# Random changepoint segmented regression with smooth transition: an example with lateral amyotrophic sclerosis data

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**Abstract**

We consider random changepoint segmented regression models to analyze data from a study conducted to verify whether treatment with stem cells may delay the onset of a symptom of amyotrophic lateral sclerosis in genetically modified mice. The proposed models capture the biological aspects of the data, accommodating a smooth transition between the periods with and without symptoms. An additional changepoint is considered to avoid negative predicted responses. Given the nonlinear nature of the model, we adapt an algorithm proposed by Muggeo et al. (2014) to estimate the fixed parameters and to predict the random effects by fitting linear mixed models via standard software at each step. We compare the variances obtained in the final step with bootstrapped and robust ones. We also average the parameters of individually fitted models in an attempt to evaluate the quality of the proposed algorithm.

*Keywords:* fitting algorithm, mixed models, random effects

# 1 Introduction

Amyotrophic Lateral Sclerosis (ALS) is one of the most common adult-onset motor neuron disease causing a progressive, rapid and irreversible degeneration of motor neurons in the cortex, brain stem and spinal cord. In the majority of cases ALS occurs sporadically; in about 10% of the cases it is caused by familial reasons. No effective treatment is available and cell therapy clinical trials are currently being conducted with ALS affected patients. The SOD1 gene encodes an important antioxidant human enzyme and mutations in this gene represent one of the most frequent causes of ALS.

Among the different animal models for ALS, SOD1 mice are the most used in preclinical studies. After the initial tremor in the limbs they develop muscle weakness in early adulthood, become fully paralyzed and die. These mice overexpress the SOD1 gene bearing the G93A mutation, a point mutation found in familial ALS. Interestingly, in this animal model the disease progression exhibits a gender effect comparable to that observed in ALS patients. Males have a shorter lifespan and a clinical condition apparently more severe than females and differences in electrophysiological parameters have also been reported.

Treatment of ALS with stem cells is a current research topic. Mesenchymal stromal cells (MSC), specially those derived from adipose tissues, and pericytes have been used in studies of neurodegenerative diseases that focus on the reduction of the speed with which symptoms progress. In this context we consider a study conducted in the Human Genome and Stem Cell Research Center, at the Biosciences Institute, University of São Paulo, Brazil with the objective of comparing MSC cells and pericytes injected in SOD1-G93A mice with respect to their effects on the evolution of some symptoms of ALS. Details may be obtained in Coatti et al. (2017).

Our objective here is to propose models for the statistical analysis of the data.

## 2 The experimental setup

A set of 34 female and 21 male 8 week old SOD1-G93A mice was divided into 3 groups. Animals in the first group (12 females and 7 males) were submitted to weekly injections of MSC cells, those in the second group (11 females and 8 males), to injection with pericytes while animals in the third group (11 females and 6 males) were submitted to the vehicle (*Hank's balanced salt solution* - HBSS). Clinical analysis of the progression of the disease was evaluated weekly up to each animal's death by means of four variables, the analysis of one of them, *rotarod* is considered in this study. The *rotarod* test was used to evaluate motor coordination and fatigue resistance. For that purpose, the length of time each animal could remain on a rotating cylinder of a *rotarod* apparatus (IITC Life Science model 755) was recorded. The initial speed of 1 rpm was increased constantly until a final speed of 30 rpm, after 180 s. Each animal was given three tries and the longest latency to fall was recorded. The specific objectives of the analysis are:

i) Identification of the moment when animals become symptomatic (symptom onset) for the six groups defined by the combination of treatment (HBSS, MSC, pericytes) and sex (male, female).

ii) Estimation of the expected variation in response after symptom onset for each group.

iii) Evaluation of the effects of treatment, sex and their interaction on the expected moment of symptom onset and post-onset variation of the expected response.

## 3 Statistical model and inference

Profile plots for the response along with LOESS curves are displayed in Figure 1.

