Early Axonal Dysfunction of the Peripheral Nervous System Influences Disease Progression of ALS: Evidences From Clinical Neuroelectrophysiology

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Research

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Abstract

Background:

Studies have indicated that more axonal dysfunction in early ALS predictive of more aggressive phenotypes. However, as an important electrophysiological index to detect motor axonal damage, the prognostic value of compound muscle action potential (CMAP) in ALS remains unclear. We aimed to determine if CMAP could be a prognostic indicator of disease progression in early ALS.

Methods: Patients were stratified into 4 groups according to the decrement of the CMAP amplitude within 12 months of symptom onset (CMAP-12 amplitudes): normal (≥the lower limit of normal, LLN), mild (<LLN but ≥50% of LLN), moderate (<50% but ≥30% of LLN) and severe (<30% of LLN). All patients were followed up every 3 months. Survival was analyzed using the Kaplan-Meier method and Cox proportional hazards regression.

Results: A total of 149 patients included in the analysis [90 male (60.4%); mean age at onset, 50.7 years]. The decrement of CMAP-12 amplitudes was normal in 24.2%, mildly in 22.1%, moderately in 15.4% and severely in 38.3%. Kaplan-Meier analysis showed there was a significant difference in the overall survival across the 4 groups ($p<0.05$). Further pairwise comparison found there were significant differences in survival between the normal *vs.* the moderately groups ($p<0.05$), and the normal *vs.* the severely groups ($p<0.01$). There was a significant inverse correlation between the CMAP-12 amplitude and overall survival. Compared to the normal group, survival in the moderately and severely decreased groups were significantly shorter (HR 3.394, 95%CI 1.292-8.917, p=0.013; and HR 4.732, 95%CI 2.032-11.017; p=0.000, respectively).

Conclusions: Our results suggested that CMAP-12 amplitude could be a prognostic indicator of disease progression in ALS. More importantly, it provided clinical evidences to the viewpoint that early axonal dysfunction of peripheral nervous system accelerates disease progression of ALS.

Background

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the degeneration of both upper and lower motor neurons. The prognosis and survival of ALS can vary between patients, as more than 60% of the patients die within 3 years of onset, while approximately 10% of the patients have a survival period of more than 8 years [1–4].

Although the fundamental mechanisms underlying ALS are not well understood, current knowledge suggests that the main processes include impaired protein homeostasis, aberrant RNA metabolism, mitochondrial dysfunction, oxidative stress, glutamate excitotoxicity, nuclear export, impaired DNA repair, dysregulated vesicle transporters, glial dysfunction, and neuroinflammation. The increased excitability of motor nerves and axonal dysfunction are also considered to be pathophysiological mechanisms of ALS [2, 5–8]. It was showed that in the early stage of the disease, synaptic integrity and the stability of the

distal cytoskeleton from the neuromuscular junction (NMJ) are destroyed, triggering a cascade of reactions toward the cell body (“dying-back” hypothesis). Also, peripheral motor axons are involved in the pathogenesis and cascade of the development of ALS [9]. It was proved that the altered axonal excitability potentially contributes to motor neuron death in ALS, and that the excitability of the axons occurred from the distal to proximal [5–7]. It means that the axonal dysfunction rather than motor neuron loss in the anterior horn of the spinal cord is the cause of disease acceleration, and that more axonal dysfunction seems predictive of a more aggressive phenotype [10–12].

The amplitude of compound muscle action potential (CMAP) is widely used in clinical practice as an important electrophysiological index to detect motor axonal damage [13]. It was found that the decrease in the CMAP amplitude of the motor nerve in early stage was significantly related to motor axonal hyperexcitability, as increased persistent sodium currents and reduced potassium currents in motor axons [5, 6, 14]. To explore the relationship between motor axonal dysfunction in early stage and disease progression in patients with ALS, we studied the CMAP amplitude within 12 months of symptom onset (CMAP-12 amplitude) in these patients. We analyzed the relationship between the decrease in CMAP-12 amplitude and survival of patients with ALS. We hope to obtain clinical evidence that supports the integrity of the peripheral nervous system (PNS) plays a protective role in the pathogenesis and progression of ALS in animal experiments [11, 12].

