THEME 10 DISEASE STRATIFICATION AND PHENOTYPING OF PATIENTS

DSP-01 Speech and pause measures as acoustic biomarkers of ALS in Canadian French

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Background: In ALS, the development of bulbar signs is associated with faster disease progression, shorter survival, and lower quality of life (1). Thus, effective measurement tools that improve early detection and progression monitoring of bulbar signs are essential for clinical management of the disease. According to recent literature, acoustic measures of speaking and pause times during passage reading show sensitivity to different stages of bulbar disease in English speakers (2,3). However, the utility of these measures in French is not known. Considering the importance of language-specific characterization of dysarthria, cross-linguistic validation of acoustic biomarkers is needed.

Objectives: Primary objective: To determine if speech and pause measures differ between French speakers with ALS and with and without bulbar symptoms and healthy controls. Secondary objective: To determine if these measures can reflect the decline in bulbar symptoms in a French cohort with ALS.

Methods: 46 Canadian French speakers (29 ALS; 17 controls) were recorded reading a passage during up to three follow-up visits (total of 92 recordings). ALS speakers were classified as bulbar symptomatic or bulbar pre-symptomatic based on their ALSFRS-R bulbar subscore (3). Recordings were analyzed using a semi-automated speech and pause segmentation procedure (4) to provide measures of speaking rate, total duration, duration of speech, and number of pauses. These measures were compared between the three groups and correlated with ALSFRS-R, total and bulbar scores.

Results: Group comparison revealed that the ALS symptomatic group significantly differed from the pre-symptomatic and control groups for speaking rate, total and speech durations, and number of pauses ($p < 0.05$). None of the measures allowed differentiating the pre-symptomatic and control groups. Speech and pause measures were all moderately correlated with ALSFRS-R total and bulbar scores ($p < 0.05$).

Discussion: As demonstrated in English speakers, measures of speech and pause behavior during passage reading are sensitive to the progression of bulbar disease in French speakers with ALS. Contrary to English, those measures were unable to detect ALS prior to the development of bulbar symptoms in French speakers. This may be due to language-specific factors (e.g. prosody) as well as our relatively small sample size. Including kinematic assessments (5) may improve early detection in French. Studies with larger cohorts of French speakers progressing from pre-symptomatic to symptomatic stages are needed to help identify early markers of bulbar disease.

Acknowledgements
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DSP-02 Assessment of wearable sensors for estimation of natural gait speed at home and in the lab

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Background: Recognized factors for falls include advanced age, muscle weakness, gait and balance problems, and

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previous falls, all of which are common in the progression of ALS. This study investigates the use of home telemonitoring using a smartphone-connected sensor to track gait as a method for rapidly responding to changes in patients’ ambulatory health.

Objectives: The first aim of the study is to determine whether walking speed measured with a wearable sensor is comparable to speed measured using standard clinical or laboratory methods regardless of whether the recording was performed at home or in the laboratory. We also ask if there is high adherence to the practice of sensor-based home gait measurement.

Methods: Patients with symptom onset in the last 3 years and an ALSFRS-R walking score of 2–3 were prospectively recruited from the Penn State Health ALS Center. At the study visit, patients completed a 10-m walk task, during which walking speed was measured using a stopwatch and a foot-worn tri-axial sensor (MMR+®, MbielieaLab Inc). Over 24 weeks, subjects completed twice-weekly, 5-min home recordings of walking using the foot sensor and a custom smartphone application. Walking speeds derived from lab recordings and the next available home recording were tested for equivalence using ANOVA. Control subjects were also recruited and completed a lab-based 10-meter walk task, followed by 4 weeks of home recordings. Control subjects underwent additional video-based walking speed analysis during lab assessments, which were compared to the other methods.

Results: 8 patients (6 male) and 2 controls (1 male) were enrolled in the study. Patients (6 ongoing) completed a median of 21.5 (3–52) recordings over 86 (7–178) days. All but 1 subject(s) submitted at least one recording per week while in the study; in total only 4.3% of recordings were sent 7 or more days after the previous recording. Four patients completed an in-person gait assessment. No differences in 10-meter walking speeds were found when comparing those calculated from stopwatch measurements versus those derived from foot sensor data. Similarly, the two control subjects had 10 m walk task speeds that were comparable across sensor, stopwatch, and video-based methods, although the video-based method had a tendency towards greater speed estimates (4–10% higher than other methods). In all subjects, walking speeds derived from home measurements were significantly lower than all lab-based measures (p < 0.001).

Discussion: Preliminary results validate the accuracy and feasibility of wearable-derived walking speed tracking in patients experiencing early gait changes due to ALS. Lab-based walking speeds were consistently greater than home assessments, perhaps as a result of subjects modifying their natural gait due to being observed. Future results will determine whether gait telemonitoring may act as a functional biomarker for fall risk.

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DSP-03 Using active digital phenotyping to quantify function and cognition in amyotrophic lateral sclerosis (ALS)

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Background: Amyotrophic lateral sclerosis (ALS) clinical trials rely on a standard set of outcome measures, including the revised ALS Functional Rating Scale (ALSFRS-R), vital capacity (VC), and handheld dynamometry (HHD). Digital Quantitative Monitoring (DQM), uses tasks performed on digital devices to obtain more frequent, quantitative and granular measurements of function alongside patient reported outcome measures in order to improve on standard ALS outcome measures.

Methods: The study originated with two intensive clinic visits separated by a week during which daily self-administered test and continuous passive data (DQM) was collected remotely. With COVID-19, the study was re-designed to a fully-remote and longitudinal format, comprising telemedicine visits at baseline, 12, and 24 weeks, weekly self-administered testing, and continuous passive data collection. During telemedicine visits, study staff administered traditional ALS outcome measures including the ALSFRS-R, neurological fatigue index – Motor Neuron Disease (NFI-MND), and a quality of life scale. DQM assessments were delivered via mobile application (Digital Artefacts) on a provided iPhone and Apple Watch, as well as via web browser on their computer. The mobile app included a symptom questionnaire, self-administered ALSFRS-R, fine motor, gait, stance, speech, and cognitive tests, and collected continuous passive data. Participants used their home computer and mouse to complete a point and click task assessing fine motor movements. Twenty-five healthy controls (HC) and 25 people with ALS (PALS) will be enrolled.

