Frequency and Clinical Correlates of Anti-Nerve Antibodies in an unselected population of CIDP patients included in the Italian Database

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ABSTRACT

Objective: to investigate the frequency and clinical correlates of anti-nerve autoantibodies in an unselected series of Italian patients with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP).

Methods: Sera from 276 CIDP patients fulfilling the EFNS/PNS criteria and included in the Italian CIDP database were examined for the presence of anti-nerve autoantibodies. Results were correlated with the clinical data collected in the database.

Results: Anti-Neurofascin155 (NF155) antibodies were found in 9/258 (3.5%) patients, anti-Contactin1 (Cont1) antibodies in 4/258 (1.6%) patients and anti-contactin-associated protein1 (Caspr1) in 1/197 (0.5%) patients while none had reactivity to Gliomedin or Neurofascin186. Predominance of IgG4 isotype was present in 7of the 9 examined patients. Anti- NF155 patients more frequently had ataxia, tremor and higher CSF protein levels than antibody negative patients. Anti-CNTN1 patients more frequently had a GBS-like onset, pain and ataxia and had more severe motor impairment at enrollment than antibody negative patients. They more frequently received plasmapheresis, possibly reflecting a less satisfactory response to IVIg or steroids. IgM antibodies against one or more gangliosides, were found in 6.5% of the patients (17/260) and were more frequently directed against GM1 (3.9%). They were frequently associated with a progressive course, with a multifocal sensorimotor phenotype and less frequent cranial nerve involvement and ataxia.

Conclusions: Anti-paranodal and anti-ganglioside antibodies are infrequent in patients with CIDP but are associated with some typical clinical association supporting the hypothesis that CIDP might be a pathogenically heterogeneous syndrome possibly explaining the different clinical presentations.

Keywords: Chronic inflammatory demyelinating polyradiculoneuropathy; CIDP; Peripheral neuropathy; anti-nerve antibodies, paranodopathy, anti-ganglioside antibodies

INTRODUCTION

Chronic inflammatory demyelinating polyneuropathy (CIDP) is the most frequent chronic immune mediated neuropathy with a prevalence ranging from 0.67 to 8.9 cases per 100 000[1]. Despite the elusiveness of its exact pathogenic mechanism,[2] the immunological involvement in CIDP is supported by its frequent improvement after immune therapies[3]. The disease course can be either relapsing-remitting or progressive and is typically characterized by a symmetric sensorimotor involvement although several variants have been described broadening the spectrum of this disorder [4].

The identification of disease-associated antibodies in other neuropathies has already entered clinical practice reshaping their clinical management and treatment strategies[5]. A number of recent studies have identified a few reactivities in CIDP patients against cell adhesion molecules at the paranodal (Neurofascin-155[6–14], Contactin-1[12,13,15–17], Contactin-associated protein 1[13,18]), or nodal domain (Neurofascin-186[13,14], Gliomedin) or against membrane Gangliosides[19–21]. Despite the variable prevalence of these reactivities in different studies, there is some evidence that some of these reactivities are associated with some typical clinical features and response to therapy.

In the present study we assessed the prevalence of anti-nerve antibodies in a large and unselected population of Italian CIDP patients to provide further information regarding the prevalence and the clinical correlates of these antibodies.

MATERIALS AND METHODS

Patients and Samples

This was a retrospective multicenter cohort study on large series of CIDP patients collected in the Italia CIDP database (CINECA, Bologna, Italy). The details of this study have been previously reported.[22] Clinical and diagnostic data were collected in each Center and the diagnostic accuracy was centrally verified according to the EFNS/PNS diagnostic criteria.[23]. Data monitoring included diagnosis revision, suspect double entries, missing data, and plausibility checks. We excluded patients with an alternative diagnosis, IgM monoclonal gammopathy and increased titers of anti-myelin-associated glycoprotein (MAG) IgM antibodies (over 7000 Unit by Buhlman method in our laboratory[24]), increased levels of circulating VEGF (> 1500 pg/mL)[25], unavailable nerve conduction studies (NCS), or data not fulfilling the EFNS/PNS diagnostic criteria[23]. The study was approved by the Ethical Committee of each participating Center. All patients gave written informed consent.