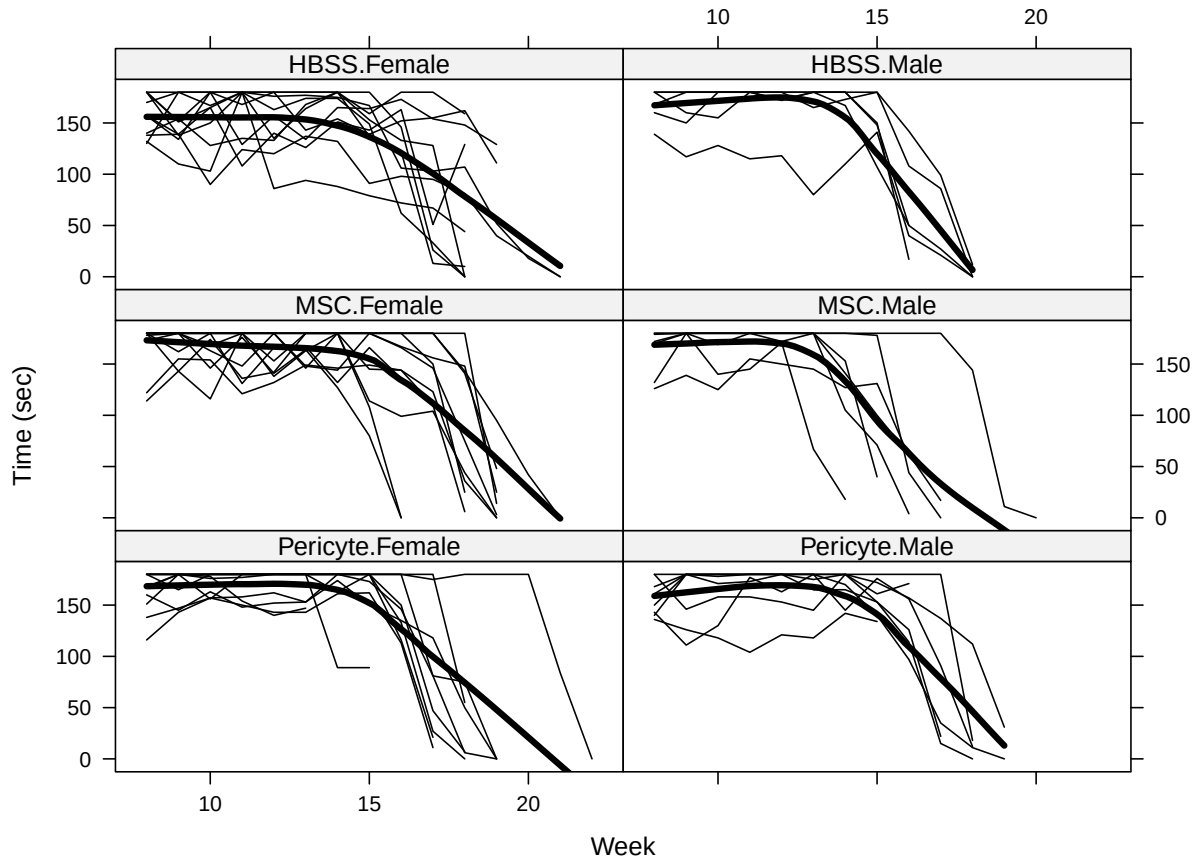


Figure 1: Profile plots for the response along with LOESS curves.

A descriptive analysis of the behaviour of the response variable corroborates its expected stable level before the onset of the symptom (a decrease in the length of time during which the animal holds on to the rotating cylinder). Furthermore, individual differences in the moment where this occurs as well as differences among the speed with which the intensity of the symptom progresses are also visible. It also seems reasonable to expect a change in the acceleration with which the intensity of the symptom progresses after the disease onset.

Given that such conclusions are in line with the expected biological behaviour, a random

changepoint polynomial segmented regression model may be considered for the analysis.

Such models have an attractive practical appeal in many fields and have been the object of statistical research for a long time as detailed in Muggeo et al. (2014). These authors consider a frequentist approach as opposed to the commonly Bayesian perspective usually employed in the statistical literature.

Keeping in mind the necessarily nonnegative nature of the response, we adopt a similar approach and consider an analysis of the ALS data based on the model

$$y_{ijk} = \alpha_{ij} I[t_k < \psi_{2ij}(\lambda_{ij})] + \gamma_{ij}[t_k - \psi_{1ij}(\lambda_{ij})]^2 I[\psi_{1ij}(\lambda_{ij}) \leq t_k < \psi_{2ij}(\lambda_{ij})] + e_{ijk} \qquad (1)$$

($i = 1, \ldots, 6$, $j = 1, \ldots, n_i$ and $k = 1, \ldots, n_{ij}$) where $y_{ijk}$ denotes the response for the $j$-th animal observed in the $i$-th group (defined by the combination of the levels of treatment and sex) at the $k$-th evaluation instant, $\alpha_{ij}$ is the corresponding stable level of the symptom, $\gamma_{ij}$ is the coefficient of the quadratic term for the curve that governs the response behaviour post-changepoint $\psi_{1ij}$, with

$$\psi_{1ij}(\lambda_{ij}) = [L_{1i} + L_{2i} \exp(\lambda_{ij})]/[1 + \exp(\lambda_{ij})]$$

to restrict the value of $\psi_{1ij}$ to the interval $(L_{1i}, L_{2i})$ in which the observations are recorded and $\psi_{2ij}$ denotes the instant where the response is null. We assume that $\alpha_{ij} = \alpha_i + a_{ij}$, $\gamma_{ij} = \gamma_i + c_{ij}$, $\lambda_{ij} = \lambda_i + \ell_{ij}$ with $\mathbf{b}_{ij} = (a_{ij}, c_{ij}, \ell_{ij})^\top \sim N(\mathbf{0}, \mathbf{G}_i)$ and $e_{ijk} \sim N(0, \sigma_i^2)$ independent of $\mathbf{b}_{ij}$.

This is an extension of the models proposed by Muggeo et al. (2014) where a smooth transition and a second changepoint are incorporated. Because of its nonlinear nature, the model must be fitted via iterative procedures.

For the sake of notational simplicity and without loss of generality, we drop the subscript $i$ to specify the the fitting algorithm.

Given that $\psi_{2j}$ corresponds to the instant $t_k$ where $y_{jk} = 0$, we have $I(t_k < \psi_{2j}) = 1$ and $I(\psi_{1j} \leq t_k < \psi_{2j}) = 1$ and consequently, that $\alpha_j + \{\gamma_j[\psi_{2j} - \psi_{1j}(\lambda_j)]^2\} = 0$, implying that

$$\psi_{2j} = \psi_{2j}(\alpha_j, \gamma_j, \psi_{1j}) = \sqrt{-\alpha_j/\gamma_j} + \psi_{1j}(\lambda_j)$$

Following Muggeo et al. (2014) and Fasola et al. (2018), the nonlinear model may be approximated by a first order Taylor expansion of

$$f[t_k, \gamma_j, \psi_{1j}(\lambda_j)] = \gamma_j[t_k - \psi_{1j}(\lambda_j)]^2 I[\psi_{1j}(\lambda_j) \leq t_k < \psi_{2j}(\lambda_j)].$$

