are associated with MBL deficiency in patients with anti-MAG neuropathy may suggest that MBL pathway inactivity may protect against anti-MAG neuropathy, but the numbers are small and this association may very well be spurious.

If future studies would further address the relevance of variation of complement activity in inflammatory neuropathies, they could benefit not only from larger numbers, but also from improved methodology. Complement assays with improved signal to noise ratios would facilitate future studies. Moreover, improved clinical tools to measure disease progression in IgM-PNP, which is notoriously difficult in patients with IgM-PNP, are needed. Although the ODSS functional scale is validated for immune-mediated neuropathies, (Merkies 2002) it probably lacks sensitivity to document disease progression in a slowly progressive primarily

sensory polyneuropathy. The development of Rasch-based disability scales may be a first step to facilitate future studies.

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5

Serum cytokine patterns in patients with polyneuropathy associated with IgM monoclonal gammopathy

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Abstract

Neuropathy associated with IgM monoclonal gammopathy (IgM-PNP) is associated with the presence of IgM antibodies against nerve constituents such as myelin associated glycoprotein (MAG) and gangliosides. To test whether B-cell stimulating cytokines are increased in these patients we measured serum concentrations of 11 cytokines in 81 IgM-PNP patients and 113 controls. IL-6 concentrations were higher in patients with IgM-PNP and IL-10 concentrations were higher in the subgroup with anti-MAG antibodies. IL-6 and IL-10 concentrations were not increased in serum from 110 patients with multifocal motor neuropathy (MMN), suggesting that this is specific for IgM-PNP and not a general feature in antibody-mediated neuropathies.

Introduction

IgM monoclonal gammopathy associated polyneuropathy (IgM-PNP) is a predominantly sensory and demyelinating neuropathy that is associated with the presence of auto-antibodies against nerve constituents such as myelin associated glycoprotein (MAG) or gangliosides. (Vlam 2011, Matà 2011) The pathogenesis of IgM-PNP is probably dominated by B cells or plasma cells, since evidence for T-cell involvement is lacking. (Dalakas 2010) The mechanisms underlying B cell activation and pathogenic antibody production that ultimately cause nerve damage are largely unknown. IgM-PNP does not respond to treatment that is successfully used for other demyelinating neuropathies including corticosteroids and intravenous immunoglobulins (IVIg), cyclophosphamide-prednisone and fludarabine. (Lunn 2012) Since IgM-PNP can cause significant limitations in daily life, there is a need for new treatment strategies.

Specific patterns of cytokine expression have been found in serum from patients with B cell mediated auto-immune diseases. (van den Ham 2009) Cytokine expression patterns may suggest specific pathophysiological pathways that could help to design novel treatment strategies. We therefore compared cytokine expression profiles in patients with IgM-PNP and healthy controls and patients with MMN, another antibody-mediated inflammatory neuropathy, as disease controls.

Patients and methods

Patients

We collected serum samples of 81 patients with IgM-PNP from a prospectively followed cohort. Haematological malignancy was excluded in all patients. (Niermeijer 2010) Two patients developed a malignancy within a year after inclusion in this study.

110 patients with MMN were also included in this study (Cats 2010). All patients had a diagnosis of probable or definite MMN according to previously published diagnostic criteria. (Joint Task Force of the EFNS and PNS, 2010) 76 (69%) patients used intravenous immunoglobulin (IVIg) maintenance therapy. 113 sera of age and sex matched healthy volunteers were used as controls. Sera were frozen directly and stored at -80°C until analysis. Patient characteristics are summarized in Table 5.1.

Table 5.1 Patient characteristics

	IgM-PNP	MMN	Controls
Age at investigation (range)	63 (41-88)	55 (27-80)	61(29-95)
Male sex (%)	57 (70%)	82 (75%)	80 (71%)
Demyelinating neuropathy (IGM-PNP) or conduction block (MMN) (%)	60 (74%)	110 (100%)	-
IgM gammopathy (%)	81 (100%)	6 (6%)	-
Anti-MAG antibodies (%)	44 (54%)	-	-
Anti-ganglioside antibodies (%)	14 (17%)	62 (57%)	-

The presence of anti-MAG antibodies was determined by means of ELISA (Bühlmann Laboratories, Switzerland). Anti-ganglioside antibodies were determined as described previously. (Cats 2010, Stork 2014)

Multiplex bead-array assays

Concentrations of 9 cytokines were using a multiplex assay with magnetic, commercially available antibody-coupled beads (Bio-Rad Corporation, Hercules, CA, USA). All 9 antibody pairs and recombinant proteins used were purchased from commercial sources as described previously. (de Jager et al., 2005) Measurements and data analysis of all assays were performed using the Bio-Plex system in combination with the Bio-Plex Manager software V.4.0 using five parametric curve fitting (Bio-Rad Laboratories, Hercules, California, USA).

Enzyme-linked immunosorbent assays (ELISA)

Serum concentrations of APRIL and BAFF were determined using commercially available ELISA kits (eBioscience, San Diego, CA, USA). Serum diluted 1:2 was added to wells pre-coated with anti-APRIL or anti-BAFF antibodies. Biotin-conjugated anti-APRIL or anti-BAFF antibodies were added, followed by Streptavidin-horseradish peroxidase. Optical densities (OD) were determined using a Fluostar ELISA reader (BMG Labtech, Ortenberg, DE) after incubation with tetramethyl-benzidine substrate, stopped by phosphoric acid.

Statistical analysis

Differences in the distribution of individual cytokine serum concentrations across disease categories and healthy controls were assessed by Mann-Whitney-tests (SPSS v22, IBM

Corporation, Armonk, New York, USA). We used Bonferroni-corrections for multiple testing of 11 different cytokines. P-values <0.0045 were significant. To assess possible clusters of cytokine expression in different diseases or subsets we used Ward's method for hierarchical clustering. We log-transformed cytokine concentrations after imposing an detection limit of 0.1 pg/ml and for the purpose of cluster analysis setting all measurement below that detection limit to the detection limit (R, R foundation for statistical computing, Vienna, Austria).

Results

Comparison of cytokine concentrations between patients and controls

Results for all IgM-PNP and controls are summarized in Table 5.2. Direct comparison of concentrations of individual cytokines between patients with IgM-PNP, MMN and controls showed significantly (p=0.00) increased IL-6 concentrations for IgM-PNP patients compared to MMN patients and healthy controls. IL-10 concentrations were significantly increased only in patients with IgM-PNP and anti-myelin associated glycoprotein (MAG) antibodies (p=0.00) (Figure 5.1). Cluster analysis did not show specific cytokine profiles in patients with IgM-PNP or MMN.

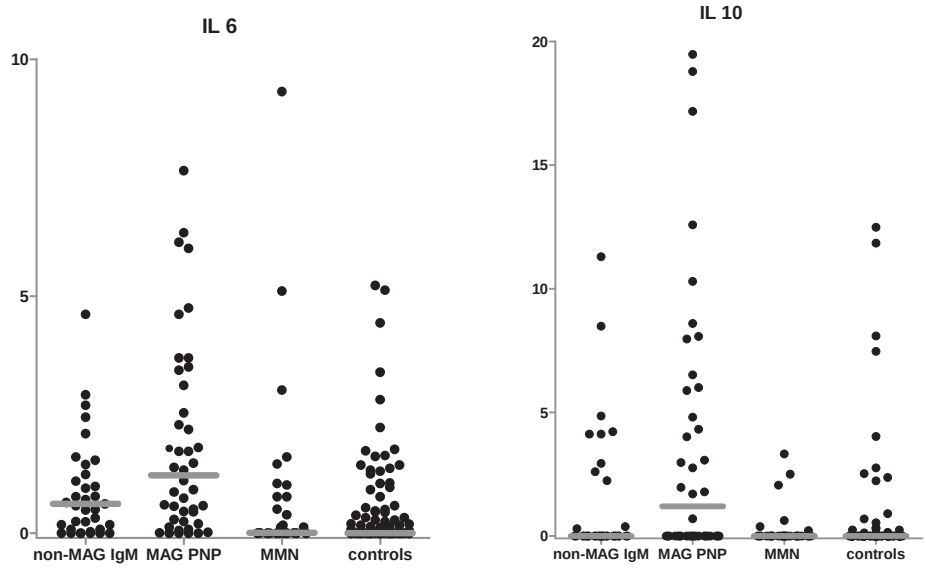

Figure 5.1 IL-6 and IL-10 serum concentrations in pg/ml for different patient groups, grey bar denoting median concentrations.

Table 5.2 Median cytokine concentrations (and range) in pg/ml (ng/ml for APRIL and BAFF) for IgM-PNP and MMN patients and controls, effect of IVIg treatment on median cytokine concentrations for MMN patients

Patient group	IL2	IL4	IL6	IL8	IL10	IL12
AntiMAG PNP	BDL (-20.55)	BDL (-2.81)	1.22 (BDL-33.91)	1.76 (0.1-16.56)	1.20 (BDL-73.09)	BDL (-10.95)
All IgM-PNP	BDL (-20.55)	BDL (-2.81)	0.77 (BDL-33.91)	1.64 (0.1-44.22)	BDL (-73.09)	BDL (-10.95)
MMN	BDL (-4.28)	BDL (-0.02)	0.01 (-30.48)	2.38(BDL-44.89)	0.11 (-131.27)	BDL (-7.1)
Controls	BDL (-21.41)	BDL (-4.31)	BDL (-5.23)	1.20 (BDL-31.42)	BDL (-12.49)	BDL (-7.49)

BDL=below detection limit

Patient group	TNFα	IFNγ	GM-CSF	BAFF	APRIL
AntiMAG PNP	BDL (-42.07)	BDL (-138.38)	BDL (-65.41)	0.35 (BDL-3.34)	3.48 (1.64-113.26)
All IgM-PNP	BDL (-42.07)	BDL (-138.38)	BDL (-65.41)	0.36 (BDL-24.98)	3.81 (1.64-113.26)
MMN	BDL (-10.55)	BDL (-175.32)	BDL (-51.41)	0.56 (0.16-109.0)	3.55 (BDL-156.5)
Controls	BDL (-91.31)	BDL (-449.75)	BDL (-116.2)	0.55 (BDL-3.59)	3.88 (BDL-127.55)

BDL=below detection limit

Discussion

Patients with IgM-PNP had higher IL-6 concentrations in serum than healthy controls and patients with MMN, while patients with IgM-PNP and anti-MAG antibodies (anti-MAG neuropathy) also had elevated IL-10 concentrations.

Elevated serum concentrations of IL-6 have been previously reported in patients with multiple myeloma (MM), Castleman or Schnitzler's syndrome and in a minority of MGUS patients without polyneuropathy. (Gironi 2010, van Deuren 2009, Rose-John 2007) Elevated IL-6 concentrations may therefore be a biomarker for plasma cell dyscrasia rather than IgM-PNP, (Bladé 2002) although patients with MMN and a concomitant M protein did not have elevated IL-6 levels (data not shown). Patients with IgM-PNP and elevated IL-6 did not have a distinct clinical (e.g. progressive) phenotype. Since follow-up in this study was relatively short, we cannot exclude the possibility that an increased IL-6 may reflect an increased risk of developing a hematological malignancy or a more progressive disease course. (Eurelings 2005) Disease activity in both auto-immune diseases like rheumatoid arthritis or systemic lupus erythematosus as well as in hematological malignancies (MM) has been shown to be correlated to IL 6 serum levels. IL-6 might have a direct stimulatory effect on plasma cells or promote B cell differentiation. Alternatively, IL-6 may skew T cells towards the pro-inflammatory Th17 subtype as opposed to T regulatory cells, (Kimura 2010) but this is unlikely given the lack of evidence of T-cel involvement in IgM-PNP. (Dalakas 2010)

Patients with anti-MAG neuropathy also had increased serum IL-10 concentrations. These findings are in line with the reported increased IL-10 production by mitogen stimulated peripheral blood mononuclear cells in 14 patients with anti-MAG neuropathy. (Gironi 2009) Similar to IL-6, elevated serum IL-10 concentrations have also been described in patients with B cell dyscrasias without neuropathy. (Stasi 1997, Myhr 2003) IL-10 not only plays a broadly inhibitory role in the production of Th1 cell and macrophage produced pro-inflammatory cytokines, but can also activate B cells and promote auto-antibody production and is as such implicated in a growing number of auto-immune diseases. (Tian 2014) Increased IL-10 production has been described as a damage-limiting reaction to nerve damage, and could therefore reflect a general phenomenon in neuropathies. However, both the fact that IL-10 serum elevation was specific for anti-MAG neuropathy as well as the absence of a parallel TNFα increase argue against this alternative explanation. (Ydens 2012) The differences in IL-10 concentration for subsets of IgM-PNP might therefore also suggest differences in pathogenesis, which may be reflected in the responses to treatment options like rituximab

for anti-MAG patients or IVIg for patients with IgM-PNP without anti-MAG but with anti-ganglioside antibodies. (Dalakas 2009, Léger 2013, Attarian 2006)

In contrast to other B-cell mediated or IVIg-responsive neurological diseases, including opsoclonus-myoclonus syndrome (OMS) and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), the specific B-cell cytokines APRIL and BAFF serum concentrations were not elevated in serum from patients with MMN or IgM-PNP compared to healthy controls. (Pranzatelli 2013, Bick 2013) The potential role of IL-6 and IL-10 modulating therapies as a treatment strategy for IgM-PNP deserves further attention.

Conclusion

Cytokine profiles differ between anti-MAG neuropathy patients and other IgM-PNP patients in comparison with healthy and neuropathy controls. These differences may indicate differences in immune-mediated disease mechanisms.

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PART III

Treatment of polyneuropathy associated with IgM paraprotein

6

Fcγ receptor IIIA genotype is associated with rituximab response in anti-myelin associated glycoprotein neuropathy

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J Neurol Neurosurg Psychiatry. 2014 Aug; 85(8): 918-20.

Abstract

Treatment with anti-B cell antibody rituximab may ameliorate disease course in a subgroup of patients with polyneuropathy associated with IgM monoclonal gammopathy. Polymorphisms of leukocyte IgG receptors (FcγR) that influence efficiency of antibody-dependent cell-mediated cytotoxicity determine rituximab efficacy in patients with lymphoma and autoimmune disease. We retrospectively investigated the association of FcγRIIA and FcγRIIIA polymorphisms with the response to rituximab treatment in a cohort of patients with polyneuropathy associated with IgM monoclonal gammopathy with and without anti-myelin associated glycoprotein antibodies. The FcγRIIIA-V/V158 genotype was associated with functional improvement (p=0.02) after one year. FcγRIIIA polymorphisms are potential biomarkers for response to rituximab treatment in polyneuropathy associated with IgM monoclonal gammopathy.