Results: All PALS participants have been enrolled and HC enrollment is projected to complete in August 2021. Thirteen PALS and 3 HC have completed participation. Of 456 scheduled sessions mobile application sessions, participants have completed 385, with 50 further partially completed sessions. Test-retest reliability at baseline varies across tests, but ICC values above 0.9 have been observed (alternating finger-tapping rate, passage reading speaking rate). Correlation with relevant baseline ALSFRS-R subscores (i.e. bulbar, fine motor, gross motor, respiratory) are moderate (0.4–0.6) or weak (0.2–0.4) for most test features analyzed.

Considering participants with at least two sessions, the median value of several computer mouse task features demonstrated strong correlations (0.6–0.9) with baseline ALSFRS-R handwriting and/or total scores. These features included normalized jerk, execution time, maximum speed, and temporal location of the main submovement. Available data will be presented.

Conclusion: This pilot study in PALS and HC is helping to clarify the utility of a variety of mobile technology-based DQM tools in ALS, to compare these tools to traditional ALS outcome measures, and to extend our ability to assess cognition in people with ALS. Early results suggest compliance is acceptable and at least a subset of the digital tests included in this study may have promise as reliable measurements of function and cognition in people with ALS.

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Disease Stratification and Phenotyping of Patients 32nd International Symposium on ALS/MND

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Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that affects both upper and lower motor neurons. We conducted the largest Taiwanese cohort to investigate the natural history and prognostic factor of ALS.

Methods: We recruited 227 patients diagnosed with definite or probable ALS. All patients were tested for common disease genes including C9ORF72, SOD1, FUS and TARDBP. Detail clinical characteristics were acquired and neurological examinations were performed after informed consent. The patients were followed up biannually for evaluation of ALSFRS-R score.

Results: The cohort consists of 127 men and 100 women. Eleven patients had a family history of ALS and 32 patients exhibited bulbar onset. Average diagnosis delay since symptoms onset was 16.8 months. On average, the functional outcome, evaluated by ALSFRS-R score, decline from 36.1 to 24.4 over the first year of diagnosis. Age of onset, presence of disease causing genes and gender did not affect the rate of functional decline. Initial ALSFRS-R score, bulbar onset, BMI and older age of onset resulted in worse survival outcome.

Discussion: This is the largest ALS cohort with an analysis of its natural history in Taiwan. This study advances the understanding of clinical characteristics, natural disease course and risk factors of ALS in Taiwanese population.

DSP-04 Natural history and clinical characteristics of ALS in Taiwan

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DSP-05 Features of the ALS patients population of a large center in central Italy

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder involving upperand lower motorneurons. The causes of neuron degeneration are still unclear, but some predisposing factor have been put in relation with an increased risk of developing the disease; these include head traumas, agonistic athletic practice, and toxic exposure. According to literature data, ALS is sporadic for 90% of cases and familiar for about 10% of the cases. The mean age at onset ranges from 58 to 63 years, and some phenotypes have a gender prevalence. These include patients with predominant bulbar involvement that are mainly women with an older age at onset, or while flail arm phenotypes that show strong male predominance. Here we describe the recent motor neuron disease (MNDs) population of the Santa Chiara Hospital, Pisa, Italy, to compare it with what the available in literature. In the last year, 153 patients (77 men and 76 women) with a mean age at onset of 63.4 years came to our attention and underwent regular neurological evaluation for MNDs. Among them, 33 had a predominant involvement of I MN at onset (15 women and 18 men with a mean age of 58.2 years). 30 men and 30 women (mean age at onset of 61.8 years) had a prevalent involvement of II MN. Among them, two male patients had a flail arm clinical picture and one woman had a monomelic arm involvement. 28 women and 5 men (mean age at onset of 68.7 years) had a bulbar picture. Two men with a mean age at onset of 60 years had a cognitive impairment and three patients (two female and one male) had a respiratory onset (mean age 68.3). 8 out of 143 ALS patients (5%) had a positive genetic test (2 SOD1 mutation, 3 C9ORF72 mutation, 3 FUS mutations). Among all patients, we had three Kennedy’s disease and seven HSP genetically confirmed. 38 patients had a familiarity for neurodegenerative diseases, mainly AD and PD, but also ALS. Among ALS patients, 100 underwent a high camp cerebral MRI to study motor cortex: 68 patients had a positive MRI. Almost all negative MRIs were in patients with lower motoneuron prevalence involvement at onset. MRI resulted negative also in four familiar ALS (2 C9ORF72-related, one FUS-related and one with NGS still ongoing but ALS familiarity). 17 patients had predisposing risk factors (7 agonistic athlete, 6 toxic exposures, 1 head trauma, 2 consanguinities among parents and one with a previous history of MG supporting a dying back hypothesis) Our database is substantially aligned with previous literature reports, supporting the validity of the current body of knowledge of ALS prevalence. Similar databases are very useful to effectively track patients and increase the efficiency of follow-ups.

DSP-06 Higher Troponin T levels positively correlated with the extent of body regions affected on EMG in ALS patients

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Background: The troponin-complex is a well-studied, small protein-complex involved in the regulatory function of skeletal and cardiac muscle contraction. The complex is subdivided into three smaller proteins – Troponin I (cTnI), Troponin T (cTnT) and Troponin C (cTnC). Both cTnT and cTnI are mainly used in the diagnosis of cardiac pathologies, where an elevated level indicates damage to the cardiomyocytes. During regeneration of skeletal muscle tissue after denervation, isoprotoins of the Troponin protein family are re-expressed in skeletal muscle (1). Several studies have detected elevated levels of cTnT in patients with neuromuscular disorders (1,2). We have also recently shown that cTnT in plasma is elevated in ALS patients compared to ALS mimics and -healthy controls and increases longitudinally as the disease progresses (3).

Objective: To determine the correlation between cTnT in plasma and levels of re-innervation analysed through neurophysiological examinations in patients with ALS.