Explicitly,

$$f[t_k, \gamma_j, \psi_{1j}(\lambda_j)] \approx f[t_k, \gamma_j, \psi_{1j}(\widehat{\lambda}_j)] + (\lambda_j - \widehat{\lambda}_j)\frac{\partial f[t_k, \gamma_j, \psi_{1j}]}{\partial \psi_{1j}}\frac{\partial \psi_{1j}(\lambda_j)}{\lambda_j}\Big|_{\lambda_j = \widehat{\lambda}_j}$$

with

$$\frac{\partial f[t_k, \gamma_j, \psi_{1j}]}{\partial \psi_{1j}} = h_j(\lambda_j) = 2\gamma_j[t_k - \psi_{1j}(\lambda_j)]I[\psi_{1j}(\lambda_j) \leq t_k < \psi_{2ij}(\lambda_j)]$$

and

$$\frac{\partial \psi_{1j}(\lambda_j)}{\partial \lambda_j} = g_j(\lambda_j) = \frac{(L_2 - L_1)\exp(\lambda_j)}{[1 + \exp(\lambda_j)]^2}.$$

Consequently we may approximate model (1) by

$$y_{jk} \approx \alpha_j I[t_k < \psi_{2j}(\widehat{\lambda}_j)] + f[t_k, \gamma_j, \psi_{1j}(\widehat{\lambda}_j)] - \widehat{\lambda}_j h_j(\widehat{\lambda}_j) g_j(\widehat{\lambda}_j) + \lambda_j h_j(\widehat{\lambda}_j) g_j(\widehat{\lambda}_j) + e_{jk}. \quad (2)$$

Considering the pseudo observations defined by $y^*_{jk} = y_{jk} + \widehat{\lambda}_j h_j(\widehat{\lambda}_j) g_j(\widehat{\lambda}_j)$, the model

$$y^*_{jk} = \alpha_j I[t_k < \psi_{2j}(\widehat{\lambda}_j)] + f[t_k, \gamma_j, \psi_{1j}(\widehat{\lambda}_j)] + \lambda_j h_j(\widehat{\lambda}_j) g_j(\widehat{\lambda}_j) + e_{jk}$$

suggests the following algorithm to fit (1)

1) Let $\psi^{(0)}_{1j} = \psi^{(0)}_1$ and $\psi^{(0)}_{2j} = \psi^{(0)}_2$.

2) Fit model $y_{jk} = \alpha_j I(t_k < \psi_{2j}^0) + \gamma_j (t_k - \psi_{2j}^{(0)})^2 I(\psi_{1j}^{(0)} \leq t_k < \psi_{2j}^{(0)}) + e_{jk}$ to obtain $\alpha^{(0)}$, $a_j^{(0)}$, $\gamma^{(0)}$, $c_j^{(0)}$, $\lambda_j^{(0)} = \log[(\psi_{1j}^{(0)} - L_1)/(L_2 - \psi_{1j}^{(0)})]$ and $\psi_{2j}^{(1)} = \sqrt{-\alpha_j^{(0)}/\gamma_j^{(0)}} + \psi_{1j}^{(0)}$.

3) Let $r = 1$.

4) Compute $y_{jk}^{(r)} = y_{jk} + \lambda_j^{(r-1)} h_j(\lambda_j^{(r-1)}) g_j(\lambda_j^{(r-1)})$.

5) Fit model

$$y_{jk}^{(r)} = \alpha_j I(t_k < \psi_{2j}^{(r)}) + \gamma_j [t_k - \psi_{1j}^{(r-1)}]^2 I(\psi_{1j}^{(r-1)} \leq t_k < \psi_{2j}^{(r)}) + \lambda_j h_j(\lambda_j^{(r-1)}) g_j(\lambda_j^{(r-1)}) + e_{jk}^{(r-1)}$$

to obtain $\alpha^{(r)}$, $a_j^{(r)}$, $\gamma^{(r)}$, $c_j^{(r)}$, $\lambda^{(r)}$, $\ell_j^{(r)}$, $\psi_{1j}^{(r)} = [L_1 + L_2 \exp(\lambda_j^{(r)})]/[1 + \exp(\lambda_j^{(r)})]$ and $\psi_{2j}^{(r+1)} = \sqrt{-\alpha_j^{(r)}/\gamma_j^{(r)}} + \psi_{1j}^{(r)}$.

6) Stop if some convergence criterion is satisfied, otherwise, let $r = r + 1$ and repeat steps 4-6.

This algorithm, adapted from Muggeo et al. (2014), essentially considers iterative fitting of standard linear mixed models by (restricted) maximum likelihood. At convergence, we expect a neglible difference between the third and fourth terms in the right hand side of (2) and as a consequence, that the pseudo observations should well approximate the original ones. Given the linear mixed model nature of the proposed fitting algorithm, we may employ the diagnostic procedures outlined in Singer et al. (2017) to check whether the adopted assumptions for the distribution of the random effects and of the random errors are reasonable.

In practice, we noted that the quality of the fitted values depends on the initial values $\psi_1^{(0)}$ and $\psi_2^{(0)}$. We suggest that the algorithm should be applied for a set of initial values (*e.g.*, 25) for $\psi_1^{(0)}$ (*e.g.*, ranging from the minimum to the maximum observed time points)

with $\psi_2^{(0)}$ set at the maximum observed time point and that the starting points obtained from the run with the smallest mean squared difference between individual observed and last iteration predicted values should be chosen.

