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Selected items from the Charcot-Marie-Tooth (CMT) Neuropathy Score and secondary clinical outcome measures serve as sensitive clinical markers of disease severity in CMT1A patients

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Abstract

This study evaluates primary and secondary clinical outcome measures in Charcot-Marie-Tooth disease type 1A (CMT1A) with regard to their contribution towards discrimination of disease severity. The nine components of the composite Charcot-Marie-Tooth disease Neuropathy Score and six additional secondary clinical outcome measures were assessed in 479 adult patients with genetically proven CMT1A and 126 healthy controls. Using hierarchical clustering, we identified four significant clusters of patients according to clinical severity. We then tested the impact of each of the CMTNS components and of the secondary clinical parameters with regard to their power to differentiate these four clusters. The CMTNS components *ulnar sensory nerve action potential (SNAP), pin sensibility, vibration and strength of arms* did not increase the discriminant value of the remaining five CMTNS components (Ulnar compound motor action potential [CMAP], leg motor symptoms, arm motor symptoms, leg strength and sensory symptoms). However, three of the six additional clinical outcome measures – the 10 m-timed walking test (T10MW), 9 hole-peg test (9HPT), and foot dorsal flexion dynamometry – further improved discrimination between severely and mildly affected patients. From these findings, we identified three different composite measures as score hypotheses and compared their discriminant power with that of the CMTNS. A composite of eight components CMAP, Motor symptoms legs, Motor symptoms arms, Strength of Legs, Sensory symptoms), displayed the strongest power to discriminate between the clusters. As a conclusion, five items from the CMTNS and three secondary clinical outcome measures improve the clinical assessment of patients with CMT1A significantly and are beneficial for upcoming clinical and therapeutic trials.

Keywords: Charcot-Marie-Tooth; CMT1A; HMSN; Secondary clinical outcome measures; CMTNS; Score generation

1. Introduction

Charcot-Marie-Tooth (CMT) diseases or hereditary motor and sensory neuropathies are the commonest inherited disorders of the peripheral nervous system with a prevalence of up to 1 in 1214 [1,2]. Affected humans develop a slowly progressive, distally pronounced muscle atrophy along with weakness, subsequent walking disability and sensory impairment. Typical signs and symptoms include steppage gait, impaired fine motor skills, distal sensory impairment, altered deep tendon reflexes and skeletal deformities (pes cavus/planus formation). The CMT disease onset, progression and severity are strikingly variable. This holds true for unrelated patients [3], but also within families [4] and even in monozygotic twins [5]. Apart from peripheral demyelination and consecutive axonal loss, onion bulb formations in peripheral nerve biopsies are distinctive findings in most demyelinating (CMT1) cases [6–9]. The most common subtype CMT1A displays an autosomal-dominant inheritance pattern and is caused by a duplication on chromosome 17p11.2 harboring the Peripheral Myelin Protein of 22 kDa (PMP22) gene [10–12]. So far – despite of several promising trials in animal models – there is no treatment available for any form of CMT [13,14].

The disease severity is currently mainly clinically assessed by the Charcot-Marie-Tooth Neuropathy Score in its first version (CMTNSv1) [15–17]. The CMTNS is a valid and reliable composite scoring system consisting of nine components, originating from the Total Neuropathy Score (TNS). It is intended to clinically measure length-dependent motor and sensory impairment in CMT patients. Each component is scored on a 0–4 point scale, positively correlating with the respective severity of each examined item [15,17], and ranges from 0 (good clinical performance) to 36 (severely affected). Shy et al. reported an increase of about 0.68 points per year in CMT1A patients [15]. An even slower progression was reported within a recent therapy trial with ascorbic acid (0.23 points per year) [18].

Lack of sensitive measures carries the risk of false negative results in clinical trials. Therefore, a novel version of the CMTNS (CMTNSv2) has been recently developed in order to standardize patient assessment, reduce floor and ceiling effects and eventually improve the scale's sensitivity to change [17]. Finally, biomarkers may also prove powerful tools to monitor therapeutic effects in clinical trials and the first transcriptional candidates derived from skin biopsies of CMT1A patients have been identified [18–20].

The CMTNS in both versions displays poor discriminatory power among the most common group of moderately affected patients. Therefore, in order to detect a therapeutic effect in future clinical trials, continuously improved clinical scoring systems are urgently needed [18,21]. In this regard, secondary clinical outcome measures including the Overall Neuropathy Limitation Scale (ONLS), 10 m-timed walking (T10MW), 9 hole-peg test (9-HPT) and the maximal voluntary isometric contraction (MVIC) of arms and legs were developed and have already shown to provide substantial reliability and excellent inter- and intrarater reliability in CMT patients, while being feasible and easy to perform [22]. Thus, these

secondary clinical outcome measures represent promising extensions to current scoring systems. We therefore addressed the discriminant validity of the CMTNS components and of six secondary outcome measures. The clinical data was obtained from two multinational prospective clinical studies of adult CMT1A patients in Europe: First, from a clinical prospective, multinational study in Italy, Czech Republic, Spain and Germany, aimed at the development of biomarkers [20] and secondly, from the initial baseline assessment in an ascorbic acid trial on CMT1A patients in Italy and the United Kingdom (CMT-TRIAAL/CMT-TRAUK) [18].

2. Methods

2.1. Patients recruitment

2.1.1. Patients were recruited in two clinical prospective trials

(1) The CMT-TRIAAL/CMT-TRAUK was a phase II/III randomized, double-blind, placebo controlled RCT assessing the efficacy of 2-year oral ascorbic acid treatment (1.5 g/day). Overall, 277 adults suffering from CMT1A were enrolled between March 2006 and September 2007. The primary endpoint was an improvement in CMTNS [15]. Secondary endpoints were the following: distal arm and leg maximal voluntary isometric contraction (MVIC); T10MW; 9 hole-peg test (9HPT); overall neuropathy limitations scale (ONLS); pain and fatigue visual analogue scales (VAS); health-related quality of life (SF-36); and electrophysiology. The CMT-TRIAAL/CMT-TRAUK initiated in 2006 and patients were recruited in Italy and the United Kingdom.

(2) In the biomarker study, patients with genetically proven CMT1A were recruited via local databases, rehabilitation centers, telephone hotlines, and web-based in Italy, Spain, Czech Republic and Germany, in order to establish biomarkers in skin biopsies and whole blood that correlate with the clinical disease severity and to facilitate future therapeutic trials with this information. Starting in 2009 202 patients were included. Data from 46 of these patients were used to identify biomarkers for disease severity [20]. Inclusion criteria were the same as for the CMT-TRIAAL/CMT-TRAUK. CMT-TRIAAL participants could not be enrolled in this study.

In both studies, the Charcot-Marie-Tooth Neuropathy Score (CMTNS) [15] was assessed together with nine secondary clinical outcome measures. Slight methodological differences distinguish the two studies (see below). One session of training took place with all participants in both trials. The CMTNS was performed without any modifications [15,18]. In the biomarker study the inclusion and exclusion criteria were identical to the CMT-TRIAAL/CMT-TRAUK apart from the exclusion of patients on the basis of ascorbic acid intake limitations [18]. Informed consent was obtained from each study participant and all involved centers received approval of their respective ethics committees.

For the comparison of the obtained clinical data with the general population, 126 healthy controls have been recruited. The healthy controls were recruited proportionately to 5 age specific groups: 18–30 years, 31–40 years, 41–50 years, 50–60 years and 60+. In each of the 5 groups two gender specific subgroups were formed. Each subgroup contained at least 10 patients.

More detailed information on the two clinical studies can be found in [18,20].

2.2. Outcome measurements

Prior to patient enrolment, investigators of both studies were trained in administration of the CMTNS and the so called secondary outcome measures [15,18,22]. There were some minor differences in the administration of the additional outcome measures between the two original trials, which are detailed below.

9 hole-peg test (Sammons Preston, Illinois, USA for patients from the biomarker study, – while a wooden custom built box was used in the baseline ascorbic acid trial): The timed test assesses the fine motor skills of patients. Patients were asked to fill in 9 preformed depressions with provided pins and remove them again after all 9 holes were filled. Only one pin at a time was allowed to be used. The time was measured as soon as the patient had removed the last pin. Verbal encouragement was allowed. In patients from the biomarker study the test started with the non-dominant side and was performed three times per side. Median values for both sides were used for further analysis of dexterity. In patients from the baseline Vitamin C study, mean values were determined from two trials.