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Methods: We conducted a retrospective cross-section analysis of plasma cTnT levels of patients diagnosed with ALS at Karolinska University Hospital during 2015–2018. We only included patients who had undergone an electromyography (EMG) examination within 6 months of the cTnT measurement. EMG-investigations had to have been examined in at least three out of the four defined regions for ALS-diagnostics. We then created an EMG-protocol which graded the amount of fibrillations or positive sharp-wave potentials from a level of 0 to 4 or in percentage (amount of muscles affected divided by number of muscles investigated).

We use Pearson’s correlation coefficient as an assessment of the correlation between cTnT levels and EMG findings.

Results: Among the 50 patients included in the study, none had any known cardiac conditions. Age at diagnosis varied between 37 and 82 years (median 64.5 years) and the cTnT levels varied between 6 and 124 ng/L (median 30.9 ng/L). cTnT levels were statistically significantly correlated with the number of EMG-regions affected 0.344; (Pearson’s 0.431; (p = 0.002).

Conclusions: To our knowledge, this is the first study to examine the correlation between plasma cTnT and neurophysiological findings in patients with ALS. We found a clear, correlation between higher levels of plasma cTnT and a greater number of body regions affected in EMG. A possible explanation is that elevated plasma cTnT levels in ALS patients are due to a re-innervation process occurring after damage to the motor neurons and the subsequent skeletal muscle tissues.

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DSP-07 Amygdala TDP-43 pathology is a sensitive pathological correlate of behavioral dysfunction in amyotrophic lateral sclerosis

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Background: Cognitive and behavioural deficits are a well-recognized symptom in up to 50% of patients with amyotrophic lateral sclerosis. Whilst cognitive deficits are thought to be driven, at least in part, by the pathological accumulation of phosphorylated TDP-43 (pTDP-43) aggregates in extra-motor brain regions, a sensitive pathological correlate of behavioural deficits is yet to be determined. The brain areas thought to predominantly be associated with behavioural dysfunction are the (i) amygdala, (ii) orbitofrontal cortex (BA11/12), (iii) ventral anterior cingulate (BA24) and (iv) medial prefrontal cortex (BAG).

Objectives: To identify a sensitive pathological correlate of behavioural dysfunction in ALS.

Methods: Here we examined post-mortem tissue from these four brain regions in a cohort of 30 sporadic ALS (sALS) patients, a proportion of which had also undergone the same neuropsychological behavioural assessment as part of the Edinburgh Cognitive ALS Screen.

Results: We show that overall, the behavioural screen done as part of the ECAS predicted TDP-43 pathology with 100% specificity and 86% sensitivity in behaviour-associated brain regions, with the amygdala demonstrating the best sensitivity and specificity when analysed alone. Furthermore, in the amygdala of sALS patients, we show variation in morphology, cell-type predominance and severity of pTDP-43 pathology and that the presence and severity of intra-neuronal, but not glial, pTDP-43 pathology is associated with a clinically detectable behavioural deficit.

Discussion: Taken together our data suggest that the behavioural questionnaire done as part of the ECAS is a reliable correlate of pTDP-43 pathology in behaviour-associated brain regions. We also show that, out of the four regions profiled, the amygdala is the most sensitive correlate of behavioural deficits and that, in this region, neuronal pTDP-43 pathology is a better correlate of behavioural dysfunction than glial pathology. These data would be supportive of recent MRI imaging studies evaluating the amygdala as a key imaging correlate of behavioural dysfunction aimed at improving monitoring and stratification of patients with behavioural symptoms.

DSP-08 Measurement of upper limb function in ALS: current methods and future directions

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Background: The Amyotrophic Lateral Sclerosis Rating Scale-revised (ALSFRS-r) is the primary outcome measure utilised in clinical trials and research in ALS. This scale is limited such that clinically meaningful changes for subjects are often missed, impacting upon the evaluation of new drugs and treatments. Technology has the potential to provide sensitive, objective outcome measurement.

Objective: To provide a state-of-the-art review of current and future trends in the measurement of upper limb function.

Methods: A general review of upper limb measurement tools including subjective paper-based questionnaires and the objective sensors that aim to supersede them was conducted. Due to the relatively low incidence of ALS, this review spanned neurological conditions in general.

Results: Current assessment methods include multi-item functional scales such as patient-reported functional scales (e.g. ABILHAND questionnaire), clinician-rated scales (e.g. the ALSFRS-r upper limb component or the DASH (Disabilities of
Background. Motor neuron diseases (MNDs) encompass a wide pathological continuum ranging from classic amyotrophic lateral sclerosis (ALS) to pure/predominant upper motor neuron (pUMN) and pure/predominant lower motor neuron (pLMN) disease forms. While it is widely accepted that these phenotypes are characterized by distinct survival rates, their longitudinal trajectories of clinical decline are still largely unknown. Additionally, the majority of prognostic studies in MNDs have mainly focused on classic ALS, while the need to evaluate distinct prognostic features in pUMN and pLMN phenotypes has been largely neglected.

Objective. To investigate longitudinal trajectories of clinical functional decline across the main MND phenotypes and to develop phenotype-specific prognostic models.

Methods. 60 patients with a clinical diagnosis of MND (26 classic ALS, 14 pUMN and 20 pLMN) were recruited and followed longitudinally with clinical evaluations approximately every 3 months, for up to 15 months. Motor examinations included the following assessments: overall degree of functional impairment (evaluated using the ALS functional rating scale revised “ALSFRS-r”), muscle strength (evaluated using the Medical Research Council “MRC” scale) and UMN involvement (evaluated using the UMN score). For each of these measures, a baseline progression rate was further estimated. Cognitive/behavioral and mood examinations included the following assessments: cognitive and behavioral impairment (evaluated using the Edinburgh Cognitive and Behavioural ALS screen “ECAS”) and mood disorders (evaluated using the Hospital and Anxiety and Depression scale “HADS”). Based on longitudinal ALSFRS-r data individual slopes of decline were generated, and linear regression models were then applied to isolate, among baseline clinical features, significant predictors of a more aggressive disease course in each clinical phenotype.

