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INVESTIGATION OF CLINICAL AND PATHOGENIC HETEROGENEITY IN CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY

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INTRODUCTION

Chronic inflammatory demyelinating polyneuropathy (CIDP) is a rare peripheral neuropathy that often responds to immune therapies *(Dyck et al., 1982; Hahn et al., 1998; Koller et al., 2005; Said, 2006; Vallat et al., 2010)*. The prevalence of CIDP is variable: 0.8/100.000 in Japan *(Kusumi et al., 1995)*, 1.32 *(Lunn et al., 1998)*-2.84/100.000 *(Mahdi-Rogers and Hughes, 2013)* in South-East of England, 1.9/100.000 in New South Wales and Australia *(McLeod et al., 1999)*, 3.5/100.000 in Piemonte, Italy *(Chiò et al., 2007)*, and 8.9/100.000 in Olmstead County, USA *(Laughlin et al., 2009)*. The incidence varies from 0.2 *(McLeod et al., 1999)* to 1.60 per 100.000 *(Laughlin et al., 2009)*. Although the variability of these data could depend on a peculiar geographical distribution of the CIDP, some studies suggest that it probably reflects the use of different diagnostic criteria. For example, in Leicestershire and Rutland, UK, the prevalence of CIDP ranged from 1.97 to 4.77/100.000 depending on the diagnostic criteria used *(Rajabally et al., 2009b)*. Since CIDP is a pharmacologically treatable pathology, this variability may also derive from the inclusion of clinical phenotypes not fulfilling the diagnostic criteria for CIDP in order to obtain the authorization to use expensive therapies. *(Busby and Donaghy, 2003; Rotta et al., 2003; Joint Task Force of the EFNS and the PNS, 2010)*. However, it remains to clarify whether these atypical phenotypes can be considered variants of CIDP or should be considered as other forms of demyelinating neuropathies with a different pathogenesis and therefore a different response to therapy. This will happen if the pathogenic mechanisms underlying the different clinical presentations will be clarified, as was the case for multifocal motor neuropathy (MMN), which was initially considered as a variant of CIDP but it is now considered as a separate disease *(Nobile-Orazio et al., 2005; Vlam et al., 2011)*. The same is happening for the forms of CIDP associated with positivity to antibodies against the paranodal proteins

Neurofascin-155, Contatin-1 and Casprin-1 *(Querol et al., 2014; Manso et al., 2016; Doppler et al., 2016).*

Clinical course can be either relapsing remitting, chronic progressive, or monophasic *(Dyck et al., 1982; Hahn et al., 2005; Koller et al., 2005; Said, 2006; Vallat et al., 2010).* As in Multiple Sclerosis, the relapsing remitting course is more typical in children and young adults, while the chronic progressive course is more frequent in old patients *(Hattori et al., 2001).* This distinction is becoming difficult, however, given the current therapeutic possibilities. In fact, in patients with a progressive course, discontinuation of therapy or dose reduction may mimic a relapse (wearing-off). The initial symptoms may progress over several weeks or months even if sometimes there is a so faster progression to induce the diagnosis of Guillain-Barrè syndrome (GBS). It has been shown that the misdiagnosis of GBS was placed in 16% of patients with CIDP. These patients were considered to have GBS with secondary evolution in CIDP but they actually had an acute onset CIDP (GBS-like) *(Ruts et al., 2010).* The diagnosis of acute-onset CIDP should be considered when a patient thought to have Guillain-Barre´ syndrome deteriorates again after 8 weeks from onset, when deterioration occurs 3 times or more *(Ruts et al., 2010)* or when sensory symptoms are present at the onset *(Dionne et al., 2010).*

Typically, the majority of CIDP patients have proximal and distal weakness and sensory symptoms. Over 90% of them have hyposthenia that can be so severe to lead to marked disability and loss of autonomy *(Simmons et al., 1993)* and over 80% have sensitive symptoms. Pain at the beginning is rare but can occasionally be a sign of onset *(Boukhris et al., 2007).* Proximal hyposthenia is one of the cornerstones of the clinical diagnosis of CIDP even if the distal muscle involvement is generally more frequent and severe than the proximal one. Osteotendinous reflexes are historically described as absent in CIDP even though total areflexia is found in 70% of patients while all the remaining 30% have hyporeflexia with the absence of only some reflexes, more frequently the

ankle jerk reflexes. Gait ataxia and upper limb tremor may occur in some patients. Cranial nerve involvement occurs in a minority of cases *(Dyck et al., 1982; McCombe et al., 1987; Barohn et al., 1989; Simmons et al., 1993).* Respiratory failure rarely occurs in the CIDP *(Henderson et al., 2005).* When questioned, over 80% of patients report fatigue as the main symptom *(Merkies et al., 1999),* and it may occasionally be the onset symptom when hyposthenia is not yet present *(Bissay et al., 2008).* Dysautonomic symptoms are infrequent *(McCombe et al., 1987*) although a mild and non-specific dysautonomia has been reported in 65% of patients *(Stamboulis et al., 2006).*

Although the diagnosis of CIDP is often easy in clinical practice, the high cost of therapies and the description of clinical variants led to the necessity of specific diagnostic criteria in order to avoid inappropriate use of expensive therapies and at the same time to treat all patients that could benefit from the therapies. That is why, although CIDP is a rare disease, at least 15 different series of diagnostic criteria have been proposed *(Bromberg, 2011; Breiner and Brannagan, 2013)*. From the literature analysis, it emerged that the European Federation of Neurological Society/Peripheral Nerve Society (EFNS/PNS) criteria have the best combination of sensitivity (73%) and specificity (90%) for the diagnosis of CIDP compared to the other criteria. The EFNS/PNS criteria have the advantage of including patients with typical and atypical presentation and to allow the diagnosis of CIDP even in patients with limited demyelinating changes and other support criteria. In fact, the EFNS/PNS criteria bring together clinical (typical or atypical CIDP) and electrophysiological criteria. Moreover, the following supporting criteria are included: increased proteins in the cerebrospinal fluid (CSF), nerve roots or plexus contrast impregnation to the MRI or findings on nerve biopsy compatible with demyelination. The typical form presents with proximal and distal symmetrical hyposthenia and sensory symptoms in the limbs that develop over a period of at least two months. Cranial nerves involvement and absent or reduced ROT in all limbs are possible. The atypical form include several variants: 1) predominantly distal (DADS-distal

acquired demyelinating symmetric neuropathy); 2) asymmetric (MADSAM-multifocal acquired demyelinating sensory and motor neuropathy or Lewis-Sumner); 3) focal (for example involvement of the brachial or lumbo-sacral plexus or of one or more peripheral nerves in an upper or lower limb); 4) pure motor; 5) pure sensory (including chronic sensitive immune polyradiculoneuropathy affecting the central process of the primary sensory neuron). In about 2/3 of cases, DADS is a paraproteinemic IgM neuropathy with antibodies against myelin-associated glycoprotein (MAG) involved in the pathogenic mechanism *(Joint Task Force of the EFNS/PNS, 2010)*. Therefore, neuropathy with anti-MAG IgM is considered different from CIDP and is less responsive to treatment *(Mathey et al., 2015).*

Overall CIDP can be a severe disease. Severe disability affect over 50% of patients at least temporarily during the course of the illness (temporary use of the wheelchair or inability to walk without support). 10% of patients have permanent disability or even die from the disease *(Lunn et al., 1999; Chiò et al., 2007).* A small number of patients, on the other hand, has a paucisintomatic course with minimal impact on functional capacity and minimal symptoms both sensory and motor. In these patients, the term of "minimal" or asymptomatic CIDP is more appropriate *(Uncini et al., 1999).*

SUMMARY AND AIM OF THE THESIS

My aim during the last three years was to create a detailed database of CIDP patients, in order to analyse the data collected in a retrospective manner and to take part to national projects on the disease. In fact, since aspects of CIDP are still unsolved, such as clinical variability, pathogenesis, prognostic factors, outcome measures and response to treatment, and considering that CIDP is a rare disease, a nation-wide collaboration is indispensable.

In Section 1 I will describe the clinical and epidemiological features of CIDP patients who referred, in the last 15 years, to our Centre of Neuromuscular Disease at University Federico II of Naples and that I selected among all the patients with disimmune diseases of peripheral nervous system.

In Section 2 I will summarise the preliminary conclusions derived from the creation of a web-based national database promoted by the Humanitas Institute that involved more than 500 patients, among which 51 come from our Neuromuscular Centre in Naples.

Section 3 is dedicated to the pathogenesis, in particular to the emerging role of antibodies against paranodal proteins in the determination of specific morphologic alterations and clinical phenotypes. In particular, I will speculate on the pathogenesis of CIDP with antibodies against Neurofascin 155. Then I will report the results derived from our collaboration with the IRCCS Mondino Foundation, Pavia, Italy, which analyzed the sera from different national centres, including our centre, in order to identify and characterize the anti-nodal and paranodal antibodies incidence and their correlation with phenotypic aspects in a large cohort of patients.

SECTION 1: DATA FROM UNIVERSITY FEDERICO II OF NAPLES COHORT OF CIDP PATIENTS

Selection of patients included in our cohort

In order to create our CIDP database, patients were selected by a retrospective review of the records of the neuromuscular laboratory of the University of Naples Federico II, Campania, in the last 15 years. We used the EFNS/PNS criteria, which put together clinical (Box 1), electrodiagnostic (Box 2) and supportive (Box 3) criteria in order to classify patients in three diagnostic categories: definite, probable and possible CIDP (Box 4).

Box 1. Clinical criteria

Inclusion criteria

- typical CIDP: chronically progressive, stepwise, or recurrent symmetric proximal and distal weakness and sensory dysfunction of all extremities, developing over at least 2 months; cranial nerves may be affected; and absent or reduced tendon reflexes in all extremities;
- atypical CIDP (still considered CIDP but with different features): one of the following:
    - predominantly distal (distal acquired demyelinating symmetric, DADS);
    - asymmetric (multifocal acquired demyelinating sensory and motor neuropathy, MADSAM or Lewis–Sumner syndrome);
    - focal (e.g., involvement of the brachial or lumbosacral plexus or of one or more peripheral nerves in one upper or lower limb);
    - pure motor;
    - pure sensory (including chronic immune sensory polyradiculopathy affecting the central process of the primary sensory neuron);

Exclusion criteria

- borrelia burgdorferi infection (Lyme disease);
- diphtheria;
- drug or toxin exposure probably to have caused the neuropathy;
- hereditary demyelinating neuropathy;
- prominent sphincter disturbance;
- diagnosis of multifocal motor neuropathy;
- IgM monoclonal gammopathy with high titre antibodies to myelin-associated glycoprotein;
- other causes for a demyelinating neuropathy including POEMS syndrome, osteosclerotic myeloma, diabetic and non-diabetic lumbosacral radiculoplexus neuropathy

PNS lymphoma and amyloidosis may occasionally have demyelinating features.

Box 2. Electrodiagnostic criteria

Definite

At least one of the following:

- motor distal latency prolongation ≥ 50% above upper limit of normal values-ULN in two nerves (excluding median neuropathy at the wrist from carpal tunnel syndrome);
- reduction of motor conduction velocity ≥ 30% below lower limit of normal values-LLN in two nerves;
- prolongation of F-wave latency ≥ 30% above ULN in two nerves (≥ 50% if amplitude of distal negative peak, compound muscle action potential-CMAP < 80% of LLN);
- absence of F-waves in two nerves if these nerves have distal negative peak CMAP amplitudes ≥ 20% of LLN + ≥ 1 other demyelinating parameter in ≥ 1 other nerve;
- partial motor conduction block: ≥ 50% amplitude reduction of the proximal negative peak CMAP relative to distal, if distal negative peak CMAP ≥ 20% of LLN, in two nerves, or in one nerve + ≥ 1 other demyelinating parameter in ≥ 1 other nerve;
- abnormal temporal dispersion (> 30% duration increase between the proximal and distal negative peak CMAP) in ≥ 2 nerves;
- distal CMAP duration (interval between onset of the first negative peak and return to baseline of the last negative peak) increase in ≥ 1 nerve (median ≥ 6.6 ms, ulnar ≥ 6.7 ms, peroneal ≥ 7.6 ms, tibial ≥ 8.8 ms) + ≥ 1 other demyelinating parameter in ≥ 1 other nerve.

