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# *Interaction of sex and onset site on the disease trajectory of Amyotrophic Lateral Sclerosis*

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## Equal Author Contribution

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## Contributions

- Juliette ORTHOLAND: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data.
- Pierre-François PRADAT: Drafting/revision of the manuscript for content, including medical writing for content;
- Sophie Tezenas du Montcel: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data.
- Stanley Durrleman: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data.

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## ABSTRACT

**Background:** Studies showed the impact of sex and onset site (spinal or bulbar) on disease onset and survival in ALS. However, they mainly result from cross-sectional or survival analysis, and the interaction of sex and onset site on the different proxies of disease trajectory has not been fully investigated.

**Methods:** We selected all patients with repeated observations in the PRO-ACT database. We divided them into four groups depending on their sex and onset site. We estimated a multivariate disease progression model, named ALS Course Map, to investigate the combined temporal changes of the four sub-scores of the revised ALS Functional Rating Scale (ALSFRS<sub>r</sub>), the forced vital capacity (FVC), and the body mass index (BMI). We then compared the progression rate, the estimated age at onset, and the relative progression of the outcomes across each group.

**Results:** We included 1,438 patients from the PRO-ACT database. They were 51% men with spinal onset, 12% men with bulbar onset, 26% women with spinal onset, and 11% women with bulbar onset. We showed a significant influence of both sex and onset site on the ALSFRS<sub>r</sub> progression. The BMI decreased 8.9 months earlier (95% CI = [3.9, 13.8]) in women than men, after correction for the onset site. Among patients with bulbar onset, FVC was impaired 2.6 months earlier (95% CI = [0.6, 4.6]) in women.

**Conclusion:** Using a multivariable disease modelling approach, we showed that sex and onset site are important drivers of the progression of motor function, BMI, and FVC decline.

## INTRODUCTION

Amyotrophic Lateral Sclerosis (ALS) is an adult-onset heterogeneous neurodegenerative disease that occurs on average between 55 and 60 years. It is characterized by a degeneration of both upper and lower motor neurons<sup>1</sup>, and the average survival time from onset to death is three to four years<sup>2</sup>. The disease shows an important phenotypic variability, particularly in terms of the site of disease onset and progression<sup>3</sup>. The difference between the disease starting with dysarthria and dysphagia (bulbar onset) and the disease with limb weakness (spinal onset) is well documented. Among other, they differ in terms of incidence<sup>3</sup>; progression rate<sup>4-6</sup>; and survival duration<sup>4</sup>.

Several epidemiological studies have shown a lower incidence of ALS in women compared to men<sup>4,7</sup> as well as differences in the onset site and age at onset<sup>8</sup>. More recently genetic<sup>9</sup> and imaging<sup>10</sup> studies have enriched the knowledge on this sexual dimorphism. However, most of these results come from cross-sectional studies<sup>7,8</sup> or survival<sup>4</sup> studies. By design, the first one failed to capture the longtime effect of clinical or demographic variables, such as age or sex<sup>11</sup>. The second only presents half of the information on the disease progression by studying mortality, leaving aside the function in daily activities. Thus how the marked sexual dimorphism influences the progression and the presentation of each ALS form, has not been fully described.

The originality of the present study is to investigate the interaction of sex and onset site on the whole progression of the following clinical proxies: BMI<sup>12</sup>, forced vital capacity (FVC)<sup>13</sup>, and sub-scores of ALS functional rating scales (ALSFRS<sub>r</sub>)<sup>14</sup>. To do so, we have used a recent longitudinal model designed for neurodegenerative diseases<sup>15,16</sup>. This enabled us to study all the outcomes at the same time, consider the non-linearity of the progression of outcomes, and capture patients' heterogeneity better than with classic models.

## MATERIALS AND METHODS

### *Study population*

We analyzed patients from the Pooled Resource Open-Access ALS Clinical Trials Consortium (PRO-ACT) database. It is composed of an aggregation of 23 phase II and III clinical trials and one observational study. Information that could lead to any identification, such as the clinical trial, tested drug, study centres, or dates, was not included in the database. As the database is an aggregation, inclusion and exclusion criteria for patients to enter the cohort are multiple and will not be described here, more information could be found in the paper that presents the database<sup>17</sup>.