Among the 662 patients included in the database, sera were only provided by 15 participating Centers who provided the sera from all their included patients for a total of 342 patients. After revision of the diagnosis, we excluded 16 patients with an alternative diagnosis, 10 patients with unavailable NCS and 40 patients not fulfilling the EFNS/PNS electrodiagnostic criteria leading to a final study population of 276 patients.

Serological analysis

Anti-node/paranode antibody testing. Antibody were measured by ELISA according to previously reported procedure.[7,15] Briefly, 96-well Nunc Polysorb ELISA plates were coated over

night at 4◦C with 1µg/ml of human recombinant NF155 protein (OriGene RC228652) or human recombinant CNTN1 protein (OriGene RC214706). Wells were saturated with 5% non-fat milk in 0.1% PBS-Tween20 solution for 1 hour at room temperature (RT) and then incubated in duplicate with sera diluted 1:100 in saturating solution for 1 hour at RT. Horse-radish peroxidase labelled polyclonal rabbit anti-human IgG/HRP was added at the dilution of 1:10,000 in saturating solution for 1 hour at RT. For antibodies to NF186 and Caspr1, plates were coated with 1µg/ml human recombinant NF186 protein (TP 329070 Origene) or 5µg/ml human recombinant Caspr1 protein (2418-CR R&D) at the same serum dilution while the polyclonal rabbit anti-human IgG/HRP was diluted 1:1000 in saturating. IgG subclasses 1-4 were determined using the appropriate horseradish peroxidase conjugated mouse-anti-human IgG with (LifeTechnologies) diluted 1:500 in saturating solution. Reactivity was detected with TMB solution (Biolegend) and the reaction was stopped with 0.1M sulphuric acid. Optical density was measured at a wavelength of 450nm by a DSX plate reader (manufactured by Technogetics). Only patients with an optical density >0.3 had their positivity confirmed in all by immunocytochemistry on transfected human embryonic kidney (HEK) 293 cells at the Neuromuscular Laboratory of the Neurology Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain (Dr. Luis Querol) [7,15].

Anti-ganglioside IgM antibodies. All collected sera were tested by ELISA for the presence of anti-ganglioside IgM antibodies (anti-GAAb) by individual assay against GM1, GM2, GD1a, GD1b, GQ1b using previously reported procedures[26] with an upper normal limit for serum antibody reactivity of 1/640.

Clinical features

All patients had a detailed clinical history including duration of weakness, sensory symptoms, ataxia, pain, autonomic dysfunction. The course of the disease was defined by the treating

neurologist as progressive or relapsing and an eventual GBS-like onset was reported.[22] Response to previously performed therapy was reported by the treating neurologist and defined as an improvement of at least 2 points at the Medical Research Council (MRC) score (range 0, worst - 60, normal) or at least 1 point on the INCAT scale (range 0, normal - 10, worst). The clinical evaluation at entry also included the INCAT sensory sum score (ISS), range 1 (normal)-20 (worst).[27] Results of cerebrospinal fluid (CSF) examination performed during the course of the disease were reported. The upper reference limit for CSF proteins was considered 50mg/dl for patients aged ≤50 years and 60 mg/dl for those aged >50 years.[28] Motor nerve conduction studies were planned to be performed bilaterally in the median, ulnar, common peroneal and tibial nerves and included distal and proximal compound muscle action potential (CMAP) amplitude (onset to peak) and duration, motor conduction velocities (MCV), distal and proximal motor latencies and in most patients F-wave latency. The results were centrally reviewed and classified according to the EFNS/PNS criteria[23].

Statistical analysis

Categorical variables were described using frequency and percentage and analyzed with the Chi-square or Fisher's exact tests. Continuous variables were described using mean and standard deviation, assessed for normality with the Shapiro-Wilk test and analyzed with the t-test (for normally distributed variables) or Wilcoxon-Mann-Whitney test (for non-parametrically distributed variables). Significance was set at an α-level of 0.05. Analyses were performed with IBM SPSS Statistics for Windows, Version 22.0 (Armonk, NY: IBM Corp, USA).