10 m-timed walking (T10MW): This timed test assesses the patients walking ability of 10 meters (32.8 feet) on even ground, without any assistance. In both studies patients were barefoot; only in the event that a walking aid (e.g., cane) was absolutely necessary, the test was performed with this aid and this circumstance was noted in the CRF. In the CMT-TRIAAL/CMT-TRAUK, mean values were determined from two trials while in the biomarker study median values from three trials were obtained.

Visual analogue scale (10 cm) for pain: The Visual analogue scale (VAS) for pain perception measures the subjectively conceived pain of patients at the time of examination [23]. Patients marked their perception of pain with a pen on a 10 cm long scale. While 0 cm represents no pain at all, a mark at 10 cm represents the worst imaginable pain.

SF-36 (Hogrefe Verlag, Göttingen; Germany, [24]): Patients were asked to fill in the Short-Form 36 questionnaire regarding quality of life in their own language.

MVIC (Citec-handheld Dynamometry, CIT Technics, Haren, NL): All examinations were performed three times. The examination started with the non-dominant side and the examinations were conducted before the skin biopsy in the biomarker study. Verbal encouragement was allowed. Different adapters of the Citec-handheld Dynamometry were used according to the manufacturer's recommendations. If the patient was unable to hold the mere weight of the device, while testing the upper extremities, a value of 0 Newton was scored. Median values (best of three trials) were used for further analysis in the biomarker study and the Vitamin C study, respectively. Any alterations to the study protocol were noted in the CRF.

Fist grip: Patients were asked to close their fist with maximum force, while holding the device up. Device was held with a 90 degree flexed arm. Patients were seated.

Pinch grip: Patients pressed the device with maximum force, using only Digitus I and II, while holding the device up. Device was held with a 90° flexed arm. Patients were seated.

Three-point-grip: Patients pressed the device with maximum force, using only with Digitus I, II and III, while holding the device up. Device was held with a 90° flexed arm. Patients were seated.

Foot dorsal flexion: Patients were lying down, while performing a dorsal flexion of the foot with maximum force. A foot fixation device was used in the ascorbic acid trial [22]. The myometer adapter rested on the distal metatarsal bones.

Foot plantar flexion: Patients were lying down, while performing a plantar flexion of the foot with maximum force. A foot fixation device was used in the ascorbic acid trial [22]. The myometer adapter rested on the metatarsal heads.

2.3. Initial filtering and scoring

In all secondary clinical parameters that were available for two limbs, the values from the dominant and the non-dominant limb correlated highly according to Spearman's ρ (correlation coefficients between 0.82 and 0.97). Therefore, by agreement the measurements from the non-dominant limb were discarded from the analysis. The correlation coefficients between primary and secondary clinical parameters are depicted in Fig. 1. Additionally, the visual analogue scale (VAS) did not show strong correlation with any other of the candidate sub-scores (maximal correlation coefficient 0.41) and was also discarded from the analysis. The measurement of the Pinch Grip was performed only in the first study (biomarker) and, thus, not available for more than half of the patients and therefore not considered in the remaining analyses.

All clinical outcome measures that were shared by the two studies (i.e., CMTNS and additional measures) were considered for the present analysis, with the exception of the SF-36. This generic health-related quality of life measure was found poorly sensitive to change in a previous 2-year prospective study regarding significant change over time [25]. These findings were also confirmed in the CMT-TRIAAL/CMT-TRAUK patients [18].

In order to achieve the best comparability with the CMTNS, the secondary clinical parameters were first transformed into z scores, which were then categorized into one of the levels *normal, very mild, mild, moderate, severe* (*0,1,2,3,4*) (Table 4). The z scores are based on the mean and standard deviation of healthy patients. These were estimated separately by gender (male/female) and age class (18–30, 31–40, 41–50, 51–60, 60+) from the control cohort which included at least 10 patients in each stratum (Table 4).

The selected 6 secondary clinical parameters were compared between the patient cohort and a cohort of healthy controls (Table 2). All 6 remaining secondary clinical parameters are significantly worse in the patient cohort than in the healthy controls (Table 2). Also the interaction with age is higher in the patient cohort (Fig. 5). As a conclusion, all 6 secondary clinical parameters can be considered potential contributors to an improved patient assessment. See the Supplementary material for the detailed results.

2.4. Statistical methods

Pairwise correlations between all available primary and secondary outcome measures were calculated using Spearman's ρ.

The secondary clinical parameters were compared between the patient cohort and the healthy controls cohort using *Fisher's exact test* for the gender and t-test for the numerical variables.

The patients were clustered according to their scores in the 9 primary the 6 selected secondary outcome measures. The clustering was performed using hierarchical clustering [26] using the complete linkage method based on the euclidean distance. The number of clusters present in the data was estimated using three common measures on the topology: the connectivity (Connectivity), the Dunn Index (Dunn) and the Silhouette Width (Silhouette) [27]. Additionally, the stability of the clusters was assessed. To do so, the clustering on the full data set was compared to the clustering on reduced data sets, where in turn each clinical parameter was omitted. The comparison of the resulting clustering was done using four measures: average proportion of non-overlap (APN), average distance (AD), average distance between means (ADM) and figure of merit (FOM) [28]. Each of these 7 methods (3 topology based and 4 stability based assessing) was used to score the clustering into 2, 3, 4, 5 or 6 groups.

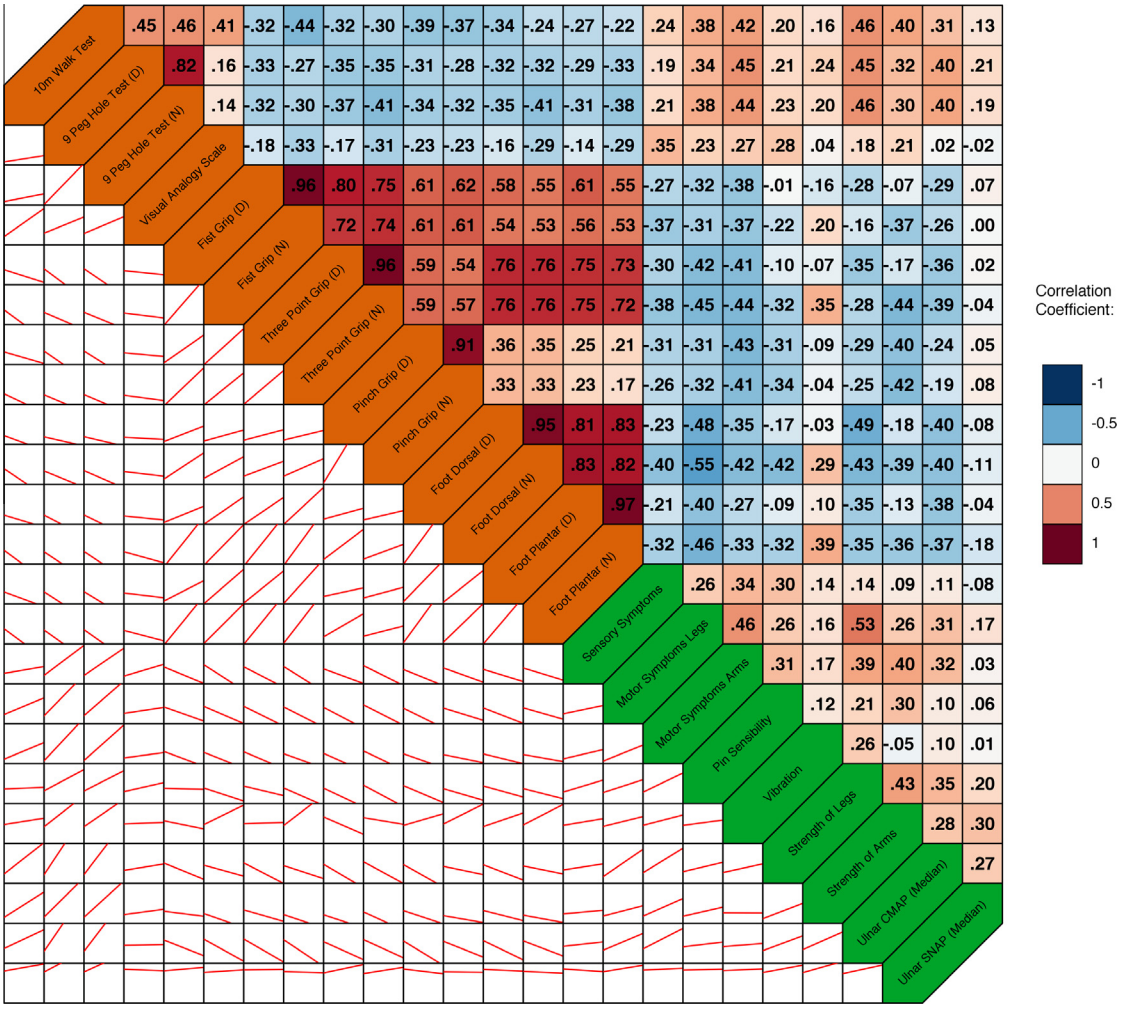


Fig. 1. Correlation of Clinical Parameters. Correlation between all, primary (printed in green) and secondary (printed in orange), clinical parameters. The lower left triangle shows a linear model fit. The upper right triangle shows the correlation coefficients where negative correlations are displayed on a blue background and positive correlations on a red background. The darkness of the background color indicates the correlation strength.

