Clinical features and outcomes of the flail arm and flail leg and pure lower motor neuron MND variants: a multicenter Italian study

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INTRODUCTION

Motor Neuron Disease (MND) is a heterogeneous group of neurodegenerative disorder defined by a progressive upper (UMN) and lower motor neuron (LMN) loss in a varying combination, encompassing a heterogeneous clinical spectrum depending on different body region involvement at onset, extent and rate of motor neuron (MN) loss and disease spreading. Amyotrophic lateral sclerosis (ALS) is the most common and severe form of MND, leading to death in about four years from symptoms onset. To date, the mainstay neuroprotective therapy is Riluzole, despite its mild efficacy, while the role of Edaravon is still debated. Phenotypic heterogeneity is increasingly recognized within the MND spectrum, ranging from selective UMN or LMN involvement, to classic ALS, when widespread combination of UMN and LMN dysfunction occurs.(1) The clinical spectrum of MND has been further detailed with the recognition of flail arm (FA), flail leg (FL) and pure lower motor neuron (PLMN) phenotypes, considered as restricted MND phenotypes characterized by a predominant or selective LMN disease (LMND), being UMN dysfunction absent or marginal.(1, 2) Furthermore, MND patients can show an extra-motor involvement such as cognitive impairment with the development, in about 10 to 15 % of cases, of frontotemporal dementia (FTD).

Few studies have previously focused on these LMN restricted phenotypes, therefore, the aim of the present study is to retrospectively investigate the differentiating features of FA, FL and PLMN phenotypes in a large Italian MND cohort.

MATERIALS AND METHODS

2648 MND patients were recruited in thirteen Italian ALS referral centers from January 2009 to December 2013 and collected in a common database, that was cleaned before data analysis. To highlight the distinguishing features of FA, FL and PLMN patients, the classic and bulbar phenotypes were used as controls. The final dataset consisted of 1944 patients. ALS diagnosis was established in accordance with Revised El Escorial criteria (EECr) at time of diagnosis. However, a subgroup of patients with FA, FL and PLMN did not fulfill EECr and were classified into the additional category "unclassified".(2) Caring neurologists collected a detailed clinical profile of each patient.(3) In a subset of patients, the results of genetic screening for mutations in common ALS-related genes were available. Information concerning non invasive mechanical ventilation (NIMV), percutaneous endoscopic gastrostomy (PEG), tracheostomy and deceased time were collected. Comparisons between groups were assessed using a binary logistic regression for categorical variables and one way analysis of variance (ANOVA), followed by Bonferroni post-hoc test, for continuous variables. The

Kaplan-Meier univariate analysis was carried out to determine the effect of phenotypes on survival and time to King's stage 4 (defined as time from symptoms onset to significant feeding or respiratory failure). Subsequently, we perform a Cox multivariate proportional hazards model corrected for well-known prognostic variables to estimate the proportional hazard ratios of phenotype on survival and time to King's stage 4.(3) *p* value was set at $p<0.05$. The study was approved by the Ethical Committees of the participating ALS centers.(3) Full description of materials and methods is avaible in the Supplementary File.

RESULTS

Demographic and clinical characteristics of each MND phenotype are summarized in Table 1 and Supplementary Table 2. We observed a significantly higher male prevalence in LMND compared with bulbar patients (Males: FA: 70.9%, FL: 59.5%, PLMN: 64.2% vs bulbar: 41.5%; $p<0.001$); moreover, male prevalence was significantly higher in FA also compared with the classic phenotype (56.8%, p=0.004) (Supplementary Table 3). The mean age at symptom onset was different between groups (FA: 62.3, FL: 63.7, PLMN: 60.6, bulbar: 68.3, classic: 63.1, p<0.001). FA, FL and PLMN patients exhibited a significantly longer mean diagnostic delay compared with both bulbar and classic patients (FA: 20.3; FL: 18.1*;* PLMN: 22.3 vs bulbar: 10.9 and classic: 12.5 months; $p<0.001$). LMND phenotypes also exhibited a reduced proportion of patients performing PEG compared with both classic and bulbar patients (FA: 20.5%, FL: 14.9%, PLMN: 11.9% vs classic: 30.5% and bulbar: 45.7%; $p<0.001$). No difference emerged in the proportion of NIMV users. Moreover, LMND patients showed lower rates of comorbid dementia (FA: 5.1%, FL: 2.0%, PLMN: 0% vs bulbar: 10.9% and classic: 7.7%; $p=0.001$). The results of genetic analysis were available for 585 patients (30.1 %) and in 23.6% of them a variant in one of the major ALS genes analyzed was detected (Supplementary Table 4). Overall mutation carrier rate was higher among classic-ALS patients (FA: 10.3%, FL: 10.8%, PLMN: 26.7% vs classic: 30.1% and bulbar: 16.5%; p<0.001). Moreover, the rate of patients harboring the *C9orf72* expansion was lower across LMND phenotypes (FA: 2.6%, FL: 1.6%, PLMN: 0% vs bulbar: 12.6% and classic: 17.1%; $p=0.001$).