Introduction

Polyneuropathy associated with IgM monoclonal gammopathy (IgM-PNP) is characterized by progressive weakness, sensory ataxia and IgM antibodies against myelin-associated glycoprotein (MAG), sulphated glucuronyl paragloboside (SGPG) or gangliosides, like GM1, GD1a, GD1b or GQ1b. IgM-PNP may lead to significant disability. (Niermeijer 2010) Despite the presence of auto-antibodies, treatment strategies that target toxic B-cell clones have been largely unsuccessful in patients with IgM-PNP. (Lunn 2012) However, results from two recent randomized placebo-controlled clinical trials suggest that rituximab treatment may lead to improvement in function in a subgroup of patients. (Dalakas 2009, Leger 2013) Rituximab is a chimeric monoclonal IgG antibody against the B cell surface protein CD20 and induces B cell depletion by several mechanisms including antibody-dependent cell-mediated cytotoxicity. (van Sorge 2003) antibody-dependent cell-mediated cytotoxicity is mediated through IgG receptors (FcγR) on specific leukocytes such as natural killer cells. (van Sorge 2003) FcγR are classified in three classes and several subclasses. (van Sorge 2003, Nimmerjahn 2010) Two FcγR subclasses, FcγRIIA and FcγRIIIA, display single nucleotide polymorphisms that correlate with the efficacy of antibody-mediated leukocyte functions such as antibody-dependent cell-mediated cytotoxicity and are associated with the response to anti-CD20 monoclonal antibody treatment in patients with lymphoma and autoimmune disease. (van Sorge 2003, Mellor 2013, Quartuccio 2013) We therefore investigated whether the FcγRIIA-R/H131 and FcγR IIIA-F/V158 polymorphisms are associated with a favourable response to treatment with rituximab in Dutch patients with IgM-PNP.

Patients and methods

Thirty patients from a prospective cohort of 169 patients with IgM-PNP were treated with rituximab monotherapy between 2004 and 2011. (Niermeijer 2010) Results of treatment in 17 patients were reported previously. (Niermeijer 2009) DNA samples of 27 patients were available for this study. All patients were examined before and one year after initiation of rituximab treatment by a physician unaware of the results of FcγR genotyping. The treatment protocol consisted of 375 mg/m^2 rituximab once weekly for 4 consecutive weeks. (Niermeijer 2009) The level of functional impairments was assessed using the 12 point inflammatory neuropathy cause and treatment overall disability sum score (ODSS). (Niermeijer 2009, Merkies 2002) Sensory deficits were scored with a 56-point sensory sum score to assess sensitivity to touch and perception of pain, vibration and passive movement. Motor deficits

were assessed using an MRC distal sum score of 4 distal muscle groups in arms and legs on both sides (maximum total score 80 points). (Niermeijer 2009) The primary outcome was defined as improvement of more than 1 point on the ODSS functional score after 1 year. Improvement of the sensory sum score and distal motor score of 5% at assessment 1 year after treatment were used as secondary outcome measures. (Niermeijer 2009)

Nerve conduction studies were performed using a standardized protocol, and were used to classify the IgM-PNP as 'demyelinating' as outlined in the AAN research criteria, or 'axonal' if these criteria were not fulfilled. The presence of anti-MAG antibodies was determined by means of ELISA (Bühlmann Laboratories, Switzerland).

FcγRIIA-R/H131and FcγRIIIA-F/V158 polymorphisms were determined using genomic DNA and allele specific polymerase chain reaction as described previously. (van Sorge 2003) Sanger sequencing was used to confirm FcγRIIIA genotyping results. (van Sorge 2003)

Differences in outcome after rituximab treatment were assessed using the Fisher's exact test. For statistical analysis FcγRIIA and FcγRIIIA genotypes were divided in efficient (FcγRIIA-H/H131, FcγRIIIA-V/V158) and less efficient (FcγRIIA-R/H131 or R/R131 and FcγRIIIA-F/V158 or F/F158) genotypes for triggering antibody-dependent cell-mediated cytotoxicity.

Results

Clinical characteristics and response to treatment

Twenty-seven patients were included in this study. Their median age was 60 (range 41 to 78). 17 patients were male. Twenty-six patients had a demyelinating polyneuropathy and 18 had antibodies against MAG. The median ODSS was 4, with a range from 2 to 7. Their median distal motor sum score was 71 (range 30-80), and their median sensory sum score 36 (range 7-50).

Twenty-five of 27 (93%) patients completed 4 weekly rituximab infusions of 375 mg/m^2. Data from all 27 patients were used for final analysis. Three out of those 27 patients (11%) improved at least one point on the ODSS and another 9 (33%) experienced improvement of sensory or motor sum scores: 2 patients improved on all 3 outcome measures, 1 patient on motor and sensory sum scores, but not on the ODSS, 1 patient improved only on the ODSS and 8 patients improved on sensory sum scores alone. Two patients, both with anti-MAG

antibodies, developed a paradoxical worsening of weakness between the second and third rituximab infusion and treatment was therefore discontinued. (Stork 2013)

Correlation of FcγRIIA-R/H131 and FcγR IIIA-F/V158 polymorphisms with treatment response

Six patients (22%) had an FcγRIIA-R/R131 genotype, 12 patients (44%) had an FcγRIIA-H/R131 genotype and 9 patients (33%) had an FcγRIIA -H/H131 genotype. FcγRIIA-R/H131 genotypes were not associated with any of the outcome measures.

Treatment outcome stratified for FcγRIIIA genotypes are summarized in Table 6.1. Eight patients (30%) had an FcγRIIIA-F/F158 genotype, 11 patients (41%) had an FcγRIIIA-F/V158 genotype and 8 patients (30%) had an FcγRIIIA-V/V158 genotype. Three of 5 (60%) patients with anti-MAG neuropathy and the FcγRIIIA-V/V158 genotype improved one or more points on the ODSS functional scale after treatment. In contrast, the ODSS did not change in any of the 13 (0%) patients with the FcγRIIIA-F/V158 or F/F158 genotypes (p=0.01). Two patients (40%) with anti-MAG antibodies and the FcγRIIIA-V/V158 genotype improved more than 5% on the distal motor sum score, whereas none of the patients with the FcγRIIIA-F/V158 or F/F158 genotypes had similar improvement (p=0.07). Three out of 5 anti-MAG neuropathy patients (60%) with the FcγRIIIA-V/V158 genotype also had improvement of the sensory sum score of more than 5%, while 2 out of 8 (25%) anti-MAG neuropathy patients with the FcγRIIIA-F/V158 genotype and 1 out of 5 (20%) with the FcγRIIIA-F/F158 genotype improved more than 5% on the sensory sum score (p=0.27).

Table 6.1 Favorable treatment response and FcγRIIIA-F/V158 genotypes in patients with IgM-PNP

Number of patients	FcγRIIIA-F/F158		FcγRIIIA-F/V158		FcγRIIIA-V/V158		p-value*
All IgM-PNP patients							
Total per genotype	8		11		8		
Improved functional score	0		0		3	(38%)	0.02
Improved motor score	0		0		3	(38%)	0.02
Improved sensory score	2	(25%)	4	(34%)	6	(75%)	0.09
Anti-MAG positive patients							
Total per genotype	5		8		5		
Improved functional score	0		0		3	(60%)	0.01
Improved motor score	0		0		2	(40%)	0.07
Improved sensory score	1	(20%)	2	(25%)	3	(60%)	0.29

* Fisher's exact test; FcγRIIIA-V/V158 versus combination of FcγRIIIA-V/F158 and F/F158 genotypes for all patients ('all') and for patients with anti-MAG antibodies ('MAG+').

The two patients who experienced paradoxical worsening had FcγRIIIA-V/V158 or FcγRIIIA-F/V158 genotypes.

The presence or titers of anti-MAG antibodies were not associated with specific FcγR genotypes and results were not different after inclusion of the 9 additional patients with polyneuropathy and IgM monoclonal gammopathy but without anti-MAG antibodies.

Discussion

Both open label and randomized studies suggest that rituximab may be effective in a subset of patients with IgM-PNP. (Niermeijer 2009, Dalakas 2009, Leger 2013) Our data suggest that inter-individual differences in treatment response may be determined by functional polymorphisms of FcγRIIIA that is expressed on important leukocyte subsets that mediate antibody-dependent cell-mediated cytotoxicity including natural killer cells. The FcγRIIIA-F/V158 polymorphism determines the affinity of this receptor to interact with IgG, including rituximab bound to CD20-expressing B-cells. (van Sorge 2003) *In vitro*, antibody-dependent cell-mediated cytotoxicity and other antibody-mediated leukocyte functions are more efficiently triggered by leukocytes from donors with at least one FcγRIIIA-V158 allele. (van Sorge 2003) The association of FcγRIIIA genotypes with treatment outcome may reflect differences in efficiency of recruitment of natural killer-cells by rituximab when it is bound to circulating B-cells. FcγRIIIA genotypes may therefore represent novel biomarkers to predict treatment response to rituximab in patients with IgM-PNP.

Improvement of the ODSS functional score, which was used as a primary outcome measure in our patients, was only seen in patients with FcγRIIIA-V/V158 genotype. Improvement of secondary outcome measures including the sensory and distal motor sum scores were also primarily seen in patients with this genotype. (Niermeijer 2009) Although the sample size of our current study is relatively small, the validity of our findings is supported by the association of FcγRIIIA-V/V158 with improvement or trends towards improvement of several outcome measures. Moreover, other studies with relatively large sample sizes found similar associations of rituximab efficacy in patients with lymphoma and generalized autoimmune disease such as rheumatoid arthritis. (Mellor 2013, Quartuccio 2013) Two patients who experienced paradoxical temporary worsening of weakness during rituximab treatment had FcγRIIIA-V/V158 and FcγRIIIA-F/V158 genotypes. We cannot exclude the possibility that paradoxical worsening is associated with specific FcγR genotypes, since associations of FcγR polymorphisms with side effects of monoclonal antibody therapy have been reported previously. (van Vollenhoven 2012, Li 2010, Vossen 1995)

In one of the randomized trials, response to rituximab in patients with IgM-PNP was associated with higher anti-MAG antibody titers and the depletion of clonally expanded memory B cells. (Dalakas 2009, Maurer 2012) Anti-MAG titers were not associated with FcγRIIIA genotypes nor response to treatment in our patients, suggesting that there may be several independent determinants of treatment efficacy. In this context, not only FcγR polymorphisms, but also FcγR expression levels on monocytes may determine rituximab response, as FcγR expression levels were recently described to be associated with disease severity in other immune-mediated neuropathies. (Tackenberg 2009) The lack of an alternative treatment option for patients with IgM-PNP, the cost of rituximab treatment and potentially serious side effects indicate that the predictive value of determinants of rituximab treatment efficacy should be studied in more detail.

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7

Rapid worsening of IgM anti-MAG demyelinating polyneuropathy during rituximab treatment

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There are no proven treatments for polyneuropathy associated with IgM monoclonal gammopathy and anti-MAG antibodies. (Lunn 2012) Two recent randomized trials (RCTs) comparing rituximab to placebo failed to demonstrate improvement on the primary endpoints. (Dalakas 2009, Leger 2013) In these trials treatment with rituximab was safe and well tolerated. The total published experience with rituximab in anti-MAG polyneuropathy encompasses fewer than 100 patients. (Renaud 2003, Rojas-Garcia 2003, Niermeijer 2012) Because rituximab is at best effective only in a small subgroup of patients, even fairly rare side-effects would be relevant to the decision to prescribe it for anti-MAG neuropathy. Some side-effects could have been missed due to the low numbers of patients in published series. We report three patients with IgM monoclonal gammopathy associated polyneuropathy and anti-MAG antibodies who rapidly worsened within weeks after the start of rituximab treatment.

One patient presented with mild weakness of the dorsiflexors of foot and toes (MRC grade 4), glove and sock-patterned hypoesthesia and sensory ataxia, which had developed slowly over the previous two years. His anti-MAG titer before treatment was 7180 BTU (Buehlmann Titer Units, normal values below 2000 BTU) total serum IgM was 13 gm/L and the M protein was 9 gm/L. Nerve conduction studies showed demyelination in nerves of both arms and legs, distally more than proximally, as well as axonal loss, more pronounced in the nerves of the legs than in those of the arms (Table 7.1). After the second of a planned four weekly rituximab infusions (375 mg/m^2) he developed proximal weakness and severe progression of the distal weakness in the legs (iliopsoas, quadriceps and hamstrings muscles MRC grade 4 and dorsiflexors of foot and toes MRC grade 2), proximal and distal weakness in the arms (MRC grade 4), and painful new sensory deficits in arms and legs. After intravenous immunoglobulin treatment (0.4 gm per kg daily for 5 days) the severity of the polyneuropathy returned to the level prior to the start of rituximab treatment. Repeated laboratory tests showed an anti-MAG titer of 5714 BTU, total serum IgM of 12 gm/L and M protein of 10 gm/L. During the three year follow-up after treatment, the distal sensory deficits were slowly progressive, but the mild weakness remained stable.

The second patient presented with a distal sensorimotor polyneuropathy in legs and hands. She had weakness of the dorsiflexors of foot and toes (MRC grade 4). Her anti-MAG titer was 49786 BTU, total serum IgM was 3.9 gm/L, an M protein was present and below 1 gm/L. Administration of intravenous immunoglobulins (0.4 gm per kg daily for 5 days), prescribed as the diagnosis chronic inflammatory demyelinating polyneuropathy (CIDP) was initially considered, did not result in improvement, and treatment with rituximab was initiated after the correct diagnosis was made. (Larue, 2010) After the second of a planned four weekly rituximab infusions (375 mg/m^2) she developed rapid deterioration of sensory deficits and

Table 7.1 Selected nerve conduction studies showing motor conduction measurements over the abductor pollicis brevis muscle for the median nerve, over the abductor digiti quinti muscle for the ulnar nerve, anterior tibial muscle for the peroneal nerve and the abductor hallucis muscle for the tibial nerve

	Patient 1		Patient 2		Patient 3	
Nerve	Before	After	Before	After	Before	After
Median nerve						
DML	8.8	12.4	6.5	9.3	8.1	8.1
MCV	32	23	37	26	33	28
TLI	0.24	0.25	0.29	0.29	0.26	0.31
CMAP	10.4	7.9	7.5	4.6	8.9	12.7
SNAP	2	Absent	2	Absent	Absent	
SCV	26		35			
Ulnar nerve						
DML	5.6	8.6	3.7	5.4	6.7	7.3
MCV	40	27	41	29	21	19
TLI	0.31	0.30	0.46	0.45	0.48	0.50
CMAP	7.1	5.4	6.2	6.3	3.3	4.5
SNAP	3	Absent	Absent	Absent	Absent	
SCV	29					
Tibial nerve						
DML	14.4					
MCV	16					
CMAP	0.2	Absent	Absent	Absent	Absent	
Peroneal nerve						
DML	9.1	15.9	5.5	14		
MCV	22	16	31	13		
CMAP	3.3	2.3	1.8	0.5	Absent	

MCVs are given for the most distal nerve segment measured. Sensory nerve potentials were measured over the second and fifth finger of the right hand, for the median and ulnar nerve respectively. NCS were conducted on the left tibial nerve, all other nerves were measured on the right.
DML: distal motor latency, in msec; MCV: motor conduction velocity, in m/sec; TLI: terminal latency index; CMAP: compound motor action potential, in mV; SNAP: sensory nerve action potential, in microV; SCV: sensory conduction velocity, in m/sec.

progression of distal weakness from mild to severe (MRC grade 3), and additional weakness in the arms (MRC grade 4). Rituximab infusion was discontinued. At follow up two months later weakness and sensory deficits had slightly improved, but remained worse than before rituximab treatment. At follow-up, the anti MAG antibody titer had dropped to 25713 BTU, serum IgM dropped to 3.2 gm/L and the M protein did not change.