These results were subjected to rank aggregation [29] which showed that the clustering into 4 groups achieved the best overall support.

Cumulative link models were used to model the patients' group membership to one of the main clusters from the hierarchical clustering. The nine primary and the six selected secondary clinical parameters were used as predictive variables. Based on this cumulative link model a forward selection scheme was applied in two phases: in a first phase to the primary clinical parameters and in a second phase additionally to the secondary clinical parameters. In the first phase, the primary clinical parameters were added to the model one by one in an order where in each step the parameter yielding the best model score was added. As model score the Akaike Information Criterion (AIC) was used, that assesses the goodness of fit as well as the model complexity. Only in the second phase, the secondary parameters are added to the model (Fig. 4).

The primary and the secondary clinical parameters were compared univariately between the two main clusters from the hierarchical clustering. The odds ratios with 95% confidence intervals were computed and the univariate logistic regression models were calculated. Additionally, we performed a Rasch analysis on the original CMTNS to fit suitable secondary outcome measures to this model. Unfortunately the original CMTNS does not comply with the Rasch model and many disorderings occur. We decided to maintain the structure of a score with 5 levels per subscore and therefore used AICs to predict the clusters.

All analyses were conducted using the statistical software package R version 3.0.2 (2013–09-25) [30].

3. Results

The descriptive statistical analysis of 479 patients with genetically proven CMT1A (Table 1) indicated that we have recruited a representative study cohort. Co-pathology was a reason for exclusion from both studies. The overall patient cohort ($n = 479$) splits into 277 (57.8%) female and 202 (42.2%) male patients. The age at the examination ranges from 18 to 71 years, with a

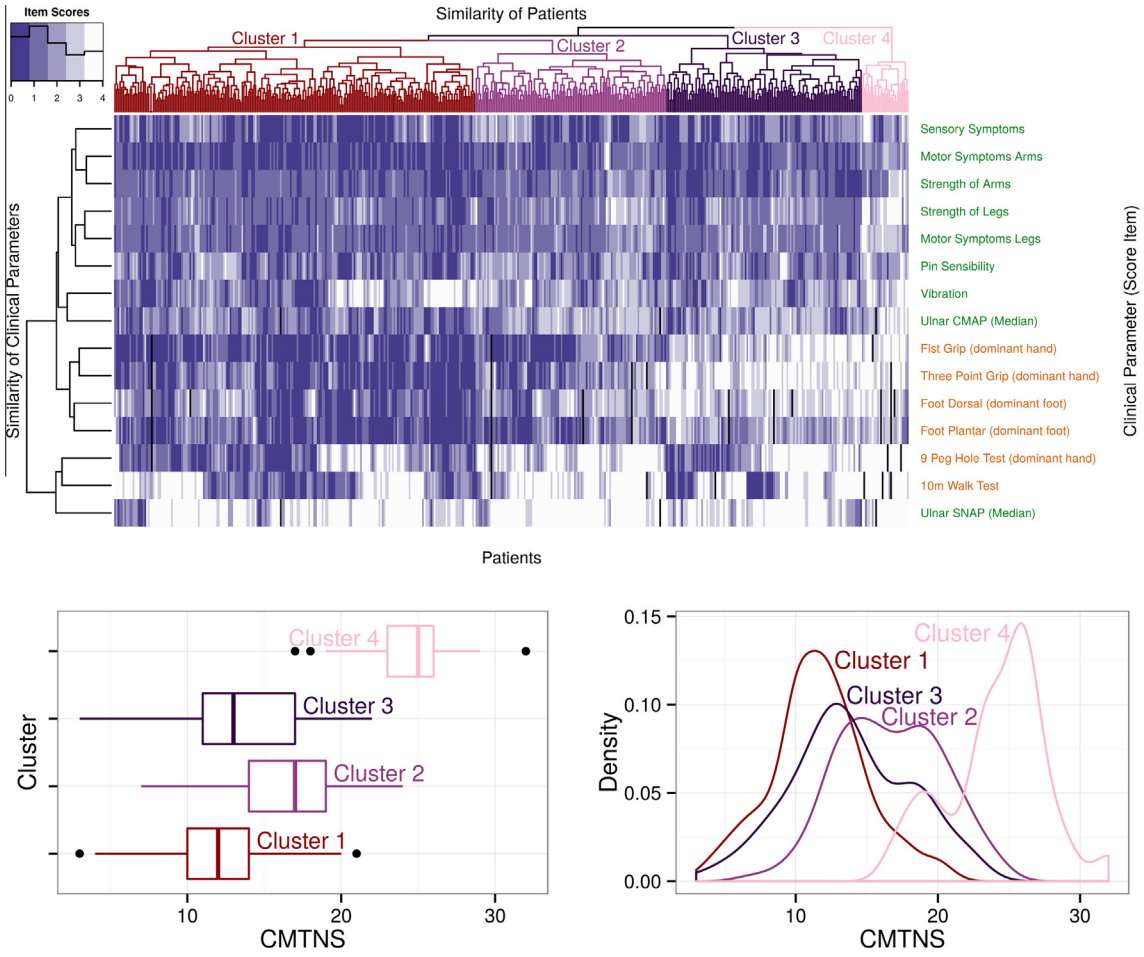


Fig. 2. Clustering of patients. The top panel shows a heatmap containing the scores of the primary and secondary clinical parameters (rows) of all patients in the study (columns). Dark shades of blue represent low scores, light shades high scores. Black colour is used for missing values. Primary clinical parameters (the sub-score of the established CMTNS) are printed in green, the secondary clinical parameters are printed in orange. Hierarchical clustering was applied to both dimensions: the patients and the parameters. The four main clusters are differently colored. The lower panels show the distribution of the standard CMTNS within these four clusters as boxplots and their density. The clustering separates a small group of highly affected patients (cluster 4) from a large group of less affected patients (cluster 1) with two intermediate groups.

mean age of 42.3 and a standard deviation (SD) of 13.2. CMTNS ranged from 3, minimal registered score, to 32, maximal observed score, with median value at 13 (Table 1). The mean Body-Mass-Index (BMI) is 25 kg/m^2. Ideally the subscores for a CMT score would fit a Rasch model. We see two main issues with using Rasch models in context of the CMTNS in adults: First, the original CMTNS does not fit a Rasch model. The Andersen likelihood-ratio test gives a p-value of 0. Only 5 subscores get an infit t-statistic in between -2 and 2 (Supplementary Fig. 1). Furthermore, vibration and SNAP get significant chisq based p-values even after Bonferroni correction. Second, there are many disorderings in the variables from the Rasch model both in the primary subscores (4 disordered in the primary CMTNS subscores) and the secondary subscores (Supplementary Fig. 2). The usual strategy here is to collapse adjacent and disordered categories, but this would again lead further away from the established CMTNS with its 5 levels per subscore. Since the overall model fit is not good, ignoring the disorderings does not present a viable solution. Nevertheless, a sample Rasch analysis was conducted despite these aforementioned limitations, where we generated a score hypothesis, where we iteratively removed the item with most significant chisq test when tested for conformance with the Rasch model (Supplementary Table 1). This approach led to the removal of all parameters of the original CMTNS and all secondary subscores except for 'Foot Plantar', 'Foot Dorsal', 'Fist Grip', and 'Pinch Grip'. As a conclusion, we agreed on the Akaike Information Criterion (AIC) as a model score.

The following data mining included a pairwise correlation of all primary and secondary clinical outcome measures according to Spearman's ρ (Fig. 1). The results indicated the poor correlation of the electrophysiological examination Ulnar sensory nerve action potential (SNAP) (median) with all other outcome measures.

