Anti-MAG IgM: differences in antibody tests and correlation with clinical findings

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Abstract

Objectives Anti-myelin-associated glycoprotein (MAG) antibody is associated with clinically heterogeneous polyneuropathies. Our purpose was to compare neuropathy phenotypes identified by different anti-MAG tests' results.

Methods Cohort study: Sera from 40 neuropathy anti-MAG EIA positive patients were tested for anti-MAG by Western blot (WB), for anti-peripheral nerve myelin (PNM) on monkey nerve by immunofluorescence assay (IFA), and for anti-HNK1 on rat CNS slices by IFA. Anti-sulfatide antibodies, for comparison, were also tested by EIA.

Results Among 40 anti-MAG EIA positive sera, 85% also had anti-PNM IFA reactivity and 67.5% bind HNK1 on rat CNS. Anti-HNK1 positive patients had the classical predominantly distal acquired demyelinating symmetric (DADS) neuropathy with a benign course, while anti-PNM positive but anti-HNK1 negative patients had predominantly axonal neuropathy with a high frequency of anti-sulfatide reactivity and the worst long-term prognosis. Anti-MAG EIA positive patients without anti-PNM or anti-HNK1 IFA reactivity had a CIDP-like polyneuropathy.

Conclusion Different methods to test for anti-MAG antibodies identify different clinical and electrophysiological findings, as well as long-term outcome. HNK1 reactivity is the strongest marker of DADS.

Keywords Autoimmune diseases · Chronic inflammatory demyelinating polyneuropathy · EMG · Myelin-associated glycoprotein · HNK-1

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Abbreviations

MAG	Myelin-associated glycoprotein
EIA	Enzyme immunosorbent assay
IFA	Immunofluorescence assay
WB	Western blot
HNK1	Human natural killer-1
DADS	Distal acquired demyelinating symmetric neuropathy
CIDP	Chronic inflammatory demyelinating polyneuropathy
PNM	Peripheral nerve myelin
SGPG	Sulfate-3-glucuronyl paragloboside
mRD	Modified Rankin disease
IVIg	Intravenous immunoglobulin
PE	Plasma exchange
MCV	Motor conduction velocity
DL	Distal latency

CMAP Compound motor action potential
SCV Sensory conduction velocity
SNAP Sensory nerve action potential

Introduction

A high proportion of patients affected by polyneuropathy and IgM monoclonal gammopathy (MG) has IgM that reacts with myelin-associated glycoprotein (MAG) [1, 2]. Anti-MAG IgM is typically associated with a distal acquired demyelinating symmetric (DADS) phenotype characterized by a slowly progressive, predominantly sensory, demyelinating neuropathy, with disproportionate distal slowing [2–4]. In many cases, a concomitant axonal loss may occur, leading to a more severe disease course [5–7]. However, several anti-MAG positive patients have been reported with atypical electrophysiological and clinical phenotypes, such as predominantly axonal sensory or sensorimotor neuropathy, motor neuropathy, or CIDP-like polyneuropathy [7–10]. Neuropathy heterogeneity could depend on heterogeneity of anti-MAG IgM reactivity, as suggested by the variable results obtained using different testing methods [6, 11]. At present, Western blot (WB) is considered the most specific test, while enzyme-immunoassay (EIA) has been proved to be the most sensitive although less specific for demyelinating polyneuropathy [11, 12]. Anti-peripheral nerve myelin (PNM) test by immunofluorescence assay (IFA) on monkey nerve has also shown higher sensitivity as compared to WB, but heterogeneity on reactivity patterns has been reported [6]. Moreover, the association of anti-MAG and anti-sulfatide IgM reactivity, as well as anti-SGPG positivity, has been shown in patients with different neuropathy phenotypes [13–17]. We have demonstrated that most patients with anti-MAG EIA positivity show anti-HNK1 reactivity as detected by IFA using rat CNS, and that HNK1 antibodies are highly associated with demyelinating polyneuropathy [18]. Now we want to compare results obtained using EIA and WB for anti-MAG, monkey nerve IFA for anti-PNM, and rat brain IFA, to detect anti-HNK1 reactivity, to verify the hypothesis whether different tests results are associated with distinct and more homogeneous subgroups of patients.