A third patient presented with sensorimotor neuropathy of hands and legs, which had developed slowly over the previous five years. Before treatment he had weakness in the

fingerflexors (MRC grade 4), kneeflexors (MRC grade 4) and plantar flexors of the ankle (MRC grade 4). Weakness of foot and toe dorsiflexion was MRC grade 2. His anti-MAG titer before treatment was 1:409600, with normal values below 1: 1600, total IgM was 4.7 gm/L and the M protein was 0.28 gm/L. Bone marrow biopsy showed less than 1% monoclonal CD20+ cells. He was treated with weekly infusions of rituximab 375 mg/m^2. After the third infusion the extent of his sensory symptoms, previously limited to his fingers and from his calves down, progressed proximally to his forearms and knees. The muscle weakness did not progress. Rituximab treatment was then terminated, and the sensory loss returned to the pre-treatment pattern in two to three weeks without further treatment. There was no improvement compared to the extent of the neuropathy before initiation of treatment. After treatment his anti-MAG titer dropped to 1:102400 and total serum IgM was 4.6 gm/L, with an M protein of 0.37 gm/L.

In all three cases nerve conduction studies were compatible with the diagnosis of anti-MAG neuropathy and in the first two worsened after rituximab infusion (Table 7.1).

These cases show that rituximab may cause deterioration in patients with polyneuropathy with monoclonal gammopathy and IgM anti-MAG antibodies. This paradoxical worsening of polyneuropathy after rituximab treatment has been described previously as a late-onset complication in the months following treatment initiation, and in patients with haematological malignancies. (Broglio 2005, Gironi 2006) These case histories show that rituximab may have significant side effects in patients with anti-MAG neuropathy. This should be carefully weighed against the suggestion based on a post-hoc analysis in two 2 RCTs that a minority of patients may benefit from rituximab treatment. Biomarkers that identify patients who possibly respond to treatment and those at risk for deterioration are needed to ensure safety and cost-effectiveness of rituximab treatment.

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PART IV

Treatment of polyneuropathy associated with IgA or IgG paraprotein

8

Treatment for neuropathies associated with paraprotein antibodies in the blood (IgG and IgA paraproteinaemic neuropathies)

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Abstract

Background Paraproteinaemic neuropathy refers to those neuropathies associated with a monoclonal gammopathy or paraprotein. The most common of these present with a chronic predominantly sensory, symmetrical neuropathy, similar to chronic inflammatory demyelinating polyradiculoneuropathy but with relatively more sensory involvement, both clinically and neurophysiologically. The optimal treatment for IgG and IgA monoclonal gammopathy of uncertain significance neuropathies is not known. This is an update of a review first published in 2006.

Objectives To assess the effects of any treatment for IgG or IgA paraproteinaemic peripheral neuropathy.

Search methods On 18 January 2014 we searched the Cochrane Neuromuscular Disease Group Trials Specialized Register, CENTRAL, MEDLINE and EMBASE. We also checked bibliographies for controlled trials of treatments for IgG or IgA paraproteinaemic peripheral neuropathy.

Selection criteria We included randomised controlled trials (RCTs) and quasi-RCTs using any treatment for IgG or IgA paraproteinaemic peripheral neuropathy. People with IgM paraproteins were excluded. We excluded participants where the monoclonal gammopathy was considered secondary to an underlying disorder. We included participants of any age with a diagnosis of monoclonal gammopathy of uncertain significance with a paraprotein of the IgG or IgA class and a neuropathy. Included participants were not required to fulfil specific electrophysiological diagnostic criteria.

Data collection and analysis The full texts of potentially relevant studies were obtained and assessed and independent data extraction was performed by three authors. Additional data and clarification were received from one trial author.

Main results We identified only one RCT with 18 participants which fulfilled the predetermined inclusion criteria. Four other trials were identified but these were not RCTs. The included trial revealed a modest benefit of plasma exchange in IgG or IgA paraproteinaemic neuropathy, over a short 3 week follow-up period, when compared to sham plasma exchange in the weakness component of the Neuropathy Disability Score (now the Neuropathy Impairment Score); improvement 17 points (95% CI 5.2 to 28.8) versus 1 point (95% CI -/-7.7 to 9.7) in the sham exchange group.. There was no significant difference in the overall NDS, vibration thresholds or neurophysiological indices. Adverse events were not reported.

Authors' conclusions The evidence from RCTs for the treatment of IgG or IgA paraproteinaemic neuropathy is currently inadequate. More RCTs of treatments are required. These should have adequate follow-up periods and contain larger numbers of participants, perhaps through multicentre collaboration, considering the relative infrequency of this condition. Observational or open trial data provide limited support for the use of treatments such as plasma exchange, cyclophosphamide combined with prednisolone, intravenous immunoglobulin and corticosteroids. These show potential therapeutic promise but the potential benefits must be weighed against adverse effects. Their optimal use and the long-term benefits need to be considered and validated with well-designed RCTs.

Plain language summary

Review question What are the benefits and harms of treatments for nerve damage associated with abnormal IgG and IgA proteins in the blood?

Background Paraproteinaemic neuropathy refers to those neuropathies associated with a paraprotein (an abnormal antibody or immunoglobulin present in relative excess in the blood). Paraproteins come from a group of blood disorders called monoclonal gammopathies. If the paraprotein is present without underlying evidence of any disease, this is known as a monoclonal gammopathy of uncertain significance (MGUS). This review looked at the treatments for neuropathy associated with and possibly caused by IgG and IgA paraproteins. The optimal treatment is not known. Treatments that act on the immune system such as plasma exchange, corticosteroids or intravenous immunoglobulin have been examined in non-randomised studies of people with IgG and IgA paraproteinaemic neuropathy.

Study characteristics We identified only one randomised controlled trial (RCT), which compared plasma exchange with sham exchange, in 18 participants with either IgA or IgG paraproteinaemic neuropathy. The results were reported after three weeks of treatment.

Key results and quality of the evidence The trial did not report our primary outcome measure, which was improvement at six months after randomisation in disability measured by a validated scale. The trial demonstrated a modest benefit in improvement of weakness over a period of three weeks. The long-term benefit is unclear. There was no improvement in this timescale in measures of sensory disturbance or electrical studies of the nerves. Adverse events were not reported. Further RCTs of this and other treatments with larger numbers of participants are needed. This is an update of a review first published in 2006. We found no additional trials for inclusion. The evidence is current to January 2014.

Background

Paraproteinaemic neuropathy refers to a group of neuropathies associated with a monoclonal gammopathy or paraprotein. A paraprotein is an immunoglobulin molecule produced by a monoclonal plasma cell expansion. The monoclonal protein is present in relative excess and is often non-functional. If the monoclonal protein is present without evidence of an underlying causative disease, this is known as a monoclonal gammopathy of uncertain significance (MGUS). Treatment for IgM paraprotein associated neuropathy has been reviewed previously. (Lunn 2012) The treatment of neuropathies occurring in people with IgG or IgA MGUS discovered in this review.

Where the only clinical manifestation of the MGUS is neuropathy, the neuropathy dictates treatment (Nobile-Orazio 2002) as themonoclonal gammopathy usually remains benign and non-progressive. Kyle found that 1% per year of all people with MGUS progressed to develop a malignant plasma cell dyscrasia. (Kyle 1993) In Ponsford's series of 50 patients with IgG or IgA MGUS neuropathy, 6% developed malignancy after a median follow-up of 14 years. (Ponsford 2000) Others have found malignant transformation occurs more frequently earlier in MGUS natural history in people with neuropathy and is associated with worsening neuropathy. (Eurelings 2001) Where MGUS transforms into myeloma, the malignancyis more likely to determine treatment.

The prevalence of MGUS increases with age. The most common paraprotein type is IgG, accounting for 61% of cases in one review. (Kyle 1992) Most people with MGUS do not have a symptomatic neuropathy. Kelly found a monoclonal protein in 10% of people with neuropathy of unknown aetiology. (Kelly 1981) Conversely, in series of people with MGUS, the prevalence of symptomatic neuropathy ranged from 1% to 36% (Gosselin 1991, Nobile-Orazio 2002, Vrethem 1993, Yeung 1991) and was higher in MGUS associated with IgM than IgG or IgA paraproteins.

Typically, paraproteinaemic neuropathy affects men in their sixth to eighth decade. It presents with a chronic predominantly sensory symmetrical neuropathy, similar to chronic inflammatory demyelinating polyradiculoneuropathy (CIDP). People with MGUS neuropathy (IgM, IgG and IgA) often have less weakness and relatively more sensory involvement, both clinically and neurophysiologically, than do people with idiopathic CIDP. (Gorson 1997b, Simmons 1993, Simmons 1995) Some have found less clinical or neurophysiological sensory involvement in IgG and IgA paraproteinaemic neuropathy compared to IgM. (Magy 2003, Notermans 2000) There is some diagnostic controversy and debate continues about whether a patient with an IgG MGUS and otherwise typical

CIDP justifies a separate diagnosis; (Bleasel 1993, Simmons 1995) some authors classify it as a concurrent illness with CIDP. (EFNS/PNS 2010, Saperstein 2001) Some have found a difference in the clinical features between people with IgM and IgG MGUS neuropathy, (Gosselin 1991, Nobile-Orazio 1992, Vrethem 2010) but others have not. (Bromberg 1992, Yeung 1991)

The majority of the cases reported in the literature are IgG as opposed to IgA. The clinical and electrophysiological features of 205 IgG and 27 IgA reported cases have been reviewed. (Nobile-Orazio 2002) The review highlighted the heterogeneity of both IgG and IgA MGUS neuropathy patients, noted previously in smaller studies by others. (Di Troia 1999, Gorson 1997a, Hermosilla 1996, Notermans 1994) Patients with IgG and IgA paraproteinaemic neuropathy have either demyelinating or axonal/mixed neuropathies, in approximately equal numbers. Those with a slowly progressive distal axonal polyneuropathy tended to show a poor response to immunotherapy (treatments that have a mechanism of action via modulation of the immune system). Others with a sensorimotor demyelinating neuropathy frequently responded to immunotherapy. (Magy 2003)

Initial screening with serum protein electrophoresis is non-specific but may identify the presence of a serum paraprotein in higher concentrations. Immunofixation is required to detect those at low concentrations (<0.2 g/L) which may not be detected by electrophoresis. Immunofixation is also necessary to identify the exact isotype of the heavy and light chains. Occasionally light chains in the urine can identify the presence of a serum paraprotein.

The pathogenic role of IgG and IgA paraproteins is debated. Monoclonal gammopathy may become apparent after the onset of neuropathy. (Nobile-Orazio 1992, Simmons 1995) Serum levels of the paraprotein fluctuate and may not correlate with the clinical course. (Bleasel 1993) Some researchers have suggested that the paraprotein is part of a secondary autoimmune response. (Di Troia 1999) Others argue that it is a coincidental finding, particularly in the setting of a chronic axonal neuropathy. (Kyle 1987, Notermans 1996a, Ritzmann 1975, Saleun 1982)

The paraprotein antibodies are sometimes found to have specific antigen targeted activity. In IgG MGUS patients, Di Troia et al. found no differences in the frequency of antibodies to various neural glycoprotein and glycolipid antigens between patients with and without neuropathy. (Di Troia 1999) Others have found antibodies to neurofilament antigens in patients with neuropathy. (Fazio 1992, Stubbs 2003) The immunological characteristics of IgG and IgA paraproteinaemic neuropathy patients have been reviewed. (Nobile-Orazio 2002) A few cases demonstrated IgA or IgG deposition in the nerves of patients (Bailey

1986, Mehndiratta 2004, Vallat 2000) but its pathogenic significance remains uncertain. In a histological study, sural nerve biopsies in eight patients with IgG paraproteins were indistinguishable from those of idiopathic CIDP. (Vital 2000) Other biopsy studies have suggested more T cell involvement in paraproteinaemic neuropathy, (Eurelings 2002, Eurelings 2003) than in CIDP without a paraprotein.

The optimal treatment for IgG and IgA MGUS neuropathies is not known. In two published observational studies 'CIDP-MGUS' patients responded less well to immunotherapy than those with idiopathic CIDP. (Simmons 1993, Simmons 1995) In a third, the responses were similar. (Gorson 1997b) In a review of 124 patients with IgG MGUS and neuropathy, treatment with immune therapies (most commonly corticosteroids and plasma exchange (PE)) was considered. (Nobile-Orazio 2002) Of these 124 patients, 67 had a demyelinating neuropathy and of these, 54 (81%) responded to immunotherapies, compared with only 7 of 34 (21%) of those with an axonal neuropathy. In the same review seven of 13 IgA cases responded to immune therapies. In a double-blind controlled trial of PE versus sham PE in 39 participants with polyneuropathy associated with MGUS (21 IgM and 18 IgG/IgA), PE produced improvement in the neuropathy disability score (now referred to as the neuropathy impairment score (Dyck 2005) and neurophysiological improvement, which was more marked in those with IgG or IgA. (Dyck 1991) Gorson reported improvement in eight of 20 IgG MGUS patients with IVIg. (Gorson 2002) In one series of patients with axonal neuropathy and IgG MGUS, improvement was reported in one out of three treated with corticosteroids. (Di Troia 1999)

Objective

To assess the effects of any treatment for IgG or IgA paraproteinaemic peripheral neuropathy.

Methods

Criteria for considering studies for this review

Types of studies We included randomised controlled trials (RCTs) and quasi-RCTs using any treatment for IgG or IgA paraproteinaemic peripheral neuropathy.

Types of participants We followed 2003 diagnostic criteria for MGUS: monoclonal protein <30 g/L and clonal plasma cell population <10% with no evidence of multiple myeloma,

other B-cell proliferative disorders or amyloidosis (International Myeloma Working Group 2003 (Myeloma 2003)). We therefore excluded patients where it was considered that the monoclonal gammopathy has arisen due to an underlying disorder, such as multiple myeloma, plasmocytoma, malignant lymphoproliferative diseases, or amyloidosis.

We included patients of any age with a diagnosis of MGUS with a paraprotein of the IgG or IgA class and a neuropathy. Patients with IgM paraproteins were excluded. Other causes of peripheral neuropathy were ruled out. The clinical picture was a recognised presentation of peripheral neuropathy, (Nobile-Orazio 2002) being typically a symmetrical sensory or sensorimotor neuropathy. Neurophysiologically the neuropathy could be demyelinating, axonal or of mixed type and therefore did not need to fit any published electrophysiological diagnostic criteria. Studies that did not exactly fulfil these criteria were included, provided the review authors agreed that IgG or IgA paraproteinaemic peripheral neuropathy was the preferred diagnosis, if necessary after consultation with the original study authors. Any departures from the diagnostic criteria were noted.

Types of interventions We included any treatment used for the treatment of IgG or IgA paraproteinaemic peripheral neuropathy. Treatments could be administered using various protocols (for example single agent, combined treatment or sequential administration). The control arm did not necessarily include a placebo, but if the control arm received a treatment then the experimental arm also had to receive that same treatment. The route of administration must have been defined but could be by any route. Dosages and the frequency and length of administration must have been defined in the studies.