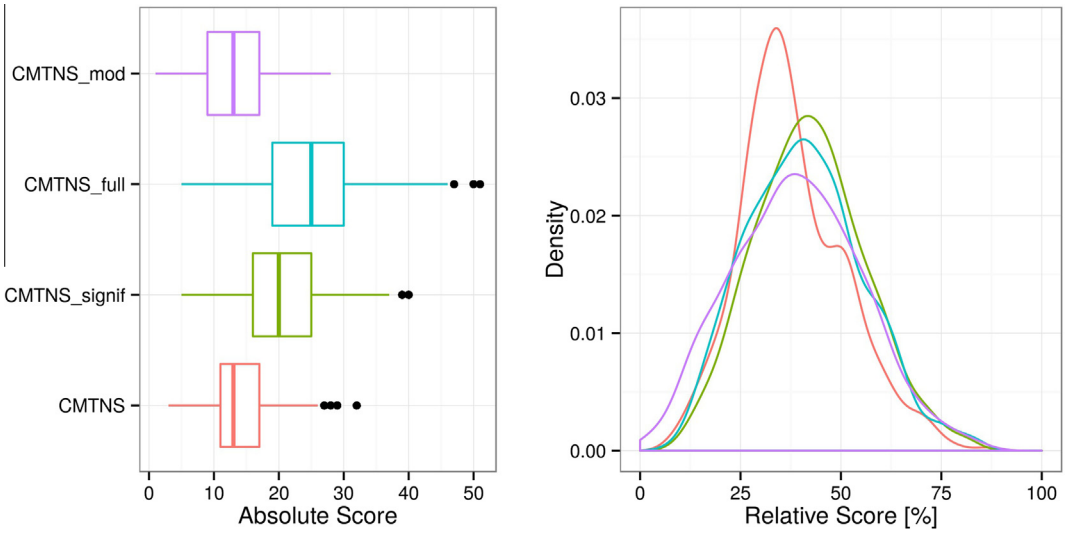


Fig. 3. Comparison of score hypotheses. The established CMTNS is compared to three new score hypotheses. Additionally to the sub-scores that make up the CMTNS, the secondary clinical parameters have been added to build the CMTNS_full score. For CMTNS_signif only the significant secondary clinical parameters have been added. In CMTNS_mod the not-significant primary parameters were exchanged to the significant secondary clinical parameters. The left panel shows all scores as box plots. The right panel shows density plots using the score values relative to the maximum possible score.

Furthermore, the handheld dynamometry of the upper limbs correlated highly among each other. The same held true for the values of the foot dynamometry. In the next step, the patients were grouped into clusters using the method of hierarchical clustering on the scored primary and secondary clinical parameters.

The data on all considered outcome measures suggest four patient clusters according to various validation methods (APN, AD, ADM, FOM, Connectivity, Dunn, Silhouette) confirmed by rank aggregation. These aforementioned clusters divide the patients with their scores accurately into four groups which show a good separation of highly affected patients from less affected patients according to the available outcome measures (Fig. 2, Table 3). Using each of the primary outcome measures in turn we modeled these to clusters using cumulative link models and assessed the goodness of fit. The Ulnar CMAP proved to be the most informative parameter. In an analysis of variance, starting from the model including only Ulnar CMAP, we subsequently added primary outcome measures to the model one at a time, in the order of how much each parameter improved the model. The model evaluation was done using the Akaike Information Criterion which measures the goodness of fit while penalizing the model complexity. After the model included all primary outcome measures, the secondary outcome measures were added to the model as well. The resulting AIC values for each outcome measure are an indicator of the additional information, which a variable is contributing to the discrimination of patients, while taking into account the variables which are already included in the previous model. Importantly, the results of this analysis indicates that the information contained in the clinical parameters "Ulnar SNAP", "Pin Sensibility", "Vibration" and "Strength of Arms" was already contained in the parameters "Ulnar CMAP", "Motor Symptoms Legs", "Motor Symptoms Arms", "Strength of Legs" and "Sensory Symptoms" (Fig. 4) and may therefore be discarded. When tested univariately, the least informative item of the primary clinical parameters proves to be "Ulnar SNAP", which stands out from the others (see also Fig. 1) as it rarely produces low scores. "Foot Dorsal", "9 hole-peg test" and "10 m-timed walking" from the secondary clinical parameters, on the other hand, added additional information not contained in the primary clinical parameters and therefore represent promising tools for patient assessment in future scoring systems.

3.1. Score hypotheses

Based on the differences in information contained in the clinical parameters (Table 4) we generated three score hypotheses for a comparison to the classical CMTNS. All three score hypotheses positively correlate to disease severity in CMT disease:

(A) *Score Hypothesis 1 (CMTNSfull):* The full CMTNS is the sum of all 15 available clinical parameters, the 9 primary and the 6 secondary clinical parameters. The CMTNSfull provides a maximum total score of 60 points.

(B) *Score Hypothesis 2 (CMTNSsignif):* Only the three secondary parameters Foot Dorsal, 9 hole-peg test and 10 m-timed walking test (T10MW), which were found to improve the model and to shrink the AIC

Table 1
Descriptive values. This table summarizes key parameters describing the study cohort. In the last column the number of missing values per parameter is given. The second last column gives the median and the range of the parameter, where applicable. The second column gives either mean ± standard deviation (for numerical variables), only the mean (for ordered categorical variables), or the occurrences per level in absolute and relative numbers (categorical variables). The first block of rows contains general parameters and the established CMTNS, the second block has the primary clinical parameters (sub-scores to the CMTNS), the third block contains the secondary clinical parameters.

Parameter	Value	Median (min; max)	Missing
Gender			0
Female	277 (58%)		
Male	202 (42%)		
Age at Examination [years]	42 ± 13	43 (18; 71)	0
Weight [kg]	70 ± 15	68 (30; 160)	4
Height [cm]	168 ± 9.1	168 (147; 193)	13
BMI [kg/(m2)]	25 ± 4.4	25 (11; 49)	16
Genetic Proof			0
No	7 (1%)		
Yes	472 (99%)		
Imprinting			327
Maternal	67 (44%)		
Paternal	78 (51%)		
Sporadic	7 (5%)		
CMTNS [0/. . ./36]	14	13 (3; 32)	3
Sensory Symptoms [0/1/2/3/4]	1.1	1 (0; 4)	4
Motor Symptoms Legs [0/1/2/3/4]	1.2	1 (0; 4)	4
Motor Symptoms Arms [0/1/2/3/4]	0.65	1 (0; 4)	4
Pin Sensibility [0/1/2/3/4]	1.5	2 (0; 4)	4
Vibration [0/1/2/3/4]	2	2 (0; 4)	4
Strength of Legs [0/1/2/3/4]	1.5	1 (0; 4)	4
Strength of Arms [0/1/2/3/4]	0.96	1 (0; 4)	4
Ulnar CMAP (Median) [0/1/2/3/4]	1.8	2 (0; 4)	7
Ulnar SNAP (Median) [0/1/2/3/4]	3.4	4 (0; 4)	7
10 m-timed walking test (T10MW) [sec]	8.4 ± 4.5	7.3 (2.8; 40)	7
Visual Analogue Scale [mm]	32 ± 30	23 (0; 100)	4
9 hole-peg test (dominant hand) [sec]	24 ± 11	22 (13; 165)	5
9 hole-peg test (non-dominant hand) [sec]	26 ± 10	23 (15; 141)	8
Fist Grip (dominant hand) [kg]	144 ± 84	128 (0; 474)	4
Fist Grip (non-dominant hand) [kg]	101 ± 64	90 (6; 401)	276
Three Point Grip (dominant hand) [kg]	59 ± 37	53 (2; 235)	6
Three Point Grip (non-dominant hand) [kg]	49 ± 45	33 (2.2; 277)	277
Pinch Grip (dominant hand) [kg]	35 ± 28	29 (0; 258)	268
Pinch Grip (non-dominant hand) [kg]	32 ± 18	29 (0; 118)	275
Foot Dorsal (dominant foot) [kg]	66 ± 71	42 (0; 446)	12
Foot Dorsal (non-dominant foot) [kg]	66 ± 88	21 (0; 312)	280
Foot Plantar (dominant foot) [kg]	98 ± 87	74 (0; 455)	9
Foot Plantar (non-dominant foot) [kg]	94 ± 110	37 (0; 382)	278

value, were added to the score. The supposedly redundant primary clinical outcome measures remained in the score, resulting in a maximum total score of 48 points.

(C) *Score Hypothesis 2 (CMTNSmod):* The four primary parameters Ulnar SNAP, Pin Sensibility, Vibration and Strength of Arms which did not yield a reduction of the AIC were replaced by the three significant secondary parameters. Thus, the modified CMTNS consists of 8 clinical parameters and amounts to a maximum total score of 32 points.