The algorithm may be applied to data with moderate sample sizes and in the last step, produces approximate covariance matrices, $Var(\widehat{\boldsymbol{\theta}}_i)$, of the fixed parameter estimators $\widehat{\boldsymbol{\theta}}_i = (\widehat{\alpha}_i, \widehat{\gamma}_i, \widehat{\lambda}_i)^\top$ which may be employed for inferential purposes. Since the interest lies in the changepoint parameters $\psi_{1i}$ and $\psi_{2i}$ instead of in the auxiliary parameters $\lambda_i$ we should consider inferences on

$$\mathbf{h}(\widehat{\boldsymbol{\theta}}_i) = [\widehat{\alpha}_i, \widehat{\gamma}_i, \psi_1(\widehat{\lambda}_i), \psi_2(\widehat{\alpha}_i, \widehat{\gamma}_i, \widehat{\lambda}_i)]^\top.$$

Approximate covariance matrices of the transformed estimators $\mathbf{h}(\widehat{\boldsymbol{\theta}}_i)$ may be obtained via the Delta method as

$$Var[\mathbf{h}(\widehat{\boldsymbol{\theta}}_i)] = \mathbf{H}(\widehat{\boldsymbol{\theta}}_i) Var(\widehat{\boldsymbol{\theta}}_i) \mathbf{H}(\widehat{\boldsymbol{\theta}}_i)^\top$$

with

$$\mathbf{H}(\widehat{\boldsymbol{\theta}}_i) = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & (L_{2i} - L_{1i}) \exp(\widehat{\lambda}_i)/[1 + \exp(\widehat{\lambda}_i)]^2 \\ (2\widehat{\alpha}_i)^{-1}(-\widehat{\alpha}_i/\widehat{\gamma}_i)^{1/2} & (2\widehat{\gamma}_i)^{-1}(-\widehat{\alpha}_i/\widehat{\gamma}_i)^{1/2} & (L_{2i} - L_{1i}) \exp(\widehat{\lambda}_i)/[1 + \exp(\widehat{\lambda}_i)]^2 \end{bmatrix}$$

In particular, comparison among the fixed parameters to identify possible effects of treatment, sex and their interactions may be carried out via Wald tests.

# 4 Results

Estimates of the parameters of model (1) obtained via fitting the approximation (2) along with the corresponding standard errors are summarized in Table 1. Standard errors for

the changepoint parameter estimates ($\widehat{\psi}_{ij}$) were obtained via the Delta method from the standard errors of the estimates of the auxiliary parameters $\widehat{\lambda}_{ij}$.

Diagnostic plots for the male group treated with MSC are displayed in Figure 2 and do not show evidences against the adopted assumptions. Diagnostic plots for the remaining groups presented similar behaviour and may be reproduced via the R-function provided in Singer et al. (2017).

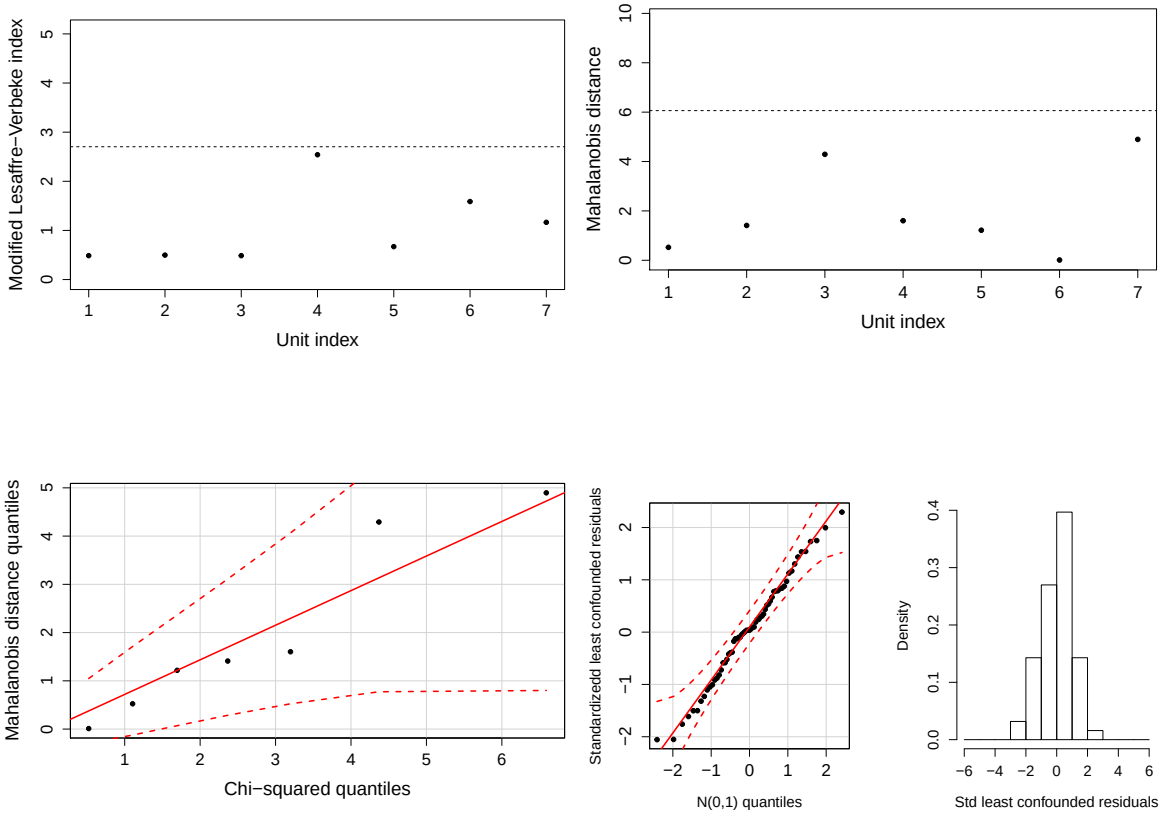


Figure 2: Diagnostic plots for males treated with MSC.