Types of outcome measures

Pre-defined primary outcome measures were:

1. Improvement at six months after randomisation in disability measured by a validated scale such as the overall disability score of Merkies et al. (Merkies 2003a)

A disability score was selected for the primary outcome, as such scores are considered to be the most relevant measures in immune-mediated neuropathies. (Merkies 2003b) They are also potentially easy to derive retrospectively from collected data. Six months was pre-defined as a favoured time point for re-evaluation, on the basis that IgG or IgA paraproteinaemic peripheral neuropathy is a chronic and slowly progressive or relapsing-remitting disorder. However to avoid limiting the scope of the review we considered trials using other trial periods and follow-up intervals and made appropriate adjustments in our analysis.

Secondary outcome measures were:

1. Improvement at six months in sensation measured by a validated scale such as the Inflammatory Neuropathy Cause and Treatment (INCAT) sensory sum score. (Merkies 2000)
2. Improvement at six months in strength measured by a validated scale such as the MRC sum score. (Kleyweg 1991)
3. Neurophysiology – improvement at six months as measured by the distally evoked summed compound muscle action potential amplitudes.
4. Neurophysiology – improvement at six months as measured by a reduction in the number of sites of conduction block, as defined by the American Association of Neurology diagnostic criteria for CIDP. (CIDP 1991)
5. Adverse events – adverse events defined as those which are fatal, life threatening or required or resulted in hospitalisation. The rate was to be adjusted for differing follow-up periods as necessary.

Search methods for identification of studies

We searched the Cochrane Neuromuscular Disease Group Trials Specialized Register (18 January 2014), CENTRAL (2013, Issue 12), MEDLINE (January 1966 to January 2014), and EMBASE (January 1980 to January 2014). Bibliographies were reviewed to identify other controlled trials. We identified no additional published or unpublished data.

Data collection and analysis

Selection of studies Titles and abstracts identified from the register, MEDLINE/EMBASE searches and bibliography identification were checked independently by two authors (ACJS and NN at this update). The full texts of potentially relevant studies were obtained and assessed independently by three authors (ACJS, MPTL and NN) to decide which trials fitted the inclusion criteria and then graded their risk of bias. There were no disagreements about inclusion criteria.

Data extraction and management Independent data extraction was performed by two authors (ACJS, NN). Some additional data and clarification were received from one of the trial authors. (Dyck 1991)

Assessment of risk of bias in included studies The 'Risk of bias' assessment took into account seven predefined domains, namely sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective outcome reporting and 'other issues'. For each domain a judgement of 'Low risk' of bias, 'High risk' of bias, or 'Unclear risk' of bias was made (Table 8.1). (Higgins 2011)

Data synthesis Meta-analysis, tests for heterogeneity across trials and planned subgroup analyses were not performed due to the lack of included trials and the lack of data available. We considered non-randomised evidence concerning adverse events, cost-effectiveness and the current utilisation of therapy in the 'Discussion' of the review.

Table 8.1 Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear bias	Quote: patients were assigned... by restricted randomization.
Allocation concealment (selection bias)	Low risk	Quote: the only investigators not blinded to treatment allocations were the patient coordinator, the biostatistician and the bloodbank consultant and personnel. The patient and the examining physician were unaware of the nature of the treatment.
Blinding of participants and personnel (performance bias)	Low risk	Quote: a curtain separated the apheresis equipment from the patient. For sham exchange, blood was drawn, separated into cells and plasma...recombined, and reinfused.
Blinding of outcome assessment (detection bias)	Low risk	Quote: the only investigators not blinded to treatment allocations were the patient coordinator, the biostatistician and the bloodbank consultant and personnel. The patient and the examining physician were unaware of the nature of the treatment.
Incomplete outcome data (attrition bias)	Low risk	Quote: one patient was found to have osteosclerotic myeloma and therefore the data on theis patient were not used in the analysis.
Selective reporting (reporting bias)	Unclear bias	Comment: Insufficient information on selection of primary outcomes.
Other bias	Low risk	Comment: none found.

Results

Description of studies The systematic search of MEDLINE, EMBASE, the Cochrane Neuromuscular Disease Group Trials Specialized Register and CENTRAL in 2005 revealed five possible trials. One trial (Dyck 1991) met the inclusion criteria (Table 8.2). The four other trials were excluded. No new trials were identified when the search was updated on 18 January 2014.

There were 18 participants with IgG or IgA paraproteinaemic neuropathy in the included trial. (Dyck 1991) This trial was a randomised double-blind, parallel sham controlled trial of plasma exchange. Participants with IgM paraproteinaemic neuropathy were also included in this trial but the results for the different types of paraprotein were discussed separately, allowing the use of these data. The criteria for the paraprotein specifically being a MGUS were not as strictly defined as those used for this review, but were still considered to fulfil the criteria adequately. The participants' neuropathies were deemed to be either stable or worsening at the time of enrolment. The intervention in this trial was a twice weekly 3.5L plasma exchange for three weeks, totalling six exchanges. No additional treatments were given. Participants remained on other treatments that they were already taking but had had no other immunotherapy for the six weeks prior. This treatment was given to eight participants with IgG or IgA paraproteinemic neuropathy initially. Ten control participants with IgG or IgA paraproteinemic neuropathy, received full sham exchanges, with plasma extraction, separation, recombination and re-infusion. Nine of these control participants subsequently underwent treatment with plasma exchange following the same protocol. The results of this open phase of the trial were also reported.

Table 8.2 Characteristics of included study (Dyck 1991)

Methods	Parallel group, randomized double-blind sham controlled trial, with subsequent open trial treatment for control patients.
Participants	39 patients completed the trial. 18 of these had either IgA or IgG paraproteinaemic neuropathy and were stable or deteriorating at the time of enrolment. 8 had plasma exchange and 10 sham exchange.
Interventions	Plasma exchange. 3.5 litre exchange, twice weekly for 3 weeks. Total of 6 exchanges.
Outcomes	Follow-up at 3 weeks. Neuropathy Impairment Score. Muscle weakness score. Vibration detection threshold. Summed neurophysiological scores of Compound Muscle Action Potentials, Motor Nerve Conduction Velocities and Sensory Nerve Action Potentials.
Notes	Adverse events not reported.

We excluded the four remaining trials for various reasons: Notermans (Notermans 1996a) performed an uncontrolled open prospective trial of intermittent cyclophosphamide and prednisolone. Five of the sixteen participants included had IgG MGUS neuropathy. A trial of pulsed high-dose dexamethasone (Notermans 1997) was also excluded as it was an uncontrolled open trial of six patients with paraproteinaemic neuropathy. Only one had an IgG MGUS, the others had IgM MGUS. Léger (Léger 1994) performed a trial of IVIg which included four participants with IgG paraproteinemic neuropathy. This was an uncontrolled open prospective trial, for which the diagnostic criteria were unclear and no clear outcome criteria were used. A trial of 60 participants, which included nine with IgG paraproteinaemic neuropathy was reported by Sghirlanzoni (Sghirlanzoni 2000). This trial included various immunosuppressant treatments. The trial was a prospective uncontrolled, non-randomised cohort study and the results for the IgG paraproteinaemic neuropathy participants were not reported separately from those with an IgM paraproteinaemic neuropathy.

Risk of bias in included studies In the Dyck trial (Dyck 1991) participants underwent 'restricted randomisation'. This was done to ensure that the baseline characteristics of age and sex were approximately equal. The authors state that the groups at baseline were 'reasonably balanced' with respect to neuropathic abnormalities. The blinding process was deemed adequate and explicit clinical and outcome criteria were used. Completeness of follow-up was considered partially adequate and there were no drop-outs. The study initially aimed to include 40 participants, including participants with IgM MGUS neuropathy. The results section describes 39 participants being enrolled in the study and one developing myeloma. The results state that the data for this participant were not used in the analysis. It is unclear whether this participant took part in the trial or even underwent treatment. It is presumed here that the participant did not receive any treatment and was not enrolled, consistent with the 39 participants that are included in the baseline and post-treatment results. The follow-up period was only three weeks.

Effects of interventions The only eligible trial (Dyck 1991) provided the results for the 18 participants with IgG or IgA paraproteinaemic neuropathy at a follow-up interval of three weeks. The results were not separated with respect to the individual IgG or IgA subgroups. The neuropathy disability score (NDS, subsequently renamed the Neuropathy Impairment Score) was used as the primary outcome measure. Scores could range from 0 to 244 points, with 244 being maximal neurological disability (impairment). Included patients had an average NDS of 60.5. Neurophysiological improvement data were provided for the group but a neurophysiological classification of the neuropathy (in terms of being predominantly axonal or demyelinating) at baseline was not.

In the randomised controlled phase of the trial, our predefined primary outcome measure was not reported, although disability was measured at three weeks. Secondary outcome measures were also addressed at three weeks, in terms of sensory (vibration) measurement, strength measurement and summed compound muscle action potential (CMAP) measurement. Overall in the trial, the 19 participants (including those with IgM as well as IgG and IgA paraproteins) who underwent plasma exchange improved, on average, more than the 20 who underwent the sham exchange. Participants with IgG or IgA paraproteinaemic neuropathy had a more statistically significant improvement in weakness reported (p=0.03) when compared to those participants with IgM paraproteins. When assessing the participants with IgG or IgA in isolation, improvement in the weakness score of the NDS was significantly higher in the eight participants given plasma exchange in comparison to the 10 given sham exchange. They showed mean score improvements of 17 (95% CI 5.2 to 28.8) in the plasma exchange group versus 1 (95% CI -/-7.7 to 9.7) in the sham exchange group. The actual number of participants who showed improvement was not specified. Comparing the overall Neuropathy Disability Score, the treatment group improved by a mean of 20 (95% CI 3.4 to 36.6) compared to 2 (95% CI -/-9.2 to 13.2) for the control group. This was not statistically significant. Vibration detection threshold mean scores were also not statistically significant. The mean scores for summed CMAP measurements were also not statistically significantly different. Subjective assessment was not recorded. Motor nerve conduction studies showed no significant differences and sensory nerve studies were not reported on follow-up.

Details of adverse events were not reported.

Discussion

Only one trial (Dyck 1991) fulfilled the predetermined inclusion criteria. Four other studies were not RCTs but we have discussed some of their findings. The Dyck trial included 39 participants of whom 18 had either IgG or IgA paraproteinaemic neuropathy. The trial design and execution were good. The blinding process was well described and performed. Clear outcome criteria were used but not all of the data were reported and the time points used were much shorter than our predefined criteria. Baseline characteristics were reasonably balanced, completeness of follow-up and randomisation were however only partially adequate, based upon the descriptions provided. Our primary outcome measure was not used in the trial, but some of our secondary outcome measures were. In particular, there was a statistically significant but modest increase in strength with plasma exchange compared to sham exchange. The small number of participants limited the power of the trial.

In the open trial stage of the trial, not included in the results section above, nine of the ten participants with IgG or IgA paraproteinaemic neuropathy who had initially received sham exchange in the controlled trial, then received plasma exchange treatment. This group subsequently showed very similar overall mean improvements when compared to those of the initial treatment group from the randomised trial phase. However when the NIS, weakness score of the NIS, vibration detection threshold score and summed CMAP scores were compared to the nine participants own original (sham control) scores, the results were not statistically significantly different. The overall results from the open trial phase did reveal some statistically significant findings but only when the results for all the IgG, IgA and IgM participants were included. The assessing physicians were unblinded at this stage.

Adverse events were not reported.

Although not included in this review, the Notermans (Notermans 1996a) trial of intermittent cyclophosphamide (300 mg/m^2 body surface daily for four days) combined with prednisone (40 mg/m^2 body surface daily for five days) in 16 participants provided relevant data. Four of the five participants with IgG paraproteinaemic neuropathy improved or stabilised following treatment, and this was maintained for three years of follow-up. Of these five participants, two had mixed axonal and demyelinating findings on motor nerve conduction studies and three had predominantly demyelinating findings. Side effects were a severe but reversible leukopenia after one cycle of cyclophosphamide and prednisolone in one patient, necessitating withdrawal of treatment. Other patients suffered hair loss and nausea.

Another trial reported by Notermans (Notermans 1997) of pulsed high dose dexamethasone (40 mg/day orally for four days, once a month, in up to six cycles) in six participants with paraproteinaemic neuropathy, showed a stable Rankin scale and a two point improvement in the MRC sum score at follow-up in the single participant with IgG paraproteinaemic neuropathy. However, this patient like two others, developed proximal lower limb weakness as a side effect. Electrophysiologically the single IgG paraproteinaemic neuropathy patient had a mixed axonal and demyelinating neuropathy. Further enrolment in the study was stopped due to serious side effects in four out of six participants, with three experiencing severe mood disturbance.

Other reviews and some of the retrospective series already discussed provide support for the use of immunotherapy. In a review which included 124 patients with IgG MGUS neuropathy, Nobile-Orazio (Nobile-Orazio 2002) found that 81% of the 67 patients with a predominantly demyelinating neuropathy responded favourably to therapies such as steroids and plasma exchange. In a retrospective review of 20 patients with IgG MGUS neuropathy,

all of whom were treated with intravenous immunoglobulin, Gorson (Gorson 2002) found a beneficial response in eight.

Other studies (Di Troia 1999, Magy 2003, Yeung 1991) have reported beneficial responses in some patients to various therapies. In a retrospective observational study, Magy reported that eight out of nine patients experienced a sustained clinical improvement with either corticosteroids, plasma exchange or intravenous immunoglobulin. Yeung reported that four out of five patients with IgG experienced a good response to corticosteroids in another retrospective observational study. Four also received cytotoxic drugs but without additional benefit. Three IgA patients treated with corticosteroids (one with a concomitant cytotoxic drug) also improved but another patient treated with plasma exchange showed no benefit. In one series of patients reported by Di Troia, with axonal neuropathy and IgG MGUS, improvement was reported in one out of three treated with corticosteroids.

This review has revealed that only one RCT relating to the treatment of IgG or IgA paraproteinaemic neuropathy exists. This may be partly due to the relatively low prevalence of this disease. Unfortunately retrospective reviews are potentially open to bias. They are not blinded, often do not consistently report useful assessment scores and are not controlled.

Sensitive and validated disability and clinical scores, that are likely to extract meaningful effects should be used in future trials. (Merkies 2006) Quality of life assessment and cost effectiveness measurements should also be considered in future studies, as the treatments that have been used and those that are likely to be used in the future are expensive. These treatments are also time consuming to receive or provide, may be invasive and are not without side effects. Trial endpoints should also be appropriate to the chronicity of the disorder and meaningful in patient terms, particularly overall disability. We had suggested a predefined endpoint of six months or a year.

Although not addressed in trials so far, the evaluation of the treatments should be made in patients with both predominantly axonal and demyelinating neuropathies associated with IgG or IgA MGUS. It is uncertain whether the presence or absence of electrophysiological characteristics predict response to treatment. The authors of this review do not consider, until proven otherwise by RCTs, that the electrophysiological finding of an axonal neuropathy should necessarily preclude treatment.

In the UK the cost of five single plasma volume PE procedures is about the same as a course of IVIg 2.0 g/kg, namely about £4000. Patients may require multiple courses of plasma exchange, each possessing inherent risks. In a large series of plasma exchange for various indications, adverse reactions including citrate toxicity (3%), vasovagal reactions and vascular access

complications, occurred in 3.9% of 17,940 procedures on 3,583 patients. (Kiprov 2001) As with any treatment the potential benefits of plasma exchange treatment should be balanced against the costs and potential side effects of that treatment.

