Blood biomarkers and prognosis of amyotrophic lateral sclerosis

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Background and purpose: The objective was to assess the ability of eight commonly measured blood markers to serve as prognostic biomarkers in amyotrophic lateral sclerosis (ALS).

Methods: A cohort study was conducted of 399 individuals with newly diagnosed ALS between 2006 and 2011 in Stockholm, Sweden. Information on eight blood markers, including creatinine, albumin, haemoglobin, C-reactive protein (CRP), glucose, potassium, sodium and calcium, measured at or after the date of ALS diagnosis, was collected. The Cox regression model and joint model were used to explore the associations between biomarkers and risk of mortality.

Results: The mean age at ALS diagnosis was 66.25 years and 58% of the patients were male. A lower than median level of serum creatinine [hazard ratio (HR) 1.67; 95% confidence interval (CI) 1.31–2.12] or albumin (HR 1.49, 95% CI 1.13–1.96) whereas a higher than median level of log-transformed CRP (HR 1.33, 95% CI 1.04–1.71) or glucose (HR 1.34, 95% CI 1.01–1.78) at baseline was associated with a higher mortality risk. Taking all available measurements after ALS diagnosis into account, an association was found between per standard deviation (SD) decrease in serum creatinine (HR 2.23, 95% CI 1.81–2.75) or albumin (HR 1.83, 95% CI 1.43–2.36) as well as per SD increase of log(CRP) (HR 1.96, 95% CI 1.58–2.43) or glucose (HR 1.61, 95% CI 1.21–2.12) and a higher mortality risk. No clear association was found for haemoglobin, potassium, sodium or calcium.

Conclusions: This study suggests that serum creatinine, albumin, CRP and glucose measured at the time of ALS diagnosis as well as their temporal changes after ALS diagnosis could serve as additional prognostic biomarkers for ALS. Their values in routine clinical practice and clinical trials of ALS need to be investigated further.

Introduction

Amyotrophic lateral sclerosis (ALS) is an incurable and relentlessly progressive neurodegenerative disease [1]. The cause remains elusive for about 80% of ALS patients [2] and the survival of ALS patients varies greatly [3]. Around 10% of patients survive 10 years or more, whereas majority of patients die within 3–5 years after symptom onset, often due to respiratory paralysis [4]. Predictors for better survival of ALS include male sex, younger age at onset and diagnosis, spinal onset, higher body mass index and weight gain after diagnosis, whereas respiratory or genitourinary comorbidities, cognitive impairment and depression, and weight loss after diagnosis are predictive of worse outcome [3]. Along with the ageing of the global population, the number of ALS cases is expected to increase by ~70% from 2015 to 2040 [5].
emphasizing the pressing need to identify both diagnostic and prognostic biomarkers for this devastating disease.

In most of the previous studies that investigated prognostic biomarkers of ALS, repeated measurement data and survival data are usually separately analysed [2]. For example, the biomarker level at a single time point (e.g. at the time of diagnosis) was most often used to predict mortality after ALS diagnosis, without accounting for the temporal change in the biomarker level after diagnosis [6–8]. Alternatively, repeated measurement data were used to quantify the correlation between the temporal change in the biomarker level and the functional decline of ALS patients, without considering the effect of such temporal change on mortality [8]. Taking advantage of the information both at baseline (diagnosis) and during follow-up (after diagnosis) has been proposed as a better approach [9]. To our knowledge, such efforts have rarely been made in identifying novel prognostic indicators for ALS.

The aim was therefore to explore the relationship between the baseline level of a biomarker (defined as the first measurement at the time of ALS diagnosis or thereafter) and mortality risk after ALS diagnosis. A joint model, which considers both the repeated measurements of a specific biomarker during the follow-up and survival data [9,10], was also used to assess the association between the temporal change in the biomarker and ALS survival.