Methods

Study setting and population

Consecutive patients referred to the Departments of Neurology of the Hospitals of Firenze and Siena, Italy, between 1997 and 2012 with a sensorimotor neuropathy testing positive for anti-MAG antibodies by EIA were included in the cohort. Medical history was obtained, and physical examination and routine laboratory analysis, immunoelectrophoresis, immunofixation, and electrophysiologic studies were carried out in all patients. Demographic and clinical variables, including modified Rankin disability (mRD) score, were obtained by reviewing the charts of the patients. The ulnar, tibial, and peroneal motor nerves and ulnar and sural sensory nerves were collected in all patients. Needle electrode examination included sampling of at least one distal and proximal muscle in the arm and leg. Electrophysiological studies were classified as indicative of demyelinating polyneuropathy if they met the criteria of the European Federation of Neurological Societies [19]; as axonal if they were mainly characterized by motor or sensory responses amplitude reduction; and mixed (demyelinating and axonal) if both demyelinating and axonal findings were found. Patients with symmetric demyelinating polyneuropathy with disproportionate distal slowing were classified as having DADS.

Laboratory methods

Patients' sera were tested by a semi-quantitative EIA (Bühlmann Laboratories, Schönenbuch, Switzerland) that uses highly purified human MAG. Sera producing titer units of 1000 BTU or greater were considered positive for MAG IgM antibody, with titers < 10,000 BTU considered as low positive and titers ≥ 10,000 considered as high positive. Anti-sulfatide IgM antibodies were determined by a home-made method as described previously [18]. The titer of anti-sulfatide antibodies was considered positive when > 1:8000. For WB analysis, human CNS myelin was isolated by sucrose gradient and delipidation, separated in 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and electro-transferred onto nitrocellulose membranes. After blocking, the membranes were incubated with diluted patients' sera overnight at 4 °C on a shaker, washed, and incubated with horseradish peroxidase–conjugated rabbit anti-human IgM (dilution 1:2000; Dako, Denmark) for 1 h at room temperature on a shaker. After washing, bound antibodies were detected by DAB. Positive sera was titrated by a dilution of 1:1000 until negative. For anti-PNM IFA test, a kit from Diamedix (Miami, FL) was used. Samples were diluted 1:10 in dilution buffer and 50 mL was applied to tissue slides and allowed to incubate 30 min at RT in a moist chamber. After washing, slides were incubated with 50 mL of IgM fluorescein-labeled conjugate for 30 min. After washing, coverslips were mounted onto slides with mounting media. Images were acquired by laser-scanning microscope (Leica Microsystems, Mannheim, Germany) and compared with anti-PNM positive and negative controls. Sera that demonstrated a fluorescence of the myelin sheath were considered positive for anti-PNM antibody.

Anti-HNK1 antibodies were measured using the IFA method, as previously described [18]. Briefly, adult Wistar rat CNS sections were washed three times with PBS solution and incubated with 0.03% Triton X-100 PBS (PBS-TX) containing 10% of fetal calf serum (FCS). Sera dilutions (1:100) in PBS-TX-FCS were then incubated for 2 h at room temperature or overnight at 4 °C. After three washes with PBS, bound IgM was detected with

fluorescein-conjugated goat antibody to human IgM (Zymed, San Francisco, CA; 1:300 in PBS-TX-FCS). Images were acquired by laser-scanning microscope (Leica Microsystems, Mannheim, Germany). Sera with the typical reactivity against HNK1 epitope were considered positive and titrated by twofold dilutions until negative. As controls for anti-HNK1, we tested sera from 93 patients with IgM MG patients with (80) or without (13) neuropathy, and from 48 patients with chronic inflammatory demyelinating polyneuropathy (CIDP) without MG, all of whom tested negative for anti-MAG EIA; sera from 30 patients with Guillain-Barrè syndrome, 65 patients with secondary polyneuropathy, 45 patients with multiple sclerosis, 15 patients with paraneoplastic syndromes, and 37 healthy donors were also tested as controls.

Statistical analysis

Statistical analyses were performed with StatView software program (SAS Institute, Cary, NC). The Mann-Whitney or the Kruskal-Wallis test was used to test the significance of the differences in the variables between groups. Fisher's exact test was used to compare categorical variables. The Spearman rank correlation coefficient assessed the correlations. The significance level was set at $p < 0.05$.

Ethics

The study was approved by the Research Ethics Committee of Careggi Hospital, which deemed that no individual informed consent was required.