Methods

Participants

Patients were recruited from January 2010 to December 2013, and each patient was given a follow-up evaluation by outpatient consultation or telephone every 3 months. For all cases, baseline demographic information and clinical data were collected during the patient’s first visit and follow-up evaluations. Survival and tracheotomy were predefined as primary outcome measures.

Patients were diagnosed and classified according to the Airlie House diagnostic criteria [15]. Those with pure lower motor syndromes were classified into an additional category of suspected ALS because they could not be classified using the established criteria [16]. All patients were interviewed and examined by board-certified neurologists from the study group who had experience with ALS. According to the site of onset and clinical features, patients were categorized with limb-onset ALS, bulbar-onset ALS, flail arm syndrome (FAS)-type ALS [17], progressive muscle atrophy (PMA), or suspected primary lateral sclerosis (PLS) (those fulfilling all the diagnostic criteria of PLS besides the course of the disease were defined) [18]. Since the etiology and prognosis of familial or juvenile ALS can be quite different from sporadic ALS, patients with familial and juvenile ALS were excluded from the analysis.

CMAP Examination and Grouping

Clinical neurophysiologic examinations were carried out by using a Key point four-channel electromyography evoked potentiometer (Medtronic, USA). The motor nerve conduction of the median

and ulnar nerves in the upper extremities as well as the peroneal and tibial nerves in the lower extremities were examined bilaterally by routine methods. The amplitude of the CMAP and nerve conduction velocity were recorded. All patients' sensory nerve conductions were also examined. The embedded pressure syndrome should be excluded. The examination of all patients was strictly confined to perform within 12 months of symptom onset.

Among the above-mentioned 8 nerves examined in each patient, the nerve with the most obvious change of the CMAP-12 amplitude was selected as the grouping basis. The amplitudes of the CMAP-12 were stratified into four groups: normal (≥ the lower limit of normal, LLN), mild decrease (< LLN but ≥ 50% of LLN), moderate decrease (<50% but ≥30%of LLN), and severe decrease (<30% of LLN). The ALS Functional Rating Score (ALS-FRS) and ALS Functional Rating Score - Revised (ALS-FRS-R) were performed at the same time.

Statistical Analysis

The data were collected to establish a database and SPSS 22.0 was used for data analysis (SPSS, Chicago, Illinois, USA). Continuous clinical and demographic variables that were normally distributed were compared using parametric tests (one-way analysis of variance, ANOVA), and categorical variables were analyzed using chi-square tests. The censoring date for the survival data was December 2017. Survival curves were estimated using Kaplan-Meier analysis, and covariates were compared using the log-rank test and Cox proportional hazards regression model.

Results

A total of 149 patients with ALS were studied, including 90 males (60.4%) and 59 females (39.6%), with a male to female ratio of 1.5:1. The oldest onset age was 83 years old, and the youngest was 22 years old, with a mean age at symptom onset of 50.7 years.

There were 16 patients (10.7%) with bulbar onset, 85 patients (57.1%) with upper-limb onset and 48 patients (32.2%) with lower-limb onset. Regarding the phenotypes, 120 patients (80.5%) had the typical limb-onset of ALS, 14 patients (9.4%) had the bulbar-onset of ALS, 8 patients (5.4%) had FAS-type ALS, 4 patients (2.7%) had PMA, and 3 patients (2%) had suspected PLS. Diagnostic grades included 37 patients (24.8%) as definite, 39 patients (26.2%) as probable, 43 patents (28.9%) as laboratory-supported probable, 25 patients (16.8%) as possible, and 5 patients (3.3%) as suspected ALS.