Authors' conclusions

Implications for practice The evidence from randomised controlled trials for the treatment of IgG or IgA paraproteinaemic neuropathy is currently inadequate. One small trial showed significant short-term benefit from plasma exchange in measures of weakness but not in a composite impairment score (NDS), sensory function or neurophysiology measures. The long-term benefits and side effects of repeated plasma exchange have not been investigated.

Implications for research More randomised controlled trials of existing and new treatments are required. These should have adequate follow-up periods and contain larger numbers of participants, perhaps through multicentre collaboration because of the relative infrequency of this condition. Some observational data provide limited support for the use of plasma exchange, cyclophosphamide combined with prednisolone, intravenous immunoglobulin and corticosteroids. Their possible potential benefits must be weighed against their sometimes severe adverse effects. Their optimal use and long-term benefits need to be considered and validated with well-designed randomised controlled trials.

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9

Effect of stem cell transplantation for B-cell malignancies on disease course of associated polyneuropathy

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Abstract

B cell dyscrasias are often refractory to medical treatments and hematological stem cell therapy (SCT) may be warranted. It is not clear whether an associated polyneuropathy may also profit from SCT. In exceptional cases SCT has been tried in patients with monoclonal gammopathy and progressive polyneuropathy refractory to medical treatments.

In a cohort of 225 patients with monoclonal gammopathy and polyneuropathy we selected the six patients who underwent SCT and retrospectively examined the effects of SCT on the disease course of the associated polyneuropathy. In all patients except one, the indication for SCT was hemato-oncological (multiple myeloma (MM) in four patients and primary AL amyloidosis in one). The remaining patient had an IgG monoclonal gammopathy of undetermined significance (MGUS) and a progressive and painful polyneuropathy for which she was treated with SCT. SCT led to improvement of motor scores and autonomic symptoms in one patient, three patients experienced improvement of neuropathic pain or sensory deficits but showed further progression of weakness. One patient showed no improvement at all. One patient died within 100 days after SCT.

In conclusion SCT as a treatment of refractory hematological malignancy, may occasionally have a positive effect on the associated progressive polyneuropathy, although the benefits are very limited and the treatment related mortality is high.

Introduction

B cell dyscrasias are associated with polyneuropathies. A variety of pathogenetic mechanisms, including cytokine-mediated damage, nerve infiltration, synthesis and deposition of noxious monoclonal proteins or binding of monoclonal antibodies to nerve constituents, may cause neuropathy in patients with B cell disorders. The disease course of these polyneuropathies may be mild, but can also be very debilitating, causing severe sensorimotor deficits and autonomic dysfunction. (Dispenzieri 2005, Kyle 2005)

Unfortunately, polyneuropathy associated with monoclonal gammopathy is often refractory to treatment. Intravenous immunoglobulins (IVIg) and the anti-CD20 monoclonal antibody rituximab may ameliorate disease course in a minority of patients. (Dalakas 2009, Niermeijer 2009) Hematological stem cell therapy (SCT) is a well-established therapy used for refractory hematological malignancies. SCT has been reported to attenuate the progressive disease course of patients with polyneuropathy with organomegaly, endocrinopathy, M-protein and skin changes (POEMS), (Jaccard 2002, Dispenzieri 2004) chronic inflammatory demyelinating polyneuropathy (CIDP), (Oyama 2007, Mahdi-Rogers 2008) primary AL amyloidosis, (Dingli 2010) and recently in a small series of patients with IgG MGUS or MM associated neuropathy, (Hummel 2011) but it is unknown whether SCT may represent a rescue therapy for a wider range of patients with polyneuropathy and B cell dyscrasias, and whether the possible beneficial effects outweigh risks associated with SCT.

Here we describe six patients with malignancy-associated polyneuropathy receiving SCT primarily for the treatment of their hematological disorders and report on the effects on the neuropathy.

Patients and methods

Patient characteristics

Six patients (2.7%) from a cohort of 225 patients (Niermeijer 2010) with polyneuropathy and B cell dyscrasia underwent SCT. The primary indication for SCT was the hematological malignancy in all patients with the exception of patient 5, in whom improvement of the polyneuropathy was the main goal. Clinical data and data from nerve conduction studies were collected prospectively following a standardized protocol that has been in use for this patient group since 1985. These data were analyzed retrospectively to assess the effect of SCT on the course of the polyneuropathy. Patient characteristics are summarized in

Table 9.1. All patients presented with complaints consistent with polyneuropathy, with B cell dyscrasia found during the neuropathy work-up. Four patients had multiple myeloma (MM) unresponsive to other treatments, (patients 1, 2, 3 and 6), one had light chain (AL) amyloidosis, and one patient had an IgG MGUS and a rapidly progressive demyelinating polyneuropathy despite treatment with IVIg and glucocorticosteroids (patient 5). With the exception of patient 1, who had a monozygous twin brother who acted as a donor, all patients were treated with autologous SCT. All transplantations were carried out in the Utrecht Medical Center, a tertiary referral center for hemato-oncology and (autologous) stem cell transplantation. Institutional morbidity and mortality lie well within international standards, as published elsewhere (10% treatment related mortality in MM patients). (Sonneveld 2007)

Neurological examination before, and one year after, stem cell transplantation was performed using a standardized protocol, described elsewhere. (Niermeijer 2010) Motor function was expressed using the modified medical research council (MRC) motor sum score of 14 muscle groups in both arms and legs, with a maximum score of 140 points. Sensory function was expressed as a sensory composite sum score consisting of scores for sense of touch (0-4), pain (0-4) and vibration (0-4) in both arms and legs and position sense (0-2) in both legs with a maximum of 56 points. (Niermeijer 2010) The modified Rankin score, (ranging from 0 for no symptoms at all to 5 for severely disabled and bedridden and 6 for dead) was used to quantify functional disability.

All patients underwent systematic nerve conduction studies using a previously described protocol. (Niermeijer 2010) Demyelination was defined according to previously established criteria. Nerve conduction studies that showed a reduction of CMAP-amplitudes to less than 20% of normal values in at least two nerves but did not meet the criteria for demyelination were scored as axonal. (Hadden 1998)

Other causes of polyneuropathy than monoclonal gammopathy were excluded following a standardized protocol. (Niermeijer 2010)

Case histories and results

Patient 1 was diagnosed with MM with IgA monoclonal gammopathy and had a progressive, predominantly motor neuropathy, with severe weakness, hypesthesia and paresthesia in the lower legs and hypesthesia in the fingertips. Nerve conduction studies showed distal demyelinisation in arms and legs. He was first treated with melfalan and dexamethasone and then, after pre-treatment with cyclophosphamide and total body irradiation, underwent

Table 9.1 **Patients' age at first visit in our neuromuscular clinic, paraprotein, hematological diagnosis, predominant clinical manifestation of the polyneuropathy**

Pt	Age	M protein	Hematological diagnosis	Polyneuropathy	Motor Sum Score		Sensory Sum Score		Rankin Score		Clinical effect on SCT on polyneuropathy
					Before	After	Before	After	Before	After	
1	44	IgAλ	Multiple myeloma	Motor	122	137	54	56	2	2	Improvement
2	60	IgAλ	Multiple myeloma	Sensorimotor	132	128	44	40	2	2	Stabilization
3	59	IgGκ	Multiple myeloma	Sensorimotor	108	(*)	24	(*)	3	(*)	Rapid further progression, death
4	43	IgGλ	AL amyloidosis	Sensory and autonomic	140	137	46	44	3	2	Improvement in autonomic neuropathy
5	58	IgGλ	MGUS	Sensorimotor	112	110	27	31	4	4	Stabilization
6	48	IgDλ	Multiple myeloma	Sensorimotor	138	136	50	50	2	2	Stabilization

Motor sum score, sensory sum score, modified rankin score, before and after treatment. Overall clinical result. (*): died before one year follow up.

allogenic stemcell transplantation with stem cells from his HLA-identical twin brother eighteen months after the first manifestation of polyneuropathy. SCT was complicated by mild graft versus host disease. One year after transplantation, strength in his legs had virtually normalized (MRC motor sum score improved from 122 to 137 out of 140).

Neurological deficits further improved slightly in the second year after SCT and then remained unchanged until eleven years after transplantation, when an exacerbation of MM was preceded by progression of sensory complaints and weakness. Nerve conduction studies abnormalities met the criteria for axonal polyneuropathy. After a second SCT with the original stem cell harvest, weakness and sensory deficits did not deteriorate any further.

Patient 2 was diagnosed with MM, an IgA-lambda gammopathy and had a sensorimotor axonal polyneuropathy, with muscle atrophy, weakness, hypesthesia and paresthesia in both lower legs and mild weakness of the intrinsic hand muscles. MM was first treated with two cycles of intermediate dose melfalan. Autologous stem cell transplantation was then performed after pre-treatment with cyclophosphamide and total body irradiation. Mucositis was the most important complication of SCT. SCT was performed one year after diagnosis of the polyneuropathy and three years after the first neuropathic complaints. After transplantation MRC motor sum scores declined a further four points to 128/140, while the sensory sum score declined from 44 to 40 out of 56. Follow up during 5 year showed no further changes in neurological examination and nerve conduction studies did not show further progression.

Patient 3 had MM, an IgG-kappa gammopathy and a rapidly progressive, sensorimotor polyneuropathy. Nerve conduction studies met the criteria for axonal polyneuropathy. Marked weakness was present distally in arms and legs, together with sock and glove patterned hypesthesia and sensory ataxia. Ambulation was lost during the diagnostic work-up. After three cycles adriamycin/dexamethasone and two cycles of intermediate dose melfalan, autologous stem cell transplantation 30 months after the first manifestation of polyneuropathy seemed to stabilize disease course for the duration of one month, but two months after transplantation the polyneuropathy deteriorated and caused severe weakness of respiratory and bulbar muscles. Three months after transplantation the patient died of respiratory insufficiency.

Patient 4 had primary amyloidosis with light chain gammopathy and progressive neuropathy with sensory ataxia and autonomic dysfunction leading to impotence, orthostatic hypotension, and loss of bowel and bladder control. Nerve conduction studies met the criteria for axonal polyneuropathy. He was also treated with adriamycine, dexamethasone and melfalan,

followed by autologous stem cell transplantation. After SCT this patient developed herpes labialis, fever and deep venous thrombosis. SCT led to a complete hematological response, and marked improvement of the autonomic complaints, regaining of bowel control and normalization of blood pressure regulation, which allowed patient to regain independence in the activities of daily life. Nerve conduction studies did not change after transplantation and motor scores decreased slightly (from 140 to 137 out of 140) while sensory sum score improved from 44 to 46 out of 56.

Patient 5 had a rapidly progressive sensory and motor demyelinating neuropathy and an IgG monoclonal gammopathy. She presented to the outpatient clinic with distal weakness in both legs, areflexia, hypesthesia of the fingers and lower legs, ataxia and very severe neuropathic pain, which did not respond to adequate dosage of either antidepressants or anticonvulsants registered for neuropathic pain treatment. The nerve conduction studies fulfilled the critera for demyelination and the diagnosis CIDP with IgG monoclonal gammopathy was made. Despite repeated IVIg infusions there was a severe progression of the neuropathy (Rankin scale 3). Treatment with high dose dexamethasone and melfalan led to a further exacerbation of the polyneuropathy. After autologous stem cell transplantation, preceded by adriamycin/cyclophosphamide and melfalan cycles, no further progression occurred and the neuropathic pain disappeared, although motor scores did not improve. SCT was complicated by neutropenic fever.

Patient 6 had MM with IgD paraproteinaemia and a polyneuropathy with painful paresthesia of the feet, mild weakness of the m. extensor hallucis longus and intact reflexes. Nerve conduction studies were consistent with an axonal polyneuropathy. One year after the first sensory complaints she underwent autologous SCT, primarly aimed at the MM. SCT was complicated by herpes simplex infection in the mouth, urinary tract infection and fluid overload. SCT led to diminished neuropathic pain, but motor, sensory sum scores and nerve conduction studies did not improve after transplantation.

Discussion

Here we report on the effect of stem cell transplantation on neuropathy in our cohort of 225 patients with monoclonal gammopathy and neuropathy. We retrospectively selected and analyzed 6 patients from this cohort who underwent SCT, primarily aimed at the malignancy underlying the monoclonal gammopathy rather than at the neuropathy. In only one patient, who received SCT for MM, the polyneuropathy improved objectively. In one

other patient, treated with SCT for primary amyliodosis, autonomic symptoms improved subjectively, allowing him to regain independence in the activities in daily life; in three other patients the neuropathy was at best stable after SCT, with further decline in motor function, but improvement in reported pain and in one patient slight improvement in sensory sum scores. However, one patient died after as a complication of the SCT. These results suggest that SCT halts progression in a minority of cases of polyneuropathy associated with B-cell dyscrasias, but that concerns about safety need to be addressed.

Efficacy of SCT for refractory polyneuropathy associated with B cell dyscrasia has not been studied systematically. Case reports have documented improvement after SCT in some patients with CIDP (7 patients), POEMS (42 patients) (Jaccard 2002, Dispenzieri 2004, Oyama 2007, Mahdi-Rogers 2008) and most recently in four patients with MGUS associated neuropathy, (Hummel 2011) but publication bias may have precluded reports of cases with an unfavorable outcome. SCT results ranged from complete remission of the neuropathy in some POEMS and CIDP patients to full relapse after initial improvement, and unresponsiveness to SCT. In comparison to the patients described in previous studies, all our patients but two had an axonal polyneuropathy. This may explain the relative lack of beneficial effect of stem cell transplantation in our group compared to the recent publication of SCT for MGUS associated neuropathy, as all patients in that study had a demyelinating neuropathy.

The potentially beneficial effect of stem cell transplantation needs to be carefully weighed against the considerable risks of treatment related mortality and morbidity. The European Group for Blood and Marrow Transplantation (EBMT) registry has reported transplantation-related mortality in 5% of 900 patients with auto-immune disease between 1995 and 2007, with mortality clearly improving in the years covered by the registry. (Farge 2010) Treatment center and the underlying disease may also represent determinants of mortality due to SCT complications. In a recent study, 2 out of 13 patients (15%) with AL amyloidosis and autonomic neuropathy died within a hundred days after transplantation and were considered transplantation-related deaths. (Dingli 2010) Results from more studies are needed to document whether SCT for B cell dyscrasias associated with polyneuropathy carries a greater than average risk for complications and death. The case history of patient 3 emphasizes that the natural disease course of some B cell dyscrasia associated neuropathies is relentless, which justifies treatment strategies with a higher risk for complications.

Due to the small number of patients with B cell dyscrasia associated neuropathy who underwent SCT we could not establish an association between efficacy of SCT and the

underlying hematological disease. The case history of patient 5 confirms findings from previous studies that autologous SCT can be considered as treatment of last resort in patients with CIDP and an associated monoclonal gammopathy, even if the B cell dyscrasia by itself does not warrant such treatment. Unfortunately, clinical trials establishing whether and when autologous stem cell transplantation should be considered are probably not feasible due to the small numbers of eligible patients. Standardized criteria of efficacy and detailed descriptions of patients treated with SCT may help to clarify whether specific nerve conduction features (i.e. demyelination, axonal loss) or underlying hematological disorder may predict outcome.