Probable

- ≥ 30% amplitude reduction of the proximal negative peak CMAP relative to distal, excluding the posterior tibial nerve, if distal negative peak CMAP ≥ 20% of LLN, in two nerves, or in one nerve + ≥ 1 other demyelinating parameter in ≥ 1 other nerve.

Possible

- as the criteria for definite CIDP but in only one nerve.

Box 3. Supportive criteria

- elevated CSF protein with leukocyte count < 10/mm^3;
- MRI showing gadolinium enhancement and/or hypertrophy of the cauda equine, lumbosacral or cervical nerve roots, or the brachial or lumbosacral plexuses;
- abnormal sensory electrophysiology in at least one nerve:
    - normal sural with abnormal median (excluding median neuropathy at the wrist from carpal tunnel syndrome);
    - radial sensory nerve action potential (SNAP) amplitudes or conduction velocity < 80% of lower limit of normal (< 70% if SNAP amplitude < 80% of lower limit of normal);
    - delayed somatosensory evoked potentials without central nervous system disease;
- objective clinical improvement following immunomodulatory treatment;
- nerve biopsy showing unequivocal evidence of demyelination and/or remyelination by electron microscopy or teased fibre analysis.

Box 4. Diagnostic categories for CIDP

Definite

- clinical criteria with electrodiagnostic criteria for definite CIDP;
- probable CIDP + at least one supportive criterion;
- possible CIDP + at least two supportive criterion.

Probable

- clinical criteria with electrodiagnostic criteria for probable CIDP;
- possible CIDP + at least one supportive criterion.

Possible

- clinical criteria with electrodiagnostic criteria for possible CIDP.

Each case was analysed in order to obtain the following data: demographic information (sex, age, age at onset), type of onset (acute, subacute or chronic), type of course (Box 5), disability expressed by the modified Rankin Scale score (Box 6) and response to treatments.

Box 5. Clinical course

- **Monophasic**
  when after the diagnosis of CIDP the disorder remained stable or improved, without further relapses during all the follow-up period;
- **Relapsing**
  when at least two episodes of rapid worsening following a period of stability or improvement of at least 4 weeks (both reported by patients or observed with clinical examination), with or without treatment, and lasting more than 7 days, were observed;
- **Progressive**
  when the disorder worsened steadily, showing no improvement with or without treatment up to the time of observation or following a previous phase of relapsing disease.

Box 6. Modified Rankin Scale for disability

- 0) asymptomatic
- 1) non-disabling symptoms that do not interfere with daily activities
- 2) slight disability (unable to carry out all activities, but still able to look after themselves)
- 3) moderate disability (requiring assistance with some activities but still able to walk without assistance)
- 4) moderate severe disability (unable to walk without assistance)
- 5) severe disability (totally dependent, requiring constant care)

Regarding first line treatments, steroids were used orally (Prednisolone) at the starting dose of 1 mg/kg/die and progressive tapering or intravenously

(Methylprednisolone) at the dose of 500 mg/die for five days. The Immunoglobulins were used intravenously at the dose of 0.4 g/Kg/die for five days.

For each patient we reported the response to treatment. In particular, patients were considered as responders to treatment when at least 1-point increase in ONLS (Overall Neuropathy Limitation Scale) and/or MRC (Medical Research Council) and/or mRS (modified Rankin Scale) scores was observed.

Clinical and epidemiological description of our cohort

We identified 103 CIDP patients. 65.05% (CI 55.21-73.75) with a diagnosis of definite CIDP, 27.18% (CI 13.23-33.72) with a diagnosis of probable CIDP and 12.77% (CI 8.9-22.93) with a diagnosis of possible CIDP. Male/female ratio was 2.55:1; females were 28.15% (CI: 20.21-37.75) and males were 71.84% (CI 62.24-79.79) of our sample. Mean age of our population was 54.64 years (CI: 51.6-57.68; median 56), mean age of onset was 43.84 years (CI: 40.56-47.12; median 44) and mean disease duration was 10.8 years (CI: 9.16-12.43; median 10). Two patients died.

Acute onset GBS-like was observed in 12.94% of our sample (CI: 7.2-22.1). The disease course was monophasic in 5.82% of patients (CI: 2.6-12.5), relapsing in 64.07% (CI: 54.22-72.87) and progressive in 30.09% (CI: 21.91-39.78).

According to the EFNS criteria, 33.98% (CI: 25.37-43.8) of our patients presented with an atypical phenotype: 10.68% (CI: 5.95-18.42) had a MADSAM neuropathy, 9.71% (CI: 5.24-17.27) had a pure sensory variant, 7.77% (CI: 3.88-14.92) presented with a DADS neuropathy, 2.91% (CI: 0.92-8.79) with a focal neuropathy and 2.91% (CI: 0.92-8.79) with a pure motor variant (Table 1).

Table 1. Demographic and clinical data

Total N°	103
M/F ratio	2.55 (M=71.84%)
Mean age (y)	54.64 (CI: 51.6-57.68; median 56)
Mean age of onset (y)	43.84 (CI: 40.56-47.12; median 44)
Mean disease duration (y)	10.8 (CI: 9.16-12.43; median10)
Dead (N°)	2 (1.9%)
Mean mRS at last visit	1.91 (CI: 1.67-2.15; median: 2)
Diagnostic categories (%)	
Definite	65.05 (CI 55.21-73.75)
Probable	22.18 (CI 13.23-33.72)
Possible	12.77 (CI 8.9-22.93)
Acute onset (%)	12.94 (CI: 7.2-22.1).
Course (%)	
Monophasic	5.82 (CI: 2.6-12.5)
Relapsing	64.07 (CI: 54.22-72.87)
Progressive	30.09 (CI: 21.91-39.78)
Phenotype (%)	
Typical	66.02 (CI 56.39-74.92)
Atypical	33.98 (CI: 25.37-43.8)
MADSAM	10.68 (CI: 5.95-18.42)
Pure Sensory	9.71 (CI: 5.24-17.27)
DADS	7.77 (CI: 3.88-14.92)
Focal	2.91 (CI: 0.92-8.79)
Pure Motor	2.91 (CI: 0.92-8.79)

Regarding disability we found that mean value of modified Rankin Scale (mRS) at the last visit was 1.91 (CI: 1.67-2.15; median: 2) and only 8.82% of patients showed severe disability (mRS $\geq$ 4).

Ninety-five out of our 103 patients (92.23%; CI: 85.07-96.11) underwent one or more than one pharmacological treatment. Among them, 85.27% (N=81; CI: 81.23-89.96) were responsive to at least one treatment (generally corticosteroids or intravenous immunoglobulin), while 14.73% (N=14; 8.84-23.54) were non responders. Among the typical CIDP patients group, 12.5% (8/64) were non responders while among the atypical ones the percentage of non responders was 19.31% (6/31). However, there was not a statistically significant difference between typical and atypical patients regarding the response to treatment.

Eighty-eight patients were treated with intravenous Immunoglobulins (IVIG), among them 78.41% (N=69; CI 68.39-85.90) responded to treatment while 21.59% (N=19; 14.09-31.61) did not. Thirteen IVIG responders (14%, CI 8.78-24.27) were shifted to Subcutaneous Immunoglobulins (SCIG): 5 IVIG responders were shifted to a continuous SCIG therapy because of a short-lasting response to IVIG, while 8 patients were shifted to a pulse SCIG therapy, 7 because of difficulties in hospitalization and 1 because of a previous allergic reaction to IVIG. Nine patients (69%, CI: 30.52-85.35) of those shifted to SCIG responded to treatment. The others worsened and returned to IVIG.

Among the patients treated with corticosteroids (N=62), instead, only 50% (N=31, CI 37.47-63.52) responded to treatment.

Twenty three patients non responders or no more responders to corticosteroids and/or IVIG were treated with second line treatments: 10.63% (N=10, CI: 5.7-18.84) with Azathioprine, 5.62% (N=4, CI: 2.17-12.20) with Cyclophosphamide, 5.62% (N=4, CI: 2.17-12.20) with Plasma Exchange, 2.12% (N=2, CI: 0.5-8.30) with Rituximab, 1.06% (N=1, CI: 0.14-7.40) with Cyclosporine, 1.06% (N=1, CI: 0.14-7.40) with Methotrexate and 1.06% (N=1, CI: 0.14-7.40) with Mitoxantrone.

Among all these patients only 10% (N=1, CI: 0.8-57.84) of those treated with Azathioprine responded. All the others remained non responders.

Comparison of the data relating to our population with those reported in literature

Clinical and epidemiological data about CIDP patients already reported in literature are variable. The reasons for this variability include the use of different diagnostic criteria and the great clinical variability of the disease. We adopted the EFNS/PNS criteria that are the most sensitive. We found a higher prevalence of the disease in males with a male/female ratio of 2.55:1. These data are in line with all the previous studies except for the studies by Lunn et al. in South-Est of England (*Lunn et al., 1999*) and Laughlin et al. in Olmsted County (*Laughlin et al., 2009*) that reported the same incidence for males and females.

Mean age of our population was 54.64 years and mean age of onset was 43.84 years, that are the lowest values reported in literature. In our patients, we calculated a mean disease duration of 10.8 years, which is the longest still described. In fact, patients reported in other studies had a disease duration ranging between 0.9 and 8.9 years. The percentage of dead patients was reported only by Viala et al. in France and it was 1.3% (*Viala et al., 2010*), substantially the same of our case report. Acute onset GBS-like was observed in 14.1% of our sample. These findings are in keeping with the literature, in fact the reported values were between 9% and 16% *(Ruts et al., 2010; Viala et al., 2010*).

The course of disease was monophasic in 5.82% of our patients. This is the lowest percentage ever reported and this could be related to the long disease duration of our patients, since some patients had a second relapse after several years from the onset, therefore a too short follow-up duration could led to classify some remittent patients as monophasic. Relapsing course occurred in 64.07% of our patients and progressive in 30.09%. The proportion of patients with these types of course is high variable in the previous studies, probably

depending on the different definition of relapsing and progressive course. An accurate description of the different courses should be made in the future in order to clarify the differences between these groups of patients. In most of the studies, however, the remitting course seems to prevail on the progressive course. The only exception is reported by Chiò et al. in Piemonte, where a progressive course occurring in a number of patients twice that of the remitting one is described *(Chiò et al., 2007)*.

In our population, the typical phenotype was more frequent than the atypical one. Also this data is variable in the literature, in particular Rotta et al. and Viala et al. reported almost the same percentage of CIDP typical and atypical, while Rajabally et al. and Mahdi-Rogers et al. reported a clear prevalence of the typical phenotype.

The distribution of atypical phenotypes in our database (Table 1) is in line with all the other studies which also reported MADSAM as the most frequent form followed by pure sensory and DADS, while the other forms appeared increasingly rare. The only exception are the study by Rajabally et al. *(Rajabally et al., 2014)* which reported pure sensory CIDP as the most frequent form followed by MADSAM, and that by Rotta et al. *(Rotta et al., 2008)* which reported DADS as the most frequent form followed by pure sensory and MADSAM.

Regarding clinical disability of patients with CIDP, mean value of Modified Rankin Scale (mRS) was 1.91 and 7% of patients showed severe disability (mRS $\geq$ 4). Only in the study by Viala et al. *(Viala et al., 2010)*, the proportion of patients who could not walk independently was higher (24%). However all the other epidemiological study, have found a proportion of patients requiring help with walking similar to ours.

Several retrospective controlled studies and some randomized controlled studies have shown in CIDP a comparable short-term efficacy of corticosteroids, IVIG and plasmapheresis as summarized in the recent Cochrane reviews *(Mehndiratta and Hughes, 2012; Mehndiratta et al., 2012; Eftimov et al., 2013)*.