All new score hypotheses lead to a wider distribution of scores and reduced the accumulation around medium score levels (12, 13) (Fig. 3). In numbers, we observe higher empirical standard deviations for all score hypotheses in comparison to the established CMTNS (CMTNS: 4.8 (13.1%), CMTNSfull: 8.8 (14.4%), CMTNSsignif: 6.7 (13.6%) and CMTNSmod: 5.3 (16.0%)). Thus, these score hypotheses display increased discriminatory power with regard to the disease severity in CMT1A patients.

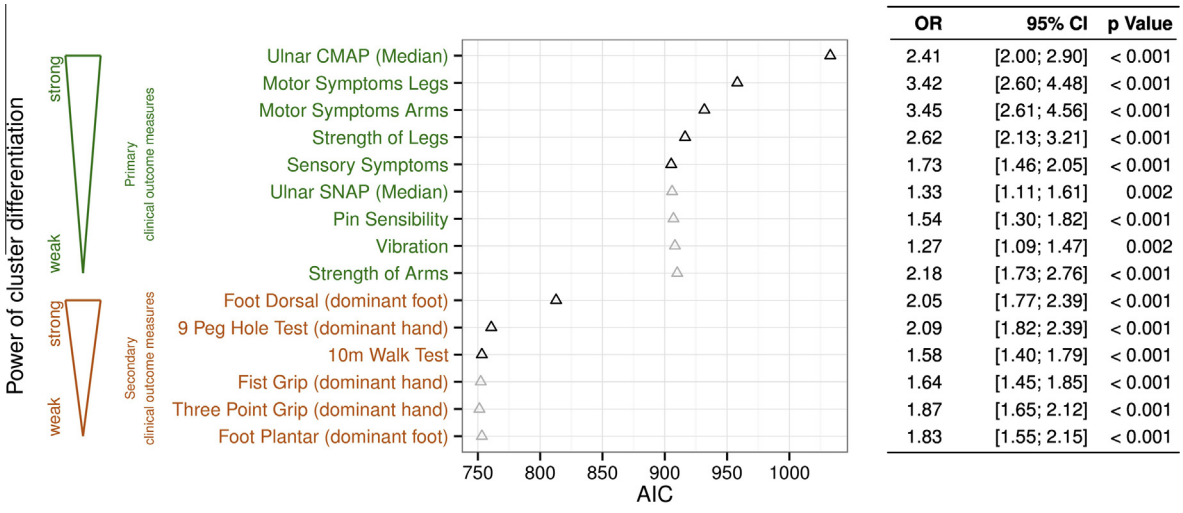
4. Discussion

We examined 479 patients suffering from genetically proven CMT1A disease recruited in two studies, a pan-European biomarker study located in 4 countries [20] and the baseline assessment of the UK/Italian based Vitamin C trial [18]. We first analyzed the primary (CMTNS) and secondary outcome measures assessed in different centers. We aimed at assessing the power of the different outcome measures to discriminate the disease

Table 2
Comparison of patient cohort with control cohort. The selected secondary clinical parameters along with age and gender were compared between the patient cohort and the cohort of healthy controls. While we do not observe significant difference in gender or age, all secondary clinical parameters are significantly worse in the cohort of affected patients, indicating that they are potentially valuable components in a score assessing CMT.

Parameter	Control cohort	Patient cohort	*p* value
n	126	479	
Gender [m/w]			0.09
Female	62 (49.2%)	277 (57.8%)	
Male	64 (50.8%)	202 (42.2%)	
Age at Examination [years]			0.36
Mean ± sd	41 ± 14	42 ± 13	
Median (min; max)	41 (20; 70)	43 (18; 71)	
10 m-timed walking test (T10MW) [sec]			**<0.01**
Mean ± sd	5 ± 0.82	8.4 ± 4.5	
Median (min; max)	4.9 (2.9; 7.6)	7.3 (2.8; 40)	
Missing	1	7	
9 hole-peg test (dominant hand) [sec]			**<0.01**
Mean ± sd	17 ± 2.4	24 ± 11	
Median (min; max)	17 (13; 26)	22 (13; 165)	
Missing	1	5	
Fist Grip (dominant hand) [kg]			**<0.01**
Mean ± sd	198 ± 62	144 ± 84	
Median (min; max)	196 (74; 384)	128 (0; 474)	
Missing	27	4	
Three Point Grip (dominant hand) [kg]			**<0.01**
Mean ± sd	95 ± 28	59 ± 37	
Median (min; max)	91 (57; 200)	53 (2; 235)	
Missing	27	6	
Foot Dorsal (dominant foot) [kg]			**<0.01**
Mean ± sd	156 ± 58	66 ± 71	
Median (min; max)	146 (51; 381)	42 (0; 446)	
Missing	28	12	
Foot Plantar (dominant foot) [kg]			**<0.01**
Mean ± sd	157 ± 59	98 ± 87	
Median (min; max)	150 (63; 485)	74 (0; 455)	
Missing	28	9	

p values smaller than 5% are printed in bold face.



OR	95% CI	p Value
2.41	[2.00; 2.90]	< 0.001
3.42	[2.60; 4.48]	< 0.001
3.45	[2.61; 4.56]	< 0.001
2.62	[2.13; 3.21]	< 0.001
1.73	[1.46; 2.05]	< 0.001
1.33	[1.11; 1.61]	0.002
1.54	[1.30; 1.82]	< 0.001
1.27	[1.09; 1.47]	0.002
2.18	[1.73; 2.76]	< 0.001
2.05	[1.77; 2.39]	< 0.001
2.09	[1.82; 2.39]	< 0.001
1.58	[1.40; 1.79]	< 0.001
1.64	[1.45; 1.85]	< 0.001
1.87	[1.65; 2.12]	< 0.001
1.83	[1.55; 2.15]	< 0.001

Fig. 4. Cluster differentiation. 15 cumulative link models were built to model the clustering. These 15 models were built step by step more and more complex by adding one clinical parameter at a time. In a first phase only the primary clinical parameters (printed in green) were considered; in the second phase the secondary clinical parameters (printed in orange) were added. In each step the clinical was chosen, that lead to the model with the lowest Akaike Information Criterion (AIC). The plot shows these AIC values. Models with lower AIC values than less complex ones are printed in black, the others are printed in gray. The table in the right panel shows the results of univariate models, where each clinical parameter was used on its own to model the clustering. The three columns show the odds ratio (OR) with its 95% confidence interval and the associated p value.