Kaplan-Meier analysis showed a significant survival difference across MND phenotypes ($X^2=85.08$, $p<0.001$) (Supplementary Figure 1A). Similarly, median time to reach King's stage 4 (Supplementary Figure 2A) was significantly different among MND phenotypes ($X^2=74.02$, $p<0.001$). Finally, multivariate Cox regression analysis (Supplementary Table 1) demonstrated an independent effect of MND phenotypes on both survival and time to reach King's stage 4 (Supplementary Figure 1B and 2B).

DISCUSSION

Our study, performed in a large cohort of Italian MND patients, demonstrates distinguishing clinical and prognostic features of FA, FL and PLMN patients. These phenotypes differed in terms of sex distribution, age at onset, diagnostic delay, rate of performing PEG and risk of developing dementia. Moreover, this group of MND patients presented a slower disease progression, calculated in terms of survival and time to reach King's stage 4.

More specifically, FA, FL and PLMN phenotypes showed a significant gender effect , occurring more frequently in males. Sex distribution was significant in the FA group, coherently with previous reports.(1, 2, 4) Importantly, FA, FL and PLMN phenotypes also showed a longer diagnostic delay and lower rate of definite diagnosis according to r-EEC, reflecting the slower disease progression and mild or absence of UMN involvement. The milder central nervous system (CNS) involvement in these phenotypes is also confirmed by the lower rates of comorbid dementia detected in these groups. Kaplan-Meier analysis showed longer survival of LMND, consistently with previous reports.(1, 2) Interestingly, in the Cox regression model curve, after adjusting for well-known prognostic clinical variables, FA and PLMN showed the longest median survival time. Previous studies demonstrated that the clinical course of FA, FL and PLMN may be benign in particular when the disease is localized in one region for a long time.(2) Analyzing the adjusted survival model, FL patients accost to the bulbar and classic survival curves, suggesting that for FL well-known ALS prognostic factors may weigh more than the phenotype. This observation might be explained by the difficult distinction between classic-ALS with lower limb onset and FL patients, due to frequent overlapping of clinical presentations at disease onset. The differences between FL and FA might also be explained by a different sex-hormones exposure, a role of the X-chromosome or neurodevelopmental differences in the cervical and lumbar regions; however, further studies are needed to confirm this finding and to elucidate potential pathophysiological differences.

To note, FA, FL and PLMN patients reached King's stage 4 about 30 months later than classic and bulbar patients. Consistently, the frequency of PEG positioning was lower. The lower rates of NIMV users across FA, FL and PLMN patients did not however reach statistical significance. The observation of a longer time to reach King's 4 in FA, FL and PLMN may be useful to guide correct patient management, follow-up and intervention timing.(5)

To date, only few large studies highlighted on LMND phenotypes. In our study, each patient underwent a detailed phenotypic classification performed by expert neurologists and we collected information of patients with an uncommon clinical manifestation, examining features that are not

much investigated in FA, FL and PLMN patients so far. However, we acknowledge that our study manifests all the limitations of a retrospective study. First, we could not include longitudinal data as ALS-Functioning rating scale, quality of life scale or spirometry testing. Second, the diagnosis of a cognitive impairment without using a common test such as ECAS is open to bias and we could not further subclassify the type of cognitive impairment. Lastly, the results of genetic analysis were not available for a considerable proportion of patients. Nonetheless, we observed a higher rate of mutation carriers for the major ALS genes among classic patients, with no differences in the ALS familiarity rate, suggesting that heritability for FA, FL and PLMN patients could be related to different genetic factors, even if almost any clinical phenotype was described as 'inherited-ALS'. Moreover, we noticed a higher rate of bulbar and classic patients harboring the *C9orf72* repeat expansion.(4)