Approximately 50-70% of patients responded to at least one of these first-line therapies, 50% of patients not responding to one of them responded to another so that approximately 80% of patients were responders to treatment *(Cocito et al., 2010; Viala et al., 2010).* The effectiveness of these therapies has also been highlighted by the EFNS/PNS guidelines *(Joint Task Force of the EFNS and the PNS, 2010),* for the IVIG in a Consensus statement of the American Association of Neuromuscolar and Electrodiagnostic Medicine (AANEM) *(Donofrio et al., 2009)*, in the guidelines of the American Academy of Neurology (AAN) on the use of IVIG in neurological diseases *(Patwa et al., 2012)* and for plasmapheresis in the guidelines of the AAN on the use of plasmapheresis in neurological diseases *(Cortese et al., 2011).*

It is often difficult to decide which therapy should be used first in the CIDP. The decision should consider the possible efficacy, the cost and the side effects in relation to the single patient. Some randomized trials have shown IVIG and oral corticosteroids to have a comparable short-term efficacy in CIDP *(Hughes et al., 2011)* as IVIG and plasmapheresis *(Dyck et al., 1994).* More recent studies have shown that both IVIG *(Hughes et al., 2008; Merkies et al., 2009)* and corticosteroids *(Van Schaik et al., 2012; Eftimov et al., 2012)* have prolonged efficacy in CIDP. A randomized controlled trial that compared efficacy at six months of the treatment with IVIG and methylprednisolone i.v. (IVMP) showed that IVIG were often more effective and better tolerated (87.5%) than corticosteroids (47.6%) during the first six months of treatment, although, when effective, corticosteroids were less frequently associated with worsening than IVIG in the six months following the interruption of therapy *(Nobile-Orazio et al., 2012).* In the extension of the follow-up of this study *(Nobile-Orazio et al., 2013a),* a similarly proportion of patients who had discontinued IVIG (87%) and IVMP (79%) worsened and required a further therapy, although the median time to relapse was significantly longer after IVMP discontinuation (14 months) compared to IVIG (4.5 months). There were no significant differences in the proportion of patients who experienced adverse

reactions. These data, together with the lower cost of corticosteroids compared to IVIG *(McCrone et al., 2003; Blackhouse et al., 2010),* can somehow compensate for the increased frequency of response to IVIG compared to the IVMP observed in the original study and thus equalize the two types of treatment. At the same time, however, it is possible that a more prolonged use of corticosteroids may lead to a higher frequency of any potential adverse events than those observed during the six months of therapy *(Dukes, 1996).* In our case report, 78.41% of patients treated with IVIG and 50% of patients treated with corticosteroids responded to treatment. Thus, globally 85.37% of the treated patients responded to one of these two treatments. No significant differences were found regarding the response to treatment between patients with a definite diagnosis of CIDP and those with a possible or probable diagnosis and between typical and atypical phenotypes.

In accordance with what already reported in literature, even our patients responding to IVIG were more numerous than those responding to corticosteroids. However, it emerged from previous studies that IVIG responders are more dependent on therapy than corticosteroids responders as they have a shorter time to relapse. For this reason in our laboratory we are adopting the general rule of administering corticosteroids first, unless contraindications, and then IVIG in case of steroid therapy failure.

The use of subcutaneous Immunoglobulins (SCIG) reduces costs and overcome the inconvenience of repeated hospital admissions. It has been shown that several patients maintain the clinical benefit achieved with IGEV by taking the same dose of SCIG at home *(Lee et al., 2008; Cocito et al., 2011b).* These observations were confirmed in a small randomized controlled trial with placebo in 15 patients which showed that SCIG were as safe and effective as IVIG in most CIDP patients *(Markvardsen et al., 2013).* SCIG can improve patients' quality of life, as they do not require the interruption of daily activities unlike intravenous infusions. In our series, 14% of patients responding to IVIG switched to SCIG therapy (N=13). Five patients responding to IVIG switched

to continuous therapy with SCIG due to a short response to IVIG; eight patients switched to pulsed therapy with SCIG, seven for the difficulty in hospitalization and one for a previous allergic reaction to IVIG. Sixtynine percent of patients switched to SCIG responded to treatment, all of those who received continuous therapy and half of those who received pulsed therapy. The others returned to IVIG. This data confirm the effectiveness of the SCIG in maintaining the effect of IVIG in patients with a short duration of response to IVIG, but also suggests the possibility of using SCIG in place of IVIG in a pulsed manner, at the time of possible clinical deterioration.

Although equally effective compared to IVIG, plasmapheresis is more invasive for the patient and has a higher prevalence of side effects, mostly related to hemodynamic alterations, which make it less suitable for long-term treatment *(Joint Task Force of the EFNS and the PNS, 2010).* It is often reserved for patients who have an insufficient response to IVIG or corticosteroids. We used plasmapheresis only as a second line therapy and in 5.62% of the total number of patients but none of them responded.

None of the randomized controlled trials with other immunosuppressive therapies confirmed their efficacy. It is however possible that the clinical scales used in these trials were not sensitive for some symptoms such as fatigue or loss of sensitivity, that generally induce the restarting of therapy. This consideration highlights the need for better rating scales in CIDP trials *(Vanhoutte et al., 2013).*

Despite the negative results emerging from these randomized studies, immunosuppressive agents are still widely used in the treatment of CIDP. This tendency probably derives from the results of uncontrolled or retrospective studies showing variable efficacy of treatment with Cyclosporine (82% of patients improved), Cyclophosphamide (75%), Rituximab (75%), Methotrexate (70%), Azathioprine (27%), Interferon alpha (64%), Alentuzumab (57%), Mycophenolate Mofetil (46%), Interferon b 1a (35%), Etanercept (30%), Tacrolimus and autologous stem cell transplantation *(Mahdi-Rogers et al.,*

2013). It should however be considered that a substantial proportion of patients after placebo discontinuation did not get worse. In a more recent multicenter retrospective study on 110 patients with CIDP not adequately responding to IVIG, corticosteroids or plasmapheresis, the proportion of patients responding to therapies with immunosoppressive agents ranged between 20 and 30%, with development of adverse events related to the use of these drugs in 10-20% of cases *(Cocito et al., 2011a).* At the Peripheral Nerve Society Meeting in Wurzburg in 2009 on the use of immunosuppressive agents in CIDP, most experts supported the use of oral Azathioprine in patients with mild to moderate CIDP and Cyclophosphamide in severely affected patients.

However, our experience with the use of immunosuppressive drugs in patients non responders to first line agents suggests some efficacy only for oral Azathioprine. In fact, oral Azathioprine given twice daily was effective in one patient out of 10 (10%) treated with this drug. Cyclophosphamide, Rituximab, Cyclosporine, Methotrexate and Mitoxantrone were ineffective.

Investigation of prognostic factors in our cohort of patients with a diagnosis of definite CIDP

The long term outcome of patients with CIDP after receiving the immune modulating treatments is unclear. There are various reports investigating long term course and outcome of CIDP patients, but follow up periods were variable among the studies, including patients with only a few year of follow up. Moreover, whether specific clinical, electrodiagnostic, and/or laboratory features are associated with different prognosis of CIDP patients is not well understood. The extent of axonal loss has been reported to be the major prognostic factor in CIDP, but this would not be prominent in the early stage of the disease. Anyway few papers have looked at early predictive factors of disability and obtained contrasting results as well. We evaluated our dataset of patients in order to choose patients with a definite diagnosis of CIDP according

to the EFNS/PNS guidelines *(Joint Task Force of the EFNS/PNS, 2010)* and with complete information regarding the onset of the disease and who had undergone a complete clinical examination within the last 6 months.

We obtained from 60 patients (Table 2) data including sex, age of onset, type of onset (acute or subacute/ chronic), phenotype (typical or atypical according to EFNS/ PNS guidelines) *(Joint Task Force of the EFNS/PNS, 2010)*, disease duration, response to treatment [intravenous immunoglobulin (IVIG) or corticosteroids (CS)], disability at the time of diagnosis and at the last examination assessed using the modified Rankin Scale (namely respectively baseline and last mRS), cerebrospinal fluid (CSF) protein levels and electrophysiological data. Disease duration (namely, follow up) was calculated as the time (years) elapsed between the first and the last clinical examination. For electrophysiological data, we evaluated the mean values of motor nerve conduction velocity (MNCV) and sensory nerve conduction velocity (SNCV), and the mean values of amplitude of compound muscular action potentials (CMAPs) and sensory action potentials (SAPs) in at least three nerves in the upper and lower limbs for each patient.

Stepwise forward logistic regression model was used to evaluate the prognostic impact of clinical, biochemical and electrophysiological parameters on the last mRS, considered as binary outcome (absence or presence of severe disability, i.e., $<4/\geq 4$ mRS) *(Nobile-Orazio 2014)*. To identify the potential predictors of long-term disability, we applied a stepwise logistic regression to test the relationship between the last mRS (dependent variable) and clinical, biochemical and electrophysiological findings (independent variables).

Spearman's rank correlation coefficient was used to evaluate the correlation between disease duration and last mRS, and Wilcoxon signed-rank test was applied to compare baseline mRS with last mRS. Statistical analysis was performed using STATA 13.0 for iOS.

Table 2. Clinical, biochemical and electrophysiological data at onset

Clinical and biochemical data	
Sex	70% Males; 30% Females
Age of Onset (years), mean ± SD [CI]	44.01 ± 17.77 [39.42 - 48.61]
Type of onset	16.7% acute; 83.3% subacute-chronic
Phenotype	73.3% typical; 26.7% atypical
Atypical Variants	62.5 % MADSAM; 31.25 % sensory; 6.25 % DADS
Disease duration (years), mean ± SD [CI]	11.05 ± 9.6 [8.57-13.53]
Responders to treatment	83.3% responders (IVIG 79.17%; CS 66.7%); 16.7% non responders[a]
Baseline mRS, mean ± SD [CI]	2.2 ± 1.23 [1.88-2.52]
Percentage of patients with baseline mRS ≥4 (ratio)	21.7% (13/60)
Percentage of patients with acute onset and baseline mRS ≥ 4 (ratio)	70% (7/10)
Last mRS, mean ± SD [CI]	2.3 ± 1.34 [1.95-2.65]
Percentage of patients with last mRS ≥ 4 (ratio)	15% (9/60)
Percentage of patients with acute onset and last mRS ≥ 4 (ratio)	10% (1/10)
CSF protein level (mg/dl), mean ± SD [CI]	110.6 ± 97.94 [84.38-136.83]
Electrophysiological findings	
Mean MNCV (m/s), mean ± SD [CI][b]	36.71 ± 9.88 [34.09-39.33]
Mean CMAP (mV), mean ± SD [CI][b]	5.7 ± 3.04 [4.91-6.48]
Mean SNCV (m/s), mean ± SD [CI][b]	43.93 ± 10.2 [41-46.86]
Mean SAP (μV), mean ± SD [CI][b]	8.82 ± 12.71 [5.45-12.20]

Legend. mRS modified Rankin Scale, MADSAM multifocal acquired demyelinating sensory and motor neuropathy, DADS distal acquired demyelinating symmetric neuropathy, CSF cerebrospinal fluid, MNCV/SNCV motor/sensory nerve conduction velocity, CMAP/SAP compound muscular/sensory action potential amplitude

[a] Some patients were treated with both therapies at different stages of the disease

[b] For each patient, mean MNCV/SNCV and mean CMAP/SAP amplitudes were evaluated in at least three motor and sensory nerves in the upper and lower limbs

Univariate and multivariate logistic regression tests disclosed a significant relationship between the last mRS and baseline mRS and between the last mRS and age of onset. Univariate logistic regression also showed an inverse association between the last mRS and response to treatment, not confirmed by multivariate logistic regression. ANOVA with repeated measures did not show a different effect on clinical disability between patients treated with IVIG and CS. There was no significant association with sex, type of onset, phenotype, CSF protein levels and electrophysiological data. There was no correlation between disease duration and the last mRS *(Spina et al., 2017)*.

The strongest predictor of long-term disability observed in our population seems to be the disability at onset. In particular, it seems that worse are patients at onset, worse patients are over time. The exception are patients with an acute onset who often reach a high level of disability in few days, as in GBS, but at the same time seem to have a greater margin of recovery. These findings are in line with those described by Chiò et al. *(Chiò et al., 2007)*, while are in contrast with those by Kuwabara et al., who however used a smaller sample of CIDP patients *(Kuwabara et al., 2006)*. We could hypothesize that acute onset is correlated to a predominant demyelinating damage of peripheral nerves, which develops rapidly but is also reversible. While it is possible that a higher disability at onset depend on an early axonal loss, that is poorly reversible with treatments *(Mygland et al., 2005; Anadani et al., 2015)*.