RESULTS

Anti-Node/Paranode IgG antibodies

Anti-NF155 and CNTN1 IgG antibodies were measured in 258 patients and resulted increased in 9 patients for anti-NF155 IgG (3.5%) and four for anti-CNTN1 IgG (1.6%). Sera from 197 patients were tested for anti-Caspr1, anti-NF186 and anti-Gliomedin IgG antibodies with reactivity observed in one patient for Caspr1 (0.5%) and none for NF186 or gliomedin. Antibody subtype analysis was performed in 5 of the nine patients with anti-NF155 antibodies and resulted positive for IgG4 in all but one patient in whom isotype characterization was not conclusive. Subtype analysis in the four anti-CNTN1 positive sera resulted positive for IgG4 in all patients with one also having IgG3 antibodies. Follow-up serological analysis in the two patients with anti-NF155 examined after therapy, revealed a decrease of antibody reactivity with an OD reduction from 1.576 to 0.900 in one and from 1.296 to 0.327 in the other in parallel with clinical improvement. In the three patients with anti-CNTN1 examined after therapy antibody decreased from 0.678, 0.775 and 0.839 of OD to of 0.100 or less paralleling clinical improvement.

Comparison of clinical features of patients with and without anti-paranodal antibodies

In table 1 are compared the clinical, diagnostic and therapeutic findings in patients with anti-NF155, anti- CNTN1 antibodies and without any of these antibodies. Patients with anti-NF155 population had a shorter disease duration at enrollment (40.44 vs. 47.41 years) and had more frequently tremor, ataxia and cranial nerve involvement consisting-in dysphagia and dysphonia in two patients as facial hypoesthesia in one. CSF analysis revealed a higher mean protein concentration with a similar frequency of increased CSF proteins. There was no significant difference in the response to IVIg and steroids. Both treated patients with anti-NF155 antibodies improved after therapy with rituximab compared to one of the three without these antibodies.

Patients with anti-CNTN1 antibodies also had a shorter disease duration at enrolment and more frequently had a GBS-like onset. They were more severely affected with a lower mean MRC score, and higher INCAT score. They also had more frequently pain at onset (75% vs. 21.6% p=0.037) and more frequently developed ataxia during the course of the disease. They also had higher mean CSF protein concentration (186.5 vs 94.35 mg/dL, p=0.037) with a similar frequency of increased CSF proteins (100% vs 72.5%). They had a less frequent response to IVIg compared to patients without these antibodies and were more likely to receive plasmapheresis (75%) than seronegative patients (9%) with a similarly frequent response. Both treated patients improved after therapy with rituximab.

Reactivity against Caspr1 was only found in a 57 years-old lady with progressive proximal and distal motor involvement of the four limbs followed by paresthesia, ataxia and subsequent tremor leading to a diagnosis of definite typical CIDP. The patient did not improve after therapy with IVIg alone or in combination to corticosteroids and with subsequent plasma exchange. The patient subsequently stabilized with physiotherapy and is now planning to receive rituximab. Pain was not a prominent symptom for the patient.

Anti-Ganglioside IgM positive CIDP

IgM antibodies to one or more ganglioside were found in 17 of the 260 examined patients (6.5%). Anti-GM1 IgM antibodies were detected in 10/260 patients (3.9%), anti-GM2 in 5/220 (2.3%), anti-GD1a in 3/224 (1.3%), anti-GD1b in 8/213 (3.8%), while none had anti-GQ1b IgM. There was a concomitant reactivity with GM1 and GD1b in two patients, with GM1 and GM2 in

one patient, with GM2 and GD1a in one patient, and with GM1, GD1b and GD1 a in two patients.