Results. Longitudinally, the ALSFRS-r delta of variation was higher in classic ALS patients (−13.67), followed by pLMN (−11.89) and pUMN cases (−5.76); significant differences were selectively observed for pUMN compared to classic ALS (p = 0.05). In classic ALS, significant predictors of a more aggressive longitudinal decline included greater baseline rates of overall functional impairment (p = 0.003) and UMN involvement (p = 0.04), as well as greater baseline lower limbs UMN involvement (p = 0.02). In pUMN, significant predictors of a more aggressive longitudinal decline were male gender (p = 0.05) and side of symptom onset (right ECAS p = 0.001, bilateral p = 0.003). In pLMN, significant predictors of a more aggressive longitudinal decline included greater baseline cognitive impairment (total ECAS score p = 0.003, ECAS ALS-specific functions score p = 0.01) and more severe mood disturbances (p = 0.01).

Discussion. In conclusion, our study confirms the urgent need for phenotype-specific prognostic models in order to improve patient’s management and clinical trials implementation in MNDs.

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(1) Does a better prognosis model imply a “higher-quality” stratification?
(2) Is it possible to settle on an agreed-upon ALS subtyping?

Methods: Five clustering algorithms were used to partition the same dataset and partitions with clusters ranging from 2 to 11 were generated. Seven Internal Clustering Validation Indexes (CVIs) were computed on the obtained partitions to assess their inherent geometrical characteristics. To quantify the resemblance of different stratifications, the Adjusted Rand Index (ARI) was calculated on each pair of partitions having the same number of clusters. The prognosis task evaluating the quality of the partitions is the prediction of the ALSFRS slope for a cohort of 2187 patients from the ALS PRO-ACT dataset. A top-ranking solution from the 2015 ALS Stratification challenge served as a baseline. The Root Mean Squared Error (RMSE) gauged the potential impact of a given stratification on the slopes prediction model.

Results: The used ensemble of CVIs was able to rule out a subset of possible clusters numbers restricting the acceptable number of groups to a maximum of 6. The analysis of CVIs set of possible clusters numbers restricting the acceptable number of clusters but with a score of 9 out of 35 possible optimal values. By the analysis of the ARIs matrices for the same cluster range, two ALS subpopulations could be selected as the most suitable subtyping given that the subpopulations are too similar to each other regarding all clustering algorithms (ARI of 0.78 out of 1).

Discussion: Choosing the “best” ALS patients’ partitioning given an “optimal” number of clusters is far from being solved. The surrogate assessment provided by the score of a prognosis model made matters worse. Partitions with the same number of clusters could have the same RMSE while being different in terms of their ARIs. In the absence of a golden standard of ALS subtypes, launching discussions to agree on computationally quantifiable set of desirable characteristics of the partitions to-be-obtained could be a solution to the ALS patients stratification problem.

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DSP-11 Reprogrammed ALS patient astrocytes reveal aberrant mitochondrial activity as a potential biomarker for therapeutic response to CuATSM

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Patient diversity and unknown disease causes are major challenges for drug development and clinical trial design for Amyotrophic Lateral Sclerosis (ALS). Unfortunately, transgenic animal models do not adequately reflect the heterogeneity of ALS patient populations. Hence, the direct translation of potential therapeutics tested in such models to the clinic has proven difficult. To address this, we developed a rapid reprogramming method to convert ALS patient skin biopsies to neuronal progenitor cells (NPCs). We subsequently differentiated NPCs into induced Astrocytes (iAs) to co-culture with mouse GFP + motor neurons and screen potential therapeutics. Here, we evaluate the effects of (SP-4-2)-(2,2-dimethyl-1,2-ethanediylidenebis[N-methylhydrazinecarbo-thioamidato-kN2,kS])[(2-)-copper (CuATSM), currently in clinical trial for ALS in Australia, on multiple sporadic (sALS) and familial (fALS) patient lines. Our data indicate a diverse patient response to CuATSM, suggesting sALS, mutant SOD1 and C9ORF72 patient subgroups have shared pathways of interest. Next, we performed a detailed analysis of the effects of CuATSM on known ALS disease markers to determine if we can identify pathways that can distinguish CuATSM responding and nonresponding lines. We found that neither ROS, RNS, reactive oxygen species, variation in soluble SOD1 levels, p62 aggregation, ER stress, or metallothioneine upregulation were potential predictors of CuATSM responsiveness. Interestingly, elevated mitochondrial respiration was the common denominator in all CuATSM-responders, a metabolic phenotype not observed in non-responders. Importantly, pre-treatment of iAs with CuATSM restored mitochondrial activity to levels comparable to healthy controls. Hence, this metabolic parameter might allow to determine patient subpopulations best suited for CuATSM treatment. Together, these findings suggest that patient iAstrocytes may be used to identify both disease modifiers and pathways dysregulated in a given individual and evaluate the impact on therapeutic response. Moreover, CuATSM might have additional therapeutic value for mitochondrial disorders. Thus, enhanced understanding of patient-specific cellular and molecular profiles could help improve clinical trial design in the future.

DSP-12 Variation in age of onset and disease progression among genetic subsets of amyotrophic lateral sclerosis (ALS) patients: results from a real-world point-in-time survey

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Introduction: Amyotrophic lateral sclerosis (ALS) is a rare, degenerative neuromuscular disease, leading to progressive muscle function loss, and ultimately death. Clinical
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Non-SOD1/C9orf72: 0.74; the patient groups (Overall: 0.80; SOD1: 0.77; C9orf72: 1.19; decline per month since symptom onset were observed across 27.47). Significant differences in the rate of ALSFRS-R score 30.31, 25.52, 26.78), and respiratory aid (27.78, 25.41, 31.59, wheelchair (20.71, 26.44, 22.74, 18.85), feeding tube (27.13, 30.31, 25.52, 26.78), and respiratory aid (27.78, 25.41, 31.59, 27.47). Significant differences in the rate of ALSFRS-R score decline per month since symptom onset were observed across the patient groups (Overall: 55.09; SOD1: 55.17; C9orf72: 59.21; Non-SOD1/C9orf72: 54.31). Non-significant differences were observed in the time taken to reach key disease milestones (all months: Overall, SOD1, C9orf72, Non-SOD1/C9orf72, respectively), including time to walking aid (17.48, 13.81, 11.66, 18.88), wheelchair (20.71, 26.44, 22.74, 18.85), feeding tube (27.13, 30.31, 25.52, 26.78), and respiratory aid (27.78, 25.41, 31.59, 27.47). Significant differences in the rate of ALSFRS-R score decline per month since symptom onset were observed across the patient groups (Overall: 0.80; SOD1: 0.77; C9orf72: 1.19; Non-SOD1/C9orf72: 0.74; p = 0.027). In particular, there was a significant difference in the rate of decline between C9orf72 and Non-SOD1/C9orf72 patients (p = 0.023) and a non-significant difference in the rate of decline between SOD1 and Non-SOD1/C9orf72 patients (p = 1.000).

