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OPTIMIZING DIAGNOSIS AND FOLLOW-UP OF HEREDITARY TRANSTHYRETIN AMYLOIDOSIS (hATTR)

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INTRODUCTION

Hereditary transthyretin amyloidosis (hATTR) is a rare genetic disease with autosomal dominant inheritance caused by mutations in the gene encoding the transthyretin (TTR) protein. It is a progressive, disabling and, if untreated, lethal disease [**Sekijima, 2001**].

Transthyretin is a plasma protein, produced mainly in the liver, and to a lesser extent in the choroid plexus and retinal pigment epithelium. TTR is assembled in tetramers and has a transport function of thyroxine and retinol [**Vieira, 2014**]. When mutated, TTR tetramer is unstable, tends to dissociation, misfolds in monomers and finally forms fibrillar aggregates of amyloid protein [**Yee, 2019**]. Deposits of amyloid fibrils accumulate in the tissue, mainly in the peripheral nervous system (somatic and autonomic) and in the myocardium, but also in the gastrointestinal tract, kidneys, eyes and leptomeningeal vessels [**Adams, 2019**].

hATTR is a worldwide disease, with over 130 known pathogenetic variants of the *TTR* gene [**Parman, 2016**]. In some areas of the world, such as Portugal, Sweden, Japan, Brazil, the island of Mallorca and Cyprus, hATTR is endemic and the Val30Met mutation is found in almost all patients and determines a phenotype with early onset (<50 years) characterized by an equal distribution in both sexes, complete penetrance, marked involvement of small fibres and cardiac involvement represented by rhythm disturbances [**Waddington-Cruz, 2021**]. In the rest of the world and therefore in Italy, hATTR is a rare (non-endemic) disease and displays a great genotypic heterogeneity, since, in addition to the Val30Met mutation, several other (non-Val30Met) pathogenetic mutations are observed [**Rapezzi, 2013;**

Russo, 2020]. Furthermore, unlike what occurs in endemic areas, the usual phenotype is typically characterized by late onset (late-onset Val30Met and non-Val30Met: ≥50 years), prevalence in males, incomplete penetrance, predominant involvement of large fibre and cardiac involvement characterized by infiltrative cardiomyopathy [**Zivkovic, 2019**].

The spectrum of clinical manifestations of hATTR is rather heterogeneous and is influenced both by the geographical area and by the type of mutation, since some variants have a predominantly "neuropathic" involvement, whilst others predominantly "cardiopathic". However, a concomitant neuropathic and cardiac involvement (known as "mixed") is frequently present [**Maurer, 2019**].

The neurological involvement is represented by a length-dependent axonal sensory-motor neuropathy [**Kollmer, 2017**; **Luigetti, 2020b**]. Some manifestations may precede the onset of neuropathy, such as carpal tunnel syndrome, secondary to amyloid deposition in the flexor retinaculum [**Severi, 2022**], and lumbar canal stenosis, due to amyloid deposition in the yellow ligament [**Wininger, 2021**]. Extremely rare are central neurological manifestations (blindness, deafness, deficits of other cranial nerves, focal neurological deficits of a transient nature, ischemic stroke and cerebral haemorrhages), secondary to the deposition of amyloid in the leptomeningeal vessels [**Sousa, 2021**].

Cardiac involvement in late-onset forms is characterized by an infiltrative cardiomyopathy with preserved ejection fraction, sometimes accompanied by valve stenosis and insufficiency, while heart rhythm disturbances are more characteristic of early-onset phenotype [**Ruberg, 2019**].

Less frequent, although possible, are ocular damage, which can manifest with abnormalities of the conjunctival vessels, vitreous opacities, secondary glaucoma, retinal microangiopathy, keratoconjunctivitis sicca and pupillary anomalies, and renal damage, with microalbuminuria, proteinuria and renal failure in advance stage [**Luigetti, 2020a**].

The hATTR is therefore a progressive pathology, which leads the patient to lose autonomous walking within 3-5 years, and to exitus, due to the onset of cardiological complications, after about 7-10 years [**Adams, 2019**]. The deterioration of the autonomous gait is used to define the stage of the disease, according to the classification of FAP (Familial Amyloid Polyneuropathy) stage: asymptomatic subjects (FAP 0), those with sensory disorders but able to walk independently (FAP 1), those who need a support for walking (FAP 2), and finally those wheelchair bound (FAP 3).

Unfortunately, in non-endemic area diagnosis can be delayed by 3-4 years [**Adams, 2019**] since a correct diagnosis is challenging for clinician. Patients with hATTR amyloidosis experience multiple neurological and/or cardiovascular testing and hospitalization prior to achieve the diagnosis [**Vera-Llonch, 2021**]. In 32-74% of cases, patients receive misdiagnoses [**Cortese, 2017**] and undergo inadequate or inappropriate treatments. Misdiagnoses are due to the lack of family history, the heterogeneous initial clinical manifestations and nerve conduction studies (NCS) that could show some demyelinating features [**Tozza, 2021**] and pathological examinations (abdominal fat and sural nerve biopsy) negative for amyloid depositions [**Luigetti, 2013**].

Based on disease's red flags, suspicion index of hATTR amyloidosis was proposed to preciously recognize hATTR and avoid diagnostic delay [**Adams, 2021**]. Suspicion index is based on the presence of a progressive polyneuropathy in addition to at least one red flag symptom suggestive of multisystemic involvement. However, sometimes the demonstration of a progressive neuropathy requires follow-up evaluations, risking wasting time. Moreover, some red flags (e.g. cardiomyopathy or vitreous opacities) need specialist evaluations that could be often lacking during the first neurological evaluation [**Luigetti, 2020a**].

While in the past the only therapeutic option was represented by liver transplantation, more recently various pharmacological alternatives have become available. The first approved drug was Tafamidis, a tetramer stabilizer, capable of hindering misfolding and fibrillogenesis, with an indication for the treatment of patients with the first stage of the disease (FAP 1) [**Coelho, 2012**]. More recently, two alternatives have become available capable of modifying the natural history of the disease: an antisense oligonucleotide (ASO), Inotersen, and a small interference RNA (siRNA), Patisiran, both effective in suppressing the hepatic synthesis of transthyretin, and thereby arresting disease progression, indicated for the treatment of patients in FAP stage 1 and 2 [**Adams, 2018; Benson, 2018b**]. All treatments demonstrated safety and efficacy in halt disease progression in real life reports as well [**Cortese, 2016, Di Stefano, 2022; Luigetti, 2022**].

The follow-up of symptomatic patients generally takes place every six months and is based on a multidisciplinary approach, including neurological and cardiological expertise, but nephrological specialists, nutritionists, otolaryngologists, ophthalmologists, physiatrists, psychologists are often needed [**Conceição, 2019**].

Neurological monitoring provides, in addition to instrumental test such as electrophysiological examination, neurological objective examination and the application of clinical scales and validated questionnaires, which allow a longitudinal comparison of follow-up in the same subject and thus an evaluation about the response to therapy.

Among these, the most used are the Neuropathy Impairment Score (NIS), a composite score that measures the muscle strength, sensations according to various sensory modalities and deep tendon reflexes [**Dyck, 2019**].

The Norfolk-quality of life-Diabetic Neuropathy scale (Norfolk QOL-DN) [**Vinik, 2014**] and the Short Form Health Survey 36 (SF-36) questionnaire are questionnaires that aim to quantify the state of health and the impact of the disease on daily activities.

The Composite Autonomic Symptom Score 31 (COMPASS-31) [**Sletten, 2012**] and the Compound Autonomic Dysfunction Test (CADT) [**Denier, 2007**] are both self-assessment questionnaires for autonomic symptoms.

Finally, the Rasch-built Overall Disability Scale (R-ODS, validated for dysimmune neuropathies) [**Pruppers, 2015**] measures the impact of the disease on autonomy in daily life activities [**Conceição, 2019; Adams 2021**].

Despite the variety of clinical scales and questionnaires used in clinical practice as indicators of the disease status in hATTR, there is no validated scale yet, which is simple to use in an outpatient clinic and is specific for this pathology, as occurs for Charcot-Marie-Tooth patient. In fact, for this condition there is a specific score (CMT Neuropathy Score), easy to apply and able to provide a whole evaluation of the patient [**Shy, 2005; Murphy, 2011**].

The Neuropathy Impairment Score (NIS) and the Neuropathy Impairment Score Lower Limbs (NIS-LL) subscore have been widely used in studies and clinical trials of hATTR patients for evaluating the efficacy of therapies [**Dick, 2005**]. Nevertheless, NIS has been developed to be used in a large group of neuropathies, so its efficacy in detecting changes of the disease progression in hATTR is limited due to the peculiar characteristics [**Dyck, 1997; Dyck, 2005**].

The NIS is a score ranging from 0 to 244 (higher score indicates a greater neurological impairment). It is a composite score of clinical impairments (weakness, reflex loss, and sensory loss) using standard assessment of muscle weakness (from 0 normal to 4 paralysis) of cranial district, upper and lower limbs, reflexes (from 0 normal, 1 reduced and 2 absent) and sensory modalities (from 0 normal, 1 reduced and 2 absent) at the distal phalanges of the hand and foot. An increase in NIS greater than 10 points from previous assessments has been proposed as an indicator of disease progression [**Conceição, 2019**].

Moreover, the NIS-LL is a subset of the NIS using measurements that quantify weakness, reflexes, and sensation in the lower limbs only, and has been primarily utilized in evaluation of length-dependent neuropathies that affect the longer nerve fibres of the lower limbs.

Nevertheless, there are several limitations relating to the use of the NIS and NIS-LL scales in the hATTR. First, the assessment of muscle weakness also includes the cranial district, barely involved in this pathology, which cannot be evaluated in the same way of the limb muscles.