For our study, we selected patients with at least two visits for all the outcomes listed below. We collected: outcome values at all available visits; the sex of the participant; and the onset site (spinal or bulbar), a label that is available in the PRO-ACT database. We divided participants into four groups: men with bulbar onset, women with bulbar onset, men with spinal onset, and women with spinal onset.

### *Outcomes*

We selected a set of outcomes that are important clinical proxies of ALS progression and are available in the PRO-ACT database. We considered the most widely used functional rating system in patients with ALS, namely the revised version of the ALS functional rating scale (ALSFRS<sub>r</sub>)<sup>14</sup>. The scale starts at a maximum theoretical value of 48 and decreases with the severity of the disease till zero. We considered the four ALSFRS<sub>r</sub> sub-scores assessing four distinct domains: bulbar, gross motor, fine motor, and respiratory functions. Each sub-scale has 12 points each<sup>14</sup>. In addition, we considered the FCV<sup>13</sup> that we normalized using the computed normal FVC<sup>18</sup> and the BMI<sup>12</sup>.

We normalized the outcomes between 0 (the healthiest value) and +1 (the maximum pathological change). The four ALSFRS<sub>r</sub> sub-scores have been normalized using their theoretical maximum and minimum values. For FVC and BMI, we first applied a box-cox transformation (estimated on the whole PRO-ACT dataset) to get a standard normal distribution<sup>19,20,21</sup>. We then clipped the resulting value between -3 and 3 points and converted them to the 0 to 1 scale.

In addition to these repeated measures, we collected the following covariates when available: riluzole use, trial arm (active, placebo, observational), age at first symptom, age at diagnosis, age at baseline, Mitos<sup>22</sup> and FT9 scores<sup>23</sup> at baseline. These data were not used as input for the disease progression model.

### *ALS Course Map*

We used a disease progression model, named ALS Course Map, which is a multivariate non-linear Bayesian mixed-effects model<sup>15,16</sup>. The model estimates the progression of a series of outcomes from a longitudinal dataset. It estimates a subject-specific set of curves for each outcome and summarizes them at the population, or sub-group level. The model does not require imputation as it is robust to missing data<sup>24</sup>. In addition, it does not require a standardized time between visits<sup>24</sup>.

ALS course map models the progression with a logistic pattern of outcomes development. As in other mixed effect models, two types of parameters are computed: fixed effect, referred to as population parameters, and random effect, referred to as individual parameters. Population parameters capture the average ALS progression from which patients deviation could be captured by three individual parameters:



The first individual parameter is the disease time shift,  $\tau_i$ , expressed in years. It represents the delay between the estimated age at disease onset for patient  $i$  and the whole population. For instance,  $\tau_i = -1$  means that the estimated disease onset of the patient occurs one year earlier than the average patient in the population. The estimation of the time shift takes all outcomes into account. There is a single time shift for a given subject.

The second one is the progression rate,  $\alpha_i$ . It represents a value that multiplies the population progression rate to get the rate of the patient  $i$ . For instance,  $\alpha_i = 1.2$  means that the disease progresses 1.2 times faster than the average patient in the population. The estimation of the progression rate also takes all outcomes into account. There is a single rate for a given subject.

Finally, a series of inter-marker spacings,  $\omega_{ij}$ , expressed in months. One  $\omega_{ij}$  changes the onset of the outcome  $j$  of the patient  $i$  while keeping other outcomes fixed. For instance,  $\omega_{ij} = +2$  means that the  $j$ -th outcome changes two months earlier than for the average patient in the population, all other things being equal. Each subject has six inter-marker spacings, one per outcome in the model. Inter-marker spacings are centred (i.e. their weighted sum equals zero) so that they do not interplay with the estimation of the time shift.

We used the open-source software, Leaspy, to estimate the model parameters from a longitudinal dataset. The software is publicly available at: [https://gitlab.com/icm-institute/aramislab/leaspy/Experimental design](https://gitlab.com/icm-institute/aramislab/leaspy/Experimental%20design). This disease progression model has been applied to other neurodegenerative conditions<sup>25,26</sup>.