In conclusion, SCT might be considered as a treatment of last resort in very severe, progressive and therapy-resistant polyneuropathies associated with B cell dyscrasias. Nevertheless, the treatment-related mortality and morbidity and the relatively high chance of a limited response at best, necessitate a careful and critical weighing of risks and benefits in each individual patient, and treatment should be limited to centers that have extensive experience with SCT and very low mortality rates.

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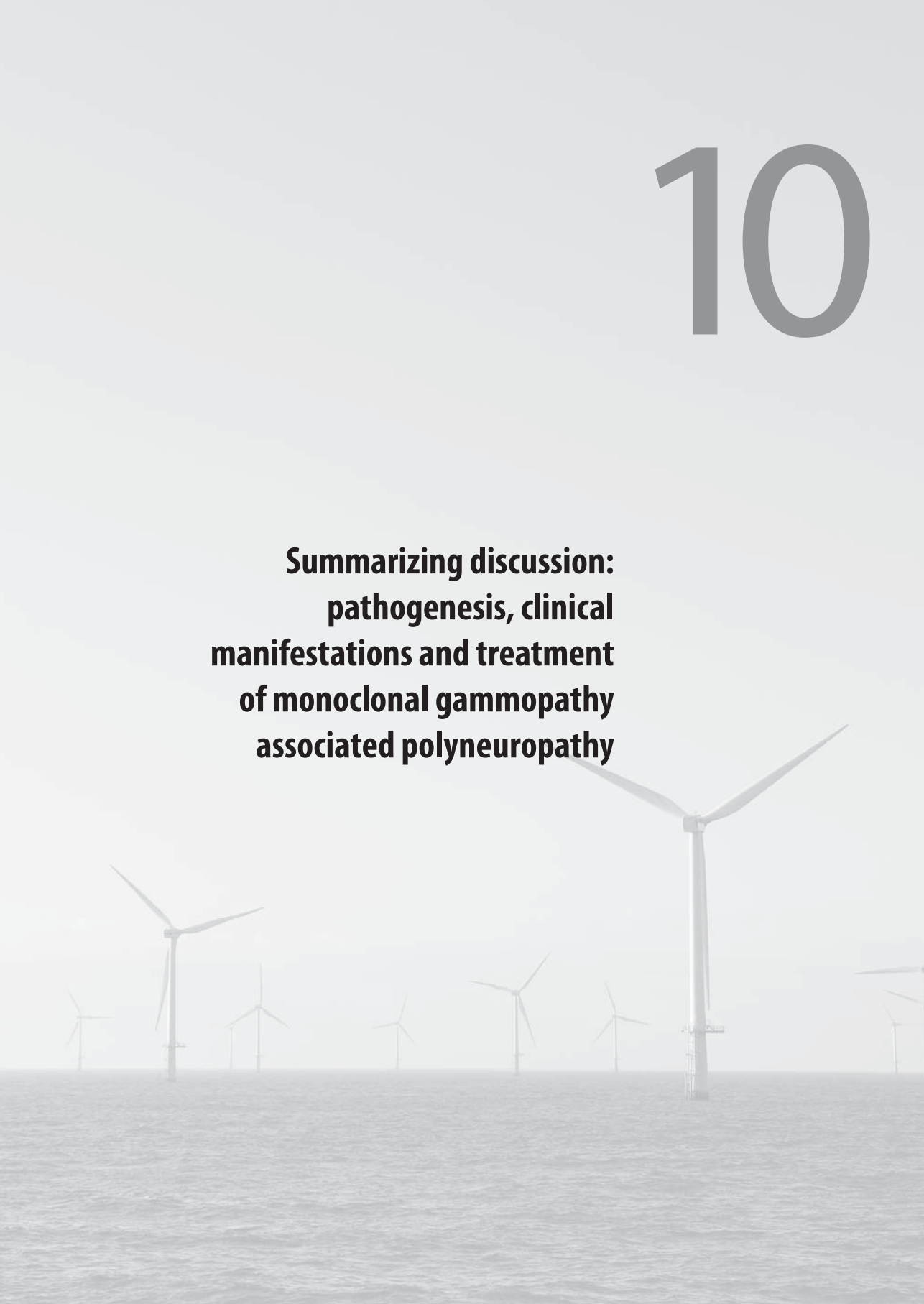
10

Summarizing discussion: pathogenesis, clinical manifestations and treatment of monoclonal gammopathy associated polyneuropathy



Several types of neuropathy are associated with monoclonal gammopathies (Table 10.1). Monoclonal gammopathies are a relatively common occurrence, with incidence increasing with age. (Kyle 2011) They can occur as expression of a hematological malignancy, but also as a non- or pre-malignant phenomenon (monoclonal gammopathy of unknown significance, MGUS). Polyneuropathies in patients with MGUS can be subdivided into three groups: patients with IgM monoclonal gammopathy (IgM-PNP) who carry antibodies against myelin associated glycoprotein (MAG), patients with IgM gammopathy without these antibodies and patients with IgG or IgA class monoclonal gammopathy. Only a small

Table 10.1 Monoclonal gammopathies and associated neuropathies

M protein	Neuropathy	Description
IgM	Anti-MAG neuropathy	Well described clinical syndrome, M protein mandatory for diagnosis
	IgM-PNP w/o anti-MAG antibodies	Clinically more heterogeneous, M protein mandatory
	CANOMAD	Well described syndrome, very similar clinical syndrome w/o M protein possible
	MMN	Well described syndrome, prevalence of M protein increased
	Waldenströms MG	Primarily hematological diagnosis, neuropathy can occur in the course of disease
	CLL	Primarily hematological diagnosis, neuropathy can occur in the course of disease
	SMA	Well described clinical syndrome, prevalence of M protein increased
IgG	POEMS	Well described multi-organ disease, M protein mandatory
	IgG/IgA MGUS-associated neuropathy	Relation between M protein and neuropathy unclear
	Amyloidosis	Several distinctive subtypes, neuropathy not mandatory in all
	Cryoglobulinemia	Both IgM and IgG gammopathies possible, neuropathy not mandatory for diagnosis
	Lymphoma	Primarily hematological diagnosis, neuropathy can occur in the course of the disease

IgM-PNP: polyneuropathy associated with IgM monoclonal gammopathy of unknown significance; CANOMAD: chronic ataxic neuropathy with ophthalmoplegia, IgM M protein, cold agglutinins and anti-di-sialosyl antibodies; MMN: multifocal motor neuropathy; MG: macroglobulinemia; CLL: chronic lymphocytic leukemia; SMA: spinal muscular atrophy; POEMS: polyneuropathy, organomegaly, endocrinopathy, M protein and skin changes; MGUS: monoclonal gammopathy of unknown significance.

minority of MGUS patients carry monoclonal antibodies of the IgM type, (Kyle 2011) and the clearest evidence for a causal relation between antibodies and neuropathies exists for IgM monoclonal antibodies, especially when antibodies against MAG are found. (Latov 2014) These are found in 70% of patients with IgM-PNP. (Kuijf 2009) A further 15% have antibodies against other glycolipids such as gangliosides or sulfatides. (Eurelings 2001, Lopate 1997). For patients with IgG or IgA monoclonal gammopathy the relevance of the gammopathy for the polyneuropathy is unclear. (Latov 2014)

Clinical and electrophysiological characteristics of IgM-PNP with and without anti-MAG antibodies

IgM gammopathy associated neuropathies (IgM-PNP) fall in a clinical spectrum of demyelinating, presumedly immune-mediated neuropathies, including multifocal motor neuropathy (MMN), multifocal inflammatory demyelinating neuropathy (MIDN), chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) and chronic ataxic neuropathy, ophthalmoplegia, monoclonal IgM gammopathy, cold agglutinins and disialosyl antibodies (CANOMAD). Many of these disorders are associated with monoclonal gammopathies. (Willison 2001, Latov 2014, Vlam 2015) These neuropathies can be clinically differentiated on several axes: predominance of sensory vs motor clinical deficits, length-dependency (distal vs combinations of distal and proximal deficits) or symmetry (diffuse, symmetrical deficits vs multifocal disease) (Figure 10.1). As a predominantly sensory, symmetrical and distal polyneuropathy anti-MAG neuropathy lies at one end of this spectrum, allowing delineation

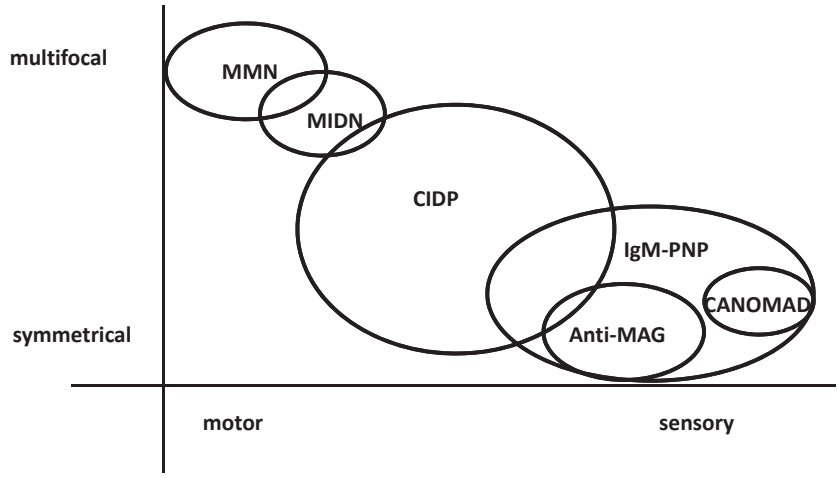


Figure 10.1 Clinical characteristics of immune-mediated neuropathies.

from CIDP, which usually leads to more weakness and more proximal involvement. (ad hoc subcommittee AAN 1991, Niermeijer 2010). In the absence of anti-MAG antibodies, differentiation between a true gammopathy-associated neuropathy and one with merely concomitant gammopathy can be difficult. Making this distinction has clear clinical relevance as polyneuropathies associated with gammopathies have a different pathogenesis and perhaps also different susceptibility to treatment. **Chapter 3** describes how the clinical and nerve conduction studies (NCS) characteristics of patients with neuropathy and an IgM MGUS, but without anti-MAG antibodies compare to those of patients with anti-MAG neuropathy.

MAG neuropathy is a well-described clinical entity: neurological deficits develop in a length-dependent symmetric neuropathic pattern, and start distally, slowly extending proximally. Weakness can be present, especially distally, but sensory deficits predominate. Tendon reflexes are often absent. (Ellie 1996, Niermeijer 2010) All sensory modalities are affected, leading to a sock-and-glove-like pattern of hypoesthesia. Worst affected, and most relevant for functional impairments, are epicretic functions, leading to impaired propriocepsis, vibration sense and 2-point discrimination. Sensory deficits may lead to postural instability and sensory ataxia, which affects a minority of patients (Niermeijer 2010) but can be severe and disabling (Nobile-Orazio 2000, Gorson 2001). A pronounced intention tremor can be present (27% in a recent cohort study) (Niermeijer 2010), and, in combination with a polyneuropathy, is highly suggestive of anti-MAG neuropathy. In contrast to the typical phenotype, isolated cases of patients with anti-MAG antibodies and a rapidly progressive, motor dominant polyneuropathy have also been described. (van den Berg 1996, Yuki 2014) NCS typically consist of demyelinating features, in >90% of patients meeting the American Academy of Neurology (AAN) criteria for demyelination, (ad hoc subcommittee AAN 1991, Niermeijer 2010) although findings consistent with axonal loss may also predominate. (Niermeijer 2010, van den Berg 1996) Both demyelination and axonal loss are length-dependent, with greater abnormalities in distal segments of nerves. (Franssen 2006) The typical length-dependent, demyelinating features become less apparent over time (Ponsford 2000, Trojaborg 1995) and demyelination of more proximal nerve segments or axonal damage may appear with disease progression.

We postulated that in the absence of anti-MAG antibodies, a similar correlation between the presence of specific auto-antibodies and clinical or NCS features might be found in patients with IgM-PNP and anti-ganglioside antibodies. That at least IgG antibodies against gangliosides may lead to specific neurological deficits has been shown in animal studies and in patients with acute neuropathies. (Kuwabara 2010) In Guillain-Barré syndrome (GBS), a strong association between anti-GM1 or anti-GD1a and a motor phenotype has been found.

(Ogawara 2003, Ho 1999) A similar association has been described between anti-GQ1b antibodies and the specific ataxic phenotype of Miller-Fisher syndrome (MFS). (Willison 1993) In chronic neuropathies high titers of IgM anti-GM1 antibodies are associated with multifocal motor neuropathy (MMN), but although IgM monoclonal gammopathy is more frequent in MMN patients than in healthy controls, it is – using stringent criteria for antibody positivity – found in only half of of MMN patients. (Cats 2010, Vlam 2015) For anti-GQ1b antibodies, a clear association exists with a distinct clinical syndrome, which, when full-blown consists of chronic ataxic neuropathy, ophthalmoplegia, monoclonal IgM gammopathy, cold agglutinins and disialosyl antibodies (CANOMAD). Combinations of several, but not all components may also occur. (Willison 2001, Kam 2011). In patients with IgM-PNP we found that the clinical phenotype of patients with anti-ganglioside antibodies was overall similar, but more heterogeneous than that of anti-MAG neuropathy patients. This heterogeneity was not randomly distributed but correlated with the presence of specific anti-ganglioside antibodies. Anti-GQ1b and anti-GD1b antibodies were associated with a sensory neuropathy and anti-asialo GM1 antibodies with an asymmetrical, predominantly motor neuropathy. These associations support a pathogenic role in the polyneuropathy for the anti-ganglioside antibodies, in parallel to *in vivo* and *in vitro* models for GBS. Different gangliosides have specific distributions over peripheral nerves, with GM1 and GD1a more frequently, but not exclusively, present in motor nerve membranes (O'Hanlon 1998, Gong 2002) and GQ1b preferentially expressed on muscle spindles, (Liu 2009) partially explaining the associations between antibodies against specific gangliosides and different neuropathy phenotypes. Most of our patients had antibodies against multiple gangliosides, probably explaining why most IgM-PNP patients with anti-ganglioside antibodies, like those with anti-MAG antibodies, had a sensorimotor neuropathy. As a result, some specific clinical phenotypes of IgM-PNP may suggest the presence of anti-ganglioside antibodies, but the differentiation of anti-ganglioside IgM-PNP from anti-MAG neuropathy remains difficult on clinical grounds alone.

Pathogenesis: from monoclonal cells to antibodies to effector functions to polyneuropathy

Much about the exact pathogenic mechanism of IgM-PNP is unknown. What we do know is mostly based on studies in anti-MAG neuropathy and it is unclear whether these findings can be extrapolated to IgM-PNP without anti-MAG antibodies.

Monoclonal cells The IgM monoclonal antibodies isolated in IgM-PNP patients are somatically hypermutated (Eurelings 2006, Maurer 2012) and are postulated to be produced

by clonally expanded IgM+ memory B cells that acquired antibody-secreting capacities. (Maurer 2012) These cells express many of the characteristics of post-germinal center memory B cells, the cells responsible for retaining immunological memory of previously encountered antigens. In contrast to most memory B cells, they did not make the usual class-switch from IgM to IgG antibodies.