Furthermore, the study of sensory impairment is limited to the distal parts of the body (distal phalanx of the second finger and big toe), and this generates a threshold

effect with rapid saturation of the score, since this type of evaluation is not able to discriminate patients with reduced sensibility limited to the distal phalanges respect to those with a reduced sensibility extended more proximally.

Lastly, the NIS includes the evaluation of reflexes, which can have a higher inter-operator variability and may be already absent in the initial stages of the disease.

Furthermore, the NIS-LL subscore, validated for the evaluation of length-dependent neuropathies, includes only the items of the lower limbs and overlooks the upper limbs, which instead can be particularly compromised in the advanced stages of the disease, realizing a further threshold effect [**Conceição, 2019**].

To better reflect the features of hATTR polyneuropathy and to identify more sensitively the disease progression or improvement, NIS was therefore modified to obtain NIS+7, and subsequently the modified NIS+7 (mNIS+7) [**Dyck, 2019**].

The NIS+7 scale uses the same weakness, reflexes, and sensation measures as the NIS, combined with seven additional assessments that were included to better characterize and estimate neuropathic impairment. Five of these additional assessments are nerve conduction studies (NCS), focused on 3 nerves in the lower limbs (tibial nerve distal motor latency; peroneal nerve compound muscle action potential amplitude, distal motor latency and conduction velocity; sural sensory nerve action potential amplitude). The additional two components of the NIS+7 are vibration detection threshold (VDT) at the great toe and heart rate response to deep breathing (HRdb), a measure of autonomic dysfunction.

mNIS+7 was developed from NIS+7 to measure neurologic impairment in controlled trials of hATTR amyloidosis. VDT was replaced by the Quantitative

Sensory Test (QST) and extends the evaluation of the nerve conduction study to the upper limbs [**Dick, 2019**].

The mNIS+7, through the inclusion of evaluation parameters of the autonomic and somatic small fibres, and the elimination of some items burdened by the threshold effect, is more sensitive, compared to previous versions of the NIS, in identifying changes in the disease state in the patient with hATTR.

Nevertheless, it is a tool hard to use in contexts other than clinical trials, since it need instrument not widely diffuse, not always feasible in the absence of highly specialized medical personnel and that are particularly time-consuming.

During my PhD program I focused my interest in hATTR disease, especially in the multidisciplinary assessment that these patients deserve and in the clinical research about some aspects still unsettled or not investigated. Therefore, the first aim of my PhD thesis (AIM #1) has been to optimize the diagnosis of hATTR neuropathy through a compound diagnostic score in order to increase the suspicion of hATTR neuropathy and thus shorten the diagnostic delay. The second aim (AIM #2) was to improve the follow-up of hATTR patient by creating a specific evaluation score for hATTR disease, drawn on disease peculiarities, that may be able to assess neurological disability, sensitive in identifying changes in the disease progression and quick and easy to apply in the daily clinical practice.

METHODS

AIM #1

To optimize the diagnosis of hATTR neuropathy, 35 hATTR patients and 55 patients with chronic idiopathic axonal polyneuropathy (CIAP) were retrospectively analysed. All patients underwent clinical assessment, nerve conduction study and Sanger sequencing of *TTR* gene. hATTR patients were defined as patients with axonal polyneuropathy carrying TTR pathogenic variant. CIAP patients were defined as patients with at least of 6-months history of axonal sensory-motor polyneuropathy resulted negative for *TTR* variant and for other causes of neuropathy through appropriate investigations [**Zis, 2016**]. In particular, all CIAP patients had not family history nor signs of hereditary neuropathy (e.i. pes cavus), no metabolic (diabetes, liver, renal or thyroid dysfunction), deficiency (vitamin B12, thiamine or pyridoxine deficiency), toxic (no history of exposure to alcohol, neurotoxic agents, drugs or chemotherapy), immunological (rheumatological, paraneoplastic or celiac disease), haematological (paraproteinemic syndrome as AL amyloidosis or POEMS) and infective (HBV, HCV, HIV) causes were identified as aetiology of neuropathy. CIAP patients were not examined for other possible hereditary late-onset chronic axonal neuropathies (e.g. CMT or CANVAS).

As clinical data, we collected gender, age of onset, disease duration (time between age of onset and first evaluation) and the presence at first evaluation of 1) family history of polyneuropathy, 2) progressive disturbance in the last 6 months as perceived by patients, 3) muscle weakness, 4) positive and negative sensory

symptoms (i.e. tingling and numbness), 5) autonomic symptoms (i.e. erectile dysfunction, diarrhoea/constipation, nausea and vomiting, sweating abnormalities) 6) carpal tunnel syndrome (CTS) history. Moreover, we collected data about the walking impairment (0= no walking difficulties; 1= walking difficulties but independent; 2= needing support; 3= wheelchair bound).

As electrophysiological features, we collected amplitude of compound muscular action potential (CMAP; mV), distal motor latency (DML; ms) and motor nerve conduction velocity (MNCV; m/s) of the median, ulnar, tibial and peroneal nerves. Moreover, we collected amplitude of sensory action potential (SAP; μV) and sensory nerve conduction velocity (SNCV; m/s) of median, ulnar, peroneal superficial and sural nerves. Moreover, SAP and CMAP amplitude values were categorized in normal (0), reduced (1) and absent (2) according to the normal value of the centre.

Statistical analysis

Descriptive statistics were based on mean±standard deviation in the case of continuous variables and on frequencies (percentage) in the case of categorical variables. Statistical differences between hATTR and CIAP groups were performed through Pearson's chi-squared test for categorical variables and Student's T test for continuous variables. P-values less than 0.05 were deemed as statistically significant. Based on the significant difference between two groups, a compound (clinical and electrophysiological) score (Figure 2) was arranged (ranging from 0 to 12) assigning the highest scores to each variable that were more frequently

abnormal in hATTR patients as shown by the comparison analysis between the two groups.

Figure 2. Composite clinical and electrophysiological score

Clinical	0	1	
Muscle weakness	No	Yes	
CTS history	No	Yes	
Electrophysiological	**0**	**1**	**2**
Median SAP	Normal	Reduced	Absent
Ulnar SAP	Normal	Reduced	Absent
Median CMAP	Normal	Reduced	Absent
Ulnar CMAP	Normal	Reduced	Absent
Tibial CMAP	Normal	Reduced	Absent

CTS= Carpal tunnel syndrome; SAP= Sensory Action Potential; CMAP= Compound Motor Action Potential.

The diagnostic score was constituted by 7 total items: motor symptoms (0=none, 1=present), CTS history (0-1), Median SAP (0= normal, 1= reduced; 2=absent), Ulnar SAP (0-2), Median CMAP (0-2), Ulnar CMAP (0-2) and Tibial CMAP (0-2). The receiving operating characteristics (ROC) analysis were used to discriminate groups using the total score. To test the difference between hATTR and CIAP patients with short disease duration, we performed a sub-analysis on the patients with disease duration <2 years through T-student test.

AIM #2

To create a score able to provide a whole assessment of the neurological impairment in hATTR patients, we used the CMTNS as a model. It is a scale that is easy to use and at the same time useful in evaluating the patient suffering from Charcot-Marie-

Tooth disease, through the subscores "symptoms", "signs" and "neurophysiology". Therefore, we included in the hATTR Neuropathy Score (hATTRNS), in addition to the neurological physical examination, some items concerning the characteristic symptoms reported by patients. Conversely, we decided to exclude neurophysiology, in order to make the score applicable even without instrumentation and medical personnel specialized in neurophysiological techniques.

For the realization of the score, we then took into consideration the critical points of the NIS. First of all, we excluded the cranial district from the assessment of muscle strength, which is rarely compromised in the hATTR, and for which it is difficult to accurately attribute the degree of weakness. After, we did not include the evaluation of deep tendon reflexes (often significantly reduced or absent already in the initial stages, and therefore poorly suited to reflect real disease progression). To better evaluate the sensation and avoid the threshold effect, we designed the related items in order to assign a progressively higher score, following the distal-proximal extension of the disorder. Lastly, to evaluate muscle strength in a standardized way we used the MRC (Medical Research Council) score, which is also the most widely used in neurological clinical practice.

hATTRNS provides a score ranging from 0 to 40 (higher score indicates greater neurological disability) and consists of 10 items, divided into a section relating to the symptoms reported by the patient, and one concerning the neurological physical examination (Figure 1).

The "symptoms" section (0-16 points) is divided into 4 items: sensory symptoms, presence of neuropathic pain, weakness and autonomic symptoms. Regarding the

first three items, a point (+1) is attributed for each body district involved reported by the patient.

Specifically, sensory symptoms referred to both positive (tingling, pins and needles) and negative (reduced sensibility) symptoms but excluded those attributable to carpal tunnel syndrome. Neuropathic pain needs to be framed with specific questions (see instructions reported in supplementary materials).

Figure 1. hATTR Neuropathy Score (hATTRNS)

		0	(+1)	(+1)	(+1)	(+1)	Total
Symptoms	**Sensory Symptoms**	None	Feet	Legs	Hands	Arms	0-16
	Neuropathic Pain	None	Feet	Legs	Hands	Arms	
	Weakness	None	Legs	Thighs	Hands	Arms	
	Autonomic Symptoms	None	Orthostatic intolerance	Gastrointestinal domain	Secretomotor domain	Genitourinary domain	
		0	**1**	**2**	**3**	**4**	
Strength	**Upper limbs**	Normal	4 on FDI or ADM	≤3 on FDI or ADM	≤4 on wrist extensor or flexor muscles	≤4 on brachial biceps or triceps muscles	0-8
	Lower limbs	Normal	≤4 on digital extensor or flexor muscles	4 on foot dorsiflexor or plantar flexor muscles	≤3 on foot dorsiflexor or plantar flexor muscles	≤4 on quadriceps o biceps femoris muscles	
Sensation	**Pinprick**	Normal	Reduced up to Wrist / Ankle	Reduced up to Mid forearm / Mid leg	Reduced up to Elbow / Knee	Reduce above Elbow / Knee	0-16
	Tactile	Normal	Reduced up to Wrist / Ankle	Reduced up to Mid forearm / Mid leg	Reduced up to Elbow / Knee	Reduce above Elbow / Knee	
	Vibration	Normal	Reduced at V finger / great toe	Reduced at MCP / Ankle	Reduced at Wrist / Knee	Reduce at Elbow / ASIS	
	Joint position sense	Normal	Reduced at V finger / great toe	Reduced at MCP / Ankle	Reduced at Wrist / Knee	Reduce at Elbow / Hip	
							0-40

The autonomic symptoms included in the score were those most frequently complained by patients with hATTR in my clinical experience: orthostatic intolerance (feeling of "empty head", lipothymia, or syncope when passing in standing position), gastrointestinal disturbances (feeling of early satiety, postprandial reflux and vomiting, development of chronic constipation or

alternating constipation-diarrhoea), genitourinary disorders (erectile difficulties or impotence, urinating difficulty, loss of sphincter control), and disorders of secretory-glandular functions (reduced sweating, heat intolerance, dry eyes or mouth). For each domain involved a point (+1) is attributed.

