Neurofilament light chain and C-reactive protein explored as predictors of survival in amyotrophic lateral sclerosis

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal disease defined by the progressive degeneration of both upper and lower motor neurons. The median survival after symptom onset is 3 to 5 years. Prognostic parameters have been identified and are associated with survival in ALS.[1,2]

Levels of neurofilament light chain (NfL) and C-reactive protein (CRP) are both altered in serum of patients with ALS.[3,4] Interestingly, these markers are linked to functional disability and survival in ALS.[3,4]

Prognostic protein biomarkers in blood are not taken into account yet when counseling patients with ALS. It is not clear to what extent these biomarkers contribute to predict survival in ALS. Here, we assessed the ability of serum NfL and CRP to predict survival in a multivariate survival model including eight other established prognostic parameters in ALS.[2]

MATERIAL & METHODS

Participants

In total 383 patients with ALS, diagnosed between 2009 and 2018 according to the revised El Escorial criteria (10.4% suspected ALS, 21.9% possible ALS, 48.6% probable ALS and 19.1% definite ALS), were included in this retrospective study. Patients were recruited at the NeuroMuscular Reference Center of the University Hospital in Leuven, Belgium and at the NEuroMuscular Omnicenter of the Niguarda Ca' Granda Hospital in Milan, Italy. The study was approved by both ethical committees: S50354 (Leuven) and 74-022019 (Milan). All participants completed the informed consent form. Blood samples were consecutively collected during the first visit, providing a wide variety of patient inclusions going from early symptomatic to second opinions. Serum was aliquoted, transported on dry ice and stored at -80°C until analysis. Assay specifications can be retrieved in the supplemental data. The forced vital capacity (FVC) and the revised ALS Functional Rating Score (ALSFRS-r) were obtained at the latest within 3 months from sampling. For each survival analysis the patients were classified into two groups

using the median of the total ALS cohort as a cut-off. Patients were censored at time of analysis if they were still alive. Survival was defined as the time from blood sampling to death.

Statistical analysis

Normality of the data was assessed with the Shapiro-Wilk test. Differences between the participating centers were determined with the Mann-Whitney U test and Chi-Squared test at a Bonferroni corrected significance level of 0.50%. Correlations were performed with the Spearman rank correlation coefficient (Bonferroni corrected at a significance level of 0.83%). The disease progression rate was calculated as $(48 - \text{ALSFRS-r})/(\text{disease duration in months})$. Survival curves were created with the Kaplan-Meier method and differences were assessed with the log-rank test. The Cox proportional-hazards regression analysis was used to estimate survival in a multivariate model including age at onset, diagnostic delay, disease progression rate, FVC, *C9orf72* repeat expansion, frontotemporal dementia, definite ALS (revised El Escorial criteria), bulbar onset and serum NfL or CRP levels. Analyses were performed with MedCalc Statistical Software (18.11.3, MedCalc Software bvba, Ostend, Belgium) and GraphPad Prism (8.1.1, GraphPad Software Inc, San Diego, CA, USA).

RESULTS

The patient cohort characterization is provided in Supplementary table 1. In contrast to serum CRP levels, serum NfL levels were significantly higher in females (152.8 pg/mL, range: 3.8 - 413.9 pg/mL; males: 101.1 pg/mL, range: 0.3 - 738.3 pg/mL; $p = 0.0007$). A significant albeit weak correlation was observed between the disease progression rate and serum NfL or CRP levels ($r_s = 0.519$ [95% CI 0.437 to 0.592] $p < 0.0001$, $r_s = 0.294$ [95% CI 0.194 to 0.387] $p < 0.0001$, respectively; Supplementary figure 1). Correlations with other parameters can be found in the supplemental data.

A significant difference was observed between the survival curves of patients with low and high serum NfL ($X^2 = 54.85$, $p < 0.0001$), but not of patients with low and high serum CRP ($X^2 = 2.83$, $p = 0.0923$)

(Fig. 1 A-B). When both biomarkers were separately introduced in the multivariate survival model, serum CRP was not associated with survival (hazard ratio (HR) = 0.86, 95% CI 0.61 to 1.23, $p = 0.4196$; Supplementary table 2). In contrast, high serum NfL levels were associated with a higher chance of a shorter survival (HR = 2.21 (95% CI 1.51 to 3.24, $p < 0.0001$; Supplementary table 2)) (Fig. 1 C-D).