Immunoglobulins In anti-MAG neuropathy, IgM expressed on clonally expanded IgM memory B cells is reactive to MAG, supporting the connection between auto-antibodies and gammopathy. (Maurer 2012)

MAG is a glycoprotein expressed on the myelin sheaths of Schwann cells and is member of the sialic-acid binding immunoglobulin-like lectin (SIGLEC) family. It functions as a ligand for axonal receptors and its interactions are assumed to play a role in the formation and maintenance of Schwann cells' myelin sheaths. (Quarles 2007, Lunn 2002, Schachner 2002) Anti-MAG antibodies bound to the myelin sheath can be demonstrated in nerve biopsies of patients. (Hays 1988)

The presence of IgM anti-ganglioside antibodies in a subset of IgM-PNP patients led to the hypothesis that they might play a pathogenic role similar to what these studies suggested for anti-MAG antibodies. Similarities in clinical manifestations of the neuropathy might be explained by the interwoven roles of MAG and gangliosides in myelin-axon interactions: gangliosides are present on neural cells in both the central and peripheral nervous system, and function in peripheral nerves as a receptor for, amongst others, MAG expressed on Schwann cells. (Lopez 2009, Quarles 2007, Vyas 2001, Lunn 2002)

In the study described in **chapter 2** we found evidence both supporting and complicating this hypothesis. On the one hand, we found that IgM-PNP patients had antibodies against gangliosides with a strongly restricted light-chain usage, suggesting mono- or at least oligoclonal cells producing them, and the light chain type (κ or λ) used by the anti-ganglioside antibodies was often the same as that of the M protein. Furthermore, we also found antibodies against complexes of gangliosides (GSC). These GSC might be a better approximation of the neural antigen than single gangliosides, as gangliosides are not expressed singularly on cell membranes, but as constituents of lipid rafts, often forming ganglioside complexes by the intertwining of two different gangliosides. In GBS, anti-GSC antibodies are associated with a more severe disease course. (Kaida 2004, 2010) But not all our findings fitted so neatly in a linear explanation from monoclonal cells to antibodies to nerve damage. Many patients had antibodies against several gangliosides or GSC. Some of these antibodies were mono- or oligoclonal, but antibodies against other gangliosides, might use both κ and λ, in

the same patient and were therefore inconsistent with a monoclonal population producing them. Other patients had anti-ganglioside antibodies with light chains usage restricted to κ, but had an IgM-λ M protein. As our study was limited to antibody specificities and light chain usage, the data cannot be used to find out *why* anti-ganglioside antibodies are so heterogeneous. One might speculate that the reason could lie in the specific role postulated for IgM memory cells, i.e. to provide a kind of starter kit of immunological memory, before small children become exposed to antigens and build their own (IgG) repertoire of memory cells. To this end IgM memory cells already express a highly diverse antibody repertoire in very young children, (Weller 2008) often reactive to polysaccharides. (Reynaud 2012, Eurelings 2006) Disturbances in the development of these IgM memory cells, e.g. because of untimely or repeated exposure to bacteria expressing glycolipids very similar to gangliosides, (Jacobs 1997) might then lead to a skewed repertoire, which after a period of dormancy leads to an oligoclonal expansion of cells whose immunoglobulins react to gangliosides and cause neuropathy. This would mean that IgM-PNP with anti-ganglioside antibodies arises from another mechanism than the pre-malignant monoclonal expansion of IgM antibody producing cells thought to lead to anti-MAG neuropathy. It might also be the case that the underlying pathogenic mechanisms are similar and that detailed examination of the anti-MAG antibody light chain usage in a large group of MAG neuropathy patients would lead to findings similar to ours in anti-ganglioside antibodies.

Returning to the hypothesized pathophysiological cascade in anti-MAG neuropathy, the antibodies bound to the myelin sheath are accompanied by complement depositions. (Hays 1988) Complement activation is the expected effector mechanism for bound IgM antibodies, based on the enhanced complement binding capabilities of IgM pentamers after their conformation change secondary to binding an antigen. (Daha 2011) The innate activity of complement activation pathways differs considerably between subjects and these differences can have clinical consequences. (Geleijns 2006) However, by measuring innate complement activity in **chapter 4** we did not find the correlation between high innate complement activation and disease severity that has been described for other neuropathies, such as MMN (classical pathway) or GBS (lectin pathway). (Vlam 2015, Geleijns 2006) What we did find was a trend towards higher activity of the mannose-binding lectin (MBL) complement activating pathway in anti-MAG neuropathy patients as well as an underrepresentation of MBL-deficient genotypes in these patients. These findings suggest that differences in complement activation might play some role in the development of IgM-PNP, or perhaps only anti-MAG neuropathy, but that if that is the case at all, its effects are blunted over the long and often indolent course of the disease. Another measure for activation of the immune system

can be found in serum cytokine profiles. In the study described in **chapter 5** we showed that IgM-PNP is associated with elevated IL-6 serum concentrations and that anti-MAG neuropathy, but not the other forms of IgM-PNP, is also associated with elevated IL-10 serum concentrations, compared to both patients with another neuropathy and healthy controls. As we measured a single time-point after the development of the neuropathy, our data do not tell us whether these findings are part of the pathogenesis of IgM-PNP or secondary to it. Some arguments for a pathogenic role exist: for IL-6 it is known that disease activity in both auto-immune diseases such as rheumatoid arthritis or systemic lupus erythematosus as well as in some hematological malignancies correlates with IL 6 serum levels. Such a pathogenic effect of IL-6 might be based on a direct stimulatory effect on plasma cells or B cell differentiation, or might be a result of IL-6 skewing T cells towards the pro-inflammatory Th17 subtype as opposed to T regulatory cells. (Kimura 2010) The elevated IL-10 concentrations might be an epiphenomenon in a damage-limiting reaction to nerve damage, but IL-10 can have B cell stimulatory effects and is, like IL-6, associated with SLE disease activity. (Chun 2007) That serum IL-10 is only elevated in anti-MAG neuropathy, as described previously, suggests it might reflect differences in pathogenesis between anti-MAG antibody negative and positive IgM-PNP. (Myhr 2003, Gironi 2009, Ydens 2012)

Summarizing, the findings described in this thesis thus far show differences in clinical characteristics, antibody clonality, complement activation and serum cytokine profiles between anti-MAG and anti-ganglioside antibodies. Those differences suggest different pathogeneses and for IgM-PNP patients with antibodies against different neural antigens, which might be reflected in different treatment responses as well.

Whom to treat, and how

IgM-PNP Disease modifying treatment of IgM-PNP has targeted both the antibody dependent immune-mediated nerve damage as well as the underlying B or plasma cell dyscrasia. For both treatment strategies retrospective studies have suggested a clinical benefit, which could not, or not consistently, be replicated in prospective, randomized trials. (Haas 1988, Ellie 1996, Nobile-Orazio 1988 and 2000, Wilson 1999, Gorson 2001, Latov 1988, Nobile-Orazio 1988, Blume 1995, Notermans 1996, Niermeijer 2006, 2007)

IVIg Intravenous immunoglobulins (IVIg) have an established role in the treatment of other immune-mediated neuropathies. (van den Bergh 2010, Eftimov Cochrane 2013) One double blind cross-over trial, again in patients with IgM-PNP showed a limited short-term improvement for 50% of patients in overall disability during the IVIg phase. (Comi 2002) Of

the 22 patients in this trial, 11 had anti-MAG antibodies. Other, unblinded or uncontrolled studies showed efficacy in lower percentage (up to 20%) of patients. (Dalakas 1996, Gorson 2001) An intriguing possibility is that this subset of IVIg-responsive IgM-PNP patients might consist of patients with anti-ganglioside antibodies. The effectiveness of IVIg in other neuropathies associated with anti-ganglioside antibodies and our data supporting IgM-PNP with ganglioside antibodies as a separate disease entity lend weight to this speculation. Treatment of IgM-PNP with anti-ganglioside antibodies has not been investigated in specific randomized trials, but case series describing successful treatment with IVIg have been published. (Attarian 2006) For example, for CANOMAD and its variant restricted to ataxic neuropathy with GQ1b antibodies (CANDA) a positive effect of IVIg and rituximab treatment has been described. (Delmont 2010, Löscher 2013, Kam 2011). Another study described the positive effect of IVIg treatment in 13 neuropathy patients with anti-GD1b antibodies, of whom 6 also had an IgM paraprotein. (Attarian 2006)

Rituximab The marked improvement in effectiveness of chemotherapeutic regimens for several B cell lymphomas after the inclusion of rituximab (Zelenetz 2012) naturally raised the question whether rituximab might also be effective in IgM-PNP.

Rituximab is a chimeric monoclonal antibody against CD20, a phosphoprotein present on B cells in all stages of development from pro-B cells to memory cells, but not on antibody producing plasma cells. (Abulayha 2014) Only a minority of monoclonally expanded B cell populations found in bone marrow biopsies of MGUS patients express CD20. (Zandecki 1995) However, CD20+, antibody-secreting IgM memory B cells have been described in anti-MAG neuropathy and could be a target for rituximab therapy. (Maurer 2012) From 1999 on several case series have described up to 60% of patients improving after rituximab treatment, (Levine 1999, Pestronk 2003, Benedetti 2007, Niermeijer 2009) with an additional benefit described after dose escalation to 750 mg per m2 instead of 375 mg per m2. (Renaud 2006) The promise of these uncontrolled studies could not be unequivocally confirmed by the two randomized trials comparing rituximab to placebo. (Dalakas 2009, Leger 2013) It is thus all the more important to carefully weigh the risks and benefits of rituximab treatment. A series of patients whose neuropathy paradoxically worsened during rituximab treatment are described in **chapter 7**. A similar serious adverse reaction has also been described for rituximab treatment of hematological malignancies. (Sala 2014, Stork 2013, Broglio 2005, Gironi 2006)

In their discussions the authors of the two randomized trials concluded that rituximab treatment may have led to a better outcome, but both trials failed to show improvement on

their pre-defined primary outcomes. (Dalakas 2009, Leger 2013, Lunn 2012) How these two trials should be interpreted is a source of contention. Their negative results have not led to a discontinuation of rituximab treatment for anti-MAG treatment. (Kawagashira 2014, Rajabally 2014) Several retrospective studies suggest that suboptimal outcome measurements and patient selection might be a better explanation for the negative results than the absence of a relevant effect of rituximab. (Kawagashira 2014, Stork 2014) As anti-MAG neuropathy is a slowly progressive disease, with predominantly sensory symptoms, variations in disease severity can be hard to quantify, even if they have relevance for the patients' daily life. (Rajabally 2014, Draak 2014) A Rasch-built disability scale could be superior to the INCAT sensory sum score or disability score in capturing these variations. (Draak 2014) On the other hand, both trials identified a subset of patients for whom rituximab led to functional improvement, as measured by the INCAT disability scale, suggesting that better pre-treatment selection might be necessary. (Dalakas 2009, Leger 2013) One approach would be by electrophysiological characteristics (demyelinating features vs axonal loss). (Kawagashira 2014) Patient selection could also be based on the differential response to therapeutic monoclonal antibodies due to Fcγ receptor polymorphisms. These polymorphisms are associated with differential affinity of effector cells for antibodies like rituximab and lead to differences in antibody dependent cellular cytotoxicity (ADCC) responses. Fcγ receptor polymorphisms have previously been shown to predict response to rituximab in several subsets of non-Hodgkin lymphoma. (Persky 2012, Kim 2006) In the study described in **chapter 6** we showed that Fcγ receptor polymorphisms could be used to predict the effectiveness of rituximab treatment in IgM-PNP as well. (Stork 2014) Our findings, in a single retrospective cohort, need corroboration, but if the subset of patients who might respond to rituximab treatment can indeed be selected by determining the Fcγ receptor genotype, cost-effectiveness of this treatment would greatly improve, and the presence of participants who, for biological reasons, cannot sufficiently respond to rituximab could explain the difference between the primary endpoint analysis and the clinical judgment of the authors in the two randomized trials.

Treatment of neuropathies associated with IgG or IgA MGUS No convincing neuronal target has been described for the monoclonal antibodies of IgG or IgA MGUS that could account for the concomitant polyneuropathy. The systematic review of the treatment of IgG or IgA monoclonal gammopathy associated neuropathy described in **chapter 8** showed that evidence for the usefulness of treating these neuropathies is very slim. The positive results of the one trial that does provide some evidence for treating these neuropathies might very well be based on the inclusion of CIDP patients with an unrelated concomitant IgG

MGUS. (Dyck 1991) As interest in the field has shifted to the more promising IgM-PNP no recent trials have been published for this patient category and none are to be expected. In **chapter 9** we showed that stem cell transplantation as a treatment for more severe hematological malignancies with a monoclonal gammopathy, had an occasional positive effect on the associated polyneuropathy. Extrapolation to the IgA/IgG MGUS population with a neuropathy would lead to the conclusion that treatment of the gammopathy could at least in theory lead to improvement, although whether this extrapolation can be made, which treatment modality that would be, and how the benefits and risks of this treatment ought to be weighed all remains to be investigated.

Conclusion and further research

In conclusion, the findings described in this thesis support both the tightening of some inclusion criteria for treatment of IgM-PNP as well as widening the group of eligible patients beyond only anti-MAG patients.

On the one hand rituximab seems ineffective in a large subset of patients currently treated, even if one accepts the optimistic interpretation of the randomized controlled trials as being suggestive of a positive effect in a subset of patients. Not addressed in this thesis but a problem probably at least partially responsible for the negative clinical trials for treatment of IgM-PNP is the difficulty of quantifying disease progression or improvement in a slowly progressive neuropathy in which sensory deficits are most likely to lead to functional disabilities. Here newly-published, Rasch-based functional scoring scales might be more helpful than the currently used functional scores. (Draak 2014)

Better and tighter patient selection for rituximab treatment would still be necessary, and the determination of Fc gamma receptor polymorphisms described in this thesis is a promising possibility. Alternatively, new immunomodulolatory drugs either targeting B cells, but less dependent on ADCC, such as ofatumumab, or targeting the complement-mediated nerve damage might lead to better results in the future, but their effectiveness in IgM-PNP remains for now unclear. (Halstead 2008, Puri 2013, Sarkozy 2015)

On the other hand recent concentration on anti-MAG antibody positive patients disregards at least 20-30% of IgM-PNP patients, most of whom have very similar clinical profile of demyelinating, sensorimotor, slowly progressive neuropathy, which has been shown to be more predictive of the disease course than the presence of specific antibodies. (Niermeijer 2010, Kuijf 2009, Nobile-Orazio 2008, Eurelings 2001) Non-randomized studies have

suggested an effect of rituximab treatment in both MAG-positive and negative IgM-PNP patients. (Niermeijer 2009, Stork 2014, Delmont 2010) This thesis provides clinical, as well as immunological support for IgM-PNP with anti-ganglioside antibodies as a separate disease entity within the monoclonal gammopathy-associated neuropathies. Based on several case series, as well as the example of other anti-ganglioside antibody associated neuropathies, IVIg is, next to rituximab, a treatment option that deserves serious consideration and further research in this group of patients.

