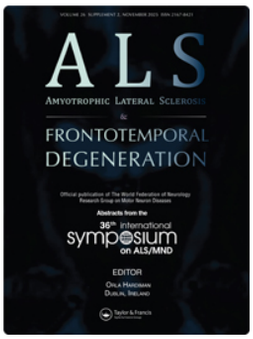

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MEETING REPORT

Utility of patient subgrouping in ALS clinical trials: a World Federation of Neurology white paper

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Abstract

The heterogeneity among the amyotrophic lateral sclerosis (ALS)/MND patient population is well recognized but not well understood. Such heterogeneity may represent a significant confound in our current and prior clinical trials as certain subgroups of patients might have a selective response (or resistance) to a novel therapeutic. The basis on which to segregate the patient population is, however, unclear. The ALS/MND Committee of the World Federation of Neurology (WFN) convened a symposium to discuss various strategies that might be considered for separating (stratifying) the population to further study. The results of that conference are presented here as a white paper, reflecting current understanding of several of the various criteria that could be implemented to divide the patient population as presented and discussed at that meeting. Consideration of grouping patients based on phenotype, cognitive involvement, imaging, or electrophysiology is presented here.

Keywords: *ALS heterogeneity, clinical trial design, ALS subtypes, stratification, subgrouping*

Introduction

Amyotrophic lateral sclerosis (ALS) is a complex, progressive neurodegenerative disease. Currently, it is widely accepted that there is significant heterogeneity, affecting the clinical presentation, the underlying pathophysiology and the potential for environmental influences contributing to the disease.

Drug development for ALS has been confounded by this well recognized heterogeneity. To date, our efforts have largely focused on ALS as a single disease entity with a shared pathophysiology and anticipated disease progression and survival. Grouping of ALS patients by their phenotype in patients entering clinical trials has been proposed as a means of managing clinical heterogeneity (1–4). The logistics of grouping patients by their phenotype for the purpose of establishing more homogeneous treatment cohorts for clinical trials remains challenging. We do not yet know what the optimal basis of such subgroup assignments should be. In December 2023, the Motor Neuron Disease/Amyotrophic Lateral Sclerosis Subcommittee of the World Federation of Neurology (WFN) convened a conference in

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Basel, Switzerland to explore potential strategies for reducing the heterogeneity and enriching the cohorts of patients entering clinical trials. The goal of the conference was to compile expert opinions and recommendations on whether the subgrouping of ALS patients should be considered for forthcoming clinical trials and furthermore, which strategies show most promise to be adopted at the present time.

Grouping patients by clinical phenotype

Defining the disease spectrum of ALS as individual entities verses a single entity has the potential to impact our ability to find an effective treatment (2–8).

As determined by the WFN diagnostic consensus guidelines, and most recently the Gold Coast Criteria, detection of ALS is determined by three independent primary attributes: body region of onset, relative mix of upper motor neuron (UMN) and lower motor neuron (LMN) deficits, combined with clinical evidence of disease progression (9–12). Defined variants of ALS include bulbar ALS (bALS, progressive bulbar palsy) which may be preferentially associated with cognitive and language impairments, and ALS presenting with frontotemporal dysfunction which presents similarly to frontotemporal dementia (FTD) (13–15). Various classification schemes have been proposed, using either phenotype or neurofilament levels, yet it remains unresolved what the optimal clinical indices, potentially defining distinct disease subtypes, are and which approach will be most productive (Figure 1) (1,2,4–6,16,17). What is clearer, however, is that ignoring phenotypic subtypes of the disease spectrum may be shortsighted as future therapeutic advances might depend on discovering which clinical indices are most discriminating in defining disease subtypes.

The case for distinct phenotypic characteristics in ALS is even more profound for UMN predominant MND, termed primary lateral sclerosis (PLS). PLS makes up approximately 3% of all MND and is rare (18). PLS is characterized by progressive UMN dysfunction with no clinically apparent involvement of the LMNs for several years (19). There is an ongoing debate as to whether ALS and PLS lie across a continuum, which is supported by the fact that some PLS patients may develop clinical and/or electrophysiological evidence of LMN degeneration years later, or may be entirely separate disorders (20,21). Interestingly, patients with PLS are found to have an inherently small variability regarding rates of increase of the ALS Functional Rating Scale (ALSFRS) scores over-time compared to other types of MND such as Charcot or flail variants (1,22–24). Patients with no EMG abnormalities four years after symptom onset can survive decades, while those with even minor evidence of LMN involvement may have poorer prognoses (18,19,25). Overall, this suggests that pure PLS or upper motor predominant ALS patients tend to follow a slow, less variable disease progression when compared to other ALS patients. This would imply a relatively homogenous subpopulation within the incredibly heterogeneous ALS population.