The second predictor of disability seems to be the age of onset. Younger patients had a reduced risk of developing severe disability over time. These findings are in line with those from previous series of CIDP patients *(Chiò et al., 2007; Sghirlanzoni et al., 2000)*. We could suppose that the immune system becomes less effective with aging while in younger patients is more efficacious in rescuing nerve damage *(Hayter et al., 2012)*. We found that long-term disability in CIDP patients is independent of disease duration. These data could depend on the low sensitivity of mRS in detecting clinical deterioration in CIDP *(Tackenberg et al., 2007; Querol et al., 2013; Kuitwaard et al., 2015; Chiò et*

al., 2007; Sghirlanzoni et al., 2000) but we can also suppose that disability remains stable over time. Stability could derive from the effectiveness of treatments available in preventing progressive deterioration, even if we did not found a correlation between long-term disability and response to treatment, or between the severity of disease and age of patients at onset. In fact, it is also unclear if the available treatments are effective in long term as well as in short term *(Cocito et al., 2010; Querol et al., 2013; Hahn et al., 1996; Eftimov et al., 2013; Dyck et al., 1994; Hughes et al., 2001; Vermeulen et al., 1993)*.

We did not found any correlation between electrophysiological data at onset and long-term disability. On nerve conduction studies, demyelination is crucial for the diagnosis of CIDP, but it does not provide information about the clinical impairment *(Joint Task Force of the EFNS/PNS 2010; Sghirlanzoni et al., 2000)*. On the contrary, as observed previously, the reduction of CMAP and/or SAP amplitudes, markers of axonal degeneration, might predict long-term disability *(Bouchard et al., 1999; Sghirlanzoni et al., 2000)*. However, the reduction of CMAP and SAP amplitudes at onset in CIDP patients should be also due to a demyelinating process such as abnormal nerve excitability, temporal dispersion or distal conduction blocks rather than to axonal loss.

Finally, CSF protein levels seems not to correlate with clinical disability in the long-term follow-up even if, higher CSF protein levels at onset have been associated with greater disability in the short-term follow-up *(Tackenberg et al., 2007; Mygland et al., 2005; Sghirlanzoni et al., 2000)*. Higher CSF protein levels at onset express greater damage to the blood–nerve-barrier at the roots and could influence the phenotype of CIDP, but not necessarily the disease severity over time *(Kuwabara et al., 2002)*.

Searching for new outcome measures for CIDP in the subgroup of patients periodically treated with the first line treatments

The assessment of response to treatment is often difficult in patients with CIDP because of the lacking of outcome measures sensitive enough in the detection of symptoms, such as fatigue, which can not be appreciated by the clinical evaluation or by the current clinimetric scales. The consequence is that it is not always simple for clinicians to consider a CIDP patient as responder or not to treatment. The doubts about the efficacy of therapy, in some cases lead to an excessive use of treatments even when not necessary. A possible strategy for this problem could be the identification of a scale able to capture minimal but important clinical variations (i.e., MCID).

Among the outcome measures commonly used in CIDP patients, only for the ONLS scale the MCID has been established, therefore this scale has been considered an "ideal" one *(Merkies et al., 2010; Hughes et al., 2016)*. However, the ONLS is an ordinal and non-linear scale, and thus, it is not able to capture a change less than 1-point. In other words, if a patient improves more than MCID values but less than 1-point in ONLS, he can not be considered as responder despite the clinical improvement. For these reasons, we decided to apply systematically in a group of CIDP patients the six-minute walk test (6MWT), before and 2 months after the treatment. The 6MWT offers continuous values (i.e., meters walked along 6 min) for calculating the MCID and to establish a cut-off for defining a patient as responder or not to therapy. This idea came from the consciousness that the 6MWT has already been successfully used to measure fatigue and explore ambulatory capacity in several neurological diseases.

We selected 42 ambulant patients with a diagnosis of definite CIDP according to the EFNS/PNS criteria and periodically treated with the same treatment, intravenous immunoglobulin (IVIG) or intravenous methylprednisolone (IVMP), in the last six months *(Joint Task Force of the EFNS/PNS 2010)*.

All patients underwent clinical evaluation comprehensive of demographic evaluation, clinimetric scales ISS, ONLS, R-ODS, mRS, MRC sum-score, and 6MWT before (baseline) treatment (IVIG or IVMP) and after (follow-up) 2 months.

At follow-up visit, patients reported whether their condition was stable, improving or getting worse compared to baseline evaluation and were also asked to indicate the degree of the improvement.

At baseline, the 6MWT correlated with ONLS supporting the sensitivity of our methodological approach in measuring clinical impairment in CIDP.

We calculated the minimal clinically important difference (MCID) for 6MWT, both using anchor-based and distribution-based approaches *(Copay et al., 2007; Merkies et al., 2010)*. In the first approach we compared the variations in outcome measure values with the self-perception of patient clinical condition changes. The second approach was based on the mathematical interpolation of baseline and/or follow-up values for each clinical outcome.

The MCID was obtained by calculating the mean value of the difference in walked meters between stable and slightly improved groups of patients.

Finally, we compared the sensitivity (the proportion of patients who were identified as responders) between the 6MWT and the other clinical measures (i.e., ONLS, MRC, and mRS combined).

By calculating the difference between “stable” and “slightly improved” groups, we obtained a mean MCID value for the 6MWT of 20.26 m, i.e., the patients walking at least 20 m more than in the previous assessment (before therapy) could be considered as responders. Interestingly, the MCID was not influenced by the degree of disability at baseline (meters walked at 6MWT). Accordingly, we decided to assume a 20-m improvement as the cut-off value to consider a patient as 6MWT-responder.

The rate of responders identified by applying this MCID for the 6MWT to the cohort of our patients increased from 58 to 66%, and by combining the 6MWT

with the other clinical measures, the rate of overall responders increased up to 74% (16% more than classic clinical measures).

Overall, the sensitivity of 6MWT was 13% greater than the other clinical measures (90% vs 77%) in identifying responder patients. Seven patients were identified as responders only through the 6MWT, thus supporting the sensitivity of the test in assessing globally gait performance, that may be influenced by both sensory and motor neuropathy. On the other hand, the 6MWT did not identify three patients otherwise classified as responders by other clinical measures because they had only upper limbs impairment. As these patients were identified by ONLS, a complete evaluation could include both these scales *(Spina et al., 2019)*.

The 6MWT has been proved to be sensitive not only for exploring the ambulatory ability but also for capturing the fatigue-related changes *(Montes et aal., 2010; Goldman et al., 2008)*.

To investigate the fatigue, we calculated at baseline, and at follow-up, the first-minute velocity (FMV), the sixth-minute velocity (SMV), and the overall mean velocity (OMV) at baseline and follow-up visit. We compared the velocities between the first and sixth minutes of 6MWT at baseline and at follow-up using the *t* student test for paired sample that showed significant differences between them both at baseline ($p<0.00$) and follow-up visit ($p<0.00$). 6MWT-responder patients showed a significant improvement at follow-up visit for FMV, SMV, and OMV compared to baseline.

In conclusion, we support that the 6MWT is a reliable and sensitive tool for monitoring CIDP patients and the combination of the 6MWT with the other clinical measures increases the chance to detect the real quote of responders to therapy. We propose to include the 6MWT in the routine neurological examination of CIDP patients and the MCID cut-off at 20 m should be used for identifying the responders and properly guiding the therapy management *(Spina et al., 2019)*.

SECTION 2: DATA FROM THE NATIONAL DATABASE

The project of a web-based national database

The wide variety of diagnostic criteria and therapies used in CIDP could depend on its rarity with only few patients followed in each Centre. For this reason, the "Humanitas" Institute promoted a project whose name was "A Lombard network for the study of Chronic Inflammatory Demyelinating Polyradicoloneuropathy (CIDP) and its variants to optimize the diagnostic and therapeutic process in light of costs and improvement of the quality of life".

The aim of the project was to improve the diagnostic approach of patients with CIDP and variants by further revision of clinical and electrophysiological diagnostic criteria. In particular, the target was to evaluate the effective diagnostic utility of the additional invasive (rachicentesis, nerve biopsy) or expensive (MRI) diagnostic tests and to evaluate the usefulness of any hospitalization, also in the light of their cost. Moreover, the plan was to analyse the possible relationship between the different variants and the classical CIDP as regard their pathogenesis and their response to therapies, and to assess the impact of therapies on improving the functionality and quality of life of patients in light of their costs and side effects.

The study was enlarged to other expert centres in Italy with the aim to reach a total number of 1000 patients.

At the time of the enrolment, detailed data were collected about the clinical history with particular attention to the type and distribution of symptoms at onset, and to their evolution with time. Data about infectious antecedents, associated pathologies, life style, eating habits, therapies performed and response perceived by patients to these therapies were collected. For this purpose, we used a detailed questioner and a set of clinimetric scales.

The diagnosis was reviewed based on the diagnostic criteria of the American Academy of Neurology (Koski and collaborators) and of the EFNS/PNS.

Patients enrolled for the study were followed for two years in order to assess disease evolution and response to pharmacological treatment, taking into account treatment duration and drugs posology. Every six months patients were administered the following scales to asses disease severity and evolution: the MRC (Medical Research Council) scale for muscle strength, the INCAT (Inflammatory Neuropathy Cause and Treatment) Sensory sumscore, the I-RODs (Inflammatory-Rash Overall Built Disability Scale) (range 1–48) and the EuroQol for the quality of life. Data on any adverse event due to drug assumption were also collected and reported in the study.

The expected results were:

- Definition of clinical, electrophysiological and laboratory criteria for the diagnosis of CIDP and above all of its variants in order to standardize the diagnosis of these pathologies. Determination of the frequency of classical CIDP and its variants in patients with inflammatory neuropathies and evaluation of the possible evolution from a phenotype to others.
- Verification of the usefulness of the diagnostic tests currently used in the light of their cost and invasiveness, and their role on the diagnosis and on the choice of the therapy. Estimation of the usefulness of measuring antibodies against nerve antigens on diagnosis and therapy.
- Verification of the efficacy of dosage and duration of therapies on the improvement of functionality and quality of life, side effects and cost. Parallel evaluation of the effectiveness of physical therapies on the functional and social improvement of patients.
- Implement in Lombardy and Italy of a collaborative network of Expert Centres in the diagnosis and therapy of CIDP and its variants.
- Creation of an optimal diagnostic and therapeutic model for patients with CIDP and its variants that takes into account costs and the improvement of patients' disability and quality of life.

- Definition of the most effective therapeutic schemes and their sequence in patients with CIDP and variants and analysis of their impact on patients' disability. Preparation of regional and national guidelines for the therapy of patients with CIDP and its variants.

Preliminary results

Characterization of frequency, evolution and response to treatment of atypical CIDP variants based on new diagnostic criteria proposed.

To determine the frequency and characteristics of the CIDP variants, their possible evolution to typical CIDP, and their treatment response, a set of diagnostic criteria for the CIDP variants was defined. Data from literature were applied to the 460 patients included in the Italian database.

The proposed set of diagnostic criteria for atypical CIDP is the following:

DADS (distal acquired demyelinating symmetric neuropathy)

Mandatory criteria

(A) with or (B) without increased distal motor latency

1. Symmetric, sensory or sensorimotor symptoms and signs starting distally in the lower limbs, without proximal limb–trunk–face involvement (length-dependent fashion).*

Other possible symptoms

1. Ataxia, neuropathic pain, cramps, fatigue, autonomic symptoms, tremor.
2. Upper-limb distal sensory or sensorimotor symptoms and signs occurring later (at least after 1 year from onset).

Exclusion criteria

1. Cranial nerve involvement.
2. Proximal limbs, trunk, face involvement.

3. Weakness without sensory symptoms.
4. Symptoms and signs starting in the upper limbs.

Pure sensory CIDP

Mandatory criteria

(A) with or (B) without abnormal motor nerve conduction studies

1. Sensory symptoms (including ataxia), without weakness, in a polyneuropathic distribution, symmetric or asymmetric.*
2. Symptoms may start anywhere in the body excluding a length-dependent pattern (included under DADS).