Table 1: Estimates and standard errors for the parameters of model (1) obtained via fitting the approximation (2) along with bootstrap and robust counterparts of the standard errors

| | | | | Std error | | |
|---|---|---|---|---|---|---|
| Parameter | Sex | Treatment | Estimate | Model | Bootstrap | Robust |
| Stable level ($\alpha$) | M | HBSS | 166.5 | 10.0 | 6.8 | 9.1 |
| | M | MSC | 170.2 | 6.2 | 6.8 | 5.8 |
| | M | Pericytes | 164.6 | 6.5 | 2.4 | 6.1 |
| Stable level ($\alpha$) | F | HBSS | 156.3 | 6.2 | 5.7 | 6.2 |
| | F | MSC | 167.5 | 3.8 | 4.0 | 3.7 |
| | F | Pericytes | 170.1 | 3.6 | 4.4 | 3.4 |
| Acceleration ($\gamma$) | M | HBSS | -18.2 | 4.7 | 4.1 | 4.4 |
| | M | MSC | -36.8 | 11.4 | 4.1 | 5.5 |
| | M | Pericytes | -23.4 | 12.3 | 20.3 | 10.7 |
| Acceleration ($\gamma$) | F | HBSS | -3.9 | 1.4 | 1.6 | 1.4 |
| | F | MSC | -25.6 | 6.1 | 23.1 | 5.9 |
| | F | Pericytes | -27.9 | 6.9 | 21.3 | 5.5 |
| Changepoint 1 ($\psi_1$) | M | HBSS | 13.8 | 0.2 | 0.4 | 0.2 |
| | M | MSC | 13.3 | 1.0 | 0.4 | 0.8 |
| | M | Pericytes | 15.1 | 0.5 | 0.4 | 0.4 |
| Changepoint 1 ($\psi_1$) | F | HBSS | 11.9 | 0.4 | 0.8 | 0.4 |
| | F | MSC | 15.4 | 0.5 | 1.0 | 0.5 |
| | F | Pericytes | 15.2 | 0.8 | 1.1 | 0.7 |
| Changepoint 2 ($\psi_2$) | M | HBSS | 16.9 | 0.3 | 0.4 | 0.3 |
| | M | MSC | 15.5 | 0.7 | 0.4 | 0.6 |
| | M | Pericytes | 17.7 | 0.5 | 0.6 | 0.5 |
| Changepoint 2 ($\psi_2$) | F | HBSS | 18.2 | 0.8 | 0.7 | 0.7 |
| | F | MSC | 18.0 | 0.4 | 1.0 | 0.4 |
| | F | Pericytes | 17.7 | 0.6 | 1.8 | 0.5 |

The results of a Wald test for the homogeneity of the six changepoints $\psi_1$ ($\chi^2 = 40.69, df = 5, p < 0.001$) suggests further analyses to identify the possible effects of treatment, sex and their interaction. A significant interaction between treatment and sex with respect to the $\psi_1$ changepoints ($\chi^2 = 12.96, df = 2, p = 0.002$) may be analysed via the multiple comparisons summarized in Table 2 and suggest that the onset of symptoms for the "typical" male in the control group (HBSS) is delayed by 1.9 [CI(95%) = 1.0, 2.9] weeks with respect to the "typical" female in the control group and that treatment with Pericytes (both sexes) or MSC (females) delay the onset of symptoms for the "typical" animals by 1.4 [CI(95%) = 0.6, 2.2] weeks with respect to the HBSS treated "typical" male. The changepoint for the MSC treated "typical" male lies between those for HBSS treated "typical" male and female but the small sample size does not lead to a significant difference in either case.

Table 2: Comparison of changepoint ($\psi_1$)

| | Changepoint | | |
|---|---|---|---|
| Comparison | $\chi^2$ | *df* | p-value |
| Sex within HBSS | 16.65 | 1 | $< 0.001$ |
| Sex within MSC | 3.46 | 1 | 0.063 |
| Sex within Pericytes | 0.02 | 1 | 0.880 |
| Pericytes = MSC(F) | 0.25 | 2 | 0.880 |
| Pericytes + MSC(F) = HBSS(M) | 10.92 | 1 | 0.001 |
| MSC(M) = HBSS(M) | 0.25 | 1 | 0.620 |
| MSC(M) = HBSS(F) | 1.74 | 1 | 0.187 |

The results for a similar analysis of the acceleration with which the symptom progresses are displayed in Table 3 and suggest no difference between sexes and an increase in the acceleration of 17.4 [CI(95%) = 16.5.5, 18.2] sec/week$^2$ for the experimental treatments (MSC and Pericytes) relatively to that of the control treatment (HBSS). Plots for the

Table 3: Comparison of post-changepoint symptom acceleration parameter ($\gamma$)

| | Acceleration coefficient | | |
|---|---|---|---|
| Comparison | $\chi^2$ | *df* | p-value |
| Homogeneity | 37.13 | 5 | $< 0.001$ |
| Sex $\times$ Treatment | 1.56 | 2 | 0.458 |
| Sex | 1.12 | 1 | 0.289 |
| Treatment | 11.17 | 2 | 0.004 |
| HBSS $\times$ MSC | 8.53 | 1 | 0.003 |
| MSC $\times$ Pericytes | 0.34 | 1 | 0.561 |

estimates of male and female "typical" animal response curves for the three treatments are displayed in Figure 3. Predicted animal specific response curves for male MSC and female HBSS treated animals are presented in Figures 4 and 5. Similar plots for the remaining groups are presented in the Supplementary Materials.