A path forward

In an effort to advance our understanding of disease subtypes, defined by clinical phenotypes, a new grading scheme was introduced that captures phenotypic heterogeneity (6). This scheme, initially introduced several years ago has been undergoing testing to highlight differences in the biomarker profile and clinical course of these subtypes (5,6). This grading scheme allows for a qualitative description of disease subtypes for potential use in clinical trials and highlights disease subtypes that may potentially respond to selective treatment (Table 1).

Thus far, using these clinical criteria (predominance of UMN, LMN, bulbar phenotype) marked differences in the disease course, measured as slope of the ALSFRS, and survival have been found (Rosenfeld, unpublished in preparation). Considering the importance of ALSFRS and survival in design of our current clinical trials, ignoring the opportunity to study individual phenotypic groups may likely contribute significantly to a type 2 statistical error, missing a treatment effect that might be present.

In summary, subgrouping ALS patients based on clinical phenotype has been underutilized as a means of defining meaningful ALS subtypes that may have a selective response to novel therapeutics under development. Despite our understanding that the most common tools used in following disease progression and trial outcomes are quite different in the various phenotypic subtypes, we regularly fail to recognize the importance of phenotypic subtyping for cohort enrichment and clinical trial analyses. These include the ALSFRS, survival and forced vital capacity (FVC).

Grouping patients by cognitive and behavioral impairment

Criteria have been developed to classify those with cognitive and or behavioral impairment (31), into ALS with cognitive impairment (ALSci), with behavioral impairment (ALSbi), or both (ALScbi), and ALS-FTD. The prevailing question is whether study of distinct subgroups of patients separately is useful for clinical trials. Will such an approach help to answer questions relevant for trials: (a)

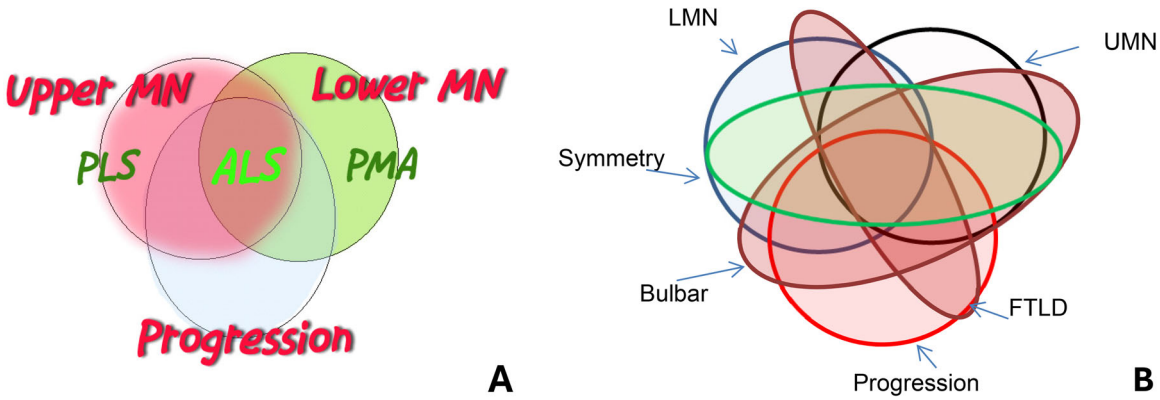

Figure 1. Stratification by clinical phenotype can be facilitated by numerous clinical criteria. (A) A traditional understanding of ALS as a generalized motor neuron disease with both upper and lower motor neuron involvement. (B) Multiple additional criteria can be considered as important variables upon which stratification can also be based (i.e. rate of progression, extent of bulbar involvement, presence of FTLD, etc.) Related motor neuron diseases, not meeting the formal criteria of ALS, include progressive muscular atrophy (PMA, lower motor neuron disease only) and primary lateral sclerosis (PLS, upper motor neuron disease only) (9). These latter entities have been challenging to define, as the clinical signs can evolve over very extended time periods.