Other possible symptoms

1. Neuropathic pain, fatigue, tremor.
2. Facial sensory symptoms.

Exclusion criteria

1. Motor symptoms/signs including cramps and motor cranial nerve palsy.
2. Multifocal distribution.
3. Autonomic dysfunction.

CISP (chronic immune sensory polyradiculopathy)

Mandatory criteria

1. Sensory symptoms with a polyneuropathic distribution without weakness.*
2. Normal motor and sensory nerve conduction and electromyograpic studies.

PLUS at least two of the following:

3. Abnormal somatosensory evoked potential not due to central nervous system involvement.
4. MRI showing gadolinium enhancement and/or hypertrophy of the cauda equina, lumbosacral or cervical nerve roots, or the brachial or lumbosacral plexuses.
5. Elevated cerebrospinal fluid protein level with normal cells.

Exclusion criteria and other possible symptoms

1. As in pure sensory CIDP .

Pure motor CIDP

Mandatory criteria

(A) with or (B) without abnormal sensory nerve conduction studies

1. Weakness, without sensory symptoms or signs, in a polyneuropathic distribution, symmetric or asymmetric.*
2. Symptoms may start anywhere in the body.

Other possible symptoms

1. Cramps, fatigue, tremor.
2. Motor cranial nerve palsy.

Exclusion criteria

1. Sensory symptoms/signs including sensory ataxia.
2. Autonomic dysfunction.
3. Neuropathic pain.
4. Multifocal distribution.

LSS (Lewis-Sumner syndrome)

Mandatory criteria

(A) with or (B) without motor conduction block

1. Sensory symptoms, with or without weakness, in a multifocal distribution (unilateral focal† CIDP included).*
2. Symptoms may start anywhere in the body.

Other possible symptoms

1. Cramps, fatigue, autonomic symptoms, ataxia, neuropathic pain.
2. Motor and/or sensory cranial nerve palsy.

Exclusion criteria

1. Weakness in isolation, without sensory symptoms.
2. Symptoms/signs in a polyneuropathic distribution.

*Clinical phenotype must have lasted at least 1 year (temporal criterion).

†Focal CIDP defined as the presence of symptoms homogeneously restricted to the nerves of one or two limbs (ipsilateral upper and lower limb).

These criteria were applied to the CIDP patients both at the disease onset and at the time of enrolment in the study. A minimum of 1-year duration of symptoms and signs specific to each atypical form was necessary to establish a diagnosis of atypical CIDP. This because typical CIDP may initially present with purely sensory or motor symptoms evolving over a few months to a typical sensorimotor form. Patients with bilateral, although asymmetric, but not multifocal, motor and sensory impairment were also considered as typical CIDP. At study entry 18% of patients had atypical CIDP, similarly to some previous studies (17.8%–19.6%). Looking at the symptom evolution from disease onset to study inclusion (mean disease duration of 5.5 years), 39% of the patients had a clinical presentation consistent with atypical CIDP that in 53% of them evolved to typical CIDP. In our study, progression to typical CIDP was significantly associated with a longer disease duration. This finding supports the idea that progression to typical CIDP is often, though not invariably, associated with the disease duration. The clinical form with the highest progression rate was the pure sensory CIDP, while DADS showed the lowest rate. Within 5 years, 48% of patients with sensory CIDP, 32% of pure motor, 36% of LSS and 24% of DADS had progressed to typical CIDP. Among the patients with atypical CIDP, 7% of total CIDP patients had DADS, 4% had purely motor CIDP, 4 % had LSS including three with focal CIDP and 3.5% had purely sensory CIDP including two (0.5%) with CISP. The relatively small prevalence of LSS in this series compared with some previous series may reflect the inclusion of patients with a multifocal neuropathy and not of those with an asymmetric polyneuropathy. This decision was supported by the absence of difference in the response to therapy between patients with symmetric or asymmetric typical CIDP and by the different response to

therapy between patients with LSS and asymmetric CIDP. We found a lower prevalence of sensory CIDP compared with previous series because we do not included patients with purely sensory DADS or LSS. DADS was the most common CIDP variant in our study. Compared with typical CIDP, patients with DADS had an older age at onset, higher MRC sum score, lower disability levels, and better QoL, confirming that DADS is clinically less disabling than typical CIDP. No significant difference between purely motor/pure sensory and typical CIDP was found in terms of severity of motor impairment, disability, and QoL.

There are some differences in the reported response to therapy in patients with atypical CIDP. In our series, patients with LSS and DADS had a less frequent response to IVIG compared with patients with typical CIDP, while there were no differences in the response to steroids. Several studies reported that purely motor CIDP did not respond or worsen after corticosteroids. We found that 43% of our patients with purely motor CIDP improved after steroids. All these patients had however a concomitant sensory electrophysiological, although not clinical, impairment. This finding suggests that the diagnosis of motor CIDP and the decision to avoid steroids should be probably restricted to patients without any clinical and electrophysiological involvement of sensory nerves.

In conclusion, our study confirms that the proportion of patients with atypical CIDP varies according to the duration of the disease and that response to therapy is different in DADS and LSS, suggesting that some difference in the pathogenic mechanisms may underlie these variants. An extensive immunological study to investigate the presence of different anti-nerve antibodies is in progress in this cohort of patients to verify whether the different clinical presentations and response to therapy might be associated with specific immunological abnormalities as was recently reported for patients with antibodies to neurofascin 155, contactin 1 and other nodal–paranodal proteins *(Doneddu et al., 2018).*

The role of antecedent events, lifestyle and dietary habits as risk factors for CIDP

The frequency and type of eventual antecedent events or infections in CIDP and the role of lifestyle and dietary habits in the development of the disease are still unknown. We used our national database to investigate whether lifestyle and dietary habits may be associated with the risk of developing CIDP and whether antecedent infections could influence the clinical presentation and course of the disease.

A total of 323 patients with a diagnosis of CIDP according to the EFNS/PNS criteria completed the study on lifestyle and dietary habits. Dietary and lifestyle data were collected also from 266 controls. We found that some dietary habits, including eating rice at least three times per week and eating fish at least once per week, are associated with a decreased risk of CIDP. None of the remaining examined variables revealed significant associations (exposure to toxic agents, smoke, alcohol consumption, illicit drugs consumption, dietary regimen, pasta, meat, raw meat, white meat, vegetables, fruits, cheese, eggs, sweets, coffee, tea, milk, soft drinks). Various ex-vivo and animal models demonstrated that rice-derived bioactive compounds have antioxidant and anti-inflammatory potential, even if less is known on the possible immunomodulatory activity of white rice. Fish-derived bioactive compounds showed remarkable anti-inflammatory and immune-modulatory activities, and fish consumption was associated with a decreased risk of autoimmune diseases. Whether this may also explain the reduced prevalence of CIDP in Japan (1.61/100.000), where the traditional diet is characterized by high consumption of rice and fish, compared to Europe and United States (range 3 to 8.9/100.000) remains unclear.

Data on antecedent events were available from 411 patients with CIDP. 8% of patients had a flu-like syndrome within 1-42 days before the onset of CIDP symptoms, 2% had an upper respiratory infection, 2% a gastrointestinal

infection, 1.5% had vaccination (all seven with flu vaccine), 1% had surgery, and 0.5% had trauma. No patients started a new immune-modulating therapy. Overall, 15.5% had an antecedent event in the 1 through 42 days prior to CIDP onset. These data were similar to that reported in literature. We found that antecedent infections were associated with an acute onset of CIDP and with cranial nerve involvement, suggesting that CIDP patients with these antecedent events might share some clinical features with GBS. No other differences were found between the two groups. Therefore, our study seems to suggest that antecedent events are unlikely to play a role in the risk of CIDP. There are few data on the associations of infections with the clinical features of CIDP.

Limitations of our study include the use of a non-validated questionnaire and the selection of patient's partners as controls. This selection bias was however attenuated by matching for sex and by randomly choosing controls for the analysis. The absence of a control group for the analysis of antecedent events is another major limitation of this study. However, the low frequency of antecedent events reported and the results of studies in other populations suggest that a role of antecedent events in CIDP risk is unlikely. More epidemiological and intervention studies are necessary to investigate in more detail the role of environmental factors in the risk of CIDP *(Doneddu et al., 2020).*

SECTION 3: INVESTIGATION OF PATHOGENESIS AND RESPONSE TO TREATMENT

The cause of CIDP is still unknown, even if the disease is mainly attributed to an autoimmune reactivity against peripheral nerve. In addition to the typical form, several clinical variants, generally responsive to immunomodulatory therapies, have been described, widening the clinical spectrum of this disease. Atypical forms are considered as variants of CIDP, representing about 30-40% of patients, and their response to treatment is often different from the classical form. Therefore, it remains the suspicion that more than variants they can be different forms of illness *(Nobile-Orazio et al., 2014)*. In parallel, other pathologies considered for some time to be variants of the CIDP have subsequently been better identified and currently considered as separated entities *(Mathey et al., 2015)*. The distinction also in this case is not only academic, as we have seen that some of these forms, as in particular the multifocal motor neuropathy (MMN) deviate from the CIDP due to the lack of response, and sometimes worsening, with steroids, that are effective in CIDP. Similarly, the demyelinating neuropathy associated with IgM monoclonal gammopathy with anti-MAG antibodies responds poorly to therapies deemed effective in CIDP *(Gorson et al., 1997)*. The availability of specific biomarkers could provide guidance for patient-tailored immunotherapeutic options. Antibodies to cell adhesion molecules of the paranodal complex, neurofascin-155 (Nfasc155), contactin-1 (CNTN1), and contactin-associated protein 1 (Caspr1), and to the nodal neurofascin-140/186 (Nfasc140/186) have been identified in various percentages of patients with CIDP, with IgG4 being the predominant isotype of these antibodies *(Devaux et al., 2012; Querol et al., 2013; Querol et al., 2014; Ogata et al., 2015; Miura et al., 2015; Doppler et al., 2013; Doppler et al., 2016)*. Moreover, anti-CNTN1 and Nfasc155 IgG4 are associated with specific alterations of the paranodal axoglial contacts in nerve biopsies *(Koike et al., 2017; Vallat et al., 2017)*, suggesting that these antibodies

induce conduction defects in patients by altering paranode integrity, hence the term paranodopathy *(Uncini et al., 2013; Uncini et al., 205; Kuwabara et al., 2017).*

Sural nerve biopsy role in understanding the pathogenesis and response to treatment of CIDP with antibody against NF155: a case from our cohort

CIDP with antibody IgG4 against paranodal protein NF155 has been better characterized then other seropositive forms. In particular, it shows characteristic aspects such as a younger age at onset, ataxia, tremor, distal weakness, CNS demyelination, and a poor response to IV Immunoglobulin *(Querol et al., 2014)*. Electrophysiological findings show markedly decreased nerve conduction velocity, motor nerve conduction blocks, prolonged distal and F-wave latencies, and more frequently than anti-NF155 antibody-negative patients the absence of sensory action potentials. Temporal dispersion of cMAP is reduced and not constantly described *(Ogata et al., 2015)*. On sural nerve biopsy, the detachment of terminal myelin loops from the axolemma at the paranode has been described as a characteristic feature. Moreover, a positive correlation between the frequency of axo-glial detachment at the paranode and axonal degeneration found in some studies suggested a primary role of IgG4 anti NF-155 antibodies not only in conduction failure but also in axonal death *(Vallat et al., 2017; Koike et al., 2017).*

Among our patients, one showed positivity for the anti NF155. This patient underwent sural nerve biopsy in 1999, two years after the disease onset and a few years before the identification of the antibodies in his serum. His clinical history, biopsy and electrophysiological studies support some hypothesis about the pathogenesis of this form of CIDP and let us do some speculations on the disease mechanisms.