### Methods

#### Study design

A retrospective cohort study was performed of newly diagnosed ALS patients during 2006–2011 in Stockholm, Sweden, based on the Stockholm CREAtinine Measurement (SCREAM) project [11]. SCREAM is a healthcare-use cohort, including all Stockholm residents at the age of 18 or above who had at least one measurement of serum creatinine during 2006–2011. For all 1118 507 individuals included in SCREAM (corresponding to 66% of the entire Stockholm population and >90% of the population above age 65 years), all other standard laboratory tests performed during this period were also extracted. The participants were individually followed from 1 January 2006 to 31 December 31 2011 through cross-linkages to both the regional or national health and population registers in Sweden, using the unique Swedish personal identification numbers. Information on demographic factors, clinical diagnoses, dispensed medicines, migration status and vital status was obtained from such linkages [11].

All participants of the SCREAM project with newly diagnosed ALS during the follow-up period were identified through the Swedish Patient Register, according to the 10th Swedish revision of the International Classification of Diseases code G12.2. Individuals with at least one hospital visit with a diagnosis of ALS were identified as ALS patients and the date when the diagnosis was issued was defined as date of ALS diagnosis. In total, 399 ALS patients were identified. These patients were then followed from the date of ALS diagnosis until emigration out of Stockholm, death or 31 December 2011, whichever came first. A validation study based in Stockholm showed a >90% positive predictive value for such register-based diagnosis of ALS [12]. The clinical diagnosis of ALS is largely based on the El Escorial criteria [13] and consists of a thorough clinical workup in Stockholm, including a magnetic resonance imaging scan of the brain and spine, a comprehensive blood analysis, lumbar puncture and electromyography, which examines both the upper and lower motor neuron involvement in bulbar, cervical, thoracic and lumbar regions and excludes mimics (personal communication with Dr Caroline Ingre, 12 May 2020). Only patients with two or more regions of upper and lower involvement are classified as definite or probable ALS.

#### Blood markers

All laboratory tests within the SCREAM project were performed by three different laboratories, namely Aleris, Unilabs and Karolinska, which provide >90% of services to the entire region of Stockholm [11]. Since these three laboratories are frequently audited for quality and harmonization by the national organization EQUALIS, the inter- and intra-laboratory variations of the laboratory tests are considered minimal [11]. Information on the date of test, test method, as well as serum or plasma levels of the eight most commonly measured blood biomarkers, including creatinine (µmol/l), albumin (g/l), haemoglobin (g/l), potassium (mmol/l), sodium (mmol/l), calcium (mmol/l), C-reactive protein (CRP, high-sensitivity type, mg/l) and glucose (mmol/l) was retrieved for all ALS patients included in the cohort. Measurements of creatinine, haemoglobin, potassium, sodium and CRP were available for the majority of the ALS patients, whereas the remaining biomarkers were available for a proportion of the patients (Table 1). Information about numbers of follow-up measurements as well as the median durations between the follow-up measurements is presented in Table S1. A logarithmic transformation was applied for CRP to better approximate a normal distribution.
Table 1 Characteristics of patients with amyotrophic lateral sclerosis

| Characteristics | Median of first measurement | Median of all measurements | Mean (SD) of first measurement | Mean (SD) of all measurements |
|-----------------|-----------------------------|-----------------------------|-------------------------------|-------------------------------|
| No. of patients  | 399                         | 399                         | 284                           | 284                           |
| Age at diagnosis, years, mean (SD) | 66.25 (12.47) | 66.25 (12.47) | 66.25 (12.47) | 66.25 (12.47) |
| Gender, n (%)    |                             |                             | Female 168 (42.11)            | Male 231 (57.89)              |
| Serum creatinine, µmol/l (n = 399) | 63.00 | 60.19 (28.16) | 63.00 | 60.19 (28.16) |
| Albumin, g/l (n = 269) | 37.00 | 32.70 (6.03) | 37.00 | 32.70 (6.03) |
| Haemoglobin, g/l (n = 395) | 140.00 | 128.84 (17.78) | 140.00 | 128.84 (17.78) |
| Potassium, mmol/l (n = 365) | 4.00 | 3.99 (0.42) | 4.00 | 3.99 (0.42) |
| Sodium, mmol/l (n = 353) | 140.00 | 139.24 (3.81) | 140.00 | 139.24 (3.81) |
| Calcium, mmol/l (n = 251) | 2.32 | 2.28 (0.12) | 2.32 | 2.28 (0.12) |
| Log(CRP), mg/l (n = 329) | 1.39 | 2.85 (1.70) | 1.39 | 2.85 (1.70) |
| Glucose, mmol/l (n = 284) | 5.75 | 6.35 (1.95) | 5.75 | 6.35 (1.95) |