The section concerning the neurological physical examination (0-24 points) is divided into the "strength" and "sensation" sections. For the examination of segmental strength, the score assigns an increasing score, from 0 to 4, based on the progressive involvement of gradually distal-proximal muscle segments, both for the upper and lower limbs. The sensation consists of 4 items, each dedicated to a specific sensory modality (tactile, pinprick, vibratory and joint position sense). In this case, the score takes into consideration the most compromised level between the upper and lower limbs, and assigns an increasing score, from 0 to 4, based on the distal-proximal extension of the sensory deficit.

We calculated the score retrospectively, collecting data from the follow-up carried out from 2011 to 2022 in the hATTR subjects followed at our centre. However, we only included patients for whom all the following clinical scales were available: NIS, Norfolk, CADT, COMPASS, R-ODS and DN4 (see supplementary materials).

Statistical analysis

The one-way ANOVA test was used to define the correlation between hATTRNS and its sub-scores (symptoms, strength and sensibility) with the FAP stage. Subsequently, Tukey's HSD was used in a post-hoc analysis to perform multiple comparisons between the total hATTRNS (and its sub-scores) and each FAP stages. Pearson's correlation coefficient was instead used to evaluate the correlation

between hATTRNS and the other outcome measures used in clinical practice (NIS, Norfolk, CADT, COMPASS, R-ODS and DN4) for hATTR patients.

Lastly, to test the ability of the score to evaluate clinical worsening, we decided to calculate the delta-hATTRNS (= last visit - previous visit) in patients for whom were available at least 2 consecutive evaluations, and compare it by Student's T-test between two groups of patients, divided on the basis of delta-NIS (= last visit - previous visit) greater than/equal (worsened patients) or less than 10 (stable patients).

RESULTS

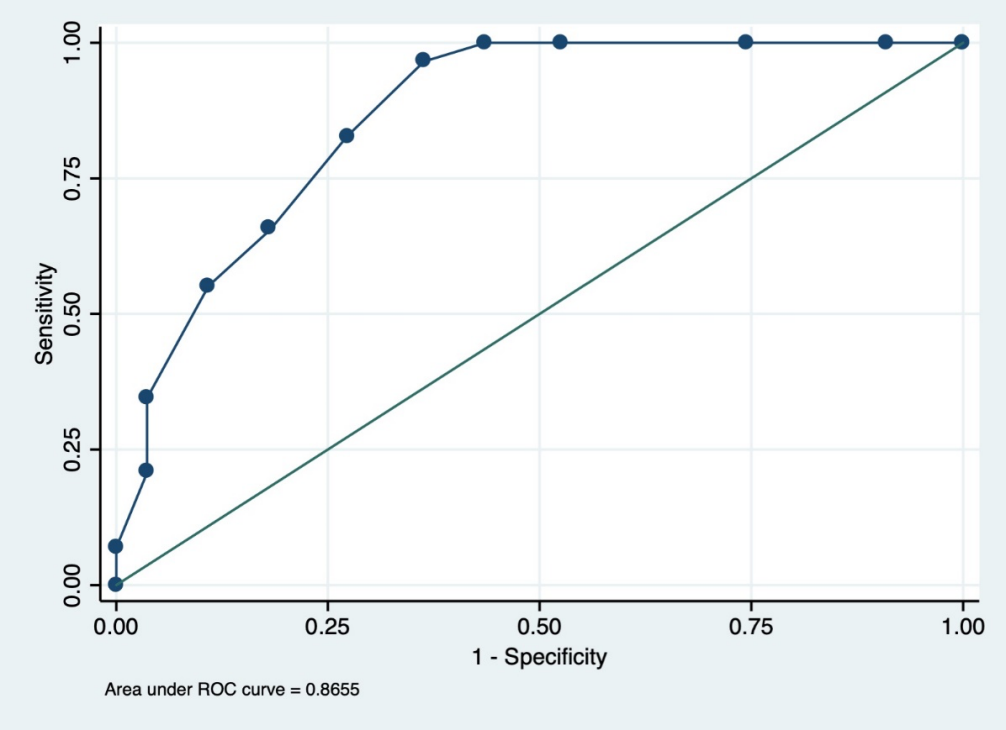
AIM #1

Clinical and electrophysiological findings were summarized in Table 1 in Tables section. Clinical data analysis showed that hATTR group had more frequently motor symptoms (p=0.002) and CTS history (p=0.001) respect CIAP patients. Moreover, patients carrying a TTR variant had a later age of onset respect patients with idiopathic neuropathy (64.3±9.9 vs 58.2±11.2; p=0.011). Conversely, no other clinical differences were found between two groups (gender, disease duration, progressive disease, sensory and autonomic symptoms, walking impairment) (Table 1 in Tables section).

Electrophysiological findings analysis showed that hATTR patients had a more reduced amplitude of SAP and CMAP in all examined nerves ($p<0.05$) (Table 2 in Tables section). In detail, hATTR group presented more frequently absent CMAP in tibial nerve (47% vs 29%), SAP in median (75% vs 24%) and ulnar (54% vs 26%) nerves, and more frequently reduced CMAP in median (75% vs 26%) and ulnar (82% vs 26%) CMAP (Table 1 in Tables section). Moreover, significant differences between two groups were MNCV across the elbow in the ulnar nerve and DML of peroneal nerve ($p<0.05$) (Table 2 in Tables section). Using ROC analysis, we established that the total score that best separated hATTR patients from CIAP was a value ≥5 (AUC = 0.86, Figure 3) with a sensitivity of 96.6% and a specificity of 63.6%. In particular, in the cohort a total score ≥5 points was present in 96.6% hATTR patients and in 36.4% CIAP patients. Lastly, the difference between the two groups with disease duration <2 years showed that the hATTR

patients had a greater score (11 patients; 7.4±1.2) respect to the CIAP patients (24 patients; 4±3.1) ($p<0.001$).

Figure 3. ROC analysis of composite score



ROC analysis of composite score in patients with HATTR and CIAP patients showing an area under the curve (AUC) of 0.8655.

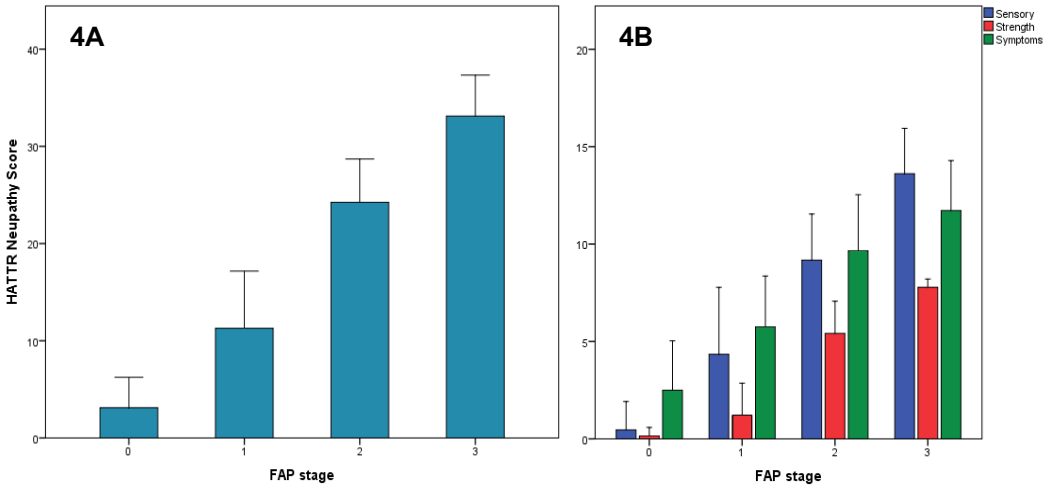
AIM #2

The demographic and clinical data are summarized in Table 3 in Tables section. Briefly, the cohort consisted of 43 individuals (31 males and 12 females). Twenty subjects carried Val30Met, 20 Phe64Leu, 2 Glu54Lys and 1 Val122Ile. Seventeen subjects were asymptomatic carriers (FAP 0), 14 patients were in FAP stage 1, 8 in FAP stage 2, and 4 in FAP stage 3. The average age at the first evaluation was 59.6±13.9 years (31-80). The total number of visits considered for computing

hATTRNS is equal to 127, with an average of 2.9±2 (1-8) visits per patient and a mean follow-up of 23.1±17 months (6-90).

The one-way ANOVA showed that the hATTRNS score is statistically associated with the FAP stage, both for the total score (F (3,123) = [211.7], p <0.001) (figure 4A), and for each subscore "symptoms" (F (3,123) = [61.4], p <0.001), "strength" (F (3,123) = [181.5], p <0.001) and "sensation" (F = 123.5, p <0.001) (figure 4B).