Results

Anti-MAG EIA

Among the 40 patients (25 males, 15 females) with anti-MAG EIA antibody titer higher than 1000 BTU (median, 11,000 BTU; range 1000−1,000,000), 33 (82.5%) had high positivity (≥ 10,000 BTU). Thirty-nine patients (97.5%) had an IgM MG, complicated by a hematological malignancy in 7 patients (17.5%). The median length of disease at the time of the study was 5.5 years (3−19 years), and the median follow-up was 3 years (1−10 years). Of the 40 positive patients, 27 (67.5%) had clinical and electrophysiological findings consistent with DADS. Of the other 13 patients, 5 had a CIDP-like phenotype including acute onset in 2 cases, 4 had axonal neuropathy, 3 a mixed polyneuropathy, and 1 had a multiple mononeuropathies. Of the 7 patients with low anti-MAG titer, 3 (43%) had DADS, including the patient without IgM MG. The median mRD score at nadir was 2 (range, 1−5). Twenty-three patients (57.5%) had been treated with plasma exchange (PE) and/or intravenous immunoglobulins (IVIg) that were effective (improvement of at least 1 point on mRD score) in 7 cases (30.4%). No significant differences were observed in demographic, clinical, and electrophysiological findings between PE/IVIg responder and non-responder patients. No correlations were observed between anti-MAG EIA titer values and clinical and electrophysiological findings.

Anti-MAG WB

Eighteen of the 34 anti-MAG EIA positive tested sera (52.9%) showed anti-MAG WB positivity. Median titer value was 1:12,800 (range,1:1000−1:1,600,000). WB positive patients had higher EIA titers as compared to WB negative patients [median, 45,000 BTU (range, 5500−1,000,000) vs 6000 BTU (range, 1000−30,000); $p = 0.0004$]. DADS was diagnosed with about the same frequency among WB positive and negative patients (77.7% vs 50%; p = ns). Two WB positive patients had an axonal polyneuropathy, purely motor, and sensorimotor, respectively; two patients had a mixed polyneuropathy. No correlation was observed between anti-MAG WB titer values and clinical and electrophysiological findings. No significant differences of these findings were found between WB positive and negative patients (Table 1).

Anti-PNM IFA

Of the 40 tested sera, 34 (85%) showed anti-PNM staining on monkey nerve (Fig. 1). All anti-MAG WB positive sera also had anti-PNM reactivity. Moreover, anti-PNM IFA positive cases had significantly higher anti-MAG EIA titer values as compared to negative patients [median, 30,000 BTU (range 3000−1,000,000) vs 7750 BTU (range, 1000−12,000); $p = 0.04$]. Anti-PNM positive patients had significantly higher mRD score and a lower response rate to PE/IVIg treatment as compared to negative patients. No significant differences in electrophysiological findings were found comparing anti-MAG EIA positive patients with and without anti-PNM IFA reactivity (Table 1).

Anti-HNK1 IFA

HNK1 reactivity was found in 27 of the 40 EIA positive patients (67.5%), with a titer ranging from 1:200 to 1:204,800 (median, 1:6400); no HNK1 reactivity was found among the 333 control sera, including those from EIA negative neuropathy patients. All anti-HNK1 positive patients had anti-PNM IFA positivity, and 71.4% of tested sera also had positive anti-MAG WB values. Anti-HNK1 positive patients had a higher titer of anti-MAG EIA as compared to the negative cases (median, 50,000 BTU (range 1000−1,000,000) vs 7000 BTU (range, 1000−12,000); $p = 0.002$] with a strong correlation between anti-MAG and anti-HNK1 titers ($p < 0.001$). Notably, 3 out of 7 patients (43%) with low anti-MAG EIA titer tested positive for anti-HNK1. IgM anti-HNK1 titers also correlated with total IgM serum concentrations ($p = 0.01$). All HNK1

Table 1 Demographic, clinical, and electrophysiological data from anti-MAG EIA positive patients grouped according to WB, monkey PNM IFA, and HNK1 IFA results