## *Statistical analysis*

We trained the model with the whole data set composed of four groups. We assessed the convergence with a rank normalized R-hat diagnostic test using a threshold of 1.2. To study the goodness-of-fit of the model to the actual data, we compared the estimated value of each visit with its real available value to compute the reconstruction error. We compared the reconstruction error among groups.

To test for the interaction of sex and onset site on the ALS course map individual parameters we used ANOVAs. We compared the individual parameters estimated by the ALS Course Map between sex and onset site interaction using ANOVAs. For significant results, we computed an independent pairwise Tukey-Kramer test<sup>27</sup>. Otherwise, we computed an ANOVA without interaction to test the influence of each covariate adjusted for the other. All the results are presented with a 95% confidence interval computed with pairwise Tukey-Kramer or ANOVA without interaction, depending on the interaction significance. We used the same comparison process to describe the cohort and compare the groups at baseline. For the cohort description, results are associated with the corrected sample standard deviation. Statistical tests were performed at a conventional 2-tailed type I error of 0.05. Data were analyzed using python software 3.8, using packages statsmodel<sup>28</sup> and bioinfokit<sup>29</sup>.

### *Standard Protocol Approvals, Registrations, and Patient Consents*

Data used in this study came from the PRO-ACT database which is institutional review board–approved. Patients originally consented to participate in clinical trials that have been aggregated in the database. Every patient has been de-identified. We accessed the data following the access procedure detailed here: <https://ncr1.partners.org/ProACT/Account/Register>.

### *Data availability*

Access to the database PRO-ACT could be asked for through the procedure above. Further information for replication of the study is available in supplementary material in eReproducibility.

## RESULTS

### *Population*

Among the 8,571 patients (78,824 visits) in the PRO-ACT database, we dropped 220 values of BMI and 45 values of FVC that were out of the normalization range. We then selected the 1,463 patients with at least two visits for all the outcomes selected. Finally, we selected the 1,438 patients with information about the onset site. Note that sex was available for all patients. Their characteristics are presented in Table 1. The cohort was composed of 51% men with spinal onset, 12% men with bulbar onset, 26% women with spinal onset, and 11% women with bulbar onset. There were no significant differences in follow-up duration ( $1.00 \pm 0.59$  years); the number of visits per patient ( $11.6 \pm 7.2$  visits); and time between visits ( $34.5 \pm 28.5$  days) over the four groups. Nevertheless, we observed significant differences among groups for ages, time durations, and scores at baseline.

### *ALS Course Map*

We can first extract qualitative results from the four groups' trajectories. Figure 1 represents the progression of ALSFRS<sub>r</sub> subscores for each subgroup. Overall, curves are shifted on the right for patients with bulbar onset (Figure 1 B and D) compared to patients with spinal onset (Figure 1 A and C). Curves are also shifted on the right for women (Figure 1 A and B) compared to men (Figure 1 C and D), illustrating later onset for women compared to men.

The model also exhibited differences in the timing of outcomes. For instance, it confirmed the progression order of ALSFRS<sub>r</sub> bulbar curve (orange) and ALSFRS<sub>r</sub> motor curves (red and green): they are switched between patients with spinal onset (Figure 1 A and C) and patients with bulbar onset (Figure 1 B and D).

Finally, we can note differences in speed. Patients with spinal onset (Figure 1 A and C and Figure 2 purple and grey) seem to have steeper curves than patients with bulbar onset (Figure 1 B and D and Figure 2 cyan and pink). All these results are confirmed below by quantitative statistical tests.

### *Estimated age at disease onset*

We found an interaction between the onset site and sex for the estimated disease onset ( $p = 0.0020$ ) (in supplementary materials eFigure 1). For patients with bulbar onset, the disease onset for men occurs 6.0 years earlier (95% CI = [2.6, 9.5]) than for women while for a woman with spinal onset, it occurs 4.7 years earlier (95% CI = [1.9, 7.4]) than for women with bulbar onset.

### *Rate of progression*

We did not find any significant interaction between the onset site and sex for the rate of progression ( $p=0.14$ ) (in supplementary materials eFigure 2). Patients with bulbar onset were found to progress 1.4 times faster (95% CI = [1.2, 1.6]) than patients with spinal onset independently of sex.