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Samenvatting in het Nederlands

Polyneuropathie, de beschadiging van vooral de zenuwuiteinden in de armen en benen, is een veelvoorkomend probleem. Het kan leiden tot zwakte van de spieren die voor bewegingen van vingers, tenen, enkel- en polsgewrichten zorgen. Daarnaast kan het gevoel in onderbenen, handen en voeten gestoord zijn, en dit kan weer tot evenwichtsproblemen leiden. In zeldzamer gevallen kunnen de gevoelsveranderingen zich ook naar bovenarmen en benen uitbreiden en kunnen ook de spieren in bovenbenen, heup- of schoudergordelspieren aangedaan zijn. Daarnaast zijn er soms ook problemen van het onwillekeurige, autonome zenuwstelsel, dat bijvoorbeeld de darmen aanstuurt. Er zijn vele verschillende oorzaken voor een polyneuropathie. In dit proefschrift worden de resultaten van onderzoek van één, relatief zeldzame oorzaak, beschreven: een woekering van immuuncellen die allemaal exact hetzelfde antilichaam produceren. Een antilichaam is een eiwit dat door het lichaam wordt gebruikt om niet-lichaamseigen indringers, zoals bacteriën of virussen te herkennen en te markeren om door andere onderdelen van het immuunsysteem te worden opgeruimd. De aanwezigheid van een meetbare hoeveelheid antilichamen die allemaal door identieke immuuncellen – klonen van een ontspoorde cel – worden geproduceerd heet een monoclonale gammopathie. Soms is dit een teken van kanker van het immuunsysteem, maar gammopathieën kunnen ook op zichzelf voorkomen, en worden dan *monoclonal gammopathy of unknown significance* (MGUS) genoemd. Het is al langer bekend dat een monoclonale gammopathie met antilichamen van het IgM subtype in plaats van alleen lichaamsvreemde ook lichaamseigen structuren herkennen kan: het *myelin-associated glycoprotein* (MAG) op de zenuwschede (isolatielaag van de zenuw). Dit leidt tot een duidelijk herkenbare polyneuropathie met vooral gevoelsstoornissen en daardoor coördinatieproblemen (ataxie). Van de patiënten met een IgM antilichaam subtype MGUS en een polyneuropathie heeft ongeveer 70% antilichamen die met MAG reageren. Van de overige 30% is minder duidelijk of en hoe de monoclonale antilichamen tot de neuropathie leiden. Sommige van deze patiënten hebben antilichamen gericht tegen een andere structuur op de zenuw: gangliosiden.

In **Deel I** (**hoofdstukken 2 en 3**) wordt onderzoek naar deze anti-ganglioside antilichamen beschreven. We vonden naast IgM antilichamen tegen afzonderlijke gangliosiden ook antilichamen tegen combinaties van gangliosiden (ganglioside-complexen) bij patiënten met polyneuropathie en een IgM MGUS (IgM-PNP). Dergelijke anti-ganglioside-complex antilichamen zijn relevant omdat gangliosiden in de zenuwen ook tot complexen in elkaar verstrengeld voorkomen en antilichamen tegen die complexen mogelijk sneller een immuunreactie uit kunnen lokken. Daarnaast vonden we dat de antilichamen tegen gangliosiden en ganglioside-complexen niet allemaal afkomstig kunnen zijn van de cellen

die de monoclonale gammopathie veroorzaken. Zo vonden we vaak antilichamen tegen onderling sterk verschillende gangliosiden, en waren die antilichamen tegen verschillende gangliosiden niet altijd uit dezelfde lichte ketens opgebouwd, hetgeen bij monoclonale antilichamen wel te verwachten is. Deze bevinding is verrassend omdat algemeen wordt aangenomen dat de antilichamen van de monoclonale gammopathie direct bestanddelen van de zenuw herkennen en uiteindelijk tot schade leiden. Dit concept lijkt dus tenminste voor patiënten met anti-ganglioside niet te kloppen.

Hoofdstuk 3 beschrijft de relatie tussen de aanwezigheid van antilichamen tegen verschillende gangliosiden en de neurologische uitval die patiënten van hun neuropathie krijgen. We vonden dat er tussen patiënten met anti-ganglioside antilichamen onderling grotere verschillen zaten dan tussen patiënten met anti-MAG antilichamen. Ook vonden we dat patiënten met antilichamen tegen de gangliosiden GQ1b en GD1b meer gevoelsproblemen leken te hebben en patiënten met anti-asialo GM1 antilichamen meer uitval van spieren, die opvallend asymmetrisch was. Deze verschillen tussen patiënten met antilichamen tegen andere gangliosiden waren niet bij alle patiënten terug te vinden, waarschijnlijk omdat patiënten met IgM-PNP vaak antilichamen tegen meerdere gangliosiden tegelijk hebben. Een vergelijkbare relatie tussen antilichamen tegen specifieke gangliosiden en het soort neurologische uitval is bekend uit het onderzoek naar het Guillain-Barré syndroom en varianten daarvan. Omdat er bij die ziekten heel overtuigende bewijzen voor de oorzakelijke rol van anti-ganglioside antilichamen in het ontstaan van de ziekte bestaan, zijn onze bevindingen een bouwsteen voor de theorie dat ook in IgM-PNP anti-ganglioside antilichamen direct bij het ontstaan van de ziekte betrokken zijn en niet alleen als een later optredend effect van zenuwschade door andere oorzaken te verklaren zijn.

Deel II (**hoofdstukken 4 en 5**) beschrijft hoe de algemene toestand van het immuunsysteem gerelateerd is aan de optredende neuropathie. **Hoofdstuk 4** beschrijft de resultaten van onderzoek naar de relatie tussen het ontstaan van de polyneuropathie en het complement systeem, een evolutionair oud onderdeel van het immuunsysteem dat onder andere als functie heeft door antilichamen als "vreemd" aangemerkte structuren kapot te maken. De hypothese vooraf was dat hogere activiteit van het complement systeem tot een ernstiger polyneuropathie zou leiden, zoals dit ook voor andere neurologische ziekten beschreven is. Hiervoor vonden we geen overtuigende bewijzen. Wel vonden we bij geen van de patiënten met een polyneuropathie en anti-MAG antilichamen het genetisch profiel dat past bij een *verminderde* activiteit van een van de drie manieren om het complement systeem te activeren (via de lectine-route). Dit zou erop kunnen wijzen dat zo'n genetisch profiel beschermt tegen

het krijgen van een anti-MAG polyneuropathie. **Hoofdstuk 5** beschrijft hoe de concentraties van 11 verschillende cytokines verschillen tussen:

- IgM-PNP patiënten met en zonder anti-MAG antistoffen,
- patiënten met een andere zenuwziekte waarvan we denken dat zij door het immuunsysteem wordt veroorzaakt (multiple motore neuropathie, MMN) en
- gezonde controles.

Cytokines zijn boodschappermoleculen die onder andere het immuunsysteem kunnen afremmen of juist aanzetten. Cytokine IL-6 bleek bij patiënten met IgM-PNP in verhoogde concentratie in het bloed aanwezig. Het is op basis van deze studie niet uit te maken of dat specifiek bij een IgM-PNP hoort, of dat de gammopathie alleen volstaat om de hogere concentratie te verklaren. Voor een relatie met IgM-PNP pleit dat we bij patiënten met MMN en een gammopathie geen verhoging van IL-6 konden vaststellen. Binnen de groep patiënten met IgM-PNP bleek dat alleen bij patiënten met anti-MAG antilichamen ook IL-10 verhoogd was, dit is een extra argument voor de theorie, ook al geopperd naar aanleiding van hoofdstuk 2, dat antilichamen tegen verschillende componenten van de zenuwen op verschillende wijze tot een polyneuropathie kunnen leiden.

Deel III (**hoofdstukken 6 en 7**) gaan over de behandeling van IgM-PNP met rituximab, een als medicijn geproduceerd antilichaam, dat B cellen, de cellen die uiteindelijk antilichamen gaan produceren, kan herkennen en zich daaraan binden kan. Na binding wordt het eigen immuunsysteem van de patiënt aangezet om die B cellen kapot te maken en daarmee de monoclonale gammopathie te bestrijden. **Hoofdstuk 6** laat zien dat variatie in het DNA (polymorfismen) dat voor de Fcγ-receptor codeert mogelijk kan voorspellen of iemand op rituximab-behandeling gaat reageren. Antilichamen maken via hun Fcγ- staart contact met andere delen van het immuunsysteem. De genetische variatie in de receptor die met die de Fcγ-staart contact moet maken, maakt het voor antilichamen moeilijker of makkelijker om met de uitvoerende (effector) cellen van het immuunsysteem te communiceren. Als hiermee inderdaad het effect van rituximab op de polyneuropathie voorspeld kan worden, kan dat alleen aan patiënten die op voorhand een goede kans hebben op die behandeling te reageren voorgeschreven worden. Dat is belangrijk omdat rituximab-behandeling duur is en niet zonder risico's. Over een van de risico's gaat **hoofdstuk 7**, dat een aantal patiënten beschrijft waarbij rituximab-behandeling op paradoxale wijze juist tot verslechtering van de polyneuropathie leidde. Het risico dat dit gebeurt is klein, maar omdat het positieve effect van rituximab relatief klein is en zeker niet bij alle behandelde patienten optreedt, moeten ook lage risico's zorgvuldig worden meegewogen bij de beslissing om iemand voor zijn IgM-PNP met rituximab te behandelen.

Tenslotte beschrijft **Deel IV** (**hoofdstukken 8 en 9**) onderzoek naar de behandeling van polyneuropathie bij patiënten met een monoclonale gammopathie, maar nu van het IgG in plaats van het IgM subtype. Bij deze patiënten is het minder duidelijk of de monoclonale gammopathie iets met de polyneuropathie te maken heeft, en daarmee ook of het dan wel zin heeft om de gammopathie te behandelen in de hoop dat de polyneuropathie opknapt. **Hoofdstuk 8** geeft een systematische samenvatting van wat daarover tot nu toe is gepubliceerd. Overtuigend bewijs dat behandeling zin heeft vonden we daarbij niet. Slechts één studie voldeed aan de tevoren opgestelde technische eisen en deze beschreef een positief effect van het wegzuiveren van de antilichamen uit het bloed door plasmaferese, zij het alleen op de korte termijn. Onduidelijk is of dit niet kwam doordat er patiënten in de onderzoeksgroep zaten met toevallig zowel een IgG gammopathie als een op een polyneuropathie lijkende neurologische ziekte waarvan we *wel* zeker weten dat die met plasmaferese behandeld kan worden. In **hoofdstuk 9** wordt beschreven of de zwaarst mogelijke behandeling voor kanker van het immuunsysteem, een stamceltransplantatie, ook tot een verbetering van een polyneuropathie kan leiden. We keken daarbij naar patiënten met een hematologische ziekte, die waarschijnlijk ten gevolge van die ziekte een polyneuropathie kregen, en voor die hematologische ziekte met een stamceltransplantatie behandeld moesten worden. We vonden bij één, hooguit twee van deze patiënten een verbetering van de polyneuropathie. Mocht er een duidelijk effect op de polyneuropathie geweest zijn, was dit vanzelfsprekend geen reden geweest om alle patiënten met een IgG-gammopathie en polyneuropathie met stamceltransplantatie te gaan behandelen, maar wel een argument om verder te kijken of er ook andere, minder ingrijpende behandelingen die ingrijpen op het immuunsysteem zijn waar die patiënten baat bij zouden kunnen hebben. De resultaten van dit hoofdstuk rechtvaardigen zelfs die voorzichtige conclusie niet, hooguit de rol van stamceltransplantatie als laatste redmiddel bij heel ernstige polyneuropathieën als alle andere behandelingen gefaald hebben.

De in dit proefschrift beschreven studies vergroten onze kennis over polyneuropathieën bij monoclonale gammopathie op verschillende vlakken: ze geven nieuwe argumenten voor de hypothese dat bij patiënten met een IgM-PNP niet alleen anti-MAG, maar ook anti-ganglioside antilichamen tot een polyneuropathie kunnen leiden. Daarnaast zijn er zowel in de opbouw van de anti-ganglioside antilichamen als in het cytokine profiel in het bloed aanwijzingen dat verschillende antilichamen mogelijk op verschillende wijze tot zenuwschade leiden. In de behandeling van IgM-PNP is bepaling van polymorfismen in de DNA-codering voor de Fcγ-receptor een veelbelovende methode om patiënten met een goede kans op de behandeling te reageren vooraf te kunnen selecteren.

Dankwoord

Geen pagina van dit proefschrift van dit proefschrift was mogelijk geweest zonder de vriendelijke medewerking van de patiënten van de polikliniek neuromusculaire ziekten. Voor velen van u ben ik al de 4e of 5e generatie van promovendi die uw ziekte onderzoekt, en nog steeds bent u bereid extra tests, komische gymnastiekoefeningen ter beoordeling van kracht en coördinatie en steeds weerkerende extra bloedafnames ten behoeve van onderzoek te ondergaan. Mocht u daar zo nu en dan wat moe van zijn geworden, heeft u dat tenminste voor mij altijd overtuigend kunnen verbergen. Ik wil u allen hartelijk danken voor zowel het meedoen aan de hier beschreven onderzoeken als voor de positieve en vriendelijke wijze waarop u dat heeft gedaan.

Mijn co-promotores, dr. W.L. van der Pol, en dr. N.C. Notermans,

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Voorzitter van de leescommissie, prof. dr. J. van Laar, beste Jaap, in ons beider leven is het een en ander veranderd sinds jij mij 13 jaar geleden in een oude Saab van Dulles International Airport op kwam halen. Het geeft een mooie symmetrie dat mijn begeleider in mijn eerste serieuze wetenschappelijke stappen nu als professor mijn proefschrift beoordeelt.

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De proeven die hebben geleid tot de hoofdstukken 2, 4, 5 en 6 hebben ieder in een eigen laboratorium plaatsgevonden. In alle vier ben ik met grote hartelijkheid en hulpvaardigheid ontvangen, en van de soms heel verschillende benaderingen van het doen van wetenschappelijk onderzoek heb ik veel geleerd.

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Curriculum vitae

Curriculum vitae

Jan Stork werd 29 maart 1979 in Vlissingen geboren. Hij deed in 1997 eindexamen VWO aan de Stedelijke Scholengemeenschap te Middelburg (cum laude). Hij studeerde geneeskunde aan de Universiteit Leiden (doctoraal examen 2002, cum laude). Voorafgaand aan zijn co-schappen werkte hij als research fellow aan de National Institutes of Health (National Institute of Arthritis, Musculoskeletal and Skin diseases, Bethesda, Maryland, USA), in het laboratorium van prof. dr. Peter Lipsky. In 2005 deed hij artsexamen en begon hij met de opleiding tot neuroloog in het Universitair Medisch Centrum Utrecht (opleiders prof. dr. Jan van Gijn en prof. dr. John Wokke). Hij onderbrak de opleiding enkele malen om het in dit proefschrift beschreven onderzoek uit te voeren. Vanaf 2013 is hij geregistreerd als neuroloog. In 2013 en 2014 werkte hij als Oberarzt in de Klinik für Neurologie van de Euregio Klinik te Nordhorn. Momenteel werkt hij gedeeltelijk weer in het UMC als fellow neuro-oncologie, en daarnaast in dezelfde functie in het Antoni van Leeuwenhoek Ziekenhuis te Amsterdam. Naast zijn werk was hij voorzitter van de vereniging van arts-assistenten en lid van de centrale opleidingscommissie in het UMC. Ook was hij coach van meerdere jeugdteams van voetbalvereniging Bunnik ’73. Hij is getrouwd met Ruth Fritsch-Stork en vader van Philip (2005) en Charlotte (2011).

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