Figure 4. Comparison of hATTRNS respect to FAP stage



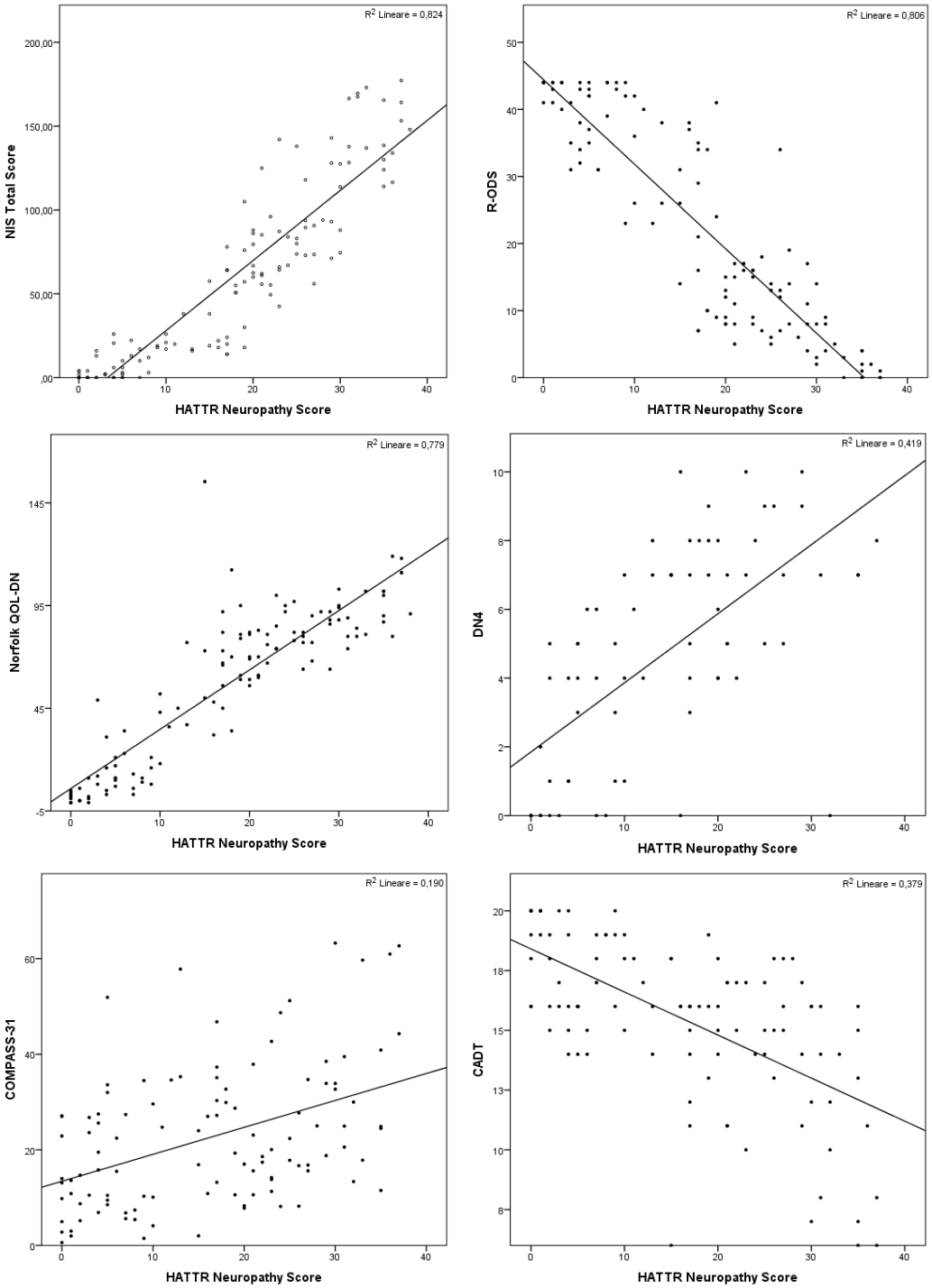
Comparison of mean hATTRNS (figure 4A) and its subscore (figure 4B) respect to FAP stages showed a significantly difference between each FAP stage.

The subsequent post-hoc analysis with Tukey's HSD test showed that the mean total hATTRNS score statistically significantly differed between the FAP 0 and FAP 1 groups (p<0.001, 95% C.I. = -11.24, -5.12), between the FAP 1 and FAP 2 groups (p<0.001, 95% C.I. = -15.66, -10.25) and between the FAP 2 and FAP 3 groups (p<0.001, 95% C.I. = -12.23, -5.52), and analogously the “symptoms”, “strength” and “sensation” sub-scores statistically significantly differed between each FAP stage.

Pearson's correlation coefficient showed that hATTRNS correlates positively with NIS ($r(124) = 0.9$, $p<0.001$), Norfolk ($r(122) = 0.88$, $p<0.001$), COMPASS ($r(107) = 0.43$, $p <0.001$) and DN4 ($r(70) = 0.64$, $p <0.001$), and negatively with R-ODS ($r(120) = -0.89$, $p <0.001$) and CADT ($r(124) = -.5$, $p <0.001$) (figure 5).

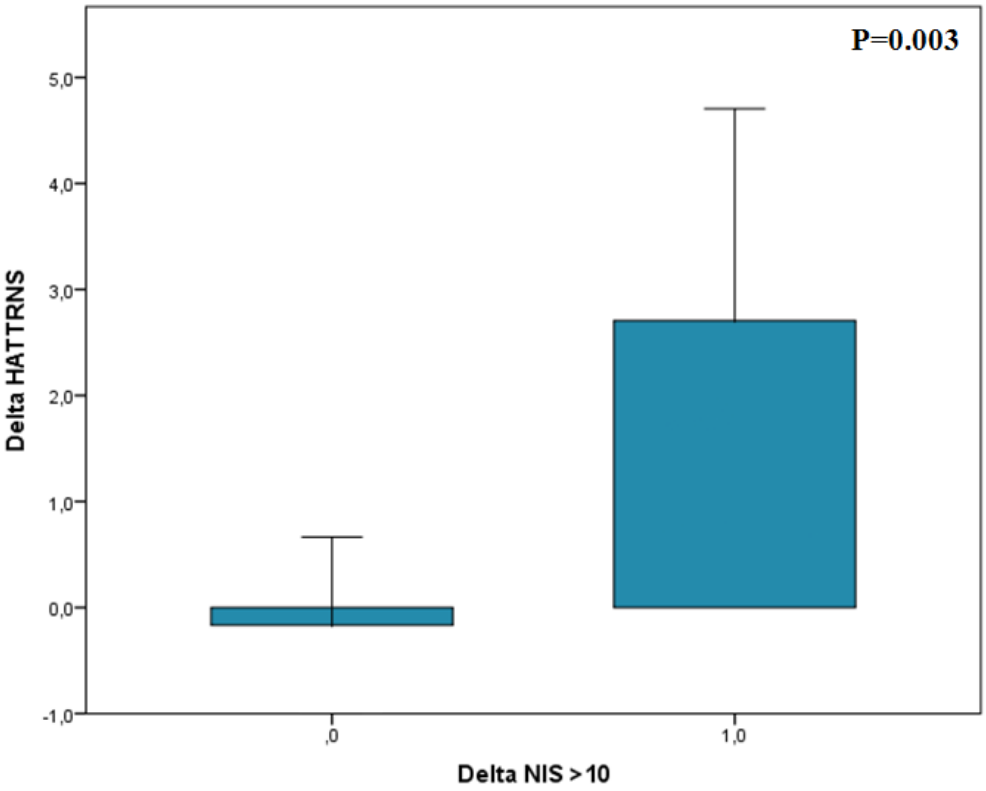
Student's T-test for independent variables showed a statistically significant difference ($p=0.003$) of the delta-hATTRNS score between patients who remained stable ($NIS<10$) at follow-up (-0.167 ± 3.3) compared to those had shown a neurological worsening ($NIS \geq 10$) (2.7 ± 3.8) at the follow-up visits (figure 6).

Figure 5. Correlation between hATTRNS and other outcome measures



hATTRNS correlates positively with NIS, Norfolk, COMPASS and DN4, and negatively with RODS and CADT.

Figure 6. Comparison of Delta hATTRNS between stable and worsen patients



The comparison of mean delta hATTRNS showed a statistically significant difference between patients that remained stable (0= NIS<10) at follow-up compared to those had shown a neurological worsening (1= NIS≥10).

DISCUSSION

hATTR represents one of few treatable hereditary rare diseases. In the last years, the possibility of having three available treatments to halt disease progression, has raised great attention about some unresolved questions. Though the clinical scientific interest on early diagnosis, patients with hATTR neuropathy still experience multiple testing and hospitalization prior to achieve the diagnosis (**Vera-Llonch, 2021**), leading to several misdiagnosis and a significant diagnostic delay.

The neuropathy in hATTR patients represents one of the most disabling and progressive condition and sometimes electrophysiological findings can misinterpreted by clinician although the neuropathy is due to a primary axonal degeneration [**Tozza, 2021**]. The first aim of this PhD thesis has targeted to mark the peculiar clinical and electrophysiological characteristics which can help clinicians to suspect hATTR among patients with axonal polyneuropathy.

Clinical findings showed that hATTR patients referred more frequently motor symptoms (86% vs 54%) and CTS history (57% vs 24%) respect patients with CIAP. These results confirmed that hATTR patients have a precocious involvement of motor system respect CIAP patients which complain especially sensory symptoms [**Singer, 2012**]. In fact, although statistical analysis missed to reach significance, only 50% (vs 70%) of hATTR patients can walk independently.

It is not surprising that no hATTR patients had a positive family history. In fact, in Italy the hATTR population is constituted by a late-onset phenotype [**Russo, 2020**],

characterized by a low penetrance and therefore a negative family history [**Manganelli, 2020**].