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In table 2 we separately compared patient with any anti-ganglioside antibody (Anti-GAAb) or with anti-GM1 antibodies alone or in combination (GM1) with seronegative patients. Patients with anti-GAAb IgM were older at disease onset (54.8 vs. 46.5 ys p=0.044) and more frequently had a progressive course (88.2% vs. 50.4% p=0.002). They were also less frequently diagnosed to have typical CIDP (41.2% vs 74.2%, p=0.009) and more frequently had Lewis-Summer syndrome (35.3% vs 6.9% p=0.002). A similar difference was also observed in patients with anti-GM2 IgM antibodies with typical CIDP in 20% of the patients (p=0.019) and Lewis-Summer syndrome in 60% (p=0.005). Pain was more frequent in patients with anti-GM1 antibodies (60% vs. 25.9% p=0.027), or anti-GD1a IgM (100% vs. 25.9% p=0.018) and in the whole anti-GAAb population (52.9% vs. 25.9% p=0.024), while cranial nerve involvement (0% vs. 22.9% p=0.027) and ataxia (5.9% vs. 28.5% p=0.047) were less frequent in patients with anti-GM1 or anti-ganglioside antibodies. Two patients with anti-GM1 antibodies had a pure motor CIDP. In both patients, motor impairment was relatively symmetric without a multineuropathic distribution making it unlikely a diagnosis of multifocal motor neuropathy. One patient had conduction block in motor nerve and responded to IVIg while the other did not have conduction block and did not improve after IVIg. There was no difference in the response to therapy according to the presence of anti-ganglioside or anti-GM1 antibodies.

DISCUSSION

The reported prevalence of anti-paranodal IgG in previous studies is quite heterogeneous (between 1% and 20.7% for anti-NF155, between 0.7% and 7.5% for anti-CNTN1, between 0,2%

and 2,9% for anti-Caspr1). These discrepancies , may reflect differences in case selection, applied diagnostic criteria, duration of disease and treatment status of screened patients[6–13,13–18,29–31]. The prevalence of these antibodies in our series of CIDP patients was lower than previously reported, This may reflect the unselected series of patients examined in our study and the fact the most of them were not treatment naïve. The latter hypothesis is possibly confirmed by the fact that in all the four patients in whom follow-up serological analysis were available after therapy there was a marked reduction in antibody titer flanking clinical remission[6,13,17] underlining the importance of screening for these autoantibodies before initiating treatment. In our series, IgG subtype analysis revealed a striking predominance of IgG4 with only one case where both IgG3 and IgG4 as also previously reported.

Despite the relatively small number of our positive patients, our findings confirm previously described features of the clinical phenotype of anti-NF155 positive patients (younger age at onset, tremor, ataxia), their electrodiagnostic features with frequently increased distal latencies and high levels of CSF proteins. Similarly, patients with high anti-CNTN1 antibodies had an advanced age at onset, a frequent GBS-like onset, a prominent motor involvement a high levels of CSF proteins. We also confirmed that these patients had a low rate of response to IVIg even if the data was not statistically different from seronegative patients. This could be also related to administration of IVIg in addition to corticosteroids in several patients recorded as responders or to the difficulty in differentiating sustained from non-sustained response in our retrospective analysis. Nevertheless, the frequent use of plasmapheresis in the anti-CNTN1 population may reflect an higher prevalence of unsatisfactory responses to first-line therapies since plasmapheresis in our CIDP population was mostly performed in patients failing to respond to steroids or IVIg. All our treated patients improved after therapy with rituximab supporting the role of this therapy

in this group of patients. Only one patient had antibodies to CASPR1 confirming the low prevalence of this reactivity. Pain was not however a prominent feature in this patient who had an otherwise typical severe CIDP poorly responsive to conventional therapies. No IgG reactivity was found against either NF186 or Gliomedin coherently with the described rarity of these autoantibodies[6–9,12–14].

We also found a consistent proportion who had increased titers of anti-ganglioside antibodies mostly directed against GM1. The majority of these patients had atypical presentation consistent with Lewis-Summer syndrome as also previously reported[20] or a pure motor symmetric CIDP. This finding may represent a link between CIDP and multifocal motor neuropathy even if the sensory impairment in those with Lewis-Sumner syndrome and the symmetric involvement in those with motor CIDP are not consistent with this diagnosis. This finding support however the opportunity to test for these antibodies in patients with atypical variant of CIDP. A similar lower rate of cranial nerve involvement was also previously reported in CIDP patients with anti-LM1 antibodies[24].