### *Outcomes onset of impairment*

#### **Respiratory function**

We found an interaction between sex and onset site for FVC ( $p<0.001$ ) but not for ALSFRS<sub>r</sub> respiratory score ( $p=0.058$ ), once corrected for the estimated age at disease onset and rate of progression (Figure 3 E and F). Among patients with bulbar onset, FVC is impaired 2.6 months earlier (95% CI = [0.6, 4.6]) in women than in men. Among men, FVC is impaired 5.4 months earlier (95% CI = [3.8, 7.0]) in patients with bulbar onset compared to spinal onset. Among women, the difference increases to 8.3 months (95% CI = [6.7, 9.9]). ALSFRS<sub>r</sub> respiratory scale changes 2.4 months earlier (95% CI = [0.7, 4.0]) for patients with bulbar onset compared to spinal onset independently of the sex.

## Motor function

We found an interaction between sex and onset site for gross motor ( $p < 0.001$ ) but not for fine motor scores ( $p = 0.12$ ), once corrected for the estimated age at disease onset and rate of progression (Figure 3 A and B). Among patients with spinal onset, ALSFRS<sub>r</sub> gross motor scale deteriorates 4.5 months later (95% CI = [3.1, 6.0]) in women than in men. But ALSFRS<sub>r</sub> fine motor scale changes 3.5 months earlier (95% CI = [2.6, 4.4]) in women than in men, independently of the onset site. Among men, ALSFRS<sub>r</sub> gross motor scale changes 10.6 months earlier (95% CI = [8.3, 12.8]) for patients with spinal onset compared to bulbar onset. Among women, the difference increases to 16.0 months earlier (95% CI = [13.7, 18.2]). ALSFRS<sub>r</sub> fine motor scale changes 10.2 months earlier (95% CI = [9.0, 11.3]) for patients with spinal onset compared to bulbar onset, independently of the sex.

## Bulbar function

We did not observe any interaction between sex and onset site for ALSFRS<sub>r</sub> bulbar score ( $p = 0.01$ ), once corrected for the estimated age at disease onset and rate of progression (Figure 3 C). Among patients with bulbar onset, ALSFRS<sub>r</sub> bulbar scale changes 6.4 months earlier (95% CI = [1.6, 11.1]) in women than men. Among men, ALSFRS<sub>r</sub> bulbar scale changes 27.2 months later (95% CI = [23.4, 31.0]) for patients with spinal onset compared to bulbar onset. Among women, the difference increases to 32.5 months later (95% CI = [28.7, 36.3]).

## BMI

We did not observe an interaction between sex and onset site for BMI ( $p = 0.17$ ), once corrected for the estimated age at disease onset and rate of progression (Figure 3 D). BMI decreases 8.9 months earlier (95% CI = [3.9, 13.8]) in women than men, independently of the onset site. BMI decreases 10.0

months earlier (95% CI = [3.8, 16.1]) for patients with bulbar onset compared to spinal onset, independently of the sex.

## DISCUSSION

Sex and onset site interact with disease progression in ALS as demonstrated on all the clinical proxies studied, including disability, weight loss, and respiratory function. In particular, several proxies were affected earlier in women than in men.

Concerning the effect on weight, we showed that BMI starts to decrease 9 months earlier in women than men, independently of the onset site. Considering the rapid progression of ALS, with a mean survival of 3-6 years, this inter-sex difference appears clinically meaningful. Weight loss is a classical consequence of bulbar impairment leading to dysphagia. But alterations in body weight are present in ALS patients already decades before clinical manifestation of ALS<sup>30</sup> and are a negative prognostic factor associated with shorter survival<sup>31</sup>. Weight loss is also associated with increased energy expenditure. It is present in almost half of the patients and is associated with shorter survival<sup>32</sup>. Inter-sex differences in metabolism may also affect the disease trajectory. The role of energy and lipid metabolism abnormalities in the pathogenesis of ALS is currently a focus of many studies in animal models and ALS patients<sup>33-36</sup>. Inter-sex differences in the percentage of body fat may also modify disease progression. It is higher in women<sup>37</sup> and may vary with menopausal status<sup>38</sup>. Studies comparing fat mass with impedancemetry or dual-energy X-ray absorptiometry are needed to better investigate the role of metabolism and the effect of sex on disease progression<sup>39,39</sup>.