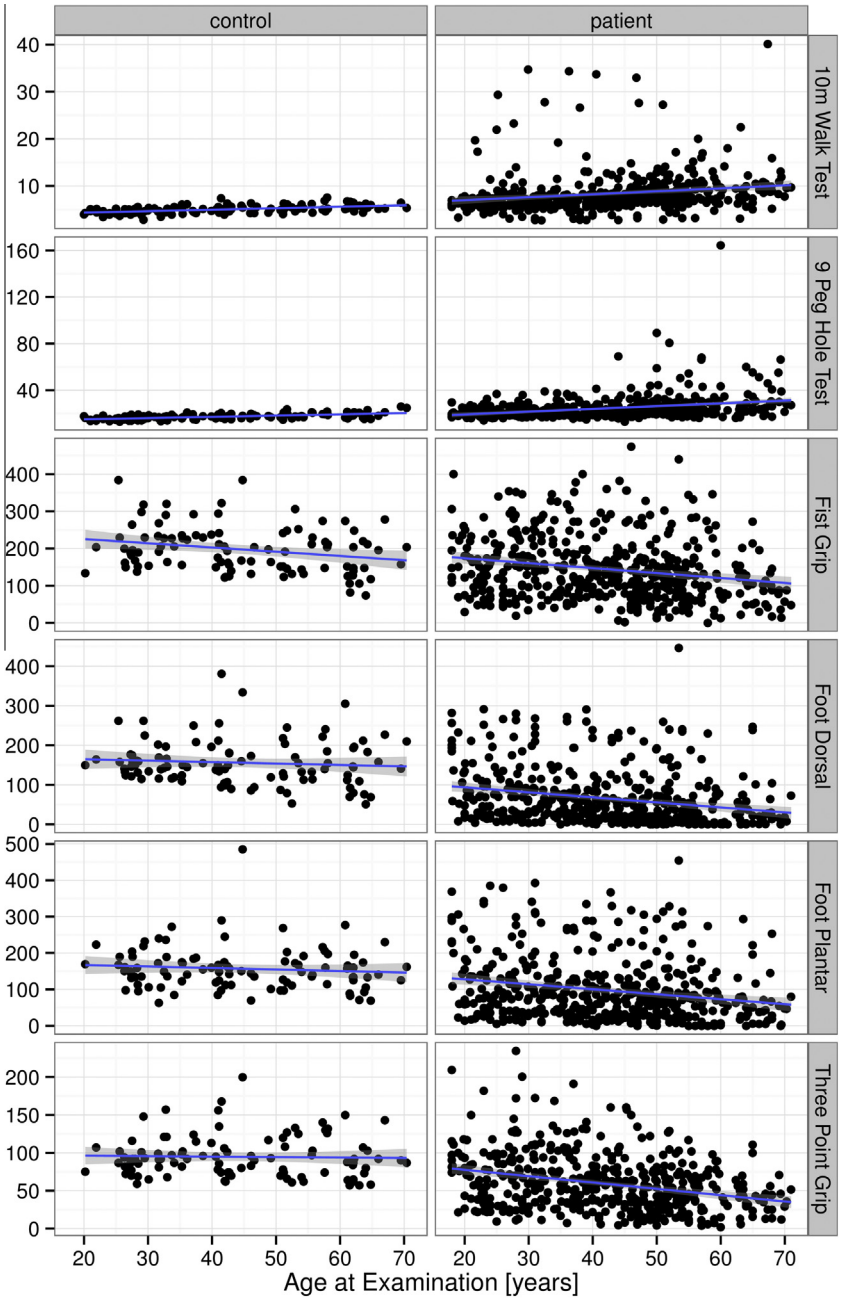


Fig. 5. Correlation with age. The selected 6 secondary clinical parameters are each plotted against the patient age. A linear regression (with confidence intervals) was fitted to the data. The left panels show the cohort of healthy controls, the right panels the patient cohort. We observe steeper regression lines in the patient cohort, indicating that these secondary clinical parameters might be suitable to asses disease progression.

severity among CMT1A patients. Our final goal was to identify items which most powerfully discriminate disease severity in CMT1A patients. These could then be used as standardized outcome measures in future CMT trials. The data sets derived from different centers and countries are comparable to future multicentre international trials. From our clinical experience our cohort reflects representative European CMT1A patients, considering e.g. BMI, age, gender ratio and disease affection. After prior filtering and correlation of clinical parameters according to Spearman's ρ, Hierarchical Clustering using 9 primary and 6 secondary clinical parameters was performed. The test revealed four main groups of patients, which was confirmed by various measurements on the topology and stability of the clustering. The four clusters represent patients with different degree of disease affection. The contribution to the clustering of each clinical parameter was then assessed by adding the parameters one by one to a model in an analysis of variance. This analysis demonstrated that the four primary parameters of the existing CMTNS Ulnar SNAP, Pin Sensibility, Vibration and Strength of Arms

Table 3
Descriptive values per cluster. This table shows again the descriptive statistics similar to Table 1. This time, the statistics are computed within each of the four main clusters from the hierarchical clustering. The last column gives the p value assessing the overall effect between all four clusters. A correlation test based on Kendall's \tau was used to assess the correlation between ordinal variables and the clustering. Fisher's exact test was applied on all categorical variables, and the Kruskal–Wallis rank sum test was performed on numerical variables.

Parameter	Cluster 1	Cluster 2	Cluster 3	Cluster 4	*p* value
n	28	117	114	216	
Gender					**<0.01**
Female	13 (46.4%)	82 (70.1%)	70 (61.4%)	111 (51.4%)	
Male	15 (53.6%)	35 (29.9%)	44 (38.6%)	105 (48.6%)	
Age					**0.01**
Mean ± sd	48 ± 12	41 ± 13	45 ± 14	41 ± 13	
Median (min; max)	50 (22; 68)	40 (19; 70)	47 (18; 71)	41 (18; 69)	
Weight					0.16
Mean ± sd	72 ± 14	68 ± 15	70 ± 16	70 ± 14	
Median (min; max)	72 (45; 106)	67 (30; 125)	68 (46; 160)	68 (38; 118)	
Height					0.42
Mean ± sd	169 ± 11	168 ± 8.4	167 ± 9.2	168 ± 9.2	
Median (min; max)	169 (151; 186)	167 (152; 192)	168 (147; 193)	167 (149; 187)	
BMI					0.18
Mean ± sd	25 ± 3.6	24 ± 4.8	25 ± 4.6	25 ± 4.2	
Median (min; max)	25 (16; 33)	23 (11; 43)	25 (17; 49)	25 (11; 37)	
Genetic Proof					0.08
No	0 (0.0%)	3 (2.6%)	1 (0.9%)	0 (0.0%)	
Yes	28 (100.0%)	114 (97.4%)	113 (99.1%)	216 (100.0%)	
Inheritance Line					0.76
Maternal	6 (40.0%)	23 (38.3%)	7 (41.2%)	28 (49.1%)	
Paternal	9 (60.0%)	34 (56.7%)	10 (58.8%)	25 (43.9%)	
Sporadic	0 (0.0%)	3 (5.0%)	0 (0.0%)	4 (7.0%)	
CMTNS [0/. . ./36]					**<0.01**
Mean ± sd	24	14	17	12	
Median (min; max)	25 (17; 32)	13 (3; 22)	17 (7; 24)	12 (3; 21)	
Sensory Symptoms [0/1/2/3/4]					**<0.01**
Mean ± sd	2	1.2	1.4	0.8	
Median (min; max)	2 (0; 4)	1 (0; 3)	1 (0; 4)	0 (0; 4)	
Motor Symptoms_legs [0/1/2/3/4]					**<0.01**
Mean ± sd	2.8	1.3	1.3	0.83	
Median (min; max)	3 (1; 4)	1 (0; 3)	1 (0; 3)	1 (0; 3)	
Motor Symptoms_arms [0/1/2/3/4]					**<0.01**
Mean ± sd	1.8	0.66	0.91	0.36	
Median (min; max)	2 (1; 3)	1 (0; 3)	1 (0; 4)	0 (0; 2)	
Pin Sensibility [0/1/2/3/4]					**<0.01**
Mean ± sd	2.3	1.3	1.8	1.3	
Median (min; max)	2 (0; 4)	1 (0; 4)	2 (0; 3)	1 (0; 4)	
Vibration [0/1/2/3/4]					**<0.01**
Mean ± sd	3	1.9	2.1	1.9	
Median (min; max)	3 (1; 4)	2 (0; 4)	2 (0; 4)	2 (0; 4)	
Strength_legs [0/1/2/3/4]					**<0.01**
Mean ± sd	3.4	1.4	1.8	1.1	
Median (min; max)	4 (1; 4)	1 (0; 4)	2 (0; 4)	1 (0; 4)	
Strength_arms [0/1/2/3/4]					**0.04**
Mean ± sd	2	0.69	1.3	0.81	
Median (min; max)	1.5 (0; 4)	1 (0; 3)	1 (0; 3)	1 (0; 3)	
CMAP_score [0/1/2/3/4]					**<0.01**
Mean ± sd	3	2	2.3	1.3	
Median (min; max)	3 (0; 4)	2 (0; 4)	3 (0; 4)	1 (0; 3)	
SNAP_score [0/1/2/3/4]					0.4
Mean ± sd	3.7	3.3	3.7	3.3	
Median (min; max)	4 (1; 4)	4 (0; 4)	4 (1; 4)	4 (0; 4)	
10 m-timed walking (T10MW)					**<0.01**
Mean ± sd	13 ± 6.2	8.4 ± 5.5	9.9 ± 5.2	7 ± 2.2	
Median (min; max)	11 (6.2; 34)	7.6 (2.8; 35)	8.3 (5.8; 40)	6.8 (2.8; 23)	
VAS					0.15
Mean ± sd	43 ± 34	32 ± 31	40 ± 29	26 ± 29	
Median (min; max)	40 (0; 91)	25 (0; 100)	40 (0; 100)	15 (0; 100)	

(*continued on next page*)

Table 3 (*continued*)