Even if this study reveals the presence of one or more anti-neural antibodies in 12 % of the examined patients, it does not provide additional data that might support their possible pathogenetic relevance. Their correlation with some characteristic clinical features support however the idea that these antibodies may somehow influence the presentation and course of the disease and possibly predict their response to therapy.

The retrospective nature of this study represents the major limitation of our work, especially in the analysis of the therapeutic response, altogether with the limited number of seropositive patients, the lack of complete antibody characterization of all included patients. The strengths of this study include the use of a national multicenter database collecting a consistent

case series reflective of the Italian CIDP population avoiding case selection related to a previous lack of response to therapy or an acute onset of CIDP and the use of well-defined and uniform inclusion and assessment criteria. This lack of selection might explain the lower frequency of these antibodies compared to a previous Italian study where some of our patients had been also included[10].

In any instance, our study supports the fact that the implementation of a pathogenetic-oriented approach in the evaluation and diagnosis of patients with CIDP may help in the identification of patients with a peculiar clinical and immunological phenotype requiring different treatment strategies and theoretically support the hypothesis that CIDP might be a syndrome that includes different chronic demyelinating neuropathies[33].

Figure 1: Flowchart description of the case population selection

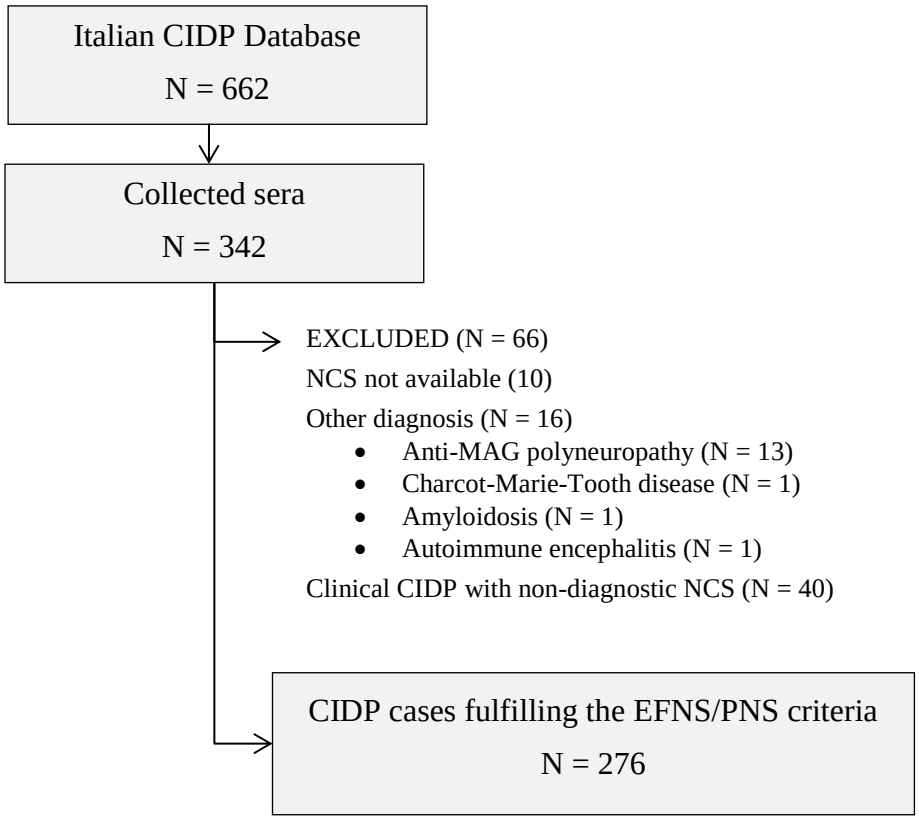

Table 1: Comparison of characteristics of patients with anti-NF155 IgG or anti-CNTN1 IgG and seronegative CIDP patients.