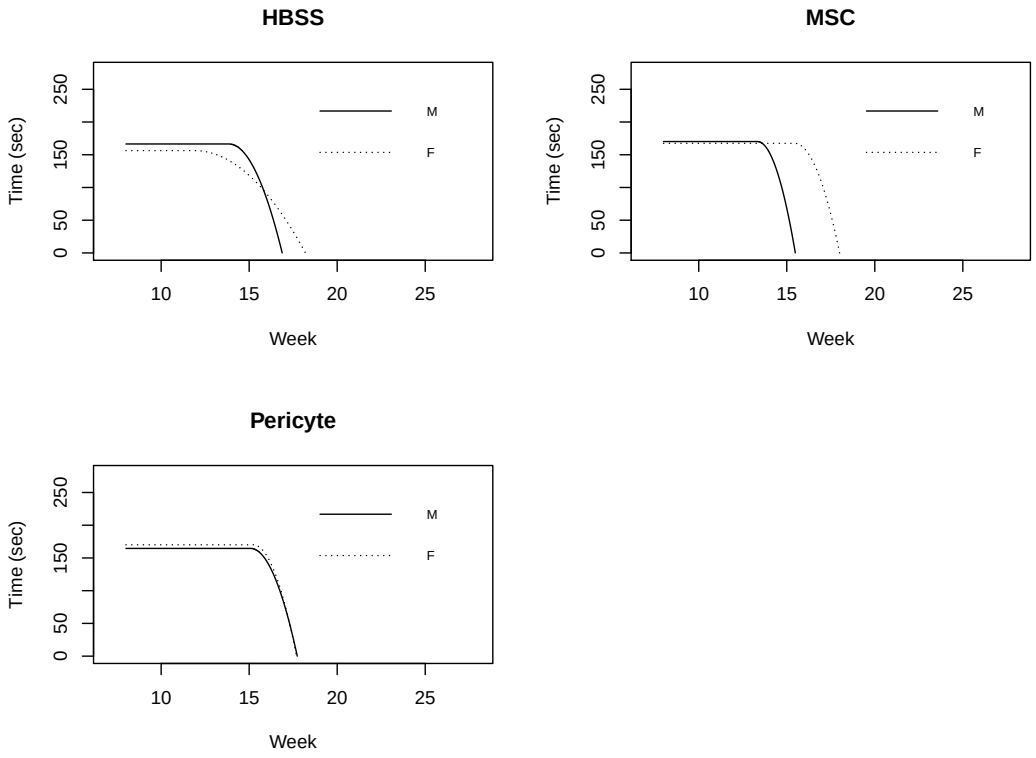


Figure 3: Estimated response curves for "typical subjects".

# 5 Discussion

We considered an extension of the algorithm proposed by Muggeo et al. (2014) to fit a segmented regression model with smooth transition to data obtained from a study designed to evaluate the effect treatment with stem cells on the delay of the onset of ALS symptoms. The proposed model is appropriate for situations where the expected pre-changepoint response is constant. Furthermore, it allows for a varying speed of symptom progress by

including a second degree polynomial component post-changepoint, a feature suggested by those authors.

Jacqmin-Gadda et al. (2006) employed a similar model with the random changepoint governed by a log-normal distribution, independent of the distribution of the remaining random effects. They mention that consideration of dependency lead to unstable results due to numerical problems. This is also an issue raised by Segalas et al. (2019). In our case, the relation between the random changepoint the subsequent variation in the response seems reasonable since a delay in the symptom onset possibly accelerates the its deterioration. We included such a dependency by considering an unstructured within-subject covariance matrix for all random terms and did not have problems in the estimation process, given the nature of the proposed algorithm that relies on iterative fitting of standard linear mixed models. This feature also allows the use of the algorithm with moderately sample sized data. Individual predicted responses are obtained with no additional effort.

Covariates may be included in model (1) along the lines outlined in Muggeo et al. (2014).

The estimated covariance matrices of the fixed parameter estimates available in the last step were employed for inferential purposes. This approach, however is not exempt from controversy. In fact, Muggeo et al. (2014) comment that the corresponding standard errors of the changepoint estimates underestimate the true standard errors and, based on a simulation study, suggest that bootstrap estimates should be employed instead. They consider a non-parametric bootstrap procedure and assume that the random effects are independent, which does not picture the most common situation where the within subject covariance matrix is unstructured. The nature of the parameters defining model (1) as well as the estimated covariance components, presented in Table 4, tend to confirm this

assumption.

Table 4: Estimates of the covariance components ($\mathbf{G}$ and $\sigma^2$) obtained via model (1)

| | | Covariance parameter | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Sex | $\sigma^2_\alpha$ | $\sigma^2_\gamma$ | $\sigma^2_\lambda$ | $\sigma_{\alpha\gamma}$ | $\sigma_{\alpha\lambda}$ | $\sigma_{\gamma\lambda}$ | $\sigma^2$ |
| HBSS | M | 512.0 | 83.1 | 0.1 | -78.0 | -0.9 | 0.3 | 13.8 |
| MSC | M | 247.4 | 849.8 | 0.8 | 211.7 | -1.5 | -24.1 | 12.4 |
| Pericytes | M | 312.6 | 1013.3 | 0.2 | -371.9 | -1.7 | -8.7 | 12.8 |
| HBSS | F | 372.8 | 21.2 | 0.2 | -38.1 | 4.9 | -2.1 | 19.9 |
| MSC | F | 148.3 | 349.7 | 0.3 | -74.6 | 1.7 | -7.0 | 14.1 |
| Pericytes | F | 115.7 | 226.4 | 0.4 | -51.6 | 1.0 | -4.7 | 14.3 |

We used model estimated fixed and dispersion parameters, namely, $\widehat{\alpha}_{ij}$, $\widehat{\gamma}_{ij}$, $\widehat{\lambda}_{ij}$, $\widehat{\mathbf{G}}$ and $\widehat{\sigma}^2$ to generate 1000 samples each with the same number of profiles as the corresponding groups, fitted model (1) model via the proposed algorithm and obtained bootstrap estimates of the standard errors of the associated fixed parameters. We also considered a robust version based on the suggestion of Liang and Zeger (1986). The estimated standard errors of the fixed parameters obtained via the three approaches are presented in Table 1. Both the estimates obtained by using the proposed algorithm and the robust version are quite similar with consistently smaller values for the latter. The bootstrapped version, however, does not suggest a consistent pattern. We conjecture that this is a consequence of assuming a trivariate normal distribution for the random effects $a_i, c_i, \ell_i$. While this seems appropriate for the individual stable level parameters and changepoints, it may not be so

for the individual acceleration component, $\gamma + \ell_i$ which may be positive, a feature that is not biologically expected. To bypass this problem, we may either impose the restriction that $\gamma + \ell_i$ be positive in the fitting algorithm or adopt a different distribution for the random effects, possibly by means of copulas. This is the object of further research.