Parameter	Cluster 1	Cluster 2	Cluster 3	Cluster 4	*p* value
9 hole-peg test_D					**<0.01**
Mean ± sd	48 ± 29	23 ± 5.6	27 ± 9.2	21 ± 3.8	
Median (min; max)	37 (24; 165)	22 (13; 45)	24 (18; 66)	20 (16; 44)	
9 hole-peg test_ND					**<0.01**
Mean ± sd	47 ± 27	26 ± 6.5	28 ± 8.8	22 ± 3.7	
Median (min; max)	39 (26; 141)	24 (17; 52)	26 (17; 72)	21 (15; 38)	
Fist Grip_D					<0.01
Mean ± sd	54 ± 40	79 ± 38	144 ± 69	192 ± 81	
Median (min; max)	44 (0; 144)	72 (2; 228)	129 (30; 400)	174 (45; 474)	
Fist Grip_ND					0.04
Mean ± sd	55 ± 44	73 ± 37	88 ± 32	152 ± 73	
Median (min; max)	36 (6; 131)	66 (15; 243)	88 (25; 147)	133 (42; 401)	
Three PointGrip_D					<0.01
Mean ± sd	21 ± 18	29 ± 13	54 ± 25	83 ± 37	
Median (min; max)	17 (2; 74)	30 (4; 59)	52 (7; 132)	76 (12; 235)	
Three PointGrip_ND					**<0.01**
Mean ± sd	16 ± 13	27 ± 12	44 ± 26	88 ± 55	
Median (min; max)	13 (2.2; 48)	27 (5.5; 55)	37 (16; 116)	77 (11; 277)	
Pinch Grip_D					**<0.01**
Mean ± sd	19 ± 21	31 ± 20	36 ± 33	44 ± 34	
Median (min; max)	14 (0; 79)	27 (6.7; 138)	31 (5.2; 184)	37 (0; 258)	
Pinch Grip_ND					**0.01**
Mean ± sd	19 ± 19	29 ± 17	28 ± 13	39 ± 17	
Median (min; max)	15 (0; 70)	26 (5; 118)	30 (0; 55)	38 (0; 94)	
Foot Dorsal_D					**<0.01**
Mean ± sd	7.7 ± 11	20 ± 17	43 ± 39	109 ± 79	
Median (min; max)	4 (0; 46)	16 (0; 101)	30 (0; 210)	86 (0; 446)	
Foot Dorsal_ND					**<0.01**
Mean ± sd	2.9 ± 4.2	16 ± 12	42 ± 55	153 ± 96	
Median (min; max)	0 (0; 14)	15 (0; 71)	21 (0; 216)	189 (2.5; 312)	
Foot Plantar_D					**<0.01**
Mean ± sd	18 ± 23	38 ± 25	88 ± 59	145 ± 96	
Median (min; max)	11 (0; 94)	30 (3; 119)	76 (0; 273)	113 (0; 455)	
Foot Plantar_ND					**<0.01**
Mean ± sd	5.6 ± 7.9	36 ± 25	74 ± 80	200 ± 120	
Median (min; max)	1.5 (0; 28)	30 (1.5; 110)	48 (0; 283)	245 (5.5; 382)	

p values smaller than 5% are printed in bold face.

do not contribute any further significant information after the parameters Ulnar CMAP, Motor Symptoms Legs, Motor Symptoms Arms, Strength of Legs and Sensory Symptoms have been assessed. However, the three secondary parameters Foot Dorsal (dynamometry), 9 hole-peg test and 10 m-timed walking test (T10MW) add significant additional discriminatory power. This statistical approach is unbiased, as even established primary clinical outcome measures were critically examined according to their capability to differentiating evidence-based clusters. Furthermore, our findings suggest that changing or supplementing the established CMTNS with significant secondary clinical items can improve its sensitivity and, thus, lead to a better distinction between different levels of severity in CMT1A.

The classical CMTNS only sub-optimally distinguishes between the aforementioned clusters. A revised version of the CMTNS [17] may be more sensitive, but is not being applied in current studies. In order to identify the best items to be included in a future scoring system, we performed three score hypotheses, each of them individually produces more widely distributed score values on our study cohort compared to the established CMTNS. Score hypothesis (A) (CMTNSfull) exceeds the classical CMTNS with regard to desired width of distribution. The score CMTNSfull provides a higher empirical standard deviation with 8.8 absolute points (14.4%) compared to 4.8 points (13.1%) of the CMTNS. The overall feasibility of the CMTNSfull is debatable, since the additional effort of examining a total of 15 parameters is not renumerated with significantly better outcome in comparison to the other two score hypotheses. This is due to the fact that the CMTNSfull includes primary and secondary clinical outcome measures that do not provide a significant gain in information. Score hypothesis (B) (CMTNSsignif) adds three significant secondary clinical outcome measures to the existing CMTNS, providing a score with 12 parameters. The distribution width in relative numbers is in between the performance of the CMTNSfull and the standard CMTNS with a standard deviation of 6.7 absolute points (13.6%). Finally, the modified CMTNS (CMTNSmod) presented as score hypothesis (C) omits non-significant primary clinical outcome measures and

Table 4
Scoring of secondary clinical parameters. For each secondary clinical parameter cutoffs at which to categorize the numerical values into the scores normal, very mild, mild, moderate, severe (0,1,2,3,4) were determined using z scores, i.e. the distance from the population mean measured in standard deviations. All scores exceeding 4 were set to 4 in order to stay on the within the comparable to the CMTNS subscores. The z scores are computed per age class and gender. This table shows the thresholds that distinguish adjacent scores in each stratum. Dashes indicate unreachable scores: The omitted threshold would be negative which is impossible for the measurement.

		18–30 years					31–40 years					41–50 years					51–60 years					61–70 years				
10 m-timed walking test (T10MW) [sec]	Female	5.1	5.6	6.1	6.6		5.8	6.6	7.3	8.1		5.9	6.5	7.1	7.8		6.3	7.1	7.8	8.5		6.4	7.0	7.5	8.1	
	Male	4.9	5.5	6.1	6.7		5.0	5.3	5.7	6.1		6.2	7.3	8.4	9.5		6.4	7.4	8.5	9.5		6.0	6.7	7.4	8.0	
	Score	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
9 hole-peg test (dominant hand) [sec]	Female	17.2	18.8	20.4	22.0		18.3	20.1	22.0	23.9		18.5	20.1	21.6	23.2		19.8	21.7	23.6	25.5		20.7	22.7	24.8	26.8	
	Male	17.4	19.1	20.7	22.3		18.5	20.1	21.7	23.3		19.3	20.6	21.9	23.2		21.9	23.7	25.6	27.4		23.9	27.5	31.2	34.8	
	Score	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Fist Grip (dominant hand) [kg]	Female	141.0	118.5	95.9	73.3		162.5	130.6	98.7	66.8		127.0	108.0	88.9	69.9		115.3	77.1	38.9	0.7		92.3	64.4	36.5	8.6	
	Male	184.1	118.3	52.6	–		221.7	187.7	153.6	119.5		172.7	103.4	34.1	–		198.9	164.0	129.2	94.3		164.6	117.1	69.7	22.2	
	Score	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Three Point Grip (dominant hand) [kg]	Female	66.2	54.4	42.5	30.6		77.3	67.4	57.5	47.6		65.1	53.7	42.2	30.7		55.9	36.4	16.9	–		57.0	45.3	33.6	21.9	
	Male	77.4	55.5	33.6	11.7		94.5	75.2	56.0	36.7		82.7	42.0	1.4	–		107.3	93.0	78.8	64.5		75.2	48.8	22.5	–	
	Score	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Foot Dorsal (dominant foot) [kg]	Female	129.6	113.1	96.7	80.2		117.6	91.0	64.5	37.9		100.7	63.7	26.6	–		79.4	46.5	13.6	–		62.6	30.3	–	–	
	Male	125.5	72.3	19.2	–		126.9	84.2	41.4	–		80.0	–	–	–		143.6	98.5	53.5	8.4		144.5	94.0	43.4	–	
	Score	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Foot Plantar (dominant foot) [kg]	Female	105.3	77.2	49.1	21.1		90.5	43.1	–	–		92.5	45.5	–	–		100.1	59.8	19.5	–		76.0	46.1	16.1	–	
	Male	145.8	108.3	70.7	33.2		121.4	66.3	11.1	–		78.1	–	–	–		133.0	87.7	42.4	–		129.4	82.6	35.7	–	
	Score	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4

adds solely significant secondary ones (Foot Dorsal (dynamometry), 9 hole-peg test and 10 m-timed walking test (T10MW)). The overall score consists of 8 parameters (max. total of 32 points). The empirical standard deviation is the statistically best fitting model with 5.3 absolute points (16.0%), while being viable in a clinical setting at the same time. The CMTNSmod is furthermore the only score, which actually reaches the value zero. This circumstance represents a strong criterion for quality. As a conclusion, in this explorative approach, the CMTNSmod serves best the purpose of identifying clinical disease severity among all mentioned score hypotheses including the classical CMTNS. We believe the CMTNSmod consisting of only 8 parameters is faster to perform than the classical CMTNS, since time consuming examinations like SNAP electrophysiology and arm dynamometry are being replaced by easy to perform examinations like the 10 m-timed walking test (T10MW) and 9 hole-peg test. While regional differences apply, we believe that the less time consuming CMTNSmod is also cheaper to perform. Re-testing in terms of time consumption and a cost-effective analysis is needed for validation.