The patient is a 40 year-old man whose clinical history began in October 1995 (when he was 18-year-old) with paresthesia and weakness of all the extremities

that appeared simultaneously to a flu-like symptoms and an increased level of TAS because of which he underwent diaminocillin therapy. Meanwhile the hyposthenic and paresthesic symptoms worsened so that in January 1996 he was admitted to the Neurology of a local Hospital, where he underwent to rachicentesis, which showed an increase in liquor proteins (400 mg/dl). The electrophysiological study showed findings compatible with a widespread sufference of motor and sensory peripheral nerve fibers of a demyelinating type. He practiced a cycle of oral steroid therapy with benefit and was discharged from hospital with a diagnosis of CIDP.

He later underwent to other hospitalizations for the worsening of symptoms and different cycles of immunoglobulin therapy were performed without success, so he began a daily cortisone therapy with a partial response until its suspension a few months later (May 1997) due to the appearance of arterial hypertension.

Because of the worsening of the symptoms in October 1997 he came to our observation for further investigations. The neurological examination showed: ambulation possible without support only for few meters with marked ataxia and bilateral steppage slightly worsened by closing the eyes. Positive Romberg's sign. Cranial nerves were not involved. Motor investigation showed bilateral distal hypotrophy and hyposthenia of the limbs, much more evident at lower limbs. ROT were all absent. Sensory investigation showed severe distal hypopallesthesia at lower limbs. Postural tremor at upper limbs was present. Cerebellar coordination tests were correctly performed.

An electrodiagnostic study at our institution revealed significant prolongation of distal motor latency and slowing of motor conduction velocities without temporal dispersion or conduction block, reduced amplification of cMAP at lower limbs and the absence of all sensory action potentials. The needle EMG examination showed chronic denervation changes in the tibialis anterior and abductor digiti minimi muscles (Table 3). At sural nerve biopsy (Fig. 1A) the nerve appeared to be composed by 5 fascicles, 3 of which had a size exceeding the norm. The epineurium and perineurium were normal. A considerable loss of

myelinated fibers, especially of the large ones, was present in the nerve fascicles. Some fibers in Wallerian-like degeneration were present. About 50 fibers had a markedly increased size (until 30 micron). This swollen fibers had a thin myelin sheath, and some of them contained a vacuole optically empty and the axon was undetectable. There were no regeneration clusters, remyelinated fibers or onion bulbs. There were no inflammatory events. The epineurial and endoneurial vasa were normal. In longitudinal sections (Fig. 1B) the swelling of the fibers with thin myelin sheath was located especially at paranode. The nodal gap was enlarged in some fibers.

The ultrastructural study on electron micrographs (Fig. 2; Fig 3) confirmed the presence of some fibers in axonal degeneration and that the enlargement of myelinated fibers was due to a great vacuole localized in the middle of the fiber that pushed peripherally the atrophic axon into the myelin. The myelin was reduced to few sheets so the fiber appeared to be taken up mostly by the vacuole. Phenomenon of active stripping of myelin by macrophages were not present.

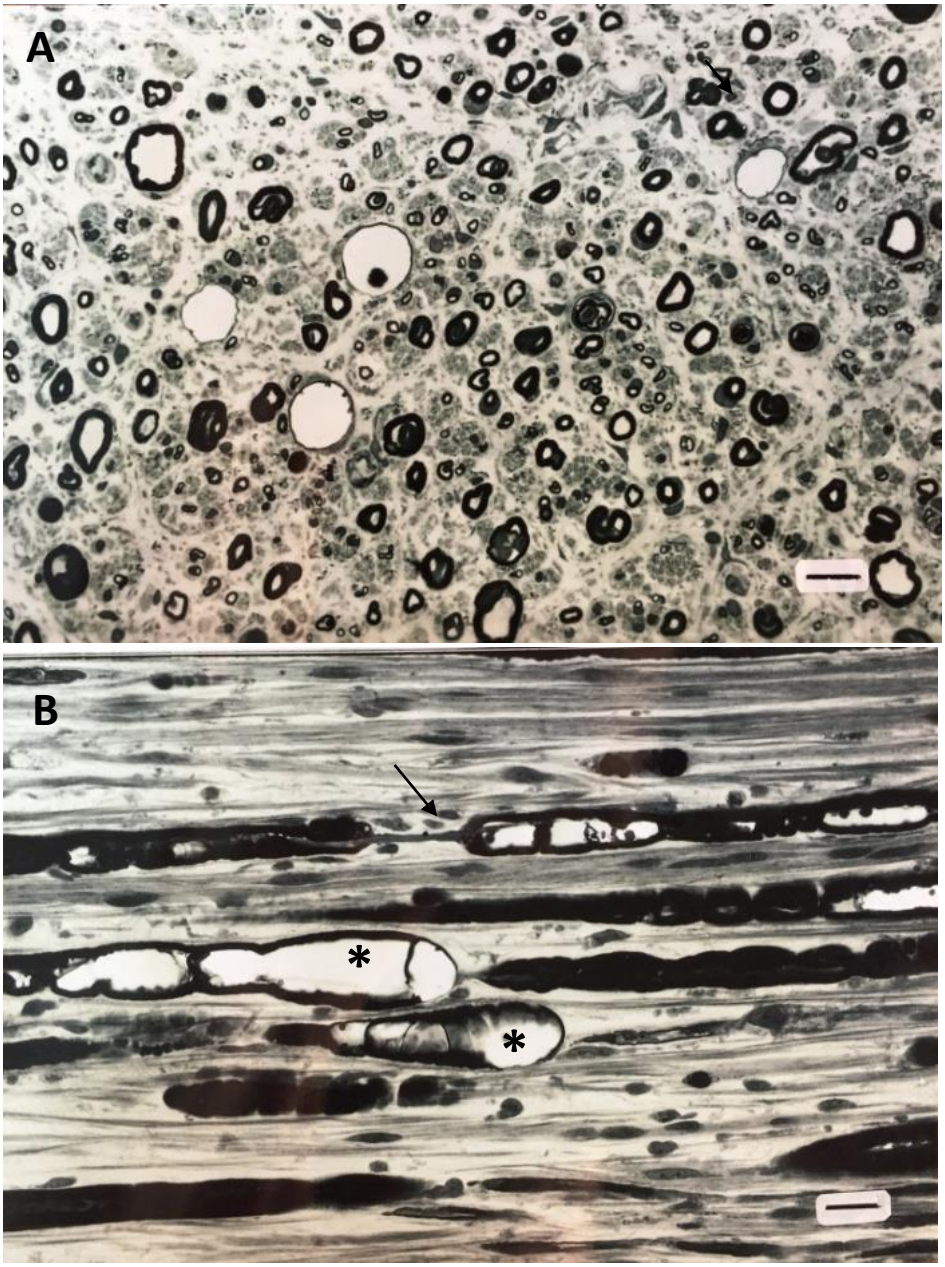


Figure 1. Light micrographs of sural nerve biopsy: transverse (A) and longitudinal (B) sections. In A note a considerable loss of large myelinated fibers, presence of some fibers in Wallerian-like degeneration and four swollen fibers with an extremely thin myelin sheath. In B the swelling is located at the paranode (asterisk) in two fibers. The nodal gap is enlarged in a fiber (arrow).
(1 mm, epoxy-embedded, semithin sections, stained with toluidine blue, x 440, bar = 20 μm)

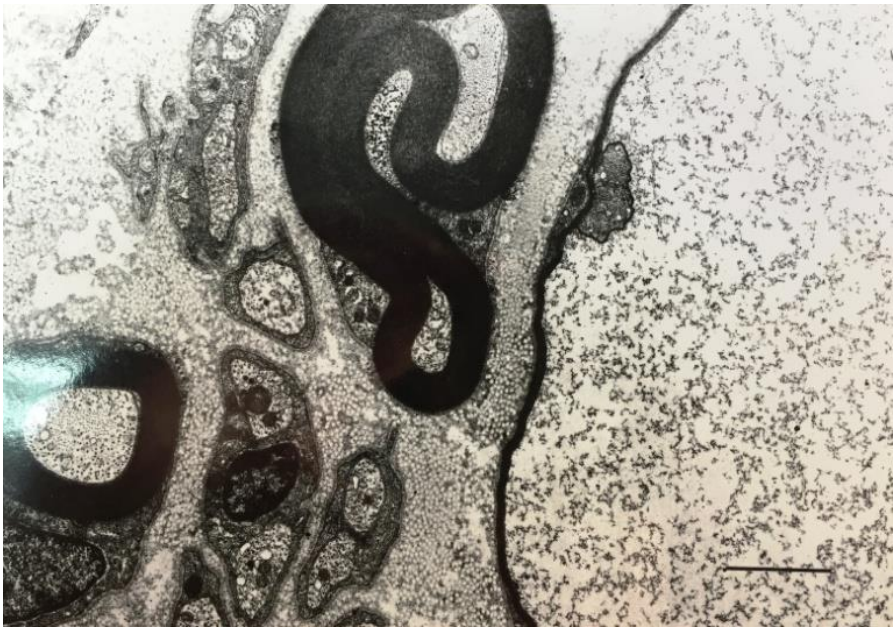


Figure 2. Electron micrograph showing an enlarged myelinated fiber due to a great vacuole, containing granular material, that pushes peripherally an atrophic axon. The myelin sheath is extremely thin. Two small myelinated fibers, lying near it, are normal as well as unmyelinated fibers.
(Thin section stained with Pb-citrate and Uranyl-acetate. X: 16.000; bar = 1 μm)

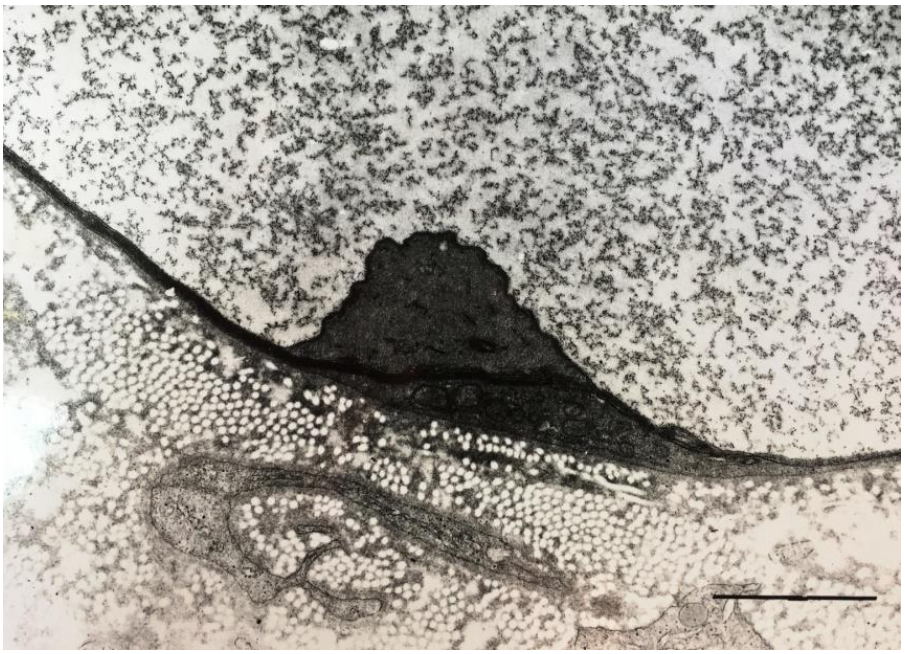


Figure 3. Electron micrograph showing a vacuolated myelinated fiber with a shrunken axon that is separated from the vacuole by a myelin leaflet.
(Thin section stained with Pb-citrate and Uracyl-acetate. X: 25.000; bar = 1 μm)

For 19 years the patient has not been visited at our Institute and came again to our observation in 2016. For years the patient had spontaneously took corticosteroids in a self-managed way even if in the last six years he had been treated with periodic cycles of intravenous corticosteroids.

At neurological examination he presented gait ataxia with marked distal hypotrophy and hyposthenia especially at lower limbs with mild proximal hypostenia of the legs, postural tremor of the arms and severe hypopallesthesia at lower limbs. Romberg's test was impossible and standing was very difficult without support.

The electrodiagnostic study showed a significant prolongation of distal motor latency and a marked slowing of motor conduction velocities without temporal dispersion in proximal segments of nerves (temporal dispersion was present in distal segments of nerves), the amplitude reduction of distal cMAP at upper limbs and the absence of the distal cMAP at lower limbs. All sensory action potentials were absent. Needle EMG confirmed the presence of chronic denervation signs (Table 3).