Within 12 months of symptom onset, the amplitudes of the CMAP, i.e. CMAP-12 amplitudes, were normal in 36 patients (24.2%), mildly decreased in 33 patients (22.1%), moderately decreased in 23 patients (15.4%), and severely decreased in 57 patients (38.3%). There was an overall difference in the onset sites among the four groups ($p < 0.01$), with more bulbar-onset patients in the normal group and more limb-onset patients in the moderately or severely decrease groups. The scores of the ALS-FRS and ALS-FRS-R were lower in the severely decreased CMAP-12 amplitude group than the normal and mildly decreased groups ($p < 0.01$), but there were no significant differences between the other groups (Table 1). There were

no significant differences in the sex ratio, onset age, phenotype of disease, and diagnostic grade among the four groups.

Table 1
Demographics and clinical characteristics of ALS patients in different severity of decrements of CMAP-12 amplitude

	Normal	Mild	Moderate	Severe	Total	p-value
Total n(%)	36 (24.2)	33 (22.1)	23 (15.4)	57 (38.3)	149	
Age at onset	51 ± 10.3	51.4 ± 13.0	50.5 ± 9.9	50.3 ± 10.0		0.964
Sex ratio(M:F)	1.4:1	1.4:1	0.9:1	2.2:1	1.5:1	0.367
Onset site, n(%)						0.000[a]
Bulbar	9 (25.0)	6 (18.2)	1 (4.3)	0	16 (10.7)	
Upper limbs	14 (38.9)	23 (69.7)	16 (69.6)	32 (56.1)	85 (57.1)	
Lower limbs	13 (36.1)	4 (12.1)	6 (26.1)	25 (43.9)	48 (32.2)	
Phenotype, n (%)						0.066
Limb-onset ALS	24 (66.7)	25 (75.8)	19 (82.6)	52 (91.2)	120 (80.5)	
Bulbar-onset ALS	8 (22.2)	5 (15.1)	1 (4.3)	0	14 (9.4)	
FAS-type ALS	1 (2.8)	2 (6.1)	2 (8.7)	3 (5.3)	8 (5.4)	
PMA	2 (5.5)	0	1 (4.3)	1 (1.8)	4 (2.7)	
Suspected PLS	1 (2.8)	1 (3.0)	0	1 (1.8)	3 (2.0)	
Airlie House category (%)						0.399
Clinically definite	7 (19.4)	7 (21.2)	7 (30.4)	16 (28.1)	37 (24.8)	
Clinically probable	6 (16.7)	7 (21.2)	9 (39.1)	17 (29.8)	39 (26.2)	
Laboratory-supported probable	15 (41.7)	9 (23.3)	3 (13.1)	16 (28.1)	43 (28.9)	

[a] $p \leq 0.01$

	Normal	Mild	Moderate	Severe	Total	p-value
Clinically possible	6 (16.7)	9 (27.3)	3 (13.1)	7 (12.3)	25 (16.8)	
Suspected	2 (5.5)	1 (3.0)	1 (4.3)	1 (1.7)	5 (3.3)	
FRS score	34.6 ± 4.6	35.3 ± 3.6	33.6 ± 4.0	31.7 ± 5.1		0.001[a]
FRS-R score	42.3 ± 5.0	43.2 ± 3.7	41.7 ± 4.0	39.1 ± 5.4		0.001[a]
[a] $p \leq 0.01$						

In Kaplan-Meier analysis, sex, onset site, phenotype of disease, and diagnostic grade had no significant impact on survival ($p > 0.05$), but the CMAP-12 amplitude had a significant effect on survival. There was a significant difference in the overall survival in the four groups ($p < 0.05$) (Table 2). Further pairwise comparisons among different categories of CMAP-12 amplitude revealed a statistically significant difference in survival between the normal group and the moderately decreased group ($p < 0.05$), and between the normal and severely decreased groups ($p < 0.01$). There was also a significant difference in survival between those with mildly decreased CMAP-12 amplitude and those with severely decreased CMAP-12 amplitude ($p < 0.05$) (Table 3). There were no other differences among the groups. The Kaplan-Meier survival plots are presented in Fig. 1.