	Anti-MAG WB		Anti-PNM IFA		Anti-NHK1 IFA	
	Positive (*n.* 18)	Negative (*n.* 16)	Positive (*n.* 34)	Negative (*n.* 6)	Positive (*n.* 27)	Negative (*n.* 13)
Female/male *n.*	7/11	5/11	13/21	2/4	10/15	4/13
Age *yrs*	71 (53–81)	67.5 (49–79)	71 (53–81)	62 (49–79)	70 (53–81)	67.5 (49–79)
Disease duration *yrs*	6 (3–10)	5 (2–19)	6.5 (3–19)	5.5 (2–15)	5.5 (3–15)	5.5 (2–19)
DADS (%) *n.*	14 (77.8%)	8 (50%)	24 (70.5%)	3 (50%)	27 (100%)	0 **
mRD score	1 (0–3)	2 (1–5)	3 (0–5)	1 (1–3)*	1 (1–3)	3 (1–5)**
IVIg/PE response *n.*	2 (28.5%)	4 (26.6%)	2 (11.1%)	4 (80%)**	1 (10%)	5 (38.4%)
Peroneal nerve						
MCV m/sec	28.5 (10.6–40)	34.4 (17–43)	29.6 (10.6–40)	36.5 (22.5–43)	27.7 (10.6–39.5)	36.8 (22.5–43)*
DL msec	10.6 (5.4–28.6)	7.27 (4.6–16.7)	8.8 (4.6–28.6)	6.2 (5.5–16.7)	10.6 (5.9–28.6)	5.9 (4.6–16.7)*
CMAP mV	1.17 (0.3–6.3)	2 (0.2–9.5)	1.2 (0.2–0.5)	1.6 (0.6–8.5)	1.1 (0.2–6.6)	2.1 (0.6–9.5)
Tibial nerve						
MCV m/sec	28.6 (11.8–46.2)	34.8 (26–42.5)	32.1 (11.8–46)	38 (23.1–42.5)	28.5 (11–39.6)	37.1 (23–46.2)*
DL msec	10 (3.7–17.6)	7 (4.3–13)	7.8 (3.7–17.6)	7.4 (4.7–12)	9.3 (3.7–17.6)	5.9 (4.3–12)*
CMAP mV	2 (0.2–12.6)	3.4 (0.2–20.8)	2.4 (0.2–12.6)	4 (0.8–20.8)	1.9 (0.2–7.3)	4.5 (0.8–20.8)*
Ulnar nerve						
MCV m/sec	44 (23.4–57)	47.2 (15.7–57.2)	46.1 (15.7–57)	44.7 (31–55)	45.4 (15.7–57)	44.8 (35.7–56.2)
DL msec	4.85 (2.5–14.5)	3.9 (2.4–16.3)	4.3 (2.4–16.3)	3.4 (2.7–4.5)	4.3 (2.8–10.5)	3.8 (2.4–16.3)
CMAP mV	10 (0.5–18.4)	9.5 (0.4–18.7)	9.6 (0.4–18.7)	9.4 (3.5–18.4)	10 (3.7–18.7)	8.6 (0.4–18.4)
Sural nerve						
SCV m/sec	34.4 (30–51.9)	42.2 (21–51)	37.2 (21–51)	42.5 (30–51)	34.3 (21–43.8)	42.5 (30–51.9)*
SNAP μV	4.4 (0.4–10.5)	5.5 (3–14)	4.4 (0.4–13.5)	5.5 (3–14)	4 (0.4–13.3)	5.5 (3–14)

Numerical values are expressed as the median (range) of observed values, where not otherwise specified. Statistics: Mann-Whitney, Kruskal-Wallis, or Fisher's exact test, as appropriate

Yrs, years; *n.*, number; *mRD*, modified Rankin disease; *IVIg*, intravenous immunoglobulin; *PE*, plasma exchange; *MCV*, motor conduction velocity; *DL*, distal latency; *CMAP*, compound motor action potential; *SCV*, sensory conduction velocity; *SNAP*, sensory nerve action potential