Because of the good response, the patient was again treated with steroids until July 2017 when a treatment with Rituximab was started (375 mg/m2 every week for four weeks). During the follow-up period he gradually improved his balance difficulty, and after six months Romberg's sign was negative. Also the ipopallesthesia and proximal weakness at lower limbs improved, while distal hyposthenia remained stable. The electrodiagnostic study at follow-up showed an improvement of the distal motor latencies and motor conduction velocities, an increase of the distal amplitude of cMAP and the reappearance of sensory action potentials at upper limbs. The absence of distal CMAPs and SAPs at lower limbs was confirmed.

Table 3. Motor Nerve Conduction Studies

	First study	Second study	Normal values
Right Median nerve			
DML (ms)	**9.6**	**14.2**	≤ 4,1
MNCV (m/sec)	**39.6**	**12.6**	≥ 50
CMAP wrist (mV)	10.5	**1**	≥ 6
F-wave latency (ms)	**51.5**	NA	≤ 31
F-wave frequency (%)	100	NA	60-100
Left Median nerve			
DML (ms)	NP	**12**	≤ 4,1
MNCV (m/sec)	NP	**11.1**	≥ 50
dCMAP wrist (mV)	NP	**1.6**	≥ 6
F-wave latency (ms)	NP	NA	≤ 31
F-wave frequency (%)	NP	NA	60-100
Right ulnar nerve			
DML (ms)	**5.3**	**8.2**	≤ 3.1
MNCV forearm (m/sec)	**47.1**	**8.6**	≥ 52
MNCV elbow (m/sec)	**23.3**	**8.3**	≥ 46
dCMAP wrist (mV)	9.4	**1.1**	≥ 7
F-wave latency (ms)	**53.3**	NA	≤ 31
F-wave frequency (%)	100	NA	70-100
Left ulnar nerve			
DML (ms)	NP	**8.5**	≤ 3.1
MNCV forearm (m/sec)	NP	**11.5**	≥ 52
MNCV elbow (m/sec)	NP	**10.1**	
dCMAP wrist (mV)	NP	**2.5**	≥ 3
F-wave frequency (%)	NP	NA	70-100
F-wave latency (ms)	NP	NA	≤ 31

Right Peroneal nerve			
DML (ms)	**16.9**	**NR**	≤ 5
MNCV (m/sec)	**29.5**	**NR**	≥ 41
dCMAP (mV)	**0.2**	**NR**	≥ 3
Left Peroneal nerve			
DML (ms)	NP	**NR**	≤ 5
MNCV (m/sec)	NP	**NR**	≥ 41
dCMAP (mV)	NP	**NR**	≥ 3
Right Tibial nerve			
DML (ms)	NP	**NR**	≤ 5
MNCV (m/sec)	NP	**NR**	≥ 40
dCMAP (mV)	NP	**NR**	≥ 5
F-wave latency (ms)	NP	NA	≤ 61
F-wave frequency (%)	NP	NA	100
Left Tibial nerve			
DML (ms)	NP	**NR**	< 5
MNCV (m/sec)	NP	**NR**	≥ 4
dCMAP (mV)	NP	**NR**	≥ 5
F-wave latency (ms)	NP	NA	≤ 61
F-wave frequency (%)	NP	NA	100

DML= distal motor latency; MNCV/SNCV= motor/sensory nerve conduction velocity; dCMAP= distal compound muscular action potential amplitude; SNAP= sensory nerve action potential amplitude; NA= not applicable; NP= not performed; NR= no response. Abnormal values are reported in bold.

Pathogenic considerations

NF155 protein is expressed at paranodes by the terminal loops of myelin and is associated with the axonal cell adhesion molecules CNTN1 and contactin-associated-protein-1 (Caspr1). This ternary complex determines the distribution of voltage-gated sodium channels (VGSCs) in clusters at the node. In particular, this junction acts as a barrier preventing lateral diffusion of VGSCs away from the node of Ranvier and also excluding the juxtaparanodal voltage-gated potassium channels from entering the nodal region *(Stathopoulos et al., 2015)*. IgG4 antibodies interfering with nodal structure can reduce the axolemmal excitability and impair saltatory conduction. In particular, the alteration of the function of the VGSCs causes the reverse of Na+ gradient across the membrane and consequent increased levels of intracellular calcium (Ca2+) mediated by Na+/Ca2+ exchanger *(Stathopoulos et al., 2015)*. The impairment of conduction at the nodes of Ranvier cause nerve conduction slowing and eventually nerve conduction block. Our patient fulfill the electrophysiological criteria for demyelination without the remyelinating processes, observed in typical CIDP. The prominent elongation of distal and F-wave latencies could be due to the prevalent Na channels inactivation mechanism in the more labile sites of the blood-brain barrier. In the predisposed axons, the massive Ca2+ entry lead to neuronal death. Probably the large fibers are more susceptible to the oxidative damage, in fact the large myelinated fibers are lost in sural nerve biopsy of this patient. On nerve conduction study proximal temporal dispersion is lacking according to morphologic data: absence of segmental demyelination/remyelination or onion bulbs and absence of macrophage mediated myelin stripping phenomena. In fact, the paranodal myelin widening is the peculiar aspect of the biopsy. The increased duration and fragmentation of the distal cMAP is not exclusively due to demyelination, because distal temporal dispersion can be induced by different factors, as cancellation between opposing phases of motor unit potentials in presence of reinnervation phenomenon. It is possible that nerve conduction blocks found in patients with

anti-NF-155 antibodies are due to paranodal dysfunction that morphologically is expressed by the terminal loops detachment *(Uncini et al., 2013)*. Axonal degeneration could be the effect of vacuole development at mesaxonal space due to myelin alteration caused by voltage-gated channels failure and not by macrophage activation. Therefore, paranodal dysfunction could be the primum movens for axonal degeneration.

The morphologic aspects found in this patient are explained by the fact that the anti-NF155 antibodies are IgG4. The mechanism of action of the IgG4s is demonstrated to be different from the other IgG, in particular the IgG4 do not activate complement, do not activate the production of pro-inflammatory cytokines and the macrophage migration from plasma to the nerve. These are the reasons why IVIG treatment is ineffective in most CIDP patients with IgG4 antibodies. In fact, IVIG have multiple mechanisms of action, in particular they reduce the expression of pro-inflammatory cytokines, prevent the interaction between T cell receptors and the APC cells, reduce the expression of endothelial cell adhesion molecules (ICAM-1) and block the complement activation by autoantibodies to their antigen. All these functions are ineffective when the autoantibodies are IgG4.

Instead, the monoclonal antibody Rituximab targets the B cell specific molecule CD20 causing the B cells depletion. In fact Rituximab has been used successfully in some of these patients *(Mahajan et al., 2014)* as in our one.

This case supports the concept of nodo/paranodopathies as a different category of CIDP that include patients with antibodies against paranodal proteins such as NF155, CASPR1 and CNTN1. The absence of proximal temporal dispersion and the presence of axonal degeneration could be the distinctive electrophysiological findings for these CIDP variants.

Our patient has the longest disease duration never reported before, twelve years. During the disease course he maintained the same characteristics at neurological examination supporting the hypothesis that even if this form of CIDP is associated with axonal degeneration and poor response to conventional

therapies, it tends to remain stable throughout the years. These data suggest that perhaps only some nerve fibers are susceptible to the IgG4 mediated damage, probably the largest one, so that the ones who are involved at the beginning, are the ones who are involved during the disease course and the ones who are responsible for the clinical phenotype. Moreover, our patient responded to Rituximab after about twenty years from the onset, suggesting that some nerve fibers are blocked in a condition of reduced excitability due to the presence of IgG4. The remotion of these antibodies make possible a partial recovery as demonstrated by electrodiagnostic study.

A national study to characterize the incidence of antibodies against paranodal proteins in a large cohort of Italian patients and to define the clinical role of the different IgG isotypes

The prevalence of anti-CNTN1 and Nfasc155 IgG4 has been well documented in cohorts of Japanese patients *(Devaux et al., 2012; Ogata et al., 2015; Miura et al., 2015; Kadoya et al., 2016; Devaux et al., 2016)*. It has been shown that anti-CNTN1 IgG4 are pathogenic in animal models and have a functional blocking activity of myelinated fibers. In Europe, the study of these antibodies has been so far limited to small cohorts of Spanish, French and German patients *(Querol et al., 2013; Querol et al., 2014; e Ng JKM et al., 2012; Delmont et al., 2017)*, and there is little information on the role of antibodies of IgG1-3 isotype. Moreover, the prevalence of anti-Caspr1 antibodies is still unknown as well as their possible pathogenic implication.

To test frequency of antibodies to nodal/paranodal proteins in CIDP we took part to a multicentric study that involved 11 Italian centers with specific expertise in neuromuscular disorders. We collected sera from 342 CIDP patients (306 typical and 36 atypical) and tested them for antibodies to Nfasc155, CNTN1, Nfasc140/186 and Caspr1 in two independent laboratories (IRCCS Mondino Foundation, National Neurological Institute of Pavia, Italy, and

Institute for Neurosciences of Montpellier, France). We also tested the antibodies in 286 controls patients, including healthy people and patients with other neuropathies or multiple sclerosis.

Out of 342 sera from CIDP patients tested, ten (3%) were positive for antibodies to anti-Nfasc155. IgG isotypes were IgG4 in seven patients, IgG3 in one and undetectable in two**.** One of these latter patients showed IgG4 reactivity against Nfasc140/186. Three patients (1%) were positive for anti-CNTN1 antibodies. IgG isotype was IgG4 in two, and mixed IgG3/IgG4 in one. Six patients (2%) showed reactivity against Caspr1. Three had IgG4 predominant isotype, two had IgG1 isotype, and one did not have a detectable isotype. Overall nineteen patients (6%) had antibodies against one of these four targets. None of healthy or pathologic controls and none of patients with atypical CIDP resulted positive. These observations mean that the tests have 100% of specificity and that the search for antibodies against nodal/paranodal component may be limited to typical CIDP cases *(Cortese et al., 2020)*.

Overall, the prevalence of anti-Nfasc155 and anti-CNTN1 antibodies in our population was similar to that reported by previous studies in European patients, but lower compared to Japanese patients *(Ogata et al., 2015; Devaux et al., 2016)* probably because of differences in inclusion criteria and non-standardized laboratory techniques. Moreover, frequency of anti-Caspr1 IgG4 antibodies was equal to that of antibodies against CNTN1, confirming that Caspr1 may also represent a relevant target of the immune-response in Caucasian CIDP patients.