Discussion: While some heterogeneity was identified across the various genetic variants of ALS, the overall results were largely non-significant indicating that age of onset and time to key milestones were largely equivalent across the overall ALS population and that all forms of the disease are severe. The rate of ALSFRS-R score decline per month showed some differences within the genetic variants, mainly driven by the C9orf72 results which showed faster progression. These subtle differences in ALS presentation and progression highlight the complexity of understanding the disease course of ALS and may contribute to delays in diagnosis and difficulty predicting symptom progression within individual patients. Strategies to better understand the varying disease courses in ALS can support earlier diagnosis and improved treatment and outcomes for patients living with this progressive, life-shortening disease.

Method: Data were collected as part of the Adelphi ALS Disease Specific Programme (DSP16), a point-in-time survey of neurologists and their ALS patients in France, Germany, Italy, Spain, the UK, and the US, between July 2020–March 2021. Neurologists were asked to complete a questionnaire reporting recently consulted patients’ demographics and clinical characteristics, including age of symptom onset, genetic testing status and results, and ALSFRS-R scores. Differences in age at symptom onset, time from symptom onset to key disease milestones, and rate of ALSFRS-R decline were assessed using ANOVA and Bonferroni-corrected t-tests.

Results: 142 neurologists completed questionnaires for 880 ALS patients, of whom 192 had a known genetic test result for both SOD1 and C9orf72 mutations (SOD1 positive: 34; C9orf72 positive: 17; Non-SOD1/C9orf72: 141). Non-significant differences were observed in the age at symptom onset across the patient groups (Overall: 55.09; SOD1: 55.17; C9orf72: 59.21; Non-SOD1/C9orf72: 54.31). Non-significant differences were observed in the time taken to reach key disease milestones (all months: Overall, SOD1, C9orf72, Non-SOD1/C9orf72, respectively), including time to walking aid (17.48, 13.81, 11.66, 18.88), wheelchair (20.71, 26.44, 22.74, 18.85), feeding tube (27.13, 30.31, 25.52, 26.78), and respiratory aid (27.78, 25.41, 31.59, 27.47). Significant differences in the rate of ALSFRS-R score decline per month since symptom onset were observed across the patient groups (Overall: 0.80; SOD1: 0.77; C9orf72: 1.19; Non-SOD1/C9orf72: 0.74; p = 0.027). In particular, there was a significant difference in the rate of decline between C9orf72 and Non-SOD1/C9orf72 patients (p = 0.023) and a non-significant difference in the rate of decline between SOD1 and Non-SOD1/C9orf72 patients (p = 1.000).

Discussion: While some heterogeneity was identified across the various genetic variants of ALS, the overall results were largely non-significant indicating that age of onset and time to key milestones were largely equivalent across the overall ALS population and that all forms of the disease are severe. The rate of ALSFRS-R score decline per month showed some differences within the genetic variants, mainly driven by the C9orf72 results which showed faster progression. These subtle differences in ALS presentation and progression highlight the complexity of understanding the disease course of ALS and may contribute to delays in diagnosis and difficulty predicting symptom progression within individual patients. Strategies to better understand the varying disease courses in ALS can support earlier diagnosis and improved treatment and outcomes for patients living with this progressive, life-shortening disease.

Background. Amyotrophic lateral sclerosis (ALS) progression is now known to be highly variable across patients (1). Given its relentless nature, the functional decline is usually expected to be continuous. However, previous studies using the revised ALS Functional Rating Scale (ALSFRSr) showed that about 25% of ALS patients experience at least one 6-month pause and that such pauses could be even longer in a smaller percentage of cases (2). ALSFRSr is a standard measure in ALS progression studies; nonetheless, it has some flaws that could undermine its ability of grasping the real disease evolution over time (3).

Objective. To assess the frequency and predictors of plateaus in ALS progression as assessed by the Medical Research Council (MRC) Scale.

Methods. All patients attending the ALS Center of Turin, with a diagnosis between 2007 and 2014 were considered. At each visit, muscle strength was evaluated in several muscles and assessed using the MRC scale. Concomitant ALSFRSr scores were retrieved. Plateaus were calculated as a stable overall MRC or ALSFRSr score lasting at least 6, 12 or 18 months.

Results. According to MRC scale scores, 122 (22.8%), 71 (13.2%) and 59 (11.0%) patients experienced a plateau lasting at least 6, 12 and 18 months. ALSFRSr scores revealed similar estimates [134, (25.0%), 89 (16.6%) and 67 (12.5%), respectively]. Plateaus were more frequent at high scores and appeared a median of 24.6 months (IQR 6.7–47.7) after the diagnosis. Only the predominant upper motor neuron phenotype (OR 4.06, 95% CI 2.06–8.10, p-value <0.001) and diagnostic delay (OR 1.03, 95% CI 1.01–10.5, p-value =0.005) were significantly correlated with their appearance.

Discussion. Plateaus in ALS progression as assessed using either ALSFRSr or MRC scale are not infrequent and should be expected especially in less aggressive phenotypes. Similar results were found both using the MRC scale and the ALSFRSr scores, suggesting a comparable reliability of these scales in grasping the disease progression. It should be considered that such results could reflect the partial inaccuracy of these scales to faithfully mirror the disease progression. However, given the widespread of both the MRC and ALSFRSr scales, we believe these findings should be nonetheless taken into account in both the clinical and experimental settings.

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DSP-14 MND, or not only? THAT is a question

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Objective: Recent studies widely demonstrate multisystem involvement of ALS. Pathology case studies, imaging studies proving above mentioned. We investigated clinical features of ALS patients, defining ALS-Plus syndromes, directed to The First University Clinic of Tbilisi State Medical University during the years of 2019–2021.