Table 2
Kaplan-Meier survival analysis for sex, onset site, phenotype, diagnostic grade, and CMAP

	Category	Median survival	χ^2	p-value
Sex	Male	55	3.73	0.053
	Female	81		
Onset site	Bulbar	87	0.083	0.893
	Upper-limb	56		
	Lower-limb	61		
Phenotype	Limb-onset	56	0.599	0.439
	Bulbar-onset	87		
	FAS-type ALS	37		
	PMA	46		
	suspected PLS			
Airlie House category	Definite	81	0.001	0.978
	Probable	55		
	Lab-supported probable	60		
	Possible	50		
	Suspected	46		
CMAP	Normal	111	17.428	0.000[a]
	Mild	N		
	Moderate	55		
	Severe	40		

[a] $p \leq 0.01$

Table 3
Log-rank (Mantel-Cox) pairwise comparisons in different categories of decreased CMAP amplitude

	Normal		Mild		Moderate		Severe	
	χ^2	p.	χ^2	p	χ^2	p	χ^2	p
Normal			1.183	0.277	4.790	0.029[a]	15.109	0.000[b]
Mild	1.183	0.277			0.967	0.325	6.465	0.011[a]
Moderate	4.790	0.029[a]	0.967	0.325			1.364	0.243
Severe	15.109	0.000[b]	6.465	0.011[a]	1.364	0.243		

[a] $p \leq 0.05$, [b]$p \leq 0.01$

The variables as sex, onset site, diagnostic grade, CMAP-12 amplitude, ALSFRS-R were included in the Cox regression model except that phenotype was not included due to fewer cases in some categories. The Cox proportional hazard regression analysis confirmed a significant inverse correlation between CMAP-12 amplitude and overall survival ($p < 0.05$), adjusting for the confounding effects of sex, onset site, and diagnostic grade. The change in CMAP-12 amplitude was correlated with prognosis, as compared to the normal group, the survival of the moderately decreased group was significantly shorter ($p < 0.05$), and the survival in the severely decreased group was the shortest ($p < 0.01$) (Table 4; Fig. 2). These data indicated that a decrease in CMAP-12 amplitude was an independent risk factor for fast progression. Cox regression model also showed a poorer prognosis of patients with lower ALSFRS-R ($p < 0.05$) (Table 4).

Table 4
Cox proportional hazard regression analysis

Variable	HR	95%CI		P value
		Lower	Upper	
Sex				
male	1			
female	0.659	0.379	1.148	0.141
Age at onset	1.014	0.988	1.040	0.288
Onset site				
Bulbar	1			
Upper-limb	0.814	0.316	2.094	0.669
Lower-limb	0.500	0.178	1.401	0.187
Airlie House category				
Clinically definite	1			.
Clinically probable	1.411	0.679	2.932	0.356
Laboratory-supported probable	1.415	0.653	3.065	0.379
Clinically possible	2.110	0.855	5.205	0.105
Suspected	1.334	0.286	6.226	0.714
ALSFRS-R	0.929	0.878	0.984	0.012[a]
CMAP-12				
Normal	1			.
Mild	1.924	0.715	5.178	0.195
Moderate	3.394	1.292	8.917	0.013[a]
Severe	4.732	2.032	11.017	0.000[b]

[a] $p \leq 0.05$, [b] $p \leq 0.01$; HR: Hazard Ratio; CI: Confidence Interval

The patients with moderately and severely decreased CMAP-12 amplitude were further divided into two subgroups: recorded within 6 months (31 cases) and within 12 months (49 cases). The Kaplan-Meier

survival analysis showed that the median survival time of the 6-month group was 27 months, and that of the 12-month group was 55 months. The survival time of the 6-month group was significantly shorter than that of the 12-month group ($p = 0.015$).