Table 1. Using this novel paradigm, a patient's presenting phenotype can be quickly categorized by the degree of UMN, LMN, bulbar, and cognitive involvement.

	A	B	C	D
Upper motor signs	Minimal or no involvement	Signs without symptoms, i.e. brisk DTR	Moderate loss of function. Increased tone, spasticity	Prominent loss of function
Lower motor signs	Minimal or no involvement	EMG changes, minimal or no weakness	Moderate weakness and/or atrophy	Prominent loss of function
Bulbar signs	Minimal or no involvement	Signs without symptoms, i.e. bulbar reflexes, fasciculations, minimal or no weakness	Mild/moderate dysarthria and/or dysphagia	More severe dysarthria and dysphagia
Cognitive score (CBS)	Minimal or no involvement	ALSci (10–16) or ALSbi (26–29)	Evidence of ALSci and ALSbi on the CBS	CBS cognitive score $\leq$10, behavioral score $\leq$32

Patients are assigned a "grade (A–D)" on each of four indices (LMN, UMN, bulbar, and cognitive) (6). Cognitive phenotype is defined here by scores on the ALS Cognitive Behavioral Screen (ALS-CBS) (30). Subgroup analyses can be specified *a priori* in an effort to identify selective responders in future clinical trials. The rate of disease progression, as defined by the ALSFRS, has already been shown to vary significantly based on the clinical phenotype, now readily identifiable with this grading scheme.

does the intervention have an effect on cognition? (b) do people with cognitive or behavioral impairments react differently to the intervention than those without? and (c) is the sample studied representative of the ALS population? For subgrouping to be useful, it may be helpful to determine whether those with cognitive and or behavioral impairment represent a distinct subgroup of ALS.

Cognition may serve as a biomarker, screening for neuropathological change, justifying the identification of a distinct more homogeneous group of patients. Brain imaging studies using a range of methods, from early functional PET, functional MRI, structural MRI, DTI, and FDG PET, reveal that cognitive impairment is associated with extramotor cerebral dysfunction in ALS (32).

Several studies have shown a specific association between types of cognitive/behavioral impairment (executive functions, language—concept formation, apathy) and localized cerebral involvement (26,27,33–35). For example patients with ALSci as defined by verbal fluency or executive dysfunction showing involvement of the dorsolateral and inferior frontal cortices. Fewer studies have examined behavioral impairment and extramotor cerebral dysfunction in ALS but apathy and disinhibition have been associated with involvement of orbitofrontal cortices.

The strongest evidence of subgroups comes from those imaging studies which have divided patients based on cognition (typically verbal fluency), into those with impairment and those without and these extra motor changes were present or more pronounced in severity and extent in the former only. These findings have included reduced blood flow during cognitively activating tasks of

verbal fluency, free movements of a joystick, structural changes in white matter volume, and metabolic changes (28,29,36,37).

This work is now further expanded using the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) a brief assessment of cognitive and behavioral impairment in ALS, with for example, a relationship to white matter integrity measured by DTI and correlating closely with disease-staging model (26,33). Robust evidence comes from post-mortem studies of 27 people with ALS who had undertaken the ECAS during the course of their illness (38). Seven of which had ALSci and all showed TDP-43 accumulation, a pathological hallmark of ALS and FTD, in prespecified extra-motor cerebral regions. For example, all three patients with deficits on the executive functions score had pathology in the dorsolateral prefrontal cortex (BA46 and BA9). This study represented the first demonstration of regional TDP43 accumulation associated with the mid-spectrum range of cognitive impairment and indicates that ECAS deficits are a biomarker for pathological change.

Longitudinal changes in cognition

How cognitive dysfunction evolves over the course of the disease has relevance for clinical trials and may also shed light on whether there are distinct subgroups. While some longitudinal studies suggest there are subgroups of individuals who decline in cognitive scores, results are mixed (39–41). Longitudinal studies are plagued by problems of attrition and findings are inconsistent; however, cross-sectional studies have shown an association between cognitive/behavioral change and disease stage (42).