Methods: Overall 47 patients with ALS were investigated, among them 24 male (51.06%), 23 female (48.9%), aged 21–84, we documented atypical clinical manifestations of those patients. Brain MRI and ENMG studies were done in all patients. A patient survey was taken using Mayo Clinic Laboratories-Neurology patient form, Cognitive changes assessed via Addenbrooke Cognitive Examination scale (ACE III), and Hamilton Anxiety and Depression Scale used for evaluating anxiety severity.

Results: ALS-Plus syndrome was found in 14 (29.8%) patients, demonstrating extrapyramidal disorders-6 (12.8%), autonomic functioning disturbances-8 (17.0%), GI system was mainly affected, and cognitive impairment in 11 patients (23.4%) evaluated with ACE III (cut-off score-83), among them only 7 fulfilled Neary criteria for FTD. Patients demonstrated mild to moderate anxiety levels. Neuroimaging revealed more cortical atrophy in ALS-Plus patients in comparison to typical ALS. No different electrophysiological patterns were observed in those subgroups.

Conclusions: Our results demonstrate strong evidence of ALS being not only MND but a multisystem disorder. Implying that ALS-plus symptoms should be screened vigorously by neurologists and managed appropriately. We are testing patients for possible genetic mutations to identify genes responsible for the development of ALS-Plus syndrome, further research is needed.

DSP-15 Patterns of longitudinal cognitive and behavioural change in ALS/MND

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Background: While symptoms of cognitive and behavioural impairment in ALS/MND (ALSci/ALSbi) are well-known (1), how these manifestations evolve over time – and who is/is not at risk for change – is unclear. Cross-sectional studies suggest that cognitive impairment is associated with advancing disease stage (2,3) and, in some cases, C9ORF72 repeat expansion (4,5). Longitudinal studies have been hampered by high attrition, small sample sizes, and the lack of neuropsychological tests suitable for repeated administration and accommodating ALS/MND-related physical disabilities.

Objectives: To explore the prevalence and temporal patterns of cognitive and behavioural change in ALS/MND patients, and the potential association with age, sex, education (years), bulbar onset, and C9ORF72 status.

Methods: Subjects were ALS, PMA, and PLS participants in CReAtE (Clinical Research in Related Disorders for Therapeutic Development) Consortium’s Phenotype-Genotype-Biomarker (PGB) study. The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) was used to assess ALS specific (ALSp; language, executive, verbal fluency) and non-specific (ALSnsp; memory, visuospatial) cognition. Informants reported behavioural symptoms through a semi-structured interview. N = 238 English speakers with ECAS at ≥3 visits (3–6 mo apart, max 5 visits) were included in latent class growth and mixed model analyses.

Results: Initial ALSci was uncommon (N = 18). Evidence for three subgroups representing different patterns of cognitive performance was found. Two showed normal scores that either remained unchanged on all ECAS measures (p = ns) or increased marginally over time for ECAS total (ECASstot; p = 0.002) and ALSp (p = 0.001), but not ALSnsp (p = 0.134), scores. One subgroup (~10% of participants) showed low initial scores on all ECAS measures and small but significant declines in ECASstot and ALSnsp (p < 0.001), but not ALSp (p = 0.057), scores over time. This profile was associated with C9ORF72 (ECASstot; OR [95% CI] = 8.69 [1.22–61.82]; ALSp: 6.93 [1.17–41.09]); lower education (ECASstot: 1.33 [1.07–1.65]; ALSp: 1.34 [1.11–1.61]; ALSnsp: 1.22 [1.11–1.61]), and male sex (ALSp: 3.43 [1.07–10.98]). Apathy was the most frequent behaviour at all time points; behaviour symptoms were uncommon and fluctuated between visits.

Discussion: A small subset of patients showed deficits in ECAS scores at initial assessment. While their language, executive functioning, and verbal fluency remained stable, memory and visuospatial functioning continued to decline. These observations suggest that ALSnp cognitive dysfunction, if present, occurs relatively early in disease–prior to first assessment, diagnosis, or perhaps even motor onset.

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DSP-16 Differentiation between MND phenotypes: the role of clinical features at the time of diagnosis

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**Background:** Motor neuron diseases (MNDs) can affect the upper motor neurons (UMN) and/or the lower motor neurons (LMN), and it is now widely accepted that pure/predominant UMN (pUMN) and pure/predominant LMN (pLMN) phenotypes have significantly better prognosis compared to classic amyotrophic lateral sclerosis (ALS). Despite this consideration, the heterogeneity of the initial manifestations often challenges an accurate differentiation between these MND phenotypes, with important consequences in terms of prognosis estimation.

**Objective:** To determine which clinical features at the time of diagnosis may help differentiating pUMN and pLMN phenotypes from classic ALS.

**Methods:** 60 MND patients were included in this retrospective study (26 classic ALS, 14 pUMN and 20 pLMN). At the time of diagnosis patients underwent a detailed clinical characterization, including site (bulbar, proximal spinal, distal spinal) and side of disease onset, disease duration, overall degree of functional impairment (assessed using the ALS Functional Rating Scale-revised [ALSFRS-r]), regional UMN involvement (graded using the UMN score), muscle strength (evaluated using the Medical Research Council [MRC] scale) and their relative progression rates. Mann-Whitney and Chi-squared tests were used to identify significant differences between pUMN and classic ALS as well as between pLMN and classic ALS. Logistic regression analyses were then applied to isolate significant predictors of pUMN and pLMN diagnoses.

**Results:** Significant predictors of a pUMN diagnosis included younger age at onset ($p<0.02$), longer disease duration ($p=0.05$), and greater cranial ($p=0.03$) and upper limbs ($p=0.03$) UMN involvement. Significant predictors of a pLMN diagnosis included longer disease duration ($p=0.01$), more severe gross motor functional impairment ($p=0.01$), lower muscle strength of the right ($p=0.001$) and left ($p=0.007$) lower limbs, less severe cranial ($p=0.02$), upper limbs ($p=0.002$) and lower limbs ($p=0.01$) UMN involvement, and slower rate of progression of the UMN signs ($p=0.01$).