Discussion

Our study found that CMAP-12 amplitudes in patients with ALS had remarkable divergences. Eighty patients (53.7%) had CMAP-12 amplitudes decreased by more than 50%, suggesting that approximately half of the patients had obvious motor axon dysfunction in the early stage of the disease. From the ALSFRS and ALSFRS-R scores, we can see that the severity of the disease in the moderately and severely decreased, but not the mildly decreased, groups was significantly worse than that of the normal group. This confirms that motor axonal dysfunction could occur in the relatively early stage of ALS and that a greater decrease in CMAP amplitude is associated with a more deterioration in clinical function.

The univariate survival analysis showed that the reduction in the CMAP-12 amplitude was significantly correlated with survival. A further decrease in the CMAP-12 amplitude was associated with a further shorter survival. Multivariate analysis, after adjusting the influence of multiple confounding factors, further found that the CMAP-12 amplitude remained closely related to survival. The survival time of patients with moderately and severely decreased CMAP-12 amplitude was significantly shorter than that of the normal group, with the severely decreased CMAP-12 amplitude to be associated with the shortest survival. The results indicated that the decrement in CMAP amplitude within 12 months of onset can be used as an independent prognostic factor. Furthermore, our subgroup analysis showed that the survival period was shorter in the patients with moderately and severely decreased CMAP amplitudes within 6 months than in patients with similar decreases within 12 months, suggesting that the earlier the amplitude of CMAP decreased, the worse the prognosis.

There have been studies evaluating CMAP amplitude, neurophysiological index (NI), motor unit number estimation, and motor unit number index as electrophysiological markers for the prognosis of the disease [12, 19–26]. Our present study differs from previous studies in defining the assessment time for CMAP changes in patients to within 12 months of onset, based on the fact that the median survival time of patients with ALS is generally 2 to 4 years, and 12 months may be regarded as a relatively early stage of the disease [27–29]. We believe that it is more meaningful to study the changes of CMAP amplitude in ALS patients in the early stages of the disease than the whole course of the disease. We speculate that the mechanism of the decline in CMAP-12 amplitude may be consist of two components: 1) hyperexcitblity of peripheral motor axons, and 2) motor axonal damage associated with earlier motor neuronal death [6–8]. Using a computerised program for multiple excitability measurements in the median nerve at the wrist, Kanai *et al*. proved that motor axonal excitability properties are strong predictors for survival in patients with ALS [7]. Besides concerning the early stage (within 12 months of onset), we used a much more routine electrophysiological method as well as selected the most obvious changeable nerve, making the highlights of our study.

Fischer *et al.* found that denervated synapses of IIb/x muscle fibers in ALS SOD1 mice (47 days old) and axonal loss of the ventral peripheral nerve (80 days old) occurred before neuronal cell body loss (100 days old) [30]. It was found that proximal axonopathy in ALS is associated with the loss of neurofilament (NF) protein in the terminal neuromuscular junction as well, and the progressive loss of NFs may evolve from distal to proximal [31]. In recent years, a dying-back hypothesis has attracted much attention [10, 30, 32, 33]. According to this hypothesis, motor nerves and nerve endings exhibit pathophysiological changes before degeneration of motor neurons and clinical symptom onset in some patients. In particular, the hypothesis suggests that ALS could be a distal axonopathy and that NMJ function may change first and then the pathophysiological changes progress proximal to the cell body [33]. Evidence of dying-back was found in an autopsy of an ALS patient with denervation and innervation of the muscles, while no pathological changes occurred in the motor neurons themselves [30]. Recent studies have found that stimulated Raman scattering microimaging can sensitively detect peripheral nerve degeneration in ALS mice and pathological specimens from ALS patients. It was also found that clear degeneration of the peripheral nerve appeared at the same time as denervated muscle in an early clinical mouse model, and that these changes occurred earlier than hypofunction of the motor nerve [34].