Moreover, in the cohort autonomic symptoms did not represent a discriminative feature. However, since the population was constituted by late-onset hATTR patients [**Russo, 2020**], the autonomic involvement at the disease onset is often subtle and undetected if not adequately investigated and become clinically prominent in advanced stage [**Manganelli, 2020**]. Another possible explanation for this lacking significant was that the autonomic dysfunction was detect through the reported symptoms during patient's interview and not by appropriate questionnaire or specific instrumental test (e.g. tilt test, Skin Sympathetic Response).

Lastly, disease progressivity unexpectedly did not differ between hATTR and CIAP patients. The reason of this result could be due to the retrospectively design of the study. In fact, the disease progression was considered as perceived by patients at first evaluation and not by follow-up evaluations. As occurs in other conditions, in which the patient perception of health status does not parallel functional and disability measures [**Tozza, 2018**], CIAP patients could perceive their neuropathy as progressive disease as well.

Electrophysiological findings showed that hATTR patients, although they had the same disease duration of CIAP patients, had a greater reduction of amplitude of potentials in all nerves with a more frequently absence of potential at lower limbs and reduction at upper limbs. The results confirmed that axonal degeneration is the primary pathomechanism in hATTR disease and suggest early involvement of upper limbs nerves respect to CIAP patients in which simultaneous development of upper and lower extremity rarely occurs [**Wolfe, 1999**]. Although hATTR

neuropathy is defined as length-dependent, the early involvement of upper limb nerves could be the expression of a ganglionopathic pattern damage [**Théaudin, 2019; Ayrignac, 2013**]. In hATTR amyloid accumulation starts in dorsal root ganglia and nerve roots and afterwards amyloid deposits spread through a proximo-distal gradient over time [**Tozza, 2021**].

Moreover, the results confirmed the role of CTS history as red flag of hATTR as it could precede by several years the onset of polyneuropathy [**Karam, 2019**]. Of interest, the electrophysiological data findings in symptomatic hATTR patients did not show a greater SNCV slowing and DML prolongation in median nerve respect to CIAP patients as expected. The findings suggest that in a patient with polyneuropathy the clinical history of CTS is important in the suspicion of ATTR rather than electrophysiological findings of CTS. In fact, the CTS physiopathology in hATTR patient seems to have a peculiar behaviour respect the idiopathic CTS. The ultrasound results showed that CTS in hATTR is characterized by a peculiar mismatch between electrophysiological and ultrasound abnormalities of the median nerve at wrist, differentiating from idiopathic CTS, in which ultrasound findings mirrors electrophysiology severity [**Salvalaggio, 2021**]. Altogether, we can suppose that the entrapment injury of the median nerve can occur in presymptomatic stage through the deposition of amyloid in the carpal ligament [**Samoes, 2017**], but contextually there is already a systemic damage of nerves that starts proximally [**Koike, 2009**].

Based on this peculiar characteristic of hATTR patients, we arranged a compound clinical and electrophysiological score. A total score $\geq$5 allows to identify with a sensitivity over 95% hATTR patients among subject with chronic axonal

polyneuropathy. A cut-off with higher sensitivity respect to the specificity was set, since the score was arranged as a screening tool. We opted to have more false positive respect to lose the possibility to detect an hATTR patient, given that the disease is debilitating but curable especially in the early stage [**Herman, 2006**]. Moreover, the score is able to discriminate hATTR also in patients with short disease duration (<2 years), strengthening that the hATTR lead a severe neuropathy since first years of disease.

The diagnostic score emphasizes the predominant motor involvement in hATTR disease respect CIAP and this difference might cause an imbalance between the two population. However, in front of a single patient with neuropathy, the severity of motor involvement can be difficult to valorise. In fact, CIAP still represents a common misdiagnosis for hATTR patients [**Gertz, 2020**]. The study identified the principal differences between the two groups and valorise them in a compound score which can be able to help clinician through a specific cut-off in order to recognize patients deserving TTR genetic analysis. Moreover, the diagnostic score is easily to perform during clinical and/or electrophysiological examination and it does not require other specialistic exam (e.g., cardiac imaging or ophthalmological examination to detect cardiomyopathy and vitreous corpus respectively).

If the score can influence marginally the choice to perform genetic analysis in a third level centre, where TTR genetic test is easily accessible, conversely it can help physician in primary centres, where the patients are evaluated for the first time and genetic test can be difficult to perform. Especially in this context, the application of the compound score in patients with sensory-motor neuropathy may have a major role, representing a first screening tool to drive the choice of referring patients in

an amyloidosis centre, avoiding wasting time and therefore shortening the time to reach a correct diagnosis.

The second aim of my PhD program has been to optimize the follow-up of hATTR patients. In fact, there is not currently clinical scale that considers all the distinguishing clinical features of hATTR, and all currently used outcome measures in clinical practice are generic and borrowed from other diseases.

Notably, the NIS, which is the most used clinical scale in hATTR, both in common clinical practice and in clinical trials, is a scale validated on patients with diabetic neuropathy [**Murphy, 2011**] and is not able to catch specific features of hATTR neuropathy as for example autonomic impairment.

Moreover, even if generally used as a primary outcome, the NIS is burdened by a considerable intra-operator variability, and it has a threshold effect on some sub-scores such as the NIS-LL and the items dedicated to sensations. On the other hand, the mNIS+7, that partially solves the limitation of the NIS, is difficult to apply, since it is time-consuming and requires adequate equipment and expert personnel. Therefore, there is not currently a clear assessment tool that is easy to use even in an outpatient setting.

We set up the hATTRNS, a clinical scale simple and quick to perform, tailored on symptoms and signs of the hATTR disease, and first providing the introduction of elements that make it more suitable for catching typical features of hATTR neuropathy. Interestingly, the hATTRNS and its sub-scores were correlated with the FAP stage. Indeed, patients with greater disability (from 0 to 3) have a higher

score. Moreover, the hATTRNS correlated with the other outcome measures used for assessing the involvement of the large (NIS, RODS) and the small (COMPASS, CADT, DN4) nerve fibres as well as the quality of life (Norfolk) in hATTR neuropathy. Lastly, the comparison analysis between the stable (NIS change <10 points) and worsened patients (NIS change >10 points) has shown that the score seems to be able to recognize the worsening of the disease among the visits. However, at present we cannot yet establish which delta-hATTRNS value between consecutive visits is able to indicate a significant progression of the neurological impairment.

A possible limitation of hATTRNS is related to the "symptoms" section. If it allows to examine the patient perception about the disease, on the other hand it introduces the subjective component, that might produce a confounding effect, sometimes "mimicking" a worsening (even in the case of stationary picture on clinical and instrumental examination), or "masking" an improvement.

Second, the hATTRNS could be characterized by inter- and intra-operator variability. Therefore, it would be desirable in the future to examine its reliability, through a study that evaluates the score difference among different operators for the same patient. After that, the score should be prospectively applied during the patient's follow-up to demonstrate its sensitivity in detecting neurological progression through the comparison with changes of the other clinical outcomes.

In conclusion, the clinical research that I conducted during my PhD program was dedicated to improve the diagnosis (AIM #1) and the management (AIM #2) of hATTR disease, through the conception of two specific and easy-to-use scales.

Firstly, a diagnostic score was shaped to establish if patients with axonal neuropathy deserve TTR genetic analysis. A compound score ≥5 points allows to discriminate hATTR from CIAP, and can be extremely useful in those area where genetic analysis is not easily accessible.

Secondly, to optimize the follow-up of patients affected by hATTR, the hATTRNS has proved to quantify the degree of neuropathic disability, to correlate with other outcome measure and to demonstrate disease progression. A further multicentric validation of the hATTRNS will allow apply the score in the future for the evaluation and follow-up of patients with hATTR neuropathy.

TABLES

Table 1. Clinical and electrophysiological findings

		ATTRv (N=35)	**CIAP** (N=55)	***p-value***
Clinical findings				
TTR gene mutation	V30M P64L V122I	42,8% 51.4% 5.7%	-	-
Gender	Male Female	91.4% 8.6%	83.6% 16.4%	p=0.289
Family history of neuropathy	No Yes	100% 0%	100% 0%	p=0.569
Progressive neuropathy	No Yes	51.4% 48.6%	47.3% 52.7%	p=0.701
Walking impairment	0 1 2 3	0% 48.6% 34.3% 17.1%	0% 69.1% 25.5% 5.4%	p=0.083
Muscle weakness	No Yes	14.3% 85.7%	45.5% 54.5%	**p=0.002**
Sensory symptoms	No Yes	2.8% 97.2%	9.1% 90.9%	p=0.248
Carpal tunnel syndrome history	No Yes	42.9% 57.1%	76.4% 23.6%	**p=0.001**
Autonomic symptoms	No Yes	68.6% 31.4%	72.7% 27.3%	p=0.672
Age of onset (years)		64.3 ± 9.9	58.2 ± 11.2	**p=0.011**
Disease duration (years)		4.3 ± 4.1	3.8 ± 2.7	p=0.534
Electrophysiological findings				
SAP Median	Normal Reduced Absent	0% 25% 75%	40.8% 34.7% 24.5%	**p<0.001**
SAP Ulnar	Normal Reduced Absent	0% 45.5% 54.5%	31.6% 42.1% 26.3%	**p<0.001**
SAP Sural	Normal Reduced Absent	5.9% 26.5% 67.6%	6.4% 34% 59.6%	p=0.671
SAP Superficial	Normal Reduced Absent	5.6% 0% 94.4%	13.9% 13.9% 72.2%	p=0.139
CMAP Median	Normal Reduced Absent	14.3% 60.7% 25%	73.3% 24.5% 2.2%	**p<0.001**
CMAP Ulnar	Normal Reduced Absent	27.6% 68.9% 3.5%	73.5% 24.5% 2%	**p<0.001**
CMAP Tibial	Normal Reduced Absent	35.3% 17.6% 47.1%	20.8% 50% 29.2%	**p=0.011**
CMAP Peroneal	Normal Reduced Absent	17.9% 39.3% 42.8%	29.2% 35.4% 35.4%	p=0.540

SAP= Sensory Action Potential; CMAP= Compound Motor Action Potential.