**Discussion:** Our findings suggest that specific clinical features at the time of diagnosis may help differentiating between more benign and more aggressive MND phenotypes. These findings have potential to facilitate appropriate stratification for clinical trials enrollment, clinical management, and prognosis estimation.

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DSP-17 Spreading pattern in ALS patients with respiratory onset

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**Background:** Spreading across anatomical regions is a hallmark of Amyotrophic Lateral Sclerosis (ALS) but disease progression in respiratory onset ALS patients has scarcely been reported. Respiratory-onset patients are a particular model to study the contiguous and predominant side involvement in ALS spreading and the role of UMN vs LMN in the spreading pattern.

**Objectives:** To assess the spreading pattern in a group of respiratory-onset patients followed in our center, taking into account their specific phenotype.

**Methods:** From a consecutive ALS population with probable/definitive ALS disease, followed in our Unit and evaluated accordingly to the ONWebDuals protocol, we included all respiratory-onset patients without concomitant involvement of other regions. We considered the phenotype (predominant UMN vs LMN) as well as the following affected regions (upper limbs, UL; lower limbs, LL; Bulbar, B; cognition; respiration, R; axil, A).

**Results:** From the 625 ALS patients included, 18 (2.88%) had respiratory onset form (15 males, mean onset age 69.2±11.6 years, mean disease duration 10.7±9.0 months). Sixteen were Caucasian and all were right-handed. Two other patients had concomitant respiratory and bulbar onset and a third one had concomitant axial onset form and were excluded. At first visit, 38.9% of the patients reported significant weight loss (>10%) while 83.3% had resting fatigue and 88.9% had orthopnea. Weak cough was present in 83.3% and paradoxical respiration in 77.8%. All patients had predominant LMN involvement. Depression was present in 88.9%. Cognitive involvement was present in only one patient. No sensory changes were noted.

The 2nd affected region was UL in 7 (4 of whom then progressed to LL involvement), LL in 3 (who progressed to UL involvement), bulbar in 4 (1 progressed to UL involvement and one to LL) and axial in 3 (1 progressed to LL involvement). An asymmetric progression was seen in 33.4%, from the respiratory region to the UL or LL (4 of them to the left segment and 1 to the right) while the remaining progressed to bilateral or middle line involvement. Progression interval to the 2nd region was 4.7±5.7 months and to a 3rd region was 6.1±8.7 months. All patients were adapted to NIV (disease duration to NIV 9.9±7 months, min 2–max 28); only two patients are alive, being total survival 31.7±21.9 months (min 8–max 91).

**Discussion:** A more medial, symmetric, rapid and contiguous spreading, with a predominant LMN pattern is observed in respiratory onset ALS patients, as compared to patients with spinal or bulbar onset. This respiratory onset phenotype was...
Cluster Validity Indices (CVIs) were used to assess the goodness-of-fit to be significantly different between patients and controls. Patients during sustained attention to response task (SART) event-related spectral perturbations taken from 24 different brain networks during EEG recordings have the potential to provide further information for ALS stratification.

**Objectives:** To explore the potential of subphenotyping based on electrophysiological data recorded during functional motor tasks, which would provide a data-driven, non-invasive approach for stratifying ALS patients. Specifically, we sought to employ cluster analysis and statistical testing to investigate whether EEG/EMG data contain subgroups that are both significant and stable.

**Methods:** Hierarchical and spectral clustering were applied to the following EEG/EMG derived data: four measures of cortico-muscular coherence (CMC) taken from 20 patients during motor task performance (1) and five measures of event-related spectral perturbations taken from 24 different patients during sustained attention to response task (SART) performance (2). These nine measures were previously found to be significantly different between patients and controls.

Cluster Validity Indices (CVIs) were used to assess the goodness-of-attachment assignment and p-values for each cluster assignment were calculated using several Monte-Carlo methods. Stability under small perturbation was quantified using Adjusted Rand Index (ARI; [0,1]) the most stable).

**Results:** Hierarchical clustering of CMC data found one significant (p = 0.004 to 0.008), stable (ARI = 0.90) solution with 5 clusters (including varying small and large sizes: one large cluster containing 11 patients, and two smaller clusters containing only 1–2 patients). Spectral clustering of SART data also found one significant (p = 0.049), stable (ARI = 0.88) solution with 5 clusters. The latter clusters were comparable in size, each containing 4–7 subjects.

**Discussion:** We found that the EEG/EMG data contain cluster structure, which was both significantly above chance level and stable. However, low sample sizes meant that some of the resulting clusters were small, making it difficult to assess whether the proposed subgroups are capturing real effects in ALS electrophysiology. While it is not possible to draw more general and strong conclusions from these specific subgroups, the findings do demonstrate that the EEG/EMG recorded during functional motor tasks contain rich information for ALS subphenotyping. With a larger sample, the cluster structure should become more apparent, and the resulting subgroups may reflect important aspects of ALS heterogeneity, which will be instrumental for stratification in clinics and for clinical trials.

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the key determinants of disability. Tracking the decline in walking capacity can provide critical clinical information; however, walking evaluation has been scarcely studied as a potential predictive factor for survival in motor neuron disease.

**Objectives:** Our goals were to assess the progression of gait decline and evaluate its association with mortality in ALS using the Timed Up and Go test (TUG). Specifically, the objectives of the study were: (1) determine the feasibility of using the Timed up and Go test (TUG) – a reliable measure of mobility – in ALS patients, (2) compare the TUG in bulbar and non-bulbar ALS patients, and (3) examine if the TUG could be a marker of disease progression similar to the ALSFRS-R score.

**Methods:** Patients with confirmed ALS according to El Escorial criteria (definite, probable or possible ALS) were recruited in the Centre for ALS and Related Disorders of Geneva University Hospitals and followed prospectively. At baseline, demographic and clinical parameters, including ALSFRS-R score and ALS form (bulbar, non-bulbar) were recorded. Exclusion criteria were the presence of other neurological or orthopedic disorders interfering with gait. The TUG was performed at baseline and subsequent evaluations occurred every 3 months. At inclusion, patients were classified as unable to perform the TUG, “slow TUG” (>10.6 s) and “fast TUG” (≤10.6 s).