The effect of sex on the respiratory decline has not been reported so far. We showed that among patients with bulbar onset, FVC is impaired 2.6 months earlier in women than in men. This difference



is also clinically meaningful since respiratory distress is the main cause of death in ALS and the decline of FVC is the main criterion for the indication of non-invasive ventilation<sup>40</sup>.

An unexpected result was that the ALSFRSr fine motor scale changes earlier in women, regardless of the onset site, but that the ALSFRSr gross motor scale changes later in women among patients with spinal onset. Further studies are needed to investigate whether these differences might be related to sexual dimorphism in the anatomic pattern of abnormalities, both in terms of susceptibility to neurodegeneration and compensatory mechanisms. The advances in neuroimaging enable an increasing number of research studies investigating cortical and subcortical structure in both health and disease conditions<sup>41,42</sup>. Note that a dimorphism of regions linked to fine motors has been shown in healthy participants<sup>43</sup>. In addition, significant sex differences in the anatomical patterns of cortical and subcortical pathology have been shown in ALS<sup>41</sup>. Finally, these sex differences might be a bias in the self-evaluation of motor abilities using ALSFRSr.

On the one hand, from a biological perspective, the mechanisms of sexual dimorphism in ALS have been shown at an anatomical level in specific brain regions, in vivo, thanks to imaging<sup>10</sup> and related to sex-specific microglial regulation<sup>44</sup>, sex hormones<sup>45</sup>, or susceptibility to oxidative stress<sup>46</sup>. A study, performed on a large number of ALS women with natural menopause and well-defined oral contraceptive usage, has demonstrated that longer exposure to female hormones has neuroprotective effects on motoneurons in ALS (positive association between a longer reproductive condition, the susceptibility to ALS, and the survival of ALS patients)<sup>45</sup>. On the other hand, the overall heritability of ALS is estimated to be of approximately 21% using genome-wide association studies (GWAS) datasets<sup>9</sup>. The effect of sex was found to be different according to the type of mutation C9orf72 or SOD1 on ALS progression<sup>9</sup>. Recent studies on presymptomatic ALS phase support the emerging hypothesis of a

general disorder in development, both in animal<sup>47</sup> and humans at cognitive<sup>48</sup> and structural level<sup>49</sup>. From a developmental origin of health and disease perspective<sup>50</sup>, the well-known sexual dimorphism in physiological development could also play a role.

A strength of our study is that our model was based on a large, multicentric, and longitudinal cohort. We used a mixed effect model following neurological research recommendation<sup>51</sup>. The model used is robust to missing data and does not require a standardized time between visits<sup>24</sup>. Logistic curves were used to overcome common limitations on score analysis: curvilinearity, and potential floor or ceiling effects<sup>52</sup>. The reconstruction error of the model was of the same range as the one of the Alzheimer's disease study<sup>25</sup>, for which the model has been originally developed (eFigure 3 and eFigure 4 in supplementary materials).

Our study has several limitations. PRO-ACT may not be representative of the real-life ALS patient population. Since the database aggregates clinical trial data, there is an overrepresentation of men, young subjects, patients with spinal onset, and slow progressors. Nevertheless, these results could help better understand the progression of placebo arms and design strategies for patient stratification in clinical trials. Due to the high level of death censoring in PRO-ACT, we were not able to analyse the survival in addition to the disease progression. The outcomes selected has also their own limits. BMI does not capture the distinction between fat, muscle, or bone mass. We have also used the sub-scores of ALSFRS<sub>r</sub> separately as recommended<sup>14</sup>, but as lower motor neurons often dominate in the overall clinical presentation<sup>5</sup>, the score might be ill-suited for other sub-form of the disease. A population-based study showed the existence of plateaus in ALSFRS<sub>r</sub> mainly shorter than 6 months, with only 10% of patients with wider plateaus<sup>53</sup>. Thus, modeling the progression of ALSFRS<sub>r</sub> subscores by logistic curves could be debated. Nevertheless, plateaus appear in high scores and are unlikely to change the

results since our patients' average length of follow-up is one year. The absence of information on genetic mutation<sup>3</sup>; neuropsychological symptoms<sup>3</sup>, exposure to metals or agricultural factor<sup>54</sup> and lifestyle<sup>55,56</sup> also limits the interpretation of the results.