Table 2. Detailed electrophysiological findings

		ATTRv	CIAP	*p-value*
Median nerve	SAP (μV)	3.2 ± 2	17 ± 13.3	**p<0.001**
	SNCV (m/s)	44.2 ± 8.2	43.6 ± 7.5	p=0.852
	DML (ms)	5 ± 1.3	4.6 ± 1.5	p=0.340
	dCMAP (mV)	2.9 ± 2.6	8.6 ± 3.4	**p<0.001**
	pCMAP (mV)	2.6 ± 2.4	7.9 ± 3.4	**p<0.001**
	MNCV (m/s)	44.2 ± 6.3	45.1 ± 8.4	p=0.108
Ulnar nerve	SAP (μV)	4 ± 2.7	15.4 ± 12.3	**p<0.001**
	SNCV (m/s)	47.6 ± 5.8	46 ± 7.6	p=0.544
	DML (ms)	3.3 ± 0.8	3.2 ± 0.8	p=0.683
	dCMAP (mV)	5.4 ± 3.7	9.5 ± 3.8	**p<0.001**
	p1CMAP (mV)	5.1 ± 3.2	8.7 ± 3.6	**p<0.001**
	p2CMAP (mV)	5 ± 2.9	8.1 ± 3.5	**p=0.001**
	MNCV1 (m/s)	49.8 ± 8	51.8 ± 7.7	p=0.304
	MNCV2 (m/s)	39.7 ± 9.2	43.9 ± 7	**p=0.042**
Tibial nerve	DML (ms)	5 ± 1.5	5.6 ± 1.5	p=0.165
	dCMAP (mV)	2.3 ± 3	5.2 ± 5.2	**p=0.022**
	pCMAP (mV)	1 ± 2.6	3.9 ± 4.4	p=0.291
	MNCV (m/s)	37.9 ± 3.9	36.7 ± 5.9	p=0.621
Peroneal nerve	DML (ms)	3.8 ± 1	5.0 ± 1.9	**p=0.024**
	dCMAP (mV)	2.0 ± 1.9	4.4 ± 3.8	**p=0.006**
	pCMAP (mV)	1.9 ± 2.1	3.8 ± 3.3	**p=0.026**
	MNCV (m/s)	39.6 ± 11.7	38.7 ± 7.2	p=0.747
Sural nerve	SAP (μV)	3.2 ± 1.8	3.9 ± 2.8	p=0.460
	SNCV (m/s)	45.4 ± 3.3	46.6 ± 7.1	p=0.523

SAP= Sensory Action Potential; SNCV= Sensory Nerve Conduction Velocity; DML= Distal Motor Latency; (d/p) CMAP= (distal/proximal) Compound Motor Action Potential; MNCV= Motor Nerve Conduction Velocity.

Table 3. Demographic and clinical features.

	Symptomatic (n= 26)	**Presymptomatic** (n= 17)	**Total** (n= 43)
Age at first evaluation	62.9±13.2 (31-80)	55.1±14 (33-80)	59.6±13.9 (31-80)
Gender (M/F)	19/7	12/5	31/12
Mutation			
Val30Met	12/26 (46.1%)	8/17 (47.1%)	20/43 (46.5%)
Phe64Leu	12/26 (46.1%)	8/17 (47.1%)	20/43 (46.5%)
Gly54Lys	2/26 (7.7%)	0/17 (0%)	2/43 (4.7%)
Val122Ile	0/26 (0%)	1/17 (5.8%)	1/43 (2.3%)
FAP stage			
0	-	17	17
1	14	-	14
2	8	-	8
3	4	-	4
Total valuation	99	28	127
N. of valuation for each patient	3.7±2.1 (1-8)	1.7±0.9 (1-4)	2.9±2 (1-8)
Follow-up duration (months)	25.5±20 (6-90)	18.2±10 (6-36)	23.1±17 (6-90)
NIS	73.3±49.8 (0-177.2)	5.2±8.2 (0-26)	52,4±58,7 (0-177)
RODS	18.1±14 (0-44)	40.4±5.9 (23-44)	23.2±15.7 (0-44)
Norfolk QOL-DN	68.7±30.8 (-1 - 155)	9.4±13.2 (-1 - 49)	56.3±37 (-1 - 155)
DN4	5.5±2.8 (0-10)	1.7±1.9 (0-5)	4.7±3.1 (0-10)
COMPASS-31	25.3±14.9 (2-63)	14.1±9.7 (1-35)	22.6±14.6 (1-63)
CADT	14.7±3.3 (6-19)	19.1±6.4 (14-20)	15.8±4.7 (6-20)
hATTRNS	21.3±9.4 (0-38)	3.1±3.1 (0-10)	17.3±11.3 (0-38)

NIS= Neuropathy Impairment Score; RODS= Rasc-Built Overall Disability Scale; COMPASS-31= Composite Autonomic Symptoms Score; CADT= Compound Autonomic Dysfunction Test; hATTRNS= hATTR Neuropathy Score.

SUPPLEMENTARY MATERIALS

Instructions for hATTR Neuropathy Score (hATTRNS)

		0	(+1)	(+1)	(+1)	(+1)	Total
Symptoms	**Sensory Symptoms**	None	Feet	Legs	Hands	Arms	0-16
	Neuropathic Pain	None	Feet	Legs	Hands	Arms	
	Weakness	None	Legs	Thighs	Hands	Arms	
	Autonomic Symptoms	None	Orthostatic intolerance	Gastrointestinal domain	Secretomotor domain	Genitourinary domain	
		0	**1**	**2**	**3**	**4**	
Strength	**Upper limbs**	Normal	4 on FDI or ADM	≤3 on FDI or ADM	≤4 on wrist extensor or flexor muscles	≤4 on brachial biceps or triceps muscles	0-8
	Lower limbs	Normal	≤4 on digital extensor or flexor muscles	4 on foot dorsiflexor or plantar flexor muscles	≤3 on foot dorsiflexor or plantar flexor muscles	≤4 on quadriceps o biceps femoris muscles	
Sensation	**Pinprick**	Normal	Reduced up to Wrist / Ankle	Reduced up to Mid forearm / Mid leg	Reduced up to Elbow / Knee	Reduce above Elbow / Knee	0-16
	Tactile	Normal	Reduced up to Wrist / Ankle	Reduced up to Mid forearm / Mid leg	Reduced up to Elbow / Knee	Reduce above Elbow / Knee	
	Vibration	Normal	Reduced at V finger / great toe	Reduced at MCP / Ankle	Reduced at Wrist / Knee	Reduce at Elbow / ASIS	
	Joint position sense	Normal	Reduced at V finger / great toe	Reduced at MCP / Ankle	Reduced at Wrist / Knee	Reduce at Elbow / Hip	
							0-40

SYMPTOMS

- Sensory symptoms. The item includes both positive symptoms and sensory loss. Ask the patient: "*Do you have loss of feeling or tingling anywhere in your body? If so, are these symptoms present most of your daytime or just occasional? In which body regions do you complain these symptoms?*". Exclude symptoms attributable to carpal tunnel syndrome.
- Neuropathic pain. Ask the patient: "*Do you experience pain? If so, does your pain have characteristic of burning pain, painful cold or electric shock? If so, in which body regions do you complain this type of pain?*".
- Weakness. Ask the patient: "*Do you have weakness in your lower limbs? If so, do you have difficult on moving up or down your feet? Do you have difficult on*

rising from a chair without assistance? Do you have weakness in your upper limbs? If so, do you have difficulty with doing and undoing buttons or zip, turning a key in a lock or cutting most food including meat and pizza with normal utensils? Do you have difficulty with activities that require extending or flexing your arms, or activities using the upper arms like wash and brush your hairs?". Exclude symptoms attributable to carpal tunnel syndrome.

- Autonomic symptoms. 1) Orthostatic intolerance. Ask the patient: "*Do you ever experience a syncopal episode? If not, have you ever felt faint, dizzy, "goofy", or had difficulty thinking soon after standing up from a sitting or lying position?*".

  2) Gastrointestinal domain. Ask the patient: "*Have you noticed if you get quickly, excessively or persistently full (bloated feeling) when eating a meal? Do you ever vomit after a meal? Have you had any bouts of diarrhoea or been constipated?*".

  3) Secretomotor domain. Ask the patient: "*Do you notice any change in your general body sweating? Do your eyes or mouth feel excessively dry?*"

  4) Genitourinary domain. Ask the patient: "*Do you experience erectile difficulties (only if male)? Have you ever lost control of your bladder function? Have you had difficulty passing urine or you had trouble completely emptying your bladder?*"

STRENGTH

Assess the segmental muscle strength through using the Medical Research Council score (0= no contraction; 1= trace of movement; 2= active movement with gravity eliminated; 3= active movement against gravity; 4= reduced muscle strength

against resistance; 5= normal strength). For segmental strength of upper limbs, explore First Dorsal Interosseous (FDI), Abductor Digiti Minimi (ADM), wrist extensor and flexor, biceps and triceps muscles. For segmental strength of lower limbs explore digital extensor and flexor, dorsiflexor (tibialis anterior) and plantar flexor (gastrocnemius and soleus), quadriceps and biceps femoris muscles. Both sides are always examined, but the score is determined by the weaker muscle on either side.

SENSATION

Assure that the patient has a reference point with normal sensibility. During the examination, patient should have their eyes closed. Both sides should be tested. Chose the worst score of the most affected side between upper and lower limbs. Always exclude signs of carpal tunnel syndrome from the evaluation.