In summary, we showed that FA, FL and PLMN patients present distinguishing features and clinical course compared with bulbar and classic patients. Our results suggest that a detailed phenotypic classification could be important in order to predict prognosis, for a more individualized approach to patient managing and to properly stratify patients in clinical trials. Moreover, phenotypic heterogeneity and the disease progression across MND phenotypes could be related to a distinct underlying pathological mechanism or different genetic factors. Further investigations are needed to clarify the pathogenesis underlying each MND spectrum phenotypes.

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Conflicts of interest:

The authors declare no conflict of interest related to this work.

Ethical standards: The study was performed in accordance with the ethical standards statement.

Table 1 Demographic and clinical characteristics of study patients

	FLAIL ARM (n=117)	FLAIL LEG (n=148)	PLMN (n=67)	CLASSIC (n=1207)	BULBAR (n=405)	*p* value
Sex (n) (M/F) (Male %)	83/34 (70.9%) *§	88/60 (59.5%) §°	43/24 (64.2%) §	686/521 (56.80%)	168/237 (41.5%) *	**<0.001**
Age at onset (y) (m) (SD)	62.27 (10.82)§	63.74 (11.27)§	60.59 (9.21)§	63.09 (11.32)	68.33 (10.20)*	**<0.001**
Diagnostic delay (m) (months) (SD)	20.26 (28.09)*§	18.11 (16.81) *§	22.28 (24.50) *§	12.53 (12.01)	10.88 (10.34)	**<0.001**
PEG yes (n) (%)	24 (20.5%) *§	22 (14.9%) *§	8 (11.9%) *§	368 (30.5%)	185 (45.7%) *	**<0.001**
NIV yes (n) (%)	42 (35.9%)	58 (39.2%)	22 (32.8%)	537 (44.5%)	151 (37.3%) *	**<0.001**
BMI (m) (SD)	24.34 (5.53)	23.77 (5.22)	23.37 (7.58)	23.61 (4.88)	23.39 (5.17)	0.685
Dementia yes (n) (%)	6 (5.1%)	3 (2.0%) * §	0 (0%) * § ° #	93 (7.7%)	44 (10.9%)	**0.001**
R-EEC						
Definite (n) (%)	12 (10.3%) *§	21 (14.2 %) *§	1 (1.5%)	413 (34.2%)	121 (29.9%)	**<0.001**
Probable (n) (%)	22 (18.8%) *	35 (23.6%) *	3 (4.5%)	412 (34.1%)	98 (24.2%) *	**<0.001**
Probable laboratory supported (n) (%)	30 (25.6%) *§	27 (18.2%) *	2 (3%)	97 (8.1%)	56 (13.8%) *	**<0.001**
Possible (n) (%)	33 (28.2%) *	37 (25.1%) *	5 (7.5%) *	155 (12.8%)	84 (20.7%) *	**<0.001**
Unclassified (n) (%)	20 (17.1%)	28 (18.9%)	56 (83.5%) *§°#	130 (10.8%)	46 (11.4%)	**<0.001**
Survival time (m) (95% CI)	91.0 46.7-135.3	62.0 50.0-73.9	63.0 51.6-74.4	38.0 35.7-40.3	31.0 28.7-33.3	**<0.001**
Time to King's 4 (m) (95% CI)	79.0 19.8-138.2	59.0 45.5-72.5	66.0 44.5-87.5	34.0 31.2-36.8	26.0 23.7-28.3	**<0.001**

All data are expressed as number (n) and percentage (%) for categorical variables and mean (m) and standard deviation (SD) for continuos variables. Mean survival time and time to King's 4, expressed as months, were calculated using the Kaplan-Meier method, and compared with the log-rank test. The following legend indicates significantly between-group

comparisons calculated trough Bonferroni test (for continuos variables) or binary logistic regression (for categorical variables): * vs classic, § vs bulbar, ° vs Flail Arm, # vs Flail Leg (see also Supplementary Table 3). M, Male; F, Female; PEG, Percutaneous Endoscopic Gastrostomy; NIV, Non-Invasive Ventilation; BMI, Body Mass Index; R-EEC, Revised El-Escorial Criteria.

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