*$p < 0.05$

**$p < 0.01$

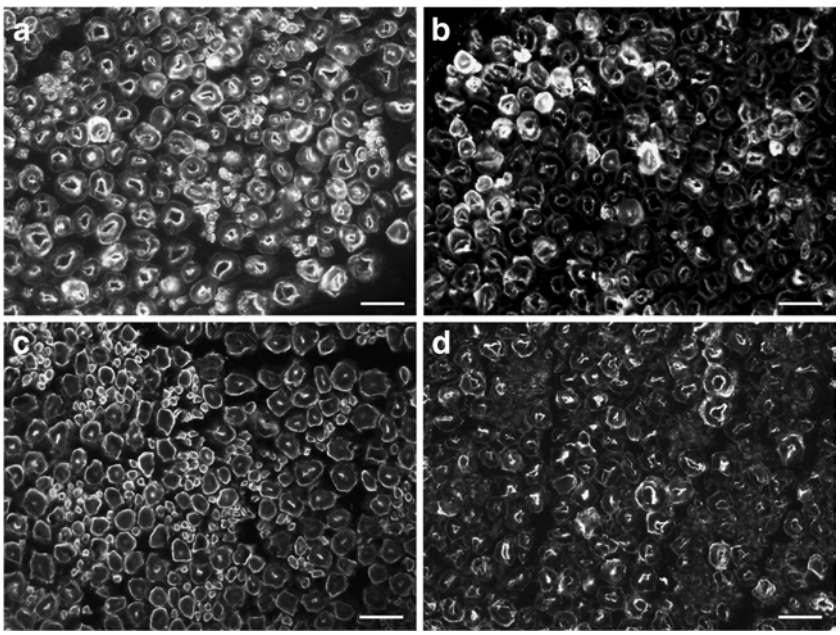

Fig. 1 Indirect immunofluorescence staining of myelin sheaths (cross section) of monkey nerve with the primary antibodies from the patient's sera IgM. a, b Images obtained with serum from a patient with high and a patient with low anti-MAG enzyme immunosorbent assay titer, both with HNK1 reactivity. c, d Images obtained using serum from two enzyme immunosorbent assay positive patients without HNK1 reactivity. The IgM stains the outer and inner border of the myelin sheet in both cases, with a pattern indistinguishable from that observed in the two HNK1 positive cases. Scale bar: 25 μm

positive patients had clinical and electrophysiological features of DADS, as compared to 67.5% of anti-MAG EIA positive patients (p = 0.003). Among anti-MAG EIA positive patients, those with anti-HNK1 positivity showed slower motor conduction velocities and more prolonged DML of peroneal and tibial nerves as compared to anti-HNK1 negative cases. Moreover, a significantly lower median mRD score was found among anti-HNK1 positive patients as compared to the negative (Table 1).

Anti-sulfatide IgM

A positive value of anti-sulfatide IgM titer (> 1:8000) was found in 5 of the 40 anti-MAG EIA positive patients (12.5%). Three patients with low anti-MAG EIA titer (43%) had anti-sulfatide reactivity, as compared with 6.1% of patients with high anti-MAG EIA titer values (p = 0.01). One of them (20%) had DADS; 2 had a mixed polyneuropathy, while the other 2 patients had an axonal neuropathy that was predominantly sensory in 1 case. Most of anti-sulfatide positive sera (60%) also show anti-PNM positivity, while only one anti-HNK1 positive case had anti-sulfatide reactivity (Table 1).

Results comparison

With regard to IFA results, we have grouped anti-MAG EIA positive patients as follows: those with both anti-HNK1 IFA and anti-PNM IFA reactivity (n. 27); those with only anti-PNM IFA reactivity (n. 7) and those without IFA reactivity (n. 6). Significant differences were observed in the frequency of demyelinating polyneuropathy diagnosis and in the frequency of response to conventional therapy, in mRS median value, in the DML of peroneal and tibial nerves, and in the percentage of WB anti-MAG and anti-sulfatide antibody positivity (Table 2). The group with only anti-PNM IFA reactivity was characterized by high frequency of anti-sulfatide positivity, high prevalence of non-demyelinating polyneuropathy,

Table 2 Demographic, clinical, and electrophysiological data from anti-MAG EIA positive patients grouped according to IFA results