- Pinprick. With a neurotip pressed for one to two seconds and ask the patient if she/he feel it as sharp or dull sensation. Abnormal finding is when patient says she/he feels definitely decreased pinprick sensation, meaning that they are certain this is decreased compared to the normal reference point.
- Tactile. Use a cotton wisp. Abnormal finding is when patient says she/he feels definitely decreased tactile sensation, meaning that they are certain this is decreased compared to the normal reference point.
- Vibration. Use Rydel-Seiffer tuning fork. Activate the tuning fork by snapping the ends together. Vibration sensibility should be tested at following sites assuring that the fork is firmly adhered on bone: distal interphalangeal joint of digit V, metacarpophalangeal (MCP) joint, wrist, elbow on upper limbs, distal head of the first metatarsal bone (great toe), medial malleolus (ankles), tibial

tuberosity (knee) and anterior superior iliac spine (ASIS) on lower limbs. Normal vibration extinction threshold is considered more or equal to 5.

- Joint position sense (JSP). It is tested by moving each joint of the patient up or down. Start examining the most distal joint and if the patient cannot identify the movements with eyes closed, test the next most proximal joints. JSP sensibility should be tested at following sites: proximal interphalangeal joint of digit V, metacarpophalangeal (MCP) joint, wrist, elbow on upper limbs, distal head of the first metatarsal bone (great toe), ankle, knee and hip on lower limbs.

Neuropathy Impairment Score (NIS)

		Destra	Sinistra	Totale
NERVI CRANICI	III nervo cranico			
	VI nervo cranico			
	Ipostenia facciale			
	Ipostenia velo			
	Ipostenia linguale			
FORZA MUSCOLARE	Respiratoria			
	Flessione collo			
	Deltoide			
	Bicipite brachiale			
	Brachioradiale			
	Tricipite brachiale			
	Flessione polso			
	Estensione polso			
	Flessione dita			
	Abduzione dita			
	Abduzione pollice			
	Flessione anca			
	Estensione anca			
	Flessione ginocchio			
	Estensione ginocchio			
	Dorsiflessione piede			
	Flessione plantare			
	Estensione dita			
	Flessione dita			
RIFLESSI	Bicipitale			
	Tricipitale			
	Brachioradiale			
	Rotuleo			
	Achilleo*			
SENSIBILITÀ ARTI SUPERIORI *(falange distale indice)*	Tattile			
	Dolorifica			
	Vibratoria			
	Statochinestesia			
SENSIBILITÀ ARTI INFERIORI *(falange distale alluce)*	**Tattile**			
	Dolorifica			
	Vibratoria			
	Statochinestesia			
Punteggio totale arti inferiori (LL-NIS)				
Punteggio totale				

FORZA MUSCOLARE: 0= normale; 1= 25% ipostenia; 2= 50% ipostenia; 3= 75% ipostenia; 3.25= riesce appena a muovere arto contro gravità; 3.50= riesce appena a muovere arto in assenza di gravità; 3.75= contrazione muscolo senza movimento segmento; 4= plegia.
RIFLESSI e SENSIBILITÀ: 0= normale; 1= ridotta; 2= assente.
*riflesso achilleo: paziente 50-69 anni: 0= ridotti; 1= assenti; paziente ≥70: 0= assenti.

Rasc-Built Overall Disability Scale (R-ODS)

	0: impossibile da eseguire	1: eseguito con difficoltà	2: eseguito facilmente
Leggere giornale			
Mangiare			
Lavarsi i denti			
Lavarsi gli arti superiori			
Sedersi sul water			
Fare un sandwich			
Vestirsi gli arti superiori			
Muovere una sedia			
Girare una chiave nella serratura			
Andare dal medico di base			
Farsi una doccia			
Lavare i piatti			
Fare shopping			
Prendere un oggetto (palla)			
Piegarsi e raccogliere un oggetto			
Salire una rampa di scale			
Viaggiare con mezzi pubblici			
Camminare per un Km			
Trasportare un oggetto pesante			
Ballare			
Stare in piedi per ore			
Correre			

Norfolk Quality of Life

Parte I: Sintomi

Ha avuto qualcuno dei seguenti sintomi nelle ultime 4 settimane? La preghiamo di fare una crocetta su tutti i sintomi che ha avuto.

	Piedi	Gambe	Mani	Braccia	No, non ho avuto questo sintomo
1. Intorpidimento	❑	❑	❑	❑	❑
2. Pizzicore, formicolii	❑	❑	❑	❑	❑
3. Sensazione di scossa elettrica	❑	❑	❑	❑	❑
4. Altre sensazioni insolite	❑	❑	❑	❑	❑
5. Dolore in superficie	❑	❑	❑	❑	❑
6. Dolore in profondità	❑	❑	❑	❑	❑
7. Debolezza	❑	❑	❑	❑	❑

Parte II: Attività quotidiane

Risponda alle domande utilizzando la seguente scala:	Non è stato un problema 0	Problema molto lieve 1	Problema lieve 2	Problema di media entità 3	Problema grave 4
8. Nelle ultime 4 settimane, a causa del dolore è rimasto/a sveglio/a o si è svegliato/a durante la notte?	❑	❑	❑	❑	❑
9. Nelle ultime 4 settimane, le ha dato fastidio il contatto con le lenzuola, gli indumenti o indossare le scarpe?	❑	❑	❑	❑	❑
10. Nelle ultime 4 settimane, le è capitato di bruciarsi o ferirsi senza sentire alcun dolore?	❑	❑	❑	❑	❑
11. Nelle ultime 4 settimane, qualche sintomo le ha impedito di svolgere le sue abituali attività durante il giorno?	❑	❑	❑	❑	❑
12. Nelle ultime 4 settimane, ha avuto difficoltà nel fare piccoli movimenti con le dita, come abbottonarsi i vestiti, girare le pagine di un libro, prendere con le dita delle monete dal tavolo?	❑	❑	❑	❑	❑
13. Nelle ultime 4 settimane, si è sentito/a malfermo/a sulle gambe quando camminava?	❑	❑	❑	❑	❑
14. Nelle ultime 4 settimane, ha avuto problemi ad alzarsi dalla sedia senza spingersi con le mani?	❑	❑	❑	❑	❑
15. Nelle ultime 4 settimane, ha avuto problemi a scendere le scale?	❑	❑	❑	❑	❑
16. Nelle ultime 4 settimane, non è stato/a capace di sentire i piedi quando camminava?	❑	❑	❑	❑	❑
17. Nelle ultime 4 settimane, non è stato/a capace di distinguere l'acqua calda da quella fredda **con le mani**?	❑	❑	❑	❑	❑
18. Nelle ultime 4 settimane, non è stato/a capace di distinguere l'acqua calda da quella fredda **con i piedi**?	❑	❑	❑	❑	❑
19. Nelle ultime 4 settimane, ha avuto problemi di vomito, in particolare dopo i pasti (ma non a causa dell'influenza o di altre malattie)?	❑	❑	❑	❑	❑

Risponda alle domande utilizzando la seguente scala:	Non è stato un problema 0	Problema molto lieve 1	Problema lieve 2	Problema di media entità 3	Problema grave 4
20. Nelle ultime 4 settimane, ha avuto problemi di diarrea e/o perdite incontrollate di feci?	❑	❑	❑	❑	❑
21. Nelle ultime 4 settimane, ha avuto problemi di svenimento o vertigini quando stava in piedi?	❑	❑	❑	❑	❑
Nelle ultime 4 settimane, quanta difficoltà ha avuto nello svolgere le seguenti attività:					
22. Farsi il bagno o la doccia?	❑	❑	❑	❑	❑
23. Vestirsi?	❑	❑	❑	❑	❑
24. Camminare?	❑	❑	❑	❑	❑
25. Sedersi o alzarsi dal water?	❑	❑	❑	❑	❑
26. Usare posate, piatti, bicchieri, ecc. per mangiare?	❑	❑	❑	❑	❑

Risponda alle domande utilizzando la seguente scala:	Per niente 0	Un po' 1	Abbastanza 2	Molto 3	Moltissimo 4
Nelle ultime 4 settimane, ha avuto qualcuno dei seguenti problemi nel suo lavoro o in altre abituali attività quotidiane a causa del suo stato di salute fisica o emotiva?					
27. Ha ridotto il tempo da dedicare al lavoro o ad altre attività?	❑	❑	❑	❑	❑
28. Ha fatto meno cose di quanto avrebbe voluto?	❑	❑	❑	❑	❑
29. È stato/a limitato/a nel tipo di lavoro o in altre attività che poteva svolgere?	❑	❑	❑	❑	❑
30. Ha avuto difficoltà nello svolgere il lavoro o altre attività (le costava più fatica del solito)?	❑	❑	❑	❑	❑

31. In generale, direbbe che adesso la sua salute è:

Eccellente	Molto buona	Buona	Passabile	Scadente
❑	❑	❑	❑	❑

32. Rispetto a 3 mesi fa, come valuterebbe la sua salute in generale adesso?

Molto migliorata	Abbastanza migliorata	Più o meno uguale	Abbastanza peggiorata	Molto peggiorata
❑	❑	❑	❑	❑

Risposta alle domande utilizzando la seguente scala:	Per niente 0	Un po' 1	Abbastanza 2	Molto 3	Moltissimo 4
33. Nelle ultime 4 settimane, in che misura la sua salute fisica ha limitato le sue abituali attività sociali con familiari, amici, vicini di casa o altri gruppi di persone?	❑	❑	❑	❑	❑
34. Nelle ultime 4 settimane, in che misura il **dolore** ha limitato le sue abituali attività (sia al lavoro che a casa)?	❑	❑	❑	❑	❑
35. Nelle ultime 4 settimane, in che misura la **debolezza** o il **tremore alle gambe e alle braccia** ha limitato le sue abituali attività (sia al lavoro che a casa)?	❑	❑	❑	❑	❑

Italian version of the Composite Autonomic Symptoms Score (COMPASS 31)