p^1 Anti-paranode vs Seronegatives

p^2 Anti-NF155 vs Seronegatives

p^3 Anti-CNTN1 vs Seronegatives

Abbreviations: RCV - Reduced conduction velocity, CB – Conduction block, PDL – Prolonged distal latency, ATD – Abnormal temporal dispersion, PFL – Prolonged F-wave latency, AF – Absent F-wave, : IVIg – Intravenous Immunoglobulins, PE – Plasma exchange, RTX - Rituximab.

	Anti-NF155 (n= 9)	**Anti-CNTN1 (n=4)**	**Seronegative (n=245)**	**P values**
Gender, Male, n (%)	5/9 (55.6%)	3/4 (75%)	171/244 (70,1%)	
Age at onset, mean (SD)	40.44 (23.61)	52.5 (15.15)	47.41 (16.84)	
Disease duration at enrollment, mean (SD)	5.02 (6.30)	4.8 (5.43)	9.51 (8.80)	p^1=0.026
CIDP subtype, n (%)				
Typical CIDP	9/9	4/4	175/244 (71.7%)	p^1=0.024
Atypical CIDP	0/9	0/0	69/245 (28.3%)	
Disease Course, n (%)				
Relapsing	5/9 (55.6%)	2/4 (50%)	115/244 (47.1%)	
Progressive	4/9 (44.4%)	2/4 (50%)	129/244 (52.9%)	
GBS-like onset	1/9 (11.1%)	2/4 (50%)	24/244 (9.8%)	p^3=0.056
Impairment, mean (SD)				
INCAT	2.25 (0.71)	6 (2.94)	2.46 (2.00)	p^3=0.001
MRC	55.25 (3.2)	42.5 (13.27)	54.83 (6.66)	p^3=0.0004
ISS	6.63 (3.85)	8.25 (5.91)	4.67 (3.89)	
Symptoms at onset, n (%)				
Motor	8/9 (88.9%)	3/4 (75%)	152/244 (62.3%)	
Sensory	8/9 (88.9%)	4/4 (100%)	197/244 (80.7%)	
Pain	2/9 (22.2%)	3/4 (75%)	53/244 (21.7%)	P^3=0.037
Fatigue	1/9 (11.1%)	2/4 (50%)	87/244 (35.7%)	
Cranial Symptoms	3/9 (33.3%)	0/4 (0%)	23/244 (9.4%)	p^2=0.053
Ataxia	4/9 (44.4%)	1/4 (25%)	28/244 (11.5%)	p^2=0.017
Cramps	1/9 (11.1%)	0/4 (0%)	30/244 (12.3%)	
All symptoms developed, n (%)				
Motor	9/9 (100%)	4/4 (100%)	218/244 (89.3%)	
Sensory	9/9 (100%)	4/4 (100%)	231/244 (94.7%)	
Pain	2/9 (22.2%)	2/4 (50%)	76/244 (31.1%)	
Fatigue	3/9 (33.3%)	2/4 (50%)	125/244 (51.2%)	
Cranial Symptoms	3/9 (33.3%)	1/4 (25%)	49/244 (20.1%)	
Ataxia	7/9 (77.8%)	3/4 (75%)	59/244 (24.2%)	p^2=0.001; p^3=0.049
Cramps	3/9 (33.3%)	0/4 (0%)	39/244 (16.0%)	
Tremor	6/9 (55.6%)	0/4 (0%)	31/244 (12.7%)	p^2=0.004
Autonomic Symptoms	0/9 (0%)	0/4 (0%)	23/244 (9.4%)	
EMG findings, n (%)				
RCV	6/9 (66.7%)	4/4 (100%)	148/244 (60.7%)	
CB	4/9 (44.4%)	2/4 (50%)	139/244 (57.0%)	