**Results:** In total, 68 patients with ALS (mean ± SD age: 69 ± 12 years; 50% female) were included. Baseline TUG were negatively correlated with the total ALSFRS-R score (r = −0.63, p < 0.001). At baseline, ALS patients with bulbar onset performed the TUG faster (9.9 ± 3.7 seconds) than non-bulbar ones (17.3 ± 14.9 seconds, p = 0.008). Thirty of 68 (44%) patients died by the end of the follow-up period. The TUG performance at the first visit did not predict mortality.

**Discussion:** While we did not find any association between mortality in ALS and gait performance, the TUG was feasible in a majority of ALS patients and was correlated with functional status. The main advantage of the TUG is its easy access in the clinical settings to provide a multiple component assessment of balance and mobility, as well as cognition. Furthermore, the TUG could be included in the battery of assessments as an additional measure in the follow-up of non-bulbar ALS patients.

**Objectives:** To determine the expression of monocyte sub-classes and the relative proportion of non-active and active CD11b-expressing monocytes in the progression of ALS.

**Methods:** ALS individuals were classified based on a slower (A-S, n = 20) and faster (A-F, n = 18) disease progression and compared to age and sex matched healthy controls (HC, n = 20). Blood samples were obtained at baseline (V1) and at two additional time points (V2 and V3) for longitudinal analysis and selected from the ALS biomarkers study cohort (East London Research Ethics Committee-REC reference 09/H0703/27). Flow cytometry was used to define the proportion of classical (CM; CD16-CD14+) and intermediate (IM; CD16+CD14+), non-classical (NCM; CD16+CD14-) monocytes together with the frequency of cells expressing non-active and active form of CD11b. Plasma CD11b levels were measured by ELISA using a commercial immunoassay.

**Results:** CD11b+NCM frequencies were higher in A-F (p = 0.0158) and presented a negative correlation with survival (p = 0.0006; R: -0.7281). At V1, above median levels of NCM were associated with reduced survival (50% reduction, p = 0.042; log rank analysis) and were also independent negative predictors of survival on multivariate analysis (p = 0.045; HR: 3.5). In contrast, active CD11b+NCM positively correlated with survival in ALS (p = 0.0168, R = 0.3862). A steep longitudinal increase of active CD11b+CM was seen particularly in A-S (ANOVA for repeated measurements, mixed models, p < 0.0001), a change between time points which strongly discriminated ALS from HC and A-F from A-S (V2/V1 ratio, AUC: 0.8971; V3/V1 ratio, AUC: 0.9583; p < 0.0001). Soluble CD11b plasma concentration correlated with all active CD11b+ monocyte expression sub-classes.

**Conclusion:** Our results indicate that high levels of NCM are associated with reduced survival in ALS. In contrast, higher frequencies of active CD11b+NCM and the increase of active CD11b+CM during disease are associated with a better prognosis. Overall, expression of NCM and the CD11b phenotype may be a major contributing factor to disease progression in ALS and a potential biomarker for disease monitoring.

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**DSP-20 Beta2-integrin CD11b-expressing monocytes and disease progression in amyotrophic lateral sclerosis**

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**Background:** Several studies have reported alterations of the immune system in ALS and a potential impact of the immune response on disease progression (1,2). Monocytes are immunoregulatory cells and extravasate into sites of neuronal degeneration. CD11b belongs to the integrin family and is a myeloid cell integrin receptor for ICAM-1 and fibrinogen. Active beta2-integrin CD11b has been reported to promote monocyte migration and to exert an anti-inflammatory effect (3).

**Objectives:** To investigate the CD11b phenotype in ALS and its potential impact on disease progression.

**Methods:** DSP-20 Beta2-integrin CD11b-expressing monocytes were isolated from healthy controls and ALS patients. DSP-20 Beta2-integrin CD11b-expressing monocytes were isolated from healthy controls and ALS patients. Neuronal degeneration. CD11b belongs to the integrin family and is a myeloid cell integrin receptor for ICAM-1 and fibrinogen. Active beta2-integrin CD11b has been reported to promote monocyte migration and to exert an anti-inflammatory effect (3).

**Objectives:** To determine the expression of monocyte sub-classes and the relative proportion of non-active and active CD11b-expressing monocytes in the progression of ALS.

**Methods:** ALS individuals were classified based on a slower (A-S, n = 20) and faster (A-F, n = 18) disease progression and compared to age and sex matched healthy controls (HC, n = 20). Blood samples were obtained at baseline (V1) and at two additional time points (V2 and V3) for longitudinal analysis and selected from the ALS biomarkers study cohort (East London Research Ethics Committee-REC reference 09/H0703/27). Flow cytometry was used to define the proportion of classical (CM; CD16-CD14+) and intermediate (IM; CD16+CD14+), non-classical (NCM; CD16+CD14-) monocytes together with the frequency of cells expressing non-active and active form of CD11b. Plasma CD11b levels were measured by ELISA using a commercial immunoassay.

**Results:** CD11b+NCM frequencies were higher in A-F (p = 0.0158) and presented a negative correlation with survival (p = 0.0006; R: -0.7281). At V1, above median levels of NCM were associated with reduced survival (50% reduction, p = 0.042; log rank analysis) and were also independent negative predictors of survival on multivariate analysis (p = 0.045; HR: 3.5). In contrast, active CD11b+NCM positively correlated with survival in ALS (p = 0.0168, R = 0.3862). A steep longitudinal increase of active CD11b+CM was seen particularly in A-S (ANOVA for repeated measurements, mixed models, p < 0.0001), a change between time points which strongly discriminated ALS from HC and A-F from A-S (V2/V1 ratio, AUC: 0.8971; V3/V1 ratio, AUC: 0.9583; p < 0.0001). Soluble CD11b plasma concentration correlated with all active CD11b+ monocyte expression sub-classes.

**Conclusion:** Our results indicate that high levels of NCM are associated with reduced survival in ALS. In contrast, higher frequencies of active CD11b+NCM and the increase of active CD11b+CM during disease are associated with a better prognosis. Overall, expression of NCM and the CD11b phenotype may be a major contributing factor to disease progression in ALS and a potential biomarker for disease monitoring.

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