DOMINIO 1: IPOTENSIONE ORTOSTATICA

1. Nell'ultimo anno, si è mai sentito/a svenire, ha mai avuto dei capogiri, si è sentito/a "intontito/a" o ha avuto difficoltà a pensare subito dopo essersi alzato/a in piedi dalla posizione seduta o sdraiata?
   - 1 (1) Sì
   - 2 (0) No (se ha risposto "No", passi per favore alla domanda 5)

2. Quando si alza, con che frequenza accusa questi sintomi o sensazioni?
   - 1 (0) Raramente
   - 2 (1) Occasionalmente
   - 3 (2) Spesso
   - 4 (3) Quasi sempre

3. Come valuterebbe la gravità di questi sintomi o sensazioni?
   - 1 (1) Leggera
   - 2 (2) Moderata
   - 3 (3) Grave

4. Nell'ultimo anno, questi sintomi o sensazioni che lei ha accusato sono:
   - 1 (3) Peggiorati/e molto
   - 2 (2) Peggiorati/e un po'
   - 3 (1) Rimasti/e più o meno uguali
   - 4 (0) Migliorati/e un po'
   - 5 (0) Migliorati/e molto
   - 6 (0) Completamente passati/e

SCORE DOMINIO 1		**X 4.0**	**SCORE CORRETTO**	

DOMINIO 2: VASOMOTORIO

5. Nell'ultimo anno, ha mai notato dei cambiamenti di colore della sua pelle, come, per esempio, la pelle che diventa rossa, bianca o violacea?
   - 1 (1) Sì
   - 2 (0) No (se ha risposto "No", passi per favore alla domanda 8)

6. Quali parti del suo corpo sono state interessate da questi cambiamenti di colore? (Faccia una crocetta su tutto quello che la riguarda)
   - 1 (1) Mani
   - 2 (1) Piedi

7. Questi cambiamenti di colore della sua pelle sono:
   - 1 (3) Peggiorati molto
   - 2 (2) Peggiorati un po'
   - 3 (1) Rimasti più o meno uguali
   - 4 (0) Migliorati un po'
   - 5 (0) Migliorati molto
   - 6 (0) Completamente passati

SCORE DOMINIO 2		**X 0.8333333**	**SCORE CORRETTO**	

DOMINIO 3: SECRETOMOTORIO

8. Negli ultimi 5 anni, ha avuto dei cambiamenti nella sudorazione generale del corpo?

1 (1) Sudo molto più rispetto a prima
2 (0) Sudo un po' più rispetto a prima
3 (0) Non ho notato alcun cambiamento nella sudorazione
4 (1) Sudo un po' meno rispetto a prima
5 (2) Sudo molto meno rispetto a prima

9. Ha la sensazione di avere gli occhi troppo asciutti?

1 (1) Sì
2 (0) No

10. Ha la sensazione di avere la bocca troppo asciutta?

1 (1) Sì
2 (0) No

11. Ora consideri il sintomo che ha da più tempo (occhi asciutti o bocca asciutta). Questo disturbo è :

1 (0) Non ho avuto nessuno di questi sintomi
2 (3) Peggiorato molto
3 (2) Peggiorato un po'
4 (1) Rimasto più o meno uguale
5 (0) Migliorato un po'
6 (0) Migliorato molto
7 (0) Completamente passato

SCORE DOMINIO 3		**X 2.1428571**	**SCORE CORRETTO**	

DOMINIO 4: GASTROINTESTINALE

12. Nell'ultimo anno, ha notato qualche cambiamento nella rapidità con cui raggiunge la sazietà (si sente pieno) quando mangia?

1 (3) Mi sazio molto più velocemente rispetto a prima
2 (2) Mi sazio più velocemente rispetto a prima
3 (1) Non ho notato alcun cambiamento
4 (0) Mi sazio meno velocemente rispetto a prima
5 (0) Mi sazio molto meno velocemente rispetto a prima

13. Nell'ultimo anno, si è sentito/a eccessivamente sazio/a o costantemente sazio/a (sensazione di gonfiore) dopo un pasto?

1 (0) Mai
2 (1) Qualche volta
3 (2) Spesso

14. Nell'ultimo anno, ha vomitato dopo un pasto?

1 (0) Mai
2 (1) Qualche volta
3 (2) Spesso

15. Nell'ultimo anno, ha avuto dolore addominale come crampi o coliche?

1 (0) Mai
2 (1) Qualche volta
3 (2) Spesso

16. Nell'ultimo anno, ha avuto attacchi di diarrea?

1 (1) Sì
2 (0) No (se ha risposto "No", passi per favore alla domanda 20)

17. Con che frequenza ciò si è verificato?
    1 (0) Raramente
    2 (1) Occasionalmente
    3 (2) Frequentemente _____ volte al mese
    4 (3) Costantemente
18. Quanto sono gravi questi attacchi di diarrea?
    1 (1) Leggeri
    2 (2) Moderati
    3 (3) Gravi
19. I suoi attacchi di diarrea sono:
    1 (3) Peggiorati molto
    2 (2) Peggiorati un po'
    3 (1) Rimasti uguali
    4 (0) Migliorati un po'
    5 (0) Migliorati molto
    6 (0) Completamente passati
20. Nell'ultimo anno, ha avuto problemi di stitichezza?
    1 (1) Sì
    2 (0) No (se ha risposto "No", passi per favore alla domanda 24)
21. Con quale frequenza ha problemi di stitichezza?
    1 (0) Raramente
    2 (1) Occasionalmente
    3 (2) Frequentemente _____ volte al mese
    4 (3) Costantemente
22. Quanto sono gravi questi episodi di stitichezza?
    1 (1) Leggeri
    2 (2) Moderati
    3 (3) Gravi
23. La sua stitichezza è:
    1 (3) Peggiorata molto
    2 (2) Peggiorata un po'
    3 (1) Rimasta uguale
    4 (0) Migliorata un po'
    5 (0) Migliorata molto
    6 (0) Completamente passata

SCORE DOMINIO 4		**X 0.8928571**	**SCORE CORRETTO**	

DOMINIO 5: URINARIO

24. Nell'ultimo anno, ha mai perso il controllo della vescica?
    1 (0) Mai
    2 (1) Occasionalmente
    3 (2) Frequentemente _____ volte al mese
    4 (3) Costantemente
25. Nell'ultimo anno, ha avuto difficoltà ad urinare?
    1 (0) Mai
    2 (1) Occasionalmente
    3 (2) Frequentemente _____ volte al mese
    4 (3) Costantemente

26. Nell'ultimo anno, ha avuto problemi a svuotare completamente la sua vescica?

1 (0) Mai
2 (1) Occasionalmente
3 (2) Frequentemente _____volte al mese
4 (3) Costantemente

SCORE DOMINIO 5		**X 1.1111111**	**SCORE CORRETTO**	

DOMINIO 6: PUPILLOMOTORIO

27. Nell'ultimo anno, senza occhiali da sole o occhiali con lenti scure, la luce intensa le ha dato fastidio agli occhi?

1 (0) Mai (se ha risposto "Mai", passi per favore alla domanda 29)
2 (1) Occasionalmente
3 (2) Frequentemente
4 (3) Costantemente

28. Quanto è grave questa sensibilità alla luce intensa?

1 (1) Leggera
2 (2) Moderata
3 (3) Grave

29. Nell'ultimo anno, ha avuto problemi a mettere a fuoco le immagini con i suoi occhi?

1 (0) Mai (se ha risposto "Mai", passi per favore alla domanda 31)
2 (1) Occasionalmente
3 (2) Frequentemente
4 (3) Costantemente

30. Quanto è grave questo problema di mettere a fuoco?

1 (1) Leggero
2 (2) Moderato
3 (3) Grave

31. Il sintomo più fastidioso per i suoi occhi (ad es., sensibilità alla luce intensa o problemi nel mettere a fuoco) è:

1 (0) Non ho avuto nessuno di questi sintomi
2 (3) Peggiorato molto
3 (2) Peggiorato un po'
4 (1) Rimasto più o meno uguali
5 (0) Migliorato un po'
6 (0) Migliorato molto
7 (0) Completamente passato

SCORE DOMINIO 6		**X 0.3333333**	**SCORE CORRETTO**	

SCORE TOTALE_____

Compound Autonomic Dysfunction Test (CADT)

	4	3	2	1	0
Ipotensione posturale	No	Asintomatico	Lipotimia	Sincopi posturali	Allettato
Nausea, Vomito	No	Nausea/ Lenta digestione	Vomito (meno di una volta a settimana)	Vomito (più di una volta a settimana)	Vomito (quotidiano)
Diarrea/Stipsi	No	Una volta al mese	Una volta a settimana	Più di due volte a settimana	Quotidianamente
Disturbi sfinterici	No	Disuria	Disuria + episodi di incontinenza	Cateterizzazione vescicale intermittente	Cateterizzazione vescicale permanente
Disfunzione erettile	No	Difficoltà	Impotenza		

DN4 questionnaire

Il questionario consiste in 4 domande alle quali si possono dare 10 risposte, ogni SI vale 1, ogni NO vale 0.

Se il punteggio è superiore a 4 siamo di fronte ad un dolore con caratteristiche di Neuropatico.

Più è alto il punteggio maggiore è la probabilità che il dolore origini nel sistema nervoso.

DOMANDA 1: il dolore presenta una o più delle seguenti caratteristiche?

	SI	NO
1. Bruciante/urente		
2. Sensazione di freddo doloroso		
3. Scariche elettriche		

DOMANDA 2: il dolore è associato, nella stessa area, a uno o più dei seguenti sintomi?

	SI	NO
4. Formicolio		
5. Punture di spillo		
6. Intorpidimento?		
7. Sensazione di prurito		

DOMANDA 3: il dolore è localizzato in un territorio dove l'esame obiettivo evidenzia:

	SI	NO
8. Ipoestesia al tatto		
9. Ipoestesia alla puntura		

DOMANDA 4: il dolore è provocato o accentuato da:

	SI	NO
10 Sfioramento della pelle		

SI = 1 punto NO = 0 punti

Punteggio del paziente: /10

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