Nardo *et al.* found that, compared with C57SOD1^{G93A} mice with slow disease progression, 129SvSOD1^{G93A} mice with rapid disease progression had significant peripheral axonal loss during the onset of the disease, which suggested that PNS damage rather than motor neuron loss itself was related to the rapid progression of the disease [11, 12]. Grouping CMAP amplitudes as determined within 12 months and comparing the differences among groups could help to sort out patients with different patterns of disease progression and to study the underlying mechanism of this progression and even apply drug trials in the future. Our study supported that peripheral motor axonal dysfunction can occur in the early stage of ALS, and that the degree of the axonal injury was related to disease progression and survival. Maintaining peripheral nerve integrity is essential to slow down the progression of the disease [12], which may provide a clue to future drug development.

In this study, the percentage of bulbar-onset patients was lower; yet, this result is consistent with our previous study [4]. It showed a higher proportion of bulbar-onset patients in the normal CMAP-12 amplitude group compared to the other ones, which seems to contradict to the common belief that this type of patients usually has a poor prognosis. However, it is noteworthy that in 14 patients with bulbar-onset of ALS, there are five patients (35.7%) conformed to the isolated bulbar phenotype of ALS [35–36]. This may partly explain why the median survival of the patients with bulbar-onset of ALS was no difference compared with those with limb-onset in this study (Table 2). On the other hand, although Kanai *et al*. showed the motor axonal excitability properties were strong and independent predictors for shorter survival in ALS patients, they did not find differences of the prognosis by multivariate analysis between the bulbar-onset and non-bulbar-onset patients in their study [7].

In addition, we noticed that the median survival of the patients with FAS-type ALS was shorter (Table 2). Consistent with it, we recently proved that 12-month duration was an important criterion for FAS, otherwise the prognosis of FAS-type ALS was significantly worse than that of FAS [17].

This study has several limitations. First, the sample size of patients was limited, especially patients in the moderately decreased CMAP-12 amplitude group. Second, important data on the use of noninvasive ventilation, riluzole or gastrostomy were absent in this study. Third, we studied only the CMAP amplitude of motor nerves. Recently, Miyaji *et al.* showed that the attenuation rate of repetitive nerve stimulation (RNS) was negatively correlated with the CMAP amplitude of the first wave. The higher the RNS attenuation rate was, the lower the CMAP amplitude, which probably indicates that there was a correlation between terminal axonal dysfunction and NMJ injury [37]. In future, we might need to combine the measurements of the CMAP-12 amplitude with NMJ changes in patients with ALS to better understand the origin and mechanisms of ALS.

Conclusions

Our data support the previous animal studies from a clinical electrophysiological point of view by showing that the severity of the decrements of CMAP amplitude in the early stage of ALS is significantly correlated with the severity of the disease. The decrement in CMAP-12 amplitude could be an electrophysiological marker to predict disease progression and survival.

Abbreviations

ALS
Amyotrophic lateral sclerosis; NMJ:Neuromuscular junction; CMAP:Compound muscle action potential; CMAP-12 amplitude:CMAP amplitude within 12 months of symptom onset; PNS:Peripheral nervous system; FAS:Flail arm syndrome; PMA:Progressive muscle atrophy; PLS:Primary lateral sclerosis; LLN; Lower limit of normal; ALS-FRS:ALS Functional Rating Score; ALS-FRS-R:ALS Functional Rating Score - Revised; NI:Neurophysiological index; NF:Neurofilament; RNS:Repetitive nerve stimulation.

Declarations

Ethics approval and consent to participate

This study was approved by the institutional ethics committee of the Peking University Third Hospital (IRB 00006761). Written informed consent was obtained from each patient.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

DF and HY conceived and designed the study. LC, SZ and JH performed the experiments. HY wrote the manuscript. DF reviewed and edited the manuscript. All authors read and approved the manuscript.