CRP, C-reactive protein; SD, standard deviation.

Statistical analyses

In the main analyses, the association of biomarker levels at baseline with the risk of mortality was first assessed using Cox regression models. Attained age was used as the underlying time scale (as an adjustment for age at follow-up), and the analysis was also adjusted for sex. The Kaplan–Meier estimate was used to compare the survival of the patients with a higher than median level of a biomarker at diagnosis to that of the patients with a lower than median level biomarker. To estimate the association between the temporal change in a biomarker after ALS diagnosis [shown as per standard deviation (SD) decrease or increase] and the risk of death, a joint model that combines a linear mixed effects model for all repeated measurements after diagnosis and a survival model for censored outcomes, as implemented in the JM package in R, was used [10]. In the longitudinal process, random intercept and random slope were considered; the time-to-event process was adjusted for age and sex; finally, in the joint model, these two processes were linked through common random effects [9].

In the secondary analysis, the temporal changes of different biomarkers prior to death amongst ALS patients with different lengths of survival after diagnosis were investigated. For this purpose, ALS patients were classified into three groups according to their survival time, namely patients who died within 1 year after diagnosis (very fast progression group, n = 122), patients who died 1–3 years after diagnosis (medium progression group, n = 88) and patients who survived more than 3 years after diagnosis (slow progression group, n = 189). The last group was used as the control group for the first two groups. Thus, for each patient in the first two groups, up to five patients were randomly selected from the slow progression group who were individually matched to them by age at diagnosis and sex. The temporal changes of different biomarkers between these groups were then compared. For each patient, the median levels of different biomarkers in each 1-month time window during the year (very fast progression group) or 2 years (medium progression group) before death or the 2 years before time of selection (slow progression group) were calculated. These median levels were then plotted by time to the date of death or selection using locally estimated scatterplot smoothing. To construct the confidence intervals (CIs) for the low progression group, the bootstrap method was used by resampling with replacement 200 times from the slow progression group.

Data analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), Stata (version 15.0; StataCorp LP, College Station, TX, USA) and R platform (version 3.6.0). A two-sided P ≤ 0.05 was considered statistically significant.

The study was approved by the Regional Ethical Review Board in Stockholm, Sweden.

Results

The mean (SD) age at ALS diagnosis was 66.25 (12.47) years, and 231 of the patients were men (58%). For each biomarker, both the median level at baseline and the mean (SD) of all measurements during follow-up are reported in Table 1.

The ALS patients were followed for an average of 2.36 years (SD 2.08 years), and 239 patients (60%) died during the follow-up. In the multivariable Cox regression models and Kaplan–Meier analyses (Fig. 1), it was found that patients with a lower than median level of serum creatinine [hazard ratio (HR) 1.67; 95% CI 1.31–2.12] or albumin (HR 1.49; 95% CI 1.13–1.96) at baseline had a higher risk of mortality. Patients with a higher than median level of log (CRP) (HR 1.33; 95% CI 1.04–1.71) or glucose (HR 1.34; 95% CI 1.01–1.78) had a higher risk of mortality. No statistically significant association was observed for haemoglobin, potassium, sodium or calcium.
Figure 1 Kaplan–Meier plots of the proportion of surviving patients from the time of ALS diagnosis, stratified by the median of first measurement at ALS diagnosis or thereafter. [Colour figure can be viewed at wileyonlinelibrary.com]
Figure 2: Association between per standard deviation (SD) biomarker change during follow-up after ALS diagnosis and mortality risk. Each hazard ratio is the summary result of the joint model between repeated measures of a biomarker and survival during follow-up. [Colour figure can be viewed at wileyonlinelibrary.com]