To summarize, we showed that sex and onset site interaction is an important driver of ALS progression considering many clinical proxies such as disability, weight loss, and respiratory function. Pathophysiological mechanisms underlying these differences are elusive and may account for an interplay of biological, lifestyle, and environmental factors. Further studies in animal models and patients are needed to better understand their respective contributions.

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**Table 1: Patient characteristics Legend: data are expressed as mean± standard deviation (percentage of available data) or frequency (percentage), p-value: ANOVA interaction p-value, BM:**

Outcomes		Spinal Man	Bulbar Man	Spinal Woman	Bulbar Woman	p-value	Subgroup relations	
Numbers	Patients	757	126	405	150			
	Visits	9236	1447	4771	1799			
	Visits per patient	12.2 ± 10.0	11.5 ± 7.4	11.8 ± 9.0	12.0 ± 7.7	0.46	-	
Riluzole use	Took at least once	533 (70%)	82 (65%)	286 (71%)	96 (64%)			
	Never took	126 (17%)	31 (25%)	72 (18%)	29 (19%)			
	No information on use	98 (13%)	13 (10%)	47 (12%)	25 (17%)			
Trial arm	Active	378 (50%)	66 (52%)	204 (50%)	65 (43%)			
	Placebo	210 (28%)	35 (28%)	115 (28%)	40 (27%)			
	Observational	169 (22%)	25 (20%)	86 (21%)	45 (30%)			
Age	First symptoms	52.3 ± 11.1 (100%)	53.1 ± 11.6 (100%)	54.1 ± 10.9 (100%)	59.5 ± 9.5 (100%)	0.0021	(SM,BM,SW)<BW	
	Diagnosis	53.0 ± 11.1 (87%)	53.7 ± 11.7 (89%)	54.7 ± 10.6 (85%)	60.5 ± 9.5 (90%)	0.0012	(SM,BM,SW)<BW	
	Baseline	54.1 ± 11.1 (100%)	54.6 ± 11.7 (100%)	56.0 ± 10.8 (100%)	61.1 ± 9.4 (100%)	0.0018	(SM,BM,SW)<BW	
Time duration	Patient time of follow-up (y)	1.0 ± 0.7 (100%)	1.0 ± 0.6 (100%)	1.0 ± 0.8 (100%)	1.0 ± 0.6 (100%)	0.58	-	
	Time between visits (d)	34 ± 28 (100 %)	35 ± 28 (100 %)	35 ± 30 (100 %)	34 ± 29 (100 %)	0.33	-	
	Disease duration (y)	1.8 ± 1.0 (100%)	1.4 ± 0.9 (100%)	1.9 ± 1.1 (100%)	1.6 ± 0.9 (100%)	0.61	B<S***	
Outcomes at baseline	Mitos	0.2 ± 0.5 (59%)	0.2 ± 0.5 (59%)	0.3 ± 0.5 (61%)	0.2 ± 0.5 (71%)	0.70	-	
	FT9	1.8 ± 0.9 (59%)	1.9 ± 1.0 (59%)	1.9 ± 0.9 (61%)	1.9 ± 1.0 (71%)	0.52	-	
	ALSFRS <sub>r</sub> score	Total	37.2 ± 5.4 (59%)	37.4 ± 5.8 (59%)	35.9 ± 5.5 (61%)	37.3 ± 5.3 (71%)	0.19	W<M***
		Respiratory	11.3 ± 1.3 (59%)	11.1 ± 1.3 (59%)	11.2 ± 1.3 (61%)	11.1 ± 1.2 (71%)	0.73	-
		Bulbar	10.8 ± 1.5 (59%)	7.6 ± 2.1 (59%)	10.5 ± 1.7 (61%)	6.7 ± 2.3 (71%)	0.025	BW<BM<(SW,SM)
		Fine motor	7.6 ± 2.8 (59%)	9.2 ± 2.6 (59%)	8.0 ± 2.7 (61%)	10.0 ± 2.1 (71%)	0.39	M<W**;B<S***
	Gross motor	7.6 ± 2.5 (59%)	9.5 ± 2.5 (59%)	6.2 ± 2.4 (61%)	9.5 ± 2.3 (71%)	0.0012	SW<SM<(BW,BM)	
	Forced vital capacity	75.9 ± 14.4 (97%)	70.3 ± 13.6 (96%)	74.6 ± 14.4 (96%)	68.7 ± 13.8 (97%)	0.83	B<S***	
	BMI	27.1 ± 4.2 (98%)	26.8 ± 4.3 (97%)	26.4 ± 5.0 (98%)	25.6 ± 4.9 (98%)	0.46	W<M***	