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Figures

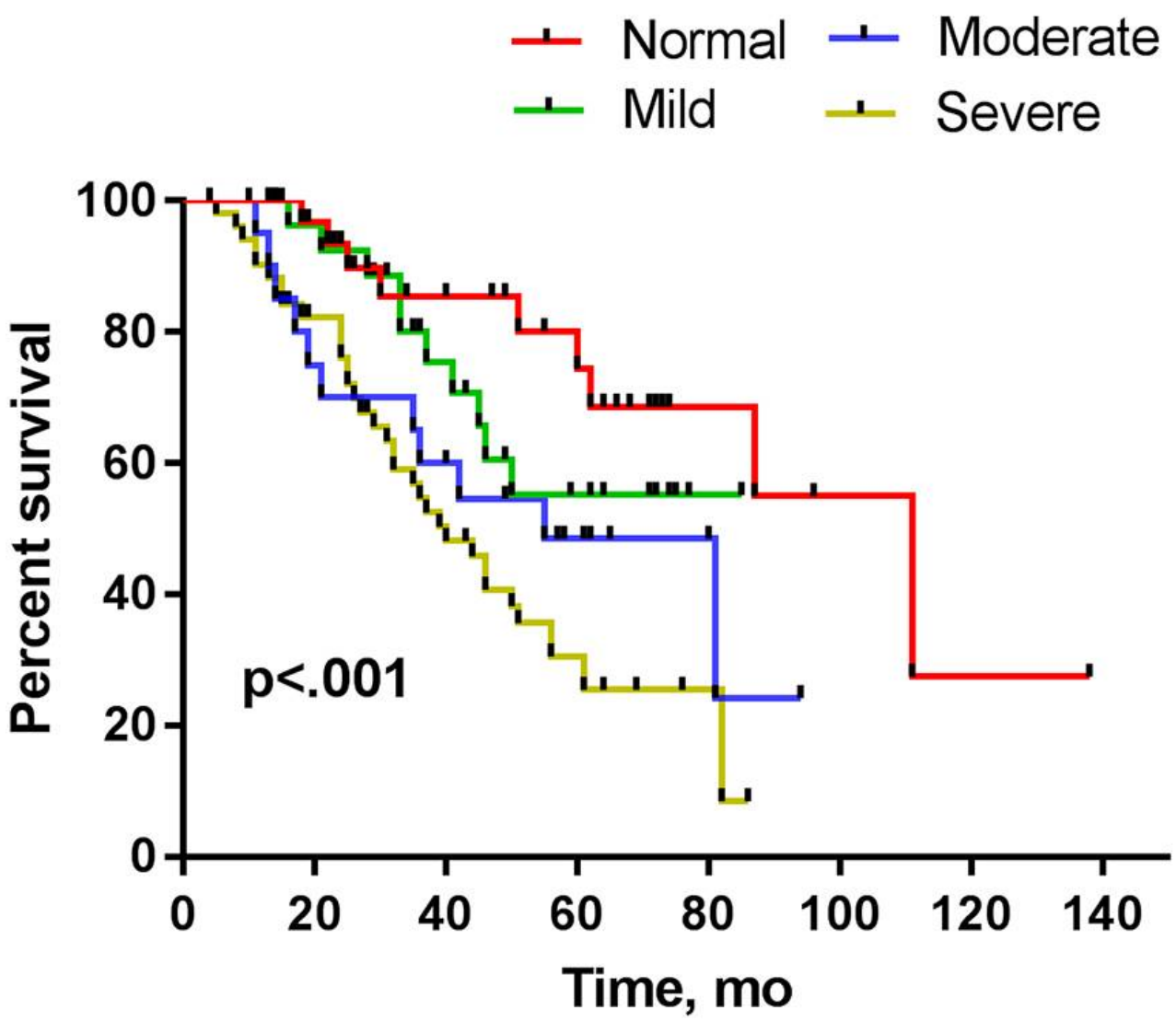

Figure 1

Kaplan-Meier plots of surviving patients stratified by decrements in CMAP amplitude