PDL	5/9 (55.6%)	1/4 (25%)	61/244 (25.0%)	
ATD	0/9 (0%)	0/4 (0%)	29/244 (11.9%)	
PFL or AF	0/9 (0%)	0/4 (0%)	24/244 (9.8%)	
CSF Analysis				
Protein concentration (mg/dL), mean (SD)	177.75 (120.91)	186.5 (114.90)	94.35 (86.35)	p^2=0.031; p^3=0.037
Age-Adjusted CSF protein positivity, n (%)	6/8 (75%)	4/4 (100%)	132/182 (72.5%)	
Biopsy, n (%)				
Demyelination	1/1 (100%)	2/2 (100%)	10/14 (71.4%)	
Axonal	0/1 (0%)	0/2 (0%)	4/14 (28.6%)	
Treatment Response, n (%)				
IVIg, Responder	5/8 (62.5%)	1/4 (25%)	135/188 (71.8%)	P^3=0.0754
Steroids, Responder	4/7 (57.1%)	1/4 (0%)	87/143 (60.8%)	
PE, Responder	0/1 (0%)	3/4 (66.7%)	16/22 (72.7%)	
RTX, Responder	2/2 (100%)	2/2 (100%)	1/3 (33.3%)	

Table 2: Comparison of characteristics of patients with anti-GAAb IgM and seronegative CIDP patients

p^1 Anti-Ganglioside positives vs Seronegatives

p^2 Anti-GM1 positives vs Seronegatives

Abbreviations: RCV - Reduced conduction velocity, CB – Conduction block, PDL – Prolonged distal latency, ATD – Abnormal temporal dispersion, PFL – Prolonged F-wave latency, AF – Absent F-wave, : IVIg – Intravenous Immunoglobulins

	aGAAb (n= 17)	**Anti-GM1 (n = 10)**	**P values**
Gender, Male, n (%)	14/17 (82.4)	9/10 (90%)	
Age at onset (years), mean (SD)	54.82 (13.45)	56.1 (12.75)	
Disease duration at enrollment (ys), mean (SD)	9.85 (8.01)	11.15 (8.58)	
CIDP subtype, n (%)			
Typical	7/17 (41.2)	6/10 (60%)	p^1=0.009
DADS	1/17 (5.9%)	0/10 (0%)	
Lewis-Summer syndrome	6/17 (35.3)	1/10 (10%)	p^1=0.002
Pure motor CIDP	2/17 (11.8)	2/10 (20%)	
Pure sensory CIDP	1/17 (5.9)	1/10 (10%)	
Disease Course, n (%)			
Relapsing	2/17 (11.8)	1/10 (10%)	
Progressive	15/17 (88.2)	9/10 (90%)	p^1=0.003; p^2=0.020
Impairment, mean (SD)			
INCAT	2.08 (1.85)	2.56 (1.94)	
MRC	55.54 (5.74)	54.11 (6.41)	
ISS	4.23 (3.66)	4 (4.36)	
All symptoms developed, n (%)			
Motor	16/17 (94.1)	10/10 (100%)	
Sensory	15/17 (88.23)	9/10 (90%)	
Pain	9/17 (52.9)	6/10 (60%)	p^1=0.024; p^2=0.027
Fatigue	8/17 (47.1)	7/10 (70%)	
Cranial Symptoms	0/17 (0)	0/10 (0%)	p^1=0.027
Ataxia	1/17 (5.9)	1/10 (10%)	p^1=0.047
Cramps	4/17 (23.5)	2/10 (20%)	
Tremor	0/17 (0)	0/10 (0%)	
Autonomic Symptoms	1/17 (5.9)	1/10 (10%)	
EMG findings, n (%)			
RCV	11/17 (64.7)	8/10 (80%)	
CB	7/17 (41.2)	4/10 (40%)	
PDL	4/17 (23.5)	4/10 (40%)	
ATD	3/17 (17.6)	2/10 (20%)	
PFL or AF	1/17 (5.9)	0/10 (0%)	
Treatment Response, n (%)			
IVIg, Responder	11/13 (84.6)	6/8 (75%)	
Steroids, Responder	2/5 (40)	1/3 (33.3%)	

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