	PNM−/HNK1− (n. 6)	PNM+/HNK1− (n. 7)	PNM+/HNK1+ (n. 27)	*p*
Age *yrs*	62 (49-79)	71 (57-77)	70 (53-81)	0.34
Disease duration *yrs*	7 (4-10)	5.5 (2-19)	6 (3-15)	0.78
DADS *n.* (%)	0	0	27 (100%)	** < 0.0001*
mRD score	3 (1-3)	2.5 (1-5)	1 (1-2)*, **	*0.0006*
IVIg/PE response *n.* (%)	4/5 (80%)	1/7 (14.2%)	1/10 (10%) **	*0.008*
Anti-sulfatide positivity *n.* (%)	1 (16.6%)	3 (42.8%)	1 (3.7%)*	*0.02*
Anti-MAG WB positivity *n.* (%)	0	3 (42.8%)	15 (71.4%) **	*0.003*
Low MAG EIA *n.* (%)	16.6%	28.5%	11.1%	0.34
Peroneal nerve				
MCV m/sec	36.5 (22.5-43)	37.2 (28-40)	27.7 (10.6-39.5)*	0.06
DL msec	6.2 (5.5-16.7.6)	5.4 (4.6-10)	10.6 (5.9-28.6) *	*0.03*
CMAP mV	1.6 (0.6-8.5)	5.3 (0.7-9.5)	2.6 (0.2-6.6)	0.15
Tibial nerve				
MCV m/sec	38.1 (23.1-42.5)	34.8 (28-46.2)	28.6 (11.8-39.6)	0.09
DL msec	7.4 (4.7-12)	5.1 (4.3-7.8)	9.3 (3.7-17.6) *	*0.03*
CMAP mV	7.7 (0.8-20.8)	4.9 (2-12.6)	1.9 (0.3-7.3) **	0.07
Ulnar nerve				
MCV m/sec	44.7 (31-55)	49 (15.7-56.2)	45.4 (15.7-57)	0.91
DL msec	3.4 (2.7-4.5)	5 (2.4-16.5)	4.3 (2.8-10.5)	0.31
CMAP mV	9.4 (3.5-18.4)	8.6 (0.4-12.8)	10 (3.7-18.7)	0.35
Sural nerve				
SCV m/sec	42.5 (30-51)	47 (42.2-51.9)	34.3 (21-43.7) *	0.10
SNAP μV	5.5 (3-14)	7.6 (4.8-10.7)	4 (0.4-13.3)	0.42
Motor conduction block				
(% of the total nerves)	25%	16%	1.8%**	*0.02*

Numerical values are expressed as the median (range) of observed values. вр^ of the right column refers to the results of the Kruskal-Wallis or chi-square test performed considering the three patient groups. *,** = $p < 0.05$ as obtained comparing anti-HNK-1 IFA positive cases with only anti-PNM IFA positive patients (*) or with patients without any IFA positivity (**) by Mann-Whitney test. No significant differences were observed between IFA negative and only anti-nerve IFA positive patients. Significant values of p are in italics

high values of mRS, and poor response to PE/IVIg treatment. One patient with anti-MAG EIA and anti-PNM IFA positivity, but without HNK1 reactivity, ultimately died of a motor neuron disease. Finally, most of the patients with only anti-MAG EIA positivity had a CIDP-like clinical and electrophysiological phenotype and the highest frequency of conventional therapy response (Table 2).

Discussion

Our study compares four methods to detect anti-MAG antibodies in relation to the clinical and electrophysiological features of a cohort of patients affected by MG-related polyneuropathy. Our findings confirm the higher sensitivity of EIA as compared to WB to detect IgM MG-associated demyelinating neuropathies; however, as reported by others [20, 21], not all anti-MAG EIA positive patients have the classical DADS phenotype with disproportionate distal slowing. EIA false positivity has been attributed to antibodies that bind to impurities found in partially purified MAG antigen [21]; however, anti-MAG EIA positivity among non-demyelinating neuropathy patients has also been detected by using highly purified proteins [12], which could suggest that anti-MAG antibodies as detected by EIA may recognize different epitopes on the MAG structure. Anti-MAG positive sera also bind to SGPG, suggesting that the antibodies are directed to the shared sulfated glucuronic acid moiety of the HNK1 molecule. Nonetheless, in some cases, anti-SGPG positive sera recognize non-myelin nerve structures, such as axons [22], which, based on some authors' hypotheses, could explain the association with non-demyelinating disorders [12]. Due to the wide overlap between anti-MAG, anti-SGPG, and anti-sulfatide antibodies, patients with either one or more of these reactivities are usually seen as a unique, although heterogeneous, disease group characterized by slowly progressive, predominantly sensory neuropathic disorder with poor response to classic immunotherapies (i.e., PE and IVIg), but overall good response to Rituximab [23]. Our results may add some further information about IgM-related neuropathy. First of all, we found that near 68% of anti-MAG EIA positive sera (82% of those with high EIA titer values) bind to HNK1 molecule on rat brain.