**bulbar men, BW: bulbar women, SM: spinal men, SW: spinal women, \*: <0.05, \*\*: <0.01, \*\*\*: <0.001**

Figure 1 Average progression of ALSFRSr subscores depending on sex and onset site: Respiratory(blue), Bulbar (orange), Fine Motor (red), Gross Motor (green)

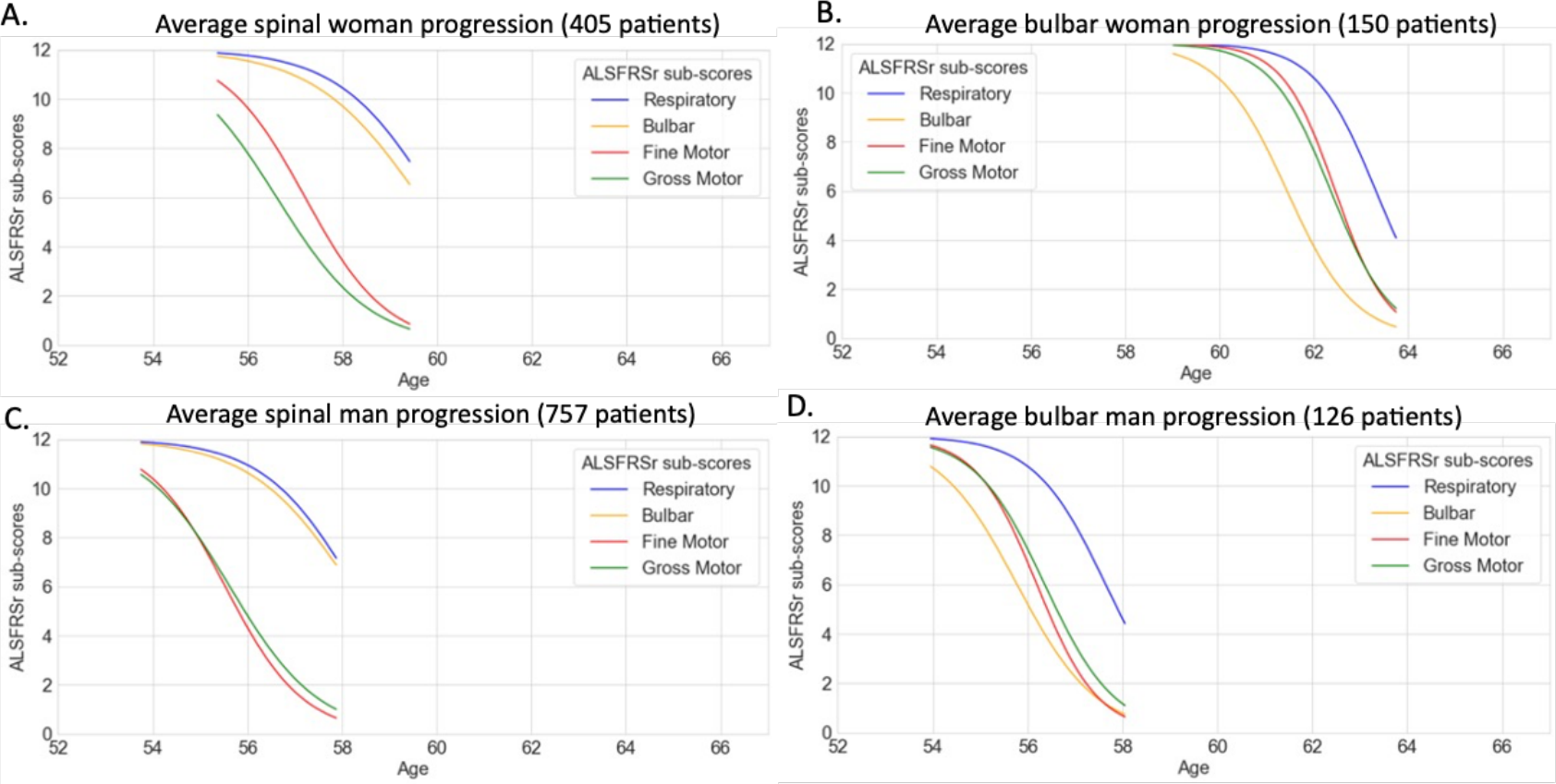
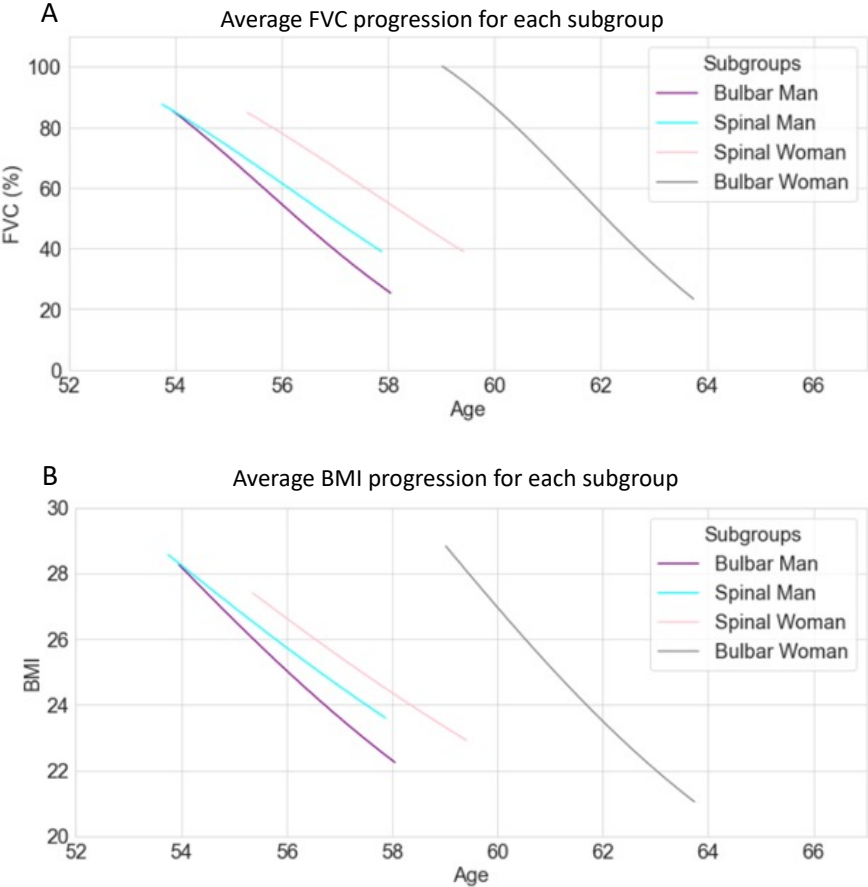


Figure 2: Average subgroup progressions of FVC and BMI for each subgroup



Legend: FVC: Forced vital capacity, BMI: Body mass index

Figure 3: Onset of impairment depending on sex and onset site

The vertical axis presents the delay of outcome impairment onset in month compared to the mean onset of the whole population. The horizontal axis is for sex with estimation for the bulbar patients in blue and the spinal patients in orange. The graph displays the mean of this delay with its confidence interval. Results are corrected for the group rate of progression and estimated age at onset. ANOVA interaction p-value is below 0.001 for ALSFRSr gross motor, ALSFRSr bulbar, and FVC and equal to 0.058 for ALSFRSr respiratory, 0.12 for ALSFRSr fine motor, and 0.17 for BMI. FVC: Forced Vital Capacity (%), \*\*: p-value for pairwise comparison <0.01, \*\*\*: p-value for pairwise comparison <0.001