Discussion

In this population-based cohort study of newly diagnosed ALS patients, it was found that lower serum creatinine and albumin, as well as higher CRP and glucose levels, at the time of diagnosis were associated with higher risk of mortality. Decreasing serum creatinine and albumin levels or increasing CRP and glucose levels during the follow-up after ALS diagnosis were also associated with a higher risk of mortality. The present analyses incorporated all available biomarker measurements for ALS patients undergoing routine care in Stockholm, and thus provide novel evidence for the usefulness of these biomarkers as potential indicators for ALS prognosis.

Our study showed that lower serum creatinine, either measured at baseline or longitudinally after diagnosis, was predictive of mortality after ALS diagnosis. Creatinine is a by-product of creatine phosphate breakdown to generate fast energy for muscle contraction, so decreasing creatinine level might be a marker of muscle wasting [7]. Three previous studies, which are in line with our findings, have also shown that baseline [6,7] or longitudinal [14] measurement of creatinine is a strong predictor of ALS prognosis. One study [14] also demonstrated longitudinal correlations of plasma creatinine with the ALS Functional Rating Scale (revised) (ALSFRS-R) score and muscle strength, in addition to overall mortality.

Lower albumin, as a biomarker of nutritional and inflammatory status, was also found to be indicative of worse prognosis in our study, which is consistent with two previous studies [7,15]. However, compared to those two studies, the effect of longitudinal change in albumin on ALS survival was additionally explored and new evidence for a link between fast albumin loss and higher mortality was demonstrated. The latter is supported by the longitudinal analysis using the PRO-FACT database [15]. For haemoglobin, it was not possible to detect a significant association when studying baseline level. However, a declining pattern of haemoglobin after diagnosis was associated with a higher mortality, although the association was borderline significant. This finding lends further support to the important role of nutritional status in the survival of ALS patients, which is multifactorial and influenced by metabolism [16], energy intake, ease of breath, and appetite [17–19].

It was observed that patients with an elevated level of CRP, the most commonly studied inflammatory biomarker, at baseline was associated with a higher risk of death after ALS diagnosis. A similar association has been reported in some, although not all,
Figure 3 Profiles of biomarkers prior to death for patients with very fast (solid red curve, including patients who died within 1 year after diagnosis) or medium (dashed blue curve, including patients who died within 1–3 years after diagnosis) progression, or prior to date of selection for patients with slow progression (dotted blue curve with 95% confidence interval, including patients who survived at least 3 years after diagnosis).
previous studies [8,20,21]. An association between increasing CRP level over time and a higher risk of death was additionally demonstrated. Furthermore, a rapidly increasing pattern of CRP during the months before death amongst patients in the very fast progression group was observed. These results provide further evidence for the involvement of altered immune responses and inflammation in ALS, especially in the later phase of disease progression [22]. High CRP levels have been shown to increase the permeability of the blood–brain barrier and trigger microglial activation [23], leading to increased release of proinflammatory cytokines and neuroinflammation [24]. It was also speculated that respiratory infections may be one of the underlying explanations for the rapid increase of CRP during the months immediately before death.

Finally, our study found that higher glucose level at baseline and increasing levels over time were both related to higher mortality risk, corroborating findings from previous studies [25,26]. These results provide further evidence for a dysregulation of glycolytic metabolism in ALS patients. As previously reported, a substantial proportion of ALS patients have glucose intolerance [27] and insulin resistance [28]. The associations noted between a higher glucose level at diagnosis and an increasing level of glucose after ALS diagnosis with a higher mortality risk might suggest that the dysregulation is more pronounced amongst patients with worse survival compared to patients with better survival. The result is also supported by the previous finding that higher baseline haemoglobin A1c (a marker of long-term glycaemic status) level is correlated with shorter survival time amongst ALS patients [29]. As type 2 diabetes has been suggested to be associated with a lower risk of ALS or later onset of ALS [12,30], further studies are needed to understand the underlying reasons for such contradiction. For instance, whilst type 2 diabetes diagnosed long before ALS diagnosis is less likely to be secondary to ALS or its treatment, glucose levels measured at the time of ALS diagnosis or thereafter might be more probably influenced by ALS itself and its treatment, including modification of diet, in particular when patients undergo enteral nutrition and especially in the terminal stage of the disease.