The choice of initial values for the iterative procedures required to fit non-linear models is usually problematic. Models that include transition functions like those proposed by Bacon and Watts (1971) and considered in Morrell et al. (1995) are non-linear despite the linearity of each component and therefore require additional attention, given the associated numerical problems. Initialization of the algorithm described in Section 3 is simple since it requires initial values only for the parameters $\psi_1$ and $\psi_2$. Although we chose these initial values by fitting the model to a grid of values for $\psi_1$ and $\psi_2$ as described in Section 3, a non-parametric bootstrap procedure as the one proposed by Wood (2001) and used in Muggeo et al. (2014) could also be employed.

Finally, we mention that model (1) inherits the interpretational difficulty associated to nonlinear mixed models: the fixed parameters correspond to the response for a "typical" subject, *i.e.*, one for which the random effects are null. The expected response must be obtained by integrating out the random effects in the likelihood. This, however, does not produce estimates of the population averaged parameters of interest (the changepoint and the acceleration coefficient in our case). We considered the algorithm proposed by Muggeo (2003) and fitted standard mixed segmented regression models with with a single random effect corresponding to the changepoint to the individual data of each animal and averaged the corresponding estimates to mimic the corresponding "population averaged" parameters. The results, also displayed in Table 5 present the same attenuation characteristic (with the obvious exception of the stable level parameter) described by other authors under

different nonlinear setups [see Diggle et al (2012) for example].

Plots of the predicted (via the segmented mixed model) and estimated profiles (via mixed and individual segmented regression models) for animals in the MSC male and HBSS female groups, respectively displayed in Figures (4) and (5). The similarity obtained for the former (the curves for the "typical units" and for the approximate "population averaged" ones the are practically superimposed) is probably due to the behavior of the observed data which follows that dictated by the model for all units. This is not so for the latter, where the observed data for units 3, 6, 8 and 10 do not follow the expected pattern, implying that, perhaps, the proposed segmented mixed model should be modified by requiring the acceleration parameter to be negative.

Table 5: Estimates for the parameters of model (1) obtained via fitting the approximation (2) along with corresponding estimates of averaged individually fitted and marginal parameters

| | | | Model | | |
|---|---|---|---|---|---|
| Parameter | Sex | Treatment | Mixed (1) | Individual | Marginal |
| Stable level ($\alpha$) | M | HBSS | 166.5 | 166.0 | 165.0 |
| | M | MSC | 170.2 | 170.0 | 173.2 |
| | M | Pericytes | 164.6 | 164.2 | 162.9 |
| Stable level ($\alpha$) | F | HBSS | 156.3 | 154.9 | 155.0 |
| | F | MSC | 167.5 | 168.4 | 165.5 |
| | F | Pericytes | 170.1 | 171.5 | 170.3 |
| Acceleration ($\gamma$) | M | HBSS | -18.2 | -30.2 | -7.5 |
| | M | MSC | -36.8 | -61.1 | -2.2 |
| | M | Pericytes | -23.4 | -45.5 | -9.9 |
| Acceleration ($\gamma$) | F | HBSS | -3.9 | -15.2 | -2.2 |
| | F | MSC | -25.6 | -27.0 | -6.1 |
| | F | Pericytes | -27.9 | -30.6 | -2.7 |
| Changepoint ($\psi_1$) | M | HBSS | 13.8 | 14.1 | 12.9 |
| | M | MSC | 13.3 | 13.6 | 9.9 |
| | M | Pericytes | 15.1 | 14.2 | 14.2 |
| Changepoint 1 ($\psi_1$) | F | HBSS | 11.9 | 13.4 | 12.3 |
| | F | MSC | 15.4 | 14.9 | 14.3 |
| | F | Pericytes | 15.2 | 14.5 | 12.1 |
| Changepoint 2 ($\psi_2$) | M | HBSS | 16.9 | 16.4 | 17.6 |
| | M | MSC | 15.5 | 15.2 | 18.8 |
| | M | Pericytes | 17.7 | 16.1 | 18.3 |
| Changepoint 2 ($\psi_2$) | F | HBSS | 18.2 | 16.6 | 20.6 |
| | F | MSC | 18.0 | 17.4 | 19.5 |
| | F | Pericytes | 17.7 | 16.9 | 20.1 |