Most importantly for clinical trials, cognitive impairment has been shown to be a negative prognostic indicator in ALS and is associated with shorter survival, resulting in increased attrition in individuals with cognitive deficits at baseline (43–45). It has been shown that it is not only cognitive impairment at baseline but also the extent of decline which is associated with shorter survival, with those who changed classification having a shorter survival than those who did not, these patients also had worsening physical deterioration than those who did not (43).

In summary, there is compelling evidence that cognitive impairment may serve as a biomarker, reflective of distinct patterns of cerebral involvement and pathology outside of the motor system.

For investigating whether an intervention has an effect on cognition, binary patient grouping may be too simplistic and prone to classification errors, and a continuous variable is more appropriate and provides rich data. The comparison of different neuropsychological tests remains challenging, but the introduction and use of the revised consensus criteria (31) and the availability of ALS-specific cognitive measures (e.g. the ECAS and the ALS Cognitive Behavior Scale (ALS-CBS) (30,46)) have improved our ability to compare study findings in a more meaningful way in the future.

Subgroup identification using neuroimaging

It has been recognized since the early 1990s that certain ALS patients had hyperintensity of the cortico-spinal tracts (CSTs) on T2, PD, and FLAIR imaging (47) and some also had a hypointense rim in the precentral gyrus called “the Hand Knob” or “the motor band sign” better seen on SWI (48).

The presence of such CST hyperintensities may help discriminate subgroups with different disease mechanisms and could be a valuable basis to discriminate amongst patients entering a clinical trial (49,50).

Decades of research have highlighted the advanced neuroimaging signature of ALS to differentiate certain phenotypes and genotypes (51,52). Motor cortical thickness was shown to be a more sensitive measure of UMN degeneration than UMN signs (53). Longitudinal studies have shown progressive imaging changes that correlate with disability and survival (52).

Functional MRI confirmed these findings with abnormal motor and extra motor resting state, and metabolic imaging using MR spectroscopy or FDG-PET showed primary motor cortex hypometabolism and metabolite alteration along the descending CST that correlated with survival (54–57).

These sophisticated imaging techniques allow us to better evaluate the spinal cord as a surrogate for both UMN and LMN degeneration. Volumetric studies showed cord atrophy in cervical and thoracic spine (58). Progressive cord atrophy on longitudinal studies mirror clinical progression and cervical cord atrophy was able to predict respiratory dysfunction and survival (58,59).

Can advanced neuroimaging techniques predict survival?

Using measures of cortical thickness and white matter analysis, Schuster et al. (60) showed that short survivors (less than 18 months) had more widespread gray and white matter degeneration compared to long survivors. In another study, Ta et al. (61) used texture analysis, a computational image-processing technique that quantifies patterns in MR images that are not detectable with visual inspection alone, and showed greater CST and faster progression of CST degeneration in the short survival subgroup. Volumetric studies of the brainstem highlighted the role of the medulla volume as a driver of survival (62,63). Using machine

learning, a subgroup of patients where low hypothalamic volume in the absence of loss of appetite or hypermetabolism was associated with low BMI and a greater risk of death

Summary. Is MRI ready to help with subgroup identification?

There is significant improvement in MRI hardware with novel head coils and new generation scanner with fast acquisition time and more powerful post-processing software that will make it easier for ALS patients to be scanned. To proceed with MRI subgrouping, we will need to implement the following steps that could be incorporated in any clinical trial: design of large cross-sectional studies that are adequately powered in a homogenous population that is deeply phenotyped with well-characterized UMN burden and cognitive changes. Include controls in longitudinal studies to account for changes due to aging. Systematic imaging of presymptomatic until pheno-conversion and beyond. Design multi-center imaging studies with data harmonization. Test phantoms and traveling heads before starting multi-center imaging studies. Establish imaging centers of excellence. Perform postmortem imaging studies to better understand the pathological driver of these MRI changes, as was demonstrated in a small rapid postmortem imaging study that the motor neuron density in motor cortex could be correctly estimated from MRI metrics.