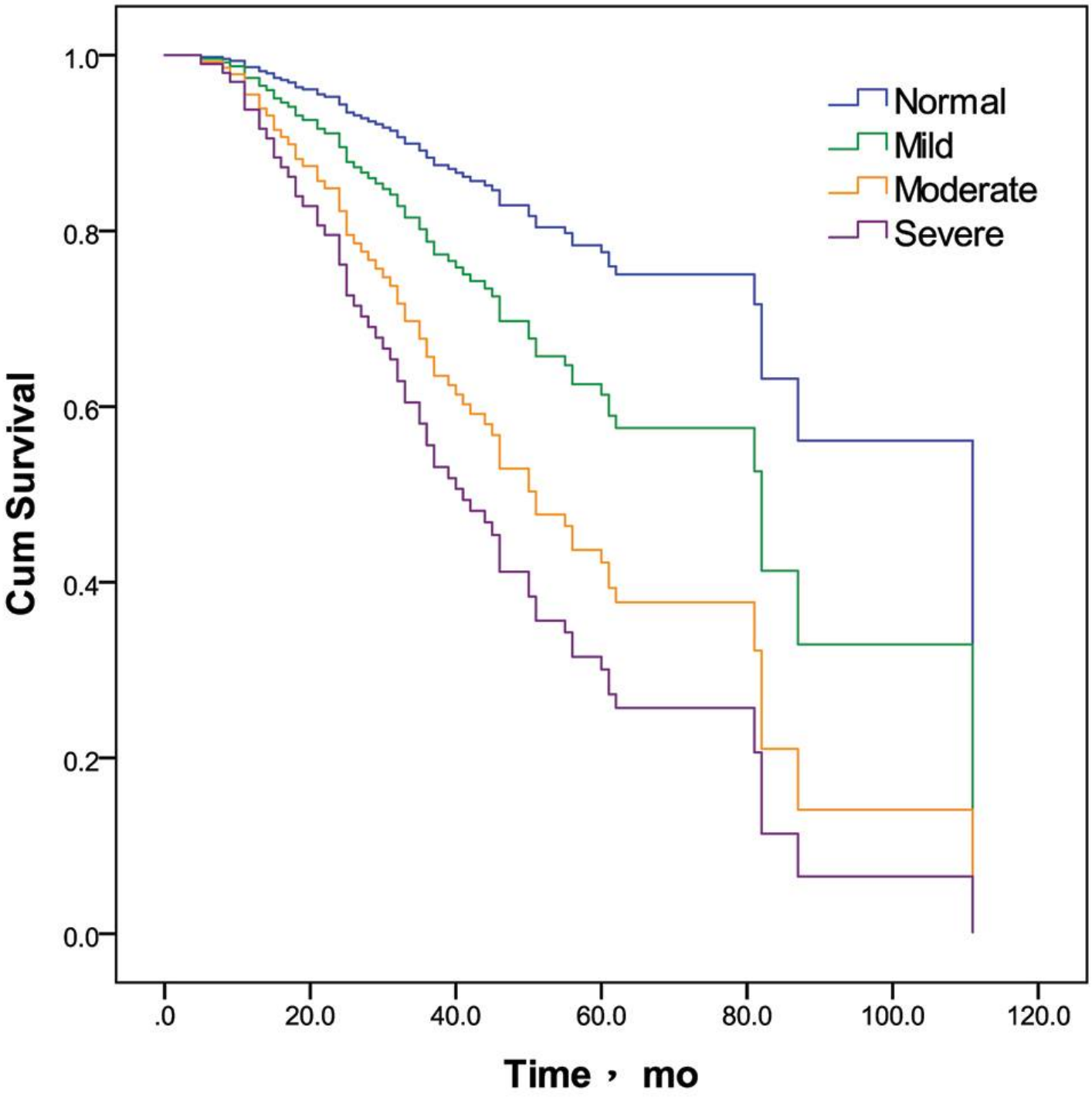

Figure 2

Survival curves stratified by decrements in CMAP amplitude in the Cox proportional hazard regression model

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