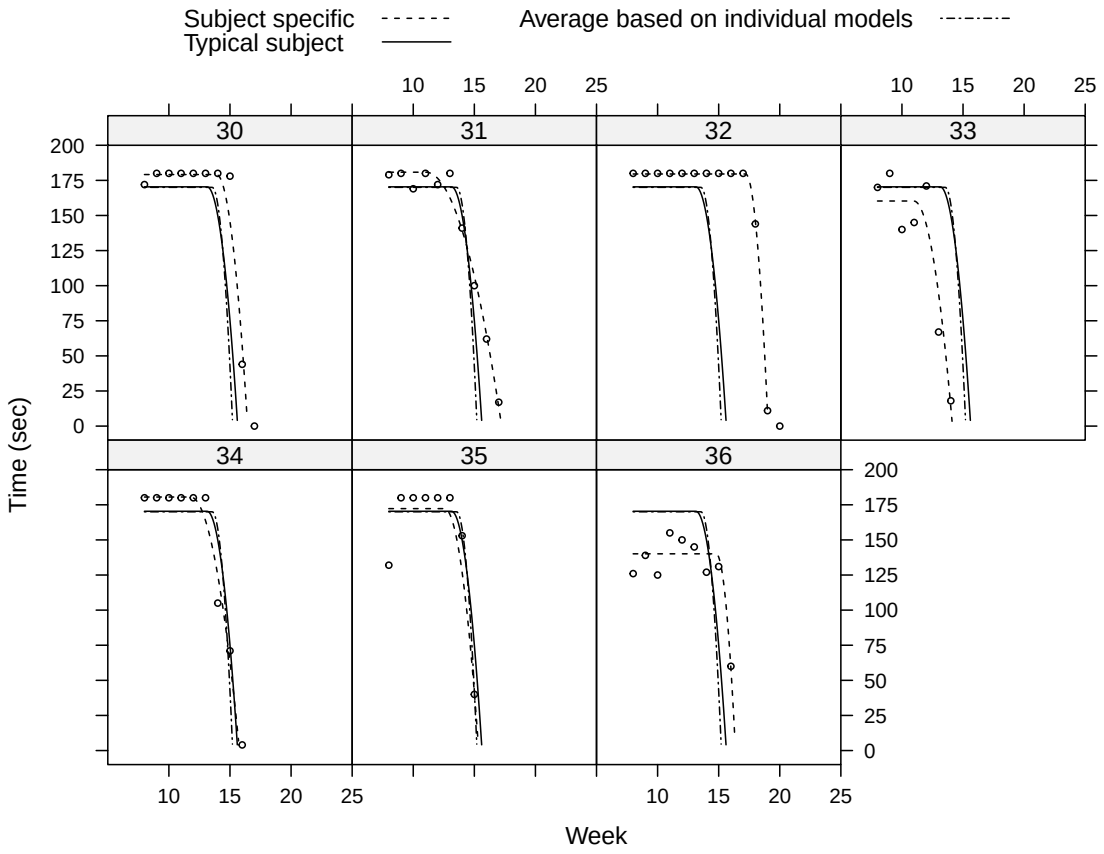


Figure 4: Predicted and estimated response curves (MSC males).

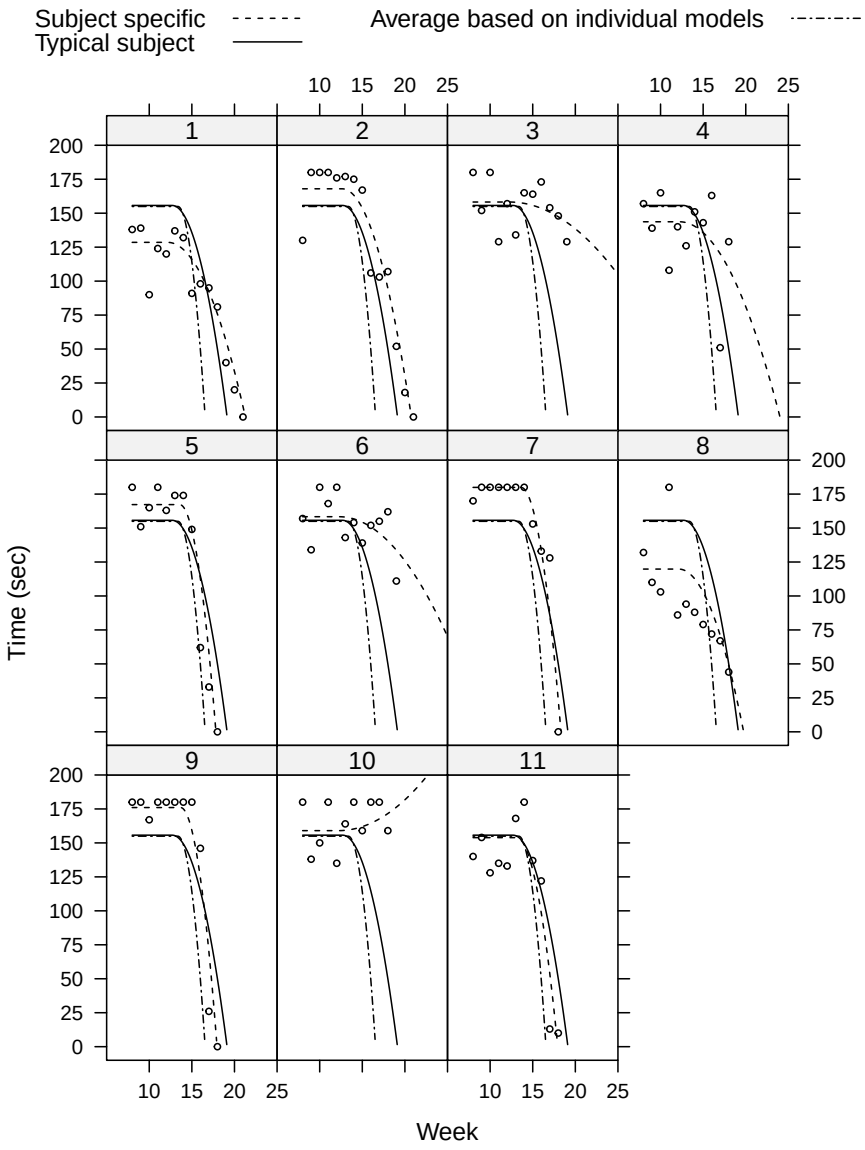


Figure 5: Predicted and estimated response curves (HBSS females).

## SUPPLEMENTARY MATERIAL

**Fitted curves:** Fitted individual and "typical" subject curves for the six groups. (.zip file)

**R-function for model fitting:** Contains the R-code to fit the segmented regression model via the proposed algorithm. File "seg2changepoint.R" contains a function used to implement the fitting algorithm. File "fittodata.R" fits the proposed model to specific groups using function "seg2changepoint.R". (.zip file)

**ALS data set:** Contains the data considered in the article. (.xlsx file)

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