HNK1 is a neural oncofetal, highly immunogenic carbohydrate epitope predominantly expressed in brain perineuronal nets and in non-rodent peripheral nervous tissue. It is composed of a sulfated trisaccharide, HSO3-3GlcAβ1-3Galβ1-4GlcNAc-, found at the non-reducing terminus of glycans. HNK1-carrying molecules identified in the nervous system include Ig-superfamily adhesion molecules such as MAG, NCAM, L1, and P0, soluble extracellular proteins like tenascin and phosphacan, and glycolipids [24–26]. In the peripheral nervous system, HNK1 is probably involved in myelin formation and stabilization acting as a cell-cell recognition ligand [27], which may explain the demyelinating phenotype, along with the characteristic histopathological finding of myelin lamellae widening, of the typical anti-MAG-associated polyneuropathy.

We used a simple indirect IFA approach that allows the recognition of HNK1 reactivity through its characteristic expression over the rodent brain [18]. The lower percentage of anti-HNK1 positivity as compared to anti-MAG EIA results could suggest a difference in the sensitivity of the two methods. However, we have found anti-HNK1 positivity in 43% of sera with low anti-MAG EIA titer values, suggesting a sufficient sensitivity even at low antibody binding activity. Most importantly, the positivity for anti-HNK1 IgM identifies the entire subgroup of patients affected by classical slowly progressive DADS with disproportionate distal slowing, in whatever EIA titer value. Recently, a new anti-HNK1 EIA method tested positive in 40/41 anti-MAG EIA positive sera [28]; however, anti-HNK1 did not differentiate between ʙtypical^ and ʙatypical^ neuropathy phenotypes, the latter representing about 15% of the total positive presented cases. Moreover, authors did not test anti-MAG EIA low positive patients, who could include a possibly treatable DADS' subgroup. We hypothesize that for the detection of anti-HNK1 antibody, IFA method is far more specific than EIA, by identifying IgMs that specifically link perineuronal nets, as demonstrated by reactivity on the brain tissue [18].

Among our anti-HNK1 IFA negative anti-MAG EIA positive patients, there is a further patients' subgroup with anti-PNM IFA positivity. These patients have a predominantly axonal neuropathy, the highest incidence of anti-sulfatide positivity as compared to the other EIA positive patients, along with the worst disability. This subgroup also comprised, in our series, of a patient with a final diagnosis of motor neuron disease, without signs of demyelination or sensory symptoms, resistant to rituximab treatment.

Finally, a restricted group of EIA positive patients (about 16%) did not have any IFA reactivity; the clinical and electrophysiological characteristics of this patients' subgroup have not any significant differences as compared to those of CIDP or MG-associated polyneuropathy patients, including the occurrence of acute onset and the frequent response to classic immunotherapy.

Overall, our results clearly show that, among anti-MAG EIA positive patients, a great proportion recognize HNK1 and a more restricted subgroup probably recognize other MAG or nerve epitopes, including sulfatides. The different antibody specificity likely parallels different disease features, being anti-HNK1 positive patients those with the classical DADS variant with disproportionate distal slowing and good long-term prognosis [29], and anti-HNK1 negative patients those with a more severe, heterogeneous disease phenotype. As this work was retrospectively performed on cases collected

until 2012, we do not have sufficient data to correlate our laboratory findings with response to therapies other than IVIg and PE, such as rituximab. However, we think that the measurement of anti-HNK1 antibodies by IFA could represent an adjunctive tool for the best treatment decision and disease monitoring. Recently, a new therapeutic approach has been proposed that uses a glycopolymer mimicking HNK1 as antibody scavenger to remove pathogenetic antibodies, thus avoiding non-specific immunosuppression [30]. If this therapy proves to be effective, HNK1 IFA reactivity evaluation could be helpful in determining treatment eligibility.

Conclusions

Anti-HNK1 IFA represents a very specific and sensitive, low-cost method to detect autoantibodies associated with DADS in MG-IgM patients. It should be used to improve the diagnosis, and treatment strategies, in anti-MAG EIA positive cases.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards All procedures involving experiments on human subjects have been done in accordance with the ethical standards of the Committee on Human Experimentation of the institution in which the experiments were done or in accordance with the Helsinki Declaration of 1975. Specific national laws have been observed.

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