Table 4. Clinical features of patients with chronic inflammatory demyelinating polyradiculoneuropathy and antibodies to Nfasc155, CNTN1, Caspr1.

	autoantibody seronegative (N=64)	anti-paranodal proteins IgG4 (N=13)	anti-Nfasc155 IgG4 (N=7)	anti-CNTN1 IgG4 (N=3)	anti-Caspr1 IgG4 (N=3)	anti-IgG1-3 or undetectable anti-Nfasc155 or anti-Caspr1 IgG subclass (N=5)
Age at Onset	39 (18 - 64)	36 (13-82)	22 (13-63)	58 (30 -82)	46 (24-57)	56 (7-74)
Early onset (<30 y)	7 (11%)	5 (38%)*	4 ***	0	1	2 (40%)
Male gender	42 (66%)	9 (69%)	4	3	2	2 (40%)
M-protein	15 (23%)	0 (0%)	0	0	0	0 (0%)
Clinical phenotype						
Typical	52 (81%)	13 (100%)	7	3	3	5 (100%)
Atypical	12 (19%)	0 (0%)	0	0	0	0 (0%)
Subacute onset	18 (28%)	9 (69%)**	5 *	2	2	1 (20%)
Weakness moderate/severe						
UL proximal	12 (19%)	4 (31%)	0	2	2	0 (0%)
UL distal	32 (50%)	8 (61%)	3	2	3	1 (20%)
LL proximal	16 (25%)	6 (46%)	1	2	3	1 (20%)
LL distal	37 (58%)	12 (92%)*	7*	2	3	3 (60%)
Pinprick sensation hallux						
Reduced	43 (67%)	10 (83%)	5	3	3	3 (60%)
Abolished	2 (3%)	1 (8%)	1	0	0	0 (0%)
Position sensation hallux						
Reduced	27 (43%)	4 (30%)**	3	0	1	2 (40%)
Abolished	8 (13%)	8 (61%)**	3	3 *	2	0 (0%)
Sensory ataxia	22 (35%)	11 (85%)**	6*	3 *	2	4 (80%)
Tremor	8 (12%)	9 (69%)***	7***	1	1	2 (40%)
Pain	23 (36%)	3 (23%)	1	2	0	0 (0%)
CSF protein	96 (24-55)	350 (128-679)***	278 (142-679)	148 (128-350)	426 (343-510)	68 (45-586)
ONLS	5 (1-11)	8 (3-10)*	5 (3-9)	8 (5-10)	8 (8-9)	3 (2-5)
Response to IVIG						
No	4 (7%)	4 (31%)***	1	2	1	0 (0%)
Partial/transi	14 (26%)	8 (61%)***	5	1	2	1 (25%)
tory	37 (67%)	1 (8%)***	1*	0**	0	3 (75%)
Good						
Response to steroids						
No	14 (28.5%)	3 (25%)	0	2	1	1 (50%)
Partial/transi	21 (43%)	6 (50%)	4	1	1	1 (50%)
tory	14 (28.5%)	3 (25%)	2	0	1	0 (0%)
Good						
Response to PEX						
No	1 (8%)	2 (67%)	0	1	1	0 (0%)
Partial/transi	6 (46%)	0 (0%)	0	0	0	0 (0%)
tory	6 (46%)	1 (33%)	0	1	0	0 (0%)
Good						
Response to immune suppressors						
No	5 (19%)	0 (0%)	0	0	0	0 (0%)
Partial/transi	13 (50%	1 (20%)	1	0	0	0 (0%)
tory	8 (31%)	4 (80%)	1	2	1	0 (0%)
Good						

*UL upper limbs, LL lower limbs, CSF cerebrospinal fluid, NCS nerve conduction studies, IVIG intravenous immunoglobulins, PEX plasma exchange. * p<0.05, ** p<0.01, ***p<0.005*

Comparing the different subclasses of patients, we found that anti-Nfasc155 and anti-Caspr1 IgG4 seropositive patients had more frequently an early disease onset compared to anti-CNTN1 IgG4 seropositive patients and controls. A subacute onset and tremor were reported in the majority of IgG4 seropositive patients compared to seronegative patients and the CIDP phenotype was always typical. A severe proprioceptive loss and sensory ataxia were observed in all patients with anti-CNTN1 IgG4 and in most of anti-Caspr1 and anti-Nfasc155 IgG4 seropositive patients (Table 4). In anti-CNTN1 and anti-Caspr1 IgG4 seropositive patients, the weakness was moderate/severe both in proximal and distal muscle groups, while in patients with anti-Nfasc155 IgG4 antibodies it was more expressed distally in the lower limbs.

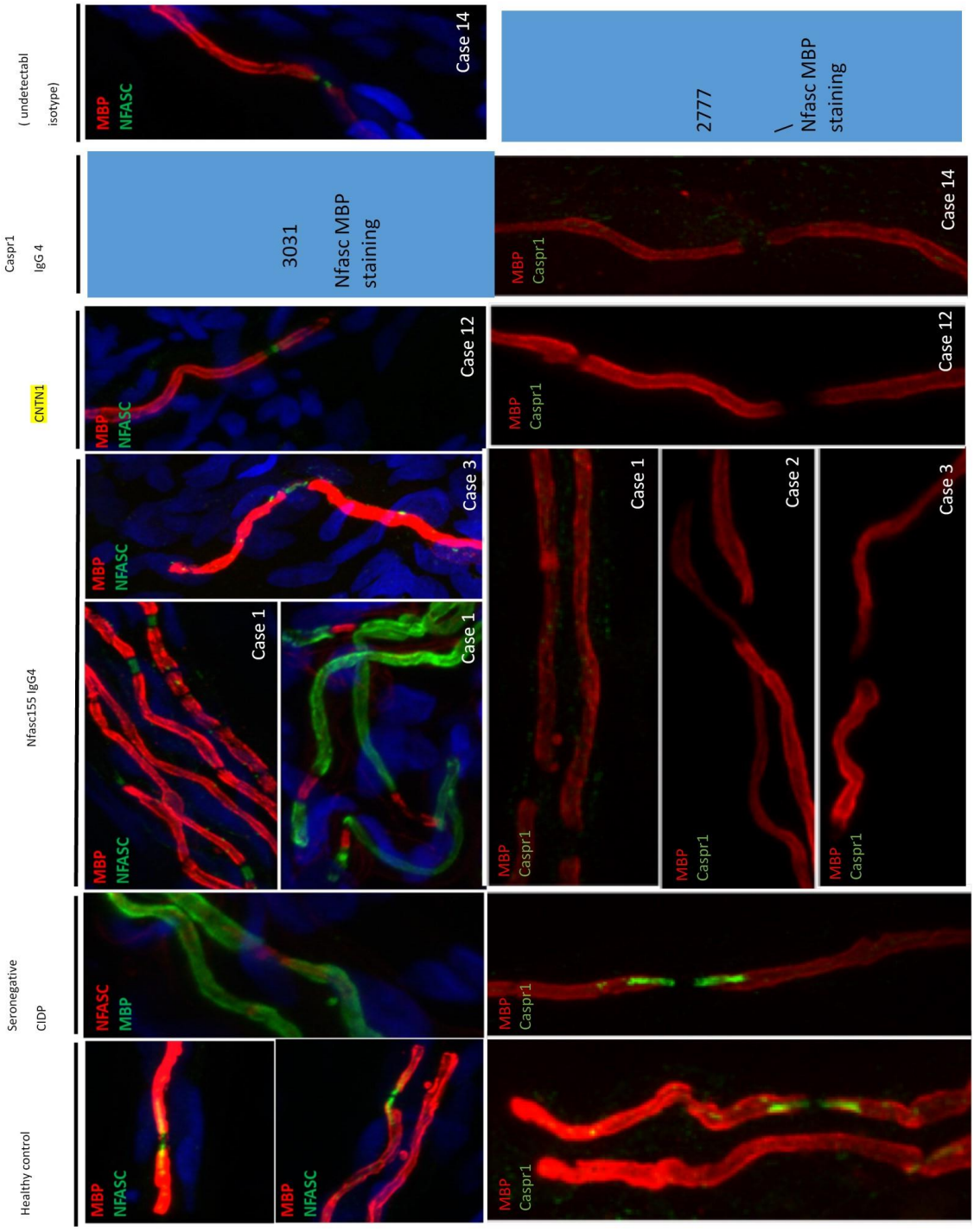
IgG4 seropositive patients had a higher level of CSF total protein and patients with anti-CNTN1 or anti-Caspr1 IgG4 antibodies showed the highest disability at onset.

Patients with IgG4 antibodies had a lower response rate to IVIG compared to seronegative patients but the same response to steroids or immunosuppressive treatment. In other words, our findings largely confirm previous observations on clinical serological correlation of antibodies to nodal/paranodal proteins and specific clinical features. In particular, we confirmed that the patients with anti-Nfasc155 IgG4 antibodies showed earlier onset, distal predominant lower limb weakness, gait disturbance and tremor, although only in one case this was disabling. We found that the patients with anti-CNTN1 antibodies were older and showed a subacute severe sensory-motor neuropathy, while previous studies found either a predominant motor or sensory impairment. Finally, our patients with anti-Caspr1 IgG4 antibodies had a highly debilitating neuropathy, but pain did not seem to be a clinical feature associated with the presence of anti-Caspr1 antibodies in our series, as opposed to the first report of two patients with Caspr1-associated inflammatory neuropathy *(Doppler et al., 2016)*.

As a novel observation of our study, we found that patients with anti-Nfasc155, CNTN1 or Caspr1 antibodies of IgG1, IgG3 or undetectable IgG isotype did not show clinical features or response to treatment distinct from seronegative patients. In particular, patients with IgG4 antibodies, but not patients with antibodies of IgG1, IgG3 or undetectable IgG isotype, showed a significantly lower response rate to IVIG compared to seronegative patients, while immunosuppressive treatment, including rituximab, cyclophosphamide and methotrexate, seemed effective in IVIG-resistant IgG4 seropositive patients, if started early in the disease course. Of note, we did not detect a significant difference in the response to steroids between patients with anti-paranodal IgG4 antibodies and seronegative patients, confirming steroids as an effective therapeutic option in CIDP cases, independently from their serological status for anti-Nfasc155, CNTN1 or Caspr1 antibodies.

We then evaluated skin biopsies from three patients with anti-Nfasc155 IgG4 antibodies, one patient with anti-CNTN1 IgG3/IgG4 antibodies, one patient with anti-Caspr1 IgG4 antibodies, one patient with anti-Nfasc155 antibodies of undetectable isotype and six seronegative CIDP patients. Analysis of myelinated fibers from patients with anti-Nfasc155 and anti-Caspr1 IgG4 showed elongation of nodes of Ranvier and loss of paranodal Nfasc155 and Caspr1 staining. Moderate elongation of node of Ranvier and loss of Nfasc155 paranodal staining were also observed in myelinated fibers of the patient with anti-CNTN1 IgG3/IgG4 antibodies. Contrarily, we did not observe similar changes in the patient with anti-Nfasc155 antibodies with undetectable isotype, in seronegative CIDP patients or healthy controls (Fig. 4). These data confirm the other recent histopathological and neurophysiological observations in patients with anti-nodal/paranodal antibodies *(Vallat et al., 2017; Uncini et al., 2018)* and indicate that isotype determination is crucial in order to correctly identify such patients and to guide treatment.

Figure 4: Morphological alterations of Ranvier node ~~of~~ in CIDP patients with IgG4 autoantibodies *(Cortese et al., 2020)*



We evaluated skin biopsies from three patients with IgG4 anti-Nfasc155 antibodies, one patient with IgG3/IgG4 anti-CNTN1 antibodies, one patient with IgG4 anti-Caspr1, one patient with undetectable isotype IgG anti-Nfasc155 and six seronegative CIDP patients. Analysis of myelinated fibers showed elongation of nodes of Ranvier

and a loss of paranodal Nfasc155 staining in skin biopsies from patients with anti-Nfasc155 (C) and Caspr1 (E) IgG4. Moderate elongation of node of Ranvier and loss of Nfasc155 paranodal staining were also observed in myelinated fibers of a CNTN1 IgG3/IgG4 positive patient (D). Contrarily, we did not observe similar changes the patient with undetectable isotype IgG anti-Nfasc155 antibodies (F), in seronegative CIDP patients (B) or healthy controls (A). A complete loss of Caspr1 staining was observed in biopsies from patients with IgG4 antibodies to paranodal proteins (I, L, M), but not in Nfasc155 seropositive patient with undetectable isotype (N), seronegative CIDP (H) or healthy patients (G).

The high frequency of anti-Caspr1 antibodies in our series prompted us to further investigate their pathogenic effects. So that, we demonstrated, throughout a cell aggregation assay, that anti-Caspr1 IgG4 and not IgG1 antibodies disrupt the interaction between CNTN1/Caspr1 and Nfasc155 *(Cortese et al., 2020)*.

These findings are in line with the previously reported ones, suggesting that IgG4 antibodies *(Manso et al., 2016; Labasque et al., 2014)* targeting the CNTN1/Caspr1 complex may have a function blocking activity and disrupt the paranodal axo-glial contact penetrating the paranodal regions. This means that therapies aiming at down-regulating the humoral immune response, such as Rituximab, can have some efficacy also in anti-Caspr1 antibody-associated CIDP *(Querol et al., 2015)*.

In conclusion, testing for the presence of antibodies against Nfasc155, CNTN1 and Caspr1 followed by IgG isotype determination in seropositive cases should be part of the diagnostic work-up in inflammatory neuropathies, in order to improve diagnostic accuracy and guide treatment. Moreover, knowledge of the mechanism underlying these CIDP subtypes, might shed light on the pathophysiology, and help further understanding of this complex and heterogeneous disease.

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