DISCUSSION

In present study, we show that levels of serum NfL are independently associated with survival in patients with ALS. Moreover, in contrast to earlier findings, serum CRP levels were not associated with survival in a multivariate model.[4]

Furthermore, age at onset, disease progression rate and FVC remained significantly associated with survival in ALS. The failure of the pulmonary function, reflected by a lowered FVC and increased serum NfL levels are both indicative of neuronal damage. As such, it is clear that the extent of neuronal damage influences survival, unlike peripheral inflammation measured by serum CRP levels. Central inflammation assessed by other protein biomarkers in cerebrospinal fluid, but not in blood, correlated with disease progression and survival in ALS.[5] Thus, our findings might indicate that the effect of central inflammation is peripherally masked by other peripheral processes in the blood. Indeed, when we performed a survival analysis per center (Supplementary table 3), serum CRP levels were still able to predict survival in patients with ALS who did not have any other concomitant acute or chronic inflammatory process (Milan cohort), as shown before.[4] Still, inflammation cannot be excluded from the survival analysis, besides CRP as a marker of the innate immune system, the adaptive immune system might contribute to the disease progression and survival as well. Moreover, our findings show that estimating the chance of a shorter survival by means of serum NfL is a promising and easily accessible alternative to cerebrospinal fluid and to other time consuming investigations.[3]

In summary, as a strong independent prognostic parameter, serum NfL could be implemented in the personalized prediction model, improving the estimation of survival in ALS. In addition, serum NfL could be used for patient counseling and as an easy tool to stratify patients in clinical trials. In contrast,

serum CRP is not associated with survival but might be used as a marker to assess anti-inflammatory treatment efficacy in ALS.

AUTHOR CONTRIBUTIONS

Concept and design: MDS, CL, PVD, KP

Drafting of the manuscript and figures: MDS, PVD, KP

Acquisition, analysis or interpretation of data: All authors

Critical revision of the manuscript for important intellectual content: All authors

ADDITIONAL INFORMATION

Competing interests

CL participated in a scientific advisory board for Neuraltus, Cytokinetics, Mitsubishi Tanabe (Europe) and Italfarmaco, received research support from Agenzia Italiana per la Ricerca sulla SLA (ARISLA) and Ministry of Health (CCM2011). AC reported serving on the editorial advisory board of Amyotrophic Lateral Sclerosis, received research support from the Italian Ministry of Health (Ricerca Finalizzata), Regione Piemonte (Ricerca Finalizzata), University of Turin, Fondazione Vialli e Mauro onlus, and the European Commission (Health Seventh Framework Programme), participated in scientific advisory boards for Biogen, Cytokinetics, Italfarmaco, Neuraltus, and Mitsubishi. PVD participated in advisory board meetings for Genzyme, Pfizer, Biogen, Cytokinetics, Mitsubishi Tanabe (Japan), CSL Behring (USA), Alexion Pharmaceuticals (USA). MDS, KP, CT and LM declare no competing interests.

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Leuven. PVD is a senior clinical investigator of the FWO-Vlaanderen and is supported by the ALS Liga België and the KU Leuven funds “een Hart voor ALS”, “Laeversfonds voor ALS Onderzoek” and “the Valéry Perrier Race against ALS fund”. No funder or sponsor had any role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

REFERENCES

- 1 Chiò A, Logroscino G, Hardiman O, *et al.* Prognostic factors in ALS: A critical review. *Amyotroph Lateral Scler* 2009;**10**:310–23. doi:10.3109/17482960802566824
- 2 Westeneng H-J, Debray TPA, Visser AE, *et al.* Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a personalised prediction model. *Lancet Neurol* 2018;**17**:423–33. doi:10.1016/S1474-4422(18)30089-9
- 3 Gille B, De Schaepdryver M, Goossens J, *et al.* Serum neurofilament light chain levels as a marker of upper motor neuron degeneration in patients with Amyotrophic Lateral Sclerosis. *Neuropathol Appl Neurobiol* 2019;**45**:291–304. doi:10.1111/nan.12511
- 4 Lunetta C, Lizio A, Maestri E, *et al.* Serum C-Reactive Protein as a Prognostic Biomarker in Amyotrophic Lateral Sclerosis. *JAMA Neurol* 2017;**74**:660–7. doi:10.1001/jamaneurol.2016.6179
- 5 Gille B, De Schaepdryver M, Dedeene L, *et al.* Inflammatory markers in cerebrospinal fluid: independent prognostic biomarkers in amyotrophic lateral sclerosis? *J Neurol Neurosurg Psychiatry* 2019;;1–9. doi:10.1136/jnnp-2018-319586

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161 **FIGURE CAPTION**

162 **Figure 1. Association of serum NfL and CRP with survival in ALS**

163 Kaplan-Meier survival curves for patients with ALS classified into two groups based upon the median
164 for serum CRP levels (A) or serum NfL levels (B). Multivariate Cox regression survival curves were
165 estimated for serum CRP levels (C) and serum NfL levels (D). Parameters included in the multivariate
166 model as continuous variable: disease progression rate, diagnostic delay, age at onset, forced vital
167 capacity, and as categorical variable: serum CRP or NfL levels, bulbar onset, frontotemporal dementia,
168 *C9orf72* repeat expansion mutation status and definite ALS El Escorial. CRP: C-reactive protein, NfL:
169 neurofilament light chain.