Even though the presented data already show that there are changes to the established CMTNS that might increase its sensitivity, there is need for more data on repeated analyses. In a small pilot trial, we re-assessed 10 patients 3 years after the first assessment. On these 10 patients, the CMTNS increases in average by 1.7 points. Using the modified version of the score, this mean difference jumps to 2.5 (165% increase). Clearly, these pilot findings need to be validated in a larger cohort. Currently, we are validating the progression data of the CMTNSmod in the setting of a longitudinal study three years after the initial score assessment. In summary continuously improved clinical scoring measures in combination with biomarkers may enable clinicians to detect small clinical changes over time in CMT patients. The usage of selected secondary outcome measures will greatly facilitate future clinical trials in Charcot-Marie Tooth disease which are so urgently needed.

5. Appendix

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6. Contribution

M.M. performed patient examinations, statistical analysis and wrote the manuscript. M.W.S. planned the biomarker trial, examined patients and wrote the manuscript.

A.S.; T.J.S.; A.L.P.-N; O.S.; N.G.-A.; G.M.F.; F.G.; L.P.; L.S.; A.Q.; G.V.; D.C.; M.L.; J.H.; R.M.; CMT-TRIAAL/CMT-TRAUK Group; performed patient examinations. P.S.; J.B.; B.S.-W.; M.C.W.; A.S. planned the biomarker trial and examined patients. D.P.; M.M.R.; M.E.S. planned the Ascorbic acid trial, examined patients and/or critically revised the manuscript. A.L.; T.B.; C.K. performed statistical analysis and wrote the manuscript. B.R.; P.Y.; W.P. planned the biomarker trial and contributed patients.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.nmd.2014.06.431.

References

[1] Skre H. Genetic and clinical aspects of Charcot-Marie-Tooth's disease. Clin Genet 1974;6:98–118.
[2] Braathen GJ. Genetic epidemiology of Charcot-Marie-Tooth disease. Acta Neurol Scand 2012;Suppl iv–22. doi: 10.1111/ane.12013.
[3] Lupski JR, Garcia CA, Parry GJ, Patel PI. Charcot-Marie-Tooth polyneuropathy syndrome: clinical electrophysiologic and genetic aspects. In: Appel SH, editor. Current Neurology. Chicago: Wiley Medical; 1991. p. 1–25.
[4] Kaku DA, Parry GJ, Malamut R, et al. Nerve conduction studies in Charcot Marie-Tooth polyneuropathy associated with a segmental duplication of chromosome 17. Neurology 1993;43:1806–8.
[5] Garcia CA, Malamut RE, England JD, et al. Clinical variability in two pairs of identical twins with the Charcot-Marie-Tooth disease type 1A duplication. Neurology 1995;45:2090–3.
[6] Pareyson D, Marchesi C. Diagnosis, natural history, and management of Charcot-Marie-Tooth disease. Lancet Neurol 2009;8:654–67. http://dx.doi.org/10.1016/S14744422(09)70110-3.
[7] Reilly MM, Murphy SM, Laurá M. Charcot-Marie-Tooth disease. J Peripher Nerv Syst Jpn 2011;16:1–14. http://dx.doi.org/10.1111/j.1529-8027.2011.00324.x.
[8] Schenone A, Nobbio L, Monti Bragadin M. Inherited neuropathies. Curr Treat Options Neurol 2011;13:160–79. http://dx.doi.org/10.1007/s11940-011-0115-z.
[9] Siskind CE, Shy ME. Genetics of neuropathies. Semin Neurol 2011;31:494–505. http://dx.doi.org/10.1055/s-0031-1299788.
[10] Lupski JR, de Oca-Luna RM, Slaugenhaupt S, et al. DNA duplication associated with Charcot-Marie-Tooth disease type 1A. Cell 1991;66:219–32.
[11] Raeymaekers P, Timmerman V, Nelis E, et al. Duplication in chromosome 17p11.2 in Charcot-Marie-Tooth neuropathy type 1a (CMT 1a). The HMSN Collaborative Research Group. Neuromuscul Disord Nmd 1991;1:93–7.
[12] Vance JM, Nicholson GA, Yamaoka LH, et al. Linkage of Charcot-MarieTooth neuropathy type 1a to chromosome 17. Exp Neurol 1989;104:186–9.
[13] Reilly MM, Shy ME, Muntoni F, Pareyson D. 168th ENMC International Workshop: outcome measures and clinical trials in Charcot-Marie-Tooth disease (CMT). Neuromuscul Disord Nmd 2010;20:839–46. http://dx.doi.org/10.1016/j.nmd.2010.08.001.
[14] Fledrich R, Stassart RM, Sereda MW. Murine therapeutic models for Charcot-Marie-Tooth (CMT) disease. Br Med Bull 2012;102:89–113. http://dx.doi.org/10.1093/bmb/lds010.
[15] Shy ME, Blake J, Krajewski K, et al. Reliability and validity of the CMT Neuropathy Score as a measure of disability. Neurology 2005;64:1209–14. http://dx.doi.org/10.1212/01.WNL.0000156517.00615.A3.
[16] Reilly MM, de Jonghe P, Pareyson D. 136th ENMC International Workshop: Charcot-Marie-Tooth disease type 1A (CMT1A)8–10 April 2005, Naarden, The Netherlands. Neuromuscul Disord Nmd 2006;16:396–402. http://dx.doi.org/10.1016/j.nmd.2006.03.008.
[17] Murphy SM, Herrmann DN, McDermott MP, et al. Reliability of the CMT Neuropathy Score (second version) in Charcot-Marie-Tooth disease. J Peripher Nerv Syst Jpn 2011;16:191–8. http://dx.doi.org/10.1111/j.1529-8027.2011.00350.x.
[18] Pareyson D, Reilly MM, Schenone A, et al. Ascorbic acid in Charcot- Marie-Tooth disease type 1A (CMT-TRIAAL and CMT-TRAUK): a double-blind randomised trial. Lancet Neurol 2011;10:320–8. http://dx.doi.org/10.1016/S1474-4422(11)700254.
[19] De Visser M, Verhamme C. Ascorbic acid for treatment in CMT1A: what's next? Lancet Neurol 2011;10:291–3.
[20] Fledrich R, Schlotter-Weigel B, Schnizer TJ, et al. A rat model of Charcot-Marie-Tooth disease 1A recapitulates disease variability and supplies biomarkers of axonal loss in patients. Brain J Neurol 2012;135:72–87. http://dx.doi.org/10.1093/brain/awr322.

[21] Shy ME, Chen L, Swan ER, et al. Neuropathy progression in CharcotMarie-Tooth disease type 1A. Neurology 2008;70:378–83. http://dx.doi.org/10.1212/01.wnl.0000297553.36441.ce.
[22] Solari A, Laurà M, Salsano E, et al. Reliability of clinical outcome measures in Charcot-Marie-Tooth disease. Neuromuscul Disord Nmd 2008;18:19–26. http://dx.doi.org/10.1016/j.nmd.2007.09.006.
[23] Huskisson EC. Measurement of pain. J Rheumatol 1982;9:768–9.
[24] Apolone G, Mosconi P. The Italian SF-36 Health Survey: translation, validation and norming. J Clin Epidemiol 1998;51(11):1025–36. http://dx.doi.org/10.1016/S08954356(98)00094-8.
[25] Padua L, Shy ME, Aprile I, et al. Correlation between clinical/neurophysiological findings and quality of life in Charcot-Marie-Tooth type 1A. J Peripher Nerv Syst 2008;13(1):64–70. http://dx.doi.org/10.1111/j.1529-8027.2008.00159.x.
[26] Venables WN, Ripley BD. Exploratory multivariate analysis. In: Venables WN, Ripley BD, editors. Modern Applied Statistics with S. New York: Springer; 2002. p. 495.
[27] Handl J, Knowles J, Kell DB. Computational cluster validation in postgenomic data analysis. Bioinformatics 2005;21:3201–12. http://dx.doi.org/10.1093/bioinformatics/bti517.
[28] Brock G, Pihur V, Datta S, Datta S. ClValid: an r package for cluster validation. J Stat Softw 2008;25:1–22.
[29] Pihur V, Datta S, Datta S. RankAggreg, an R package for weighted rank aggregation. BMC Bioinf 2009;10:62.
[30] Core Team R. R: A Language and Environment for Statistical Computing. Vienna: Austria; 2013.