Subgrouping by electrophysiology

Any disease biomarker measure applied for grouping cohorts of patients in clinical trials and/or used as a progression marker would be predictive and fulfill the following criteria:

- Quantifiable, reliable using standard operating procedures across multiple centers, accounting for relevant sources of variability including intra- and inter subject, intra- and inter assessment and inter laboratory (64).

Only a few neurophysiological measures, however, have so far been tested through rigorous test/retest paradigms (65–67).

Motor unit number estimate (MUNE) techniques have been suggested to be used for identifying separate groups of patients and not surprisingly, many of the neurophysiological studies have shown an excellent correlation with other clinical markers such as the ALSFRS-R (68–71).

However, this usually requires longitudinal data (69,71). For grouping cohorts of patients into clinical trials, however, a baseline measure that predicts progression rate (e.g. neurofilaments) without the need to generate longitudinal data would be much better suited. Motor unit number index (MUNIX) is a modified motor unit number estimation (MUNE) technique based on surface recordings of voluntary muscle activity. It can be recorded in any muscle in which a maximal compound muscle action potential (CMAP) can be elicited (72). A MUNIX-Score, taken at baseline shortly after the diagnosis, has been demonstrated to be a predictor for disease progression, but the ALSFRS does the same (70) which raises the question of why applying a neurophysiological method if a simple clinical measure or a combination of clinical characteristics (e.g. the ENCALS-Prediction-Model) can achieve the same (73).

The much greater value of neurophysiological markers is their sensitivity to change over time in terms of progression markers. As detected by MUNIX, the decline is faster as compared to standard measures like the ALSFRS-R and slow vital capacity; it can detect motor unit loss before clinical weakness occurs and it can dissect loss of motor units and amount of reinnervation (66,69). Importantly, when applying a MUNIX-score combining several muscles, a power calculation revealed that based on 100 patients (25% difference between placebo and verum, each arm 50 patients) trial duration would be 26.5 months for the ALSFRS but only 11.7 months for the MUNIX-score. From a central (brain) functional perspective, assessment of cortical motor function, and the advent of hyperexcitability in ALS/MND, as reflected by reduction in short interval intracortical excitability, has developed as a diagnostic and prognostic biomarker in ALS (74). Confirmation of cortical motor dysfunction would promote earlier diagnosis of ALS and recruitment into therapeutic trials (17,75).

Conclusions

The variability among patients affected with ALS has been recognized since the earliest descriptions, yet, historically, has not resulted in changes in the way we design our clinical trials or conceptualize the diagnosis. Studying ALS as a single entity without regard to significance of the clinical heterogeneity, or the variability in imaging, pathophysiology or electrophysiology, risks the possibility of missing a therapeutic advance that may be relevant to a specific subgroup of affected patients.

Given the paucity of disease-modifying therapies despite the multiple attempts of well-intentioned clinical trials, exploring various novel strategies, such as grouping patients (*a priori*) on one or more criteria, is an alternative approach that may be both timely and necessary. In addition to the criteria summarized in this report, enriching our treatment cohort groups based on selection for

a specific biomarker that has relevance to the mechanism being tested is another strategy that has not been regularly employed in the prior ALS trials.

Our clinical trials in the future might optimally employ the use of ALS subgroups in phase 2 trials to better inform decisions in phase 3 trial design. This strategy of subgrouping patients, along with data on target engagement and outcomes, would likely result in better, more successful phase 3 trials. Alternatively, if a subgroup analysis were designed in a phase 3 trial that did not result in significant differences, an *a priori* statistical plan could be proposed to then collapse the data between groups, increasing sample size and returning to the more traditional analysis that is currently used.

While the use of patient subgroups in the design of our future phase 2 and 3 trials might be perceived as an impediment to enrollment and trial efficiency, it might also be considered as a necessary advancement in a disorder as complex and heterogeneous as ALS. Such strategy is regularly adopted with success in other neurological conditions such as multiple sclerosis (relapsing-remitting MS is usually tested separately), epilepsy (generalized versus focal versus complex partial seizures are usually tested separately), and headache (type of migraine tested separately). These examples should be valuable considerations as we reassess trial designs in the future.

Our current understanding is not sufficient to know which strategy is optimal. Our current reality, however, is that the need for a paradigm changing approach in our design and understanding of disease heterogeneity cannot be ignored.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article. No funding was received in the preparation of this manuscript.

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