Altogether, our findings lend support to the idea of monitoring both the baseline levels and the temporal changes of serum creatinine, albumin, CRP and glucose in the clinical care of ALS patients to better understand disease progression. In clinical trials, these biomarkers could also be considered as surrogate endpoints to reduce cost and increase statistical power [31], as suggested by an earlier study [14].

The strengths of this study include the population-based study design, repeated measurements of study biomarkers, prospective and independent outcome ascertainment, and complete follow-up, which together could greatly reduce concerns of information and selection biases. Besides, by using joint models, it was possible to incorporate longitudinal measurements whilst accounting for the within-subject variability and time-to-event outcome. Joint modelling of longitudinal and survival data is advantageous and is particularly useful when the goal is the prediction of an event (e.g. mortality) by using repeated measures of biomarkers. Furthermore, the present study, to our knowledge, is the first to visualize the temporal pattern of biomarkers prior to death amongst ALS patients with different lengths of survival.

Our study, however, has a number of limitations. First, because of the nature of register-based study, little information was available on clinical characteristics of ALS patients. As a result, it was not possible to assess whether the noted associations were independent of other prognostic indicators for ALS other than age and sex, such as ALSFRS-R, vital capacity or site of onset [3]. It is important, however, to note that the main purpose of our study is to describe statistical associations instead of proving causation. Secondly, the lack of genetic information for patients with ALS precluded the possibility to assess the results for ALS with a known genetic cause, because ALS patients with different genetic causes might have different progression rates after diagnosis [4]. However, our best efforts were made to describe the temporal changes of the biomarkers amongst patients with very fast, medium and slow progression. Thirdly, levels of the studied biomarkers (e.g. glucose and creatinine) can be affected by time of blood collection (e.g. before or after a meal) and other factors (e.g. diet or medicine use). Lack of such information in our study precluded the possibility of assessing the roles of these factors on the study findings. The fact that the vast majority of glucose measures should have been taken in fasting conditions and that similar results are noted between our study and a previous one where fasting status was adjusted [29] argues against a substantial influence by the time of blood collection, however. By contrast, because of its relatively small diurnal or post-prandial [32] variation, fasting is typically not a requirement for plasma creatinine testing. However, some medicines can falsely elevate creatinine levels and it is acknowledged that it is not possible to account for those. This being said, these conditions would increase rather than decrease plasma creatinine concentrations, allowing speculation that this bias may result, if anything, in an
underestimation of our observed changes. Finally, the studied population was representative of the ALS patients in Stockholm, Sweden, during 2006–2011, and the generalization of our findings to other areas, ethnicities and calendar periods should be done with caution.

In conclusion, in this population-based retrospective cohort of ALS patients, it is found that lower levels of serum creatinine and albumin, as well as higher levels of CRP and glucose, at baseline were associated with a higher risk of death after diagnosis. Decreasing levels of creatinine and albumin, and increasing levels of CRP and glucose, after ALS diagnosis were also indicative of poor prognosis. Given their easy access and low cost, the usefulness of these routinely measured blood biomarkers in clinical practice and clinical trials of ALS warrants further assessment.

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Disclosure of conflicts of interest
The authors declare no financial or other conflicts of interest.

Data availability statement
Data are available for collaborative studies with qualified investigators after inquiry.

Supporting Information
Additional Supporting Information may be found in the online version of this article:

Table S1. Information about biomarker measurements in patients with amyotrophic lateral sclerosis.

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