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Exploring clinical chemistry markers in amyotrophic lateral sclerosis: insights into survival and disease trajectories

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Abstract

Objective Commonly measured clinical chemistry markers might be indicative of survival and disease progression in amyotrophic lateral sclerosis (ALS).

Methods In a cohort study of 270 ALS patients diagnosed from April 2014 to May 2021 in Stockholm, Sweden, we examined the link between 29 clinical chemistry markers at diagnosis and mortality risk at 6 months, 1 year, and 3 years after diagnosis. Summary variables from exploratory factor analysis (EFA) assessed the markers' collective impact on survival. We integrated ALS functional rating scale-revised (ALSFRS-R) scores with survival data using a joint latent class model to identify patterns of functional decline. Multinomial logistic regression determined how the EFA-derived factors predicted the decline trajectories post-diagnosis.

Results Higher levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), apolipoprotein B, and albumin at diagnosis were linked to lower mortality in ALS patients, while increased neurofilament light chain (NfL), leukocyte count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and carbon dioxide (CO₂) levels indicated higher mortality. The 'Red blood cell profile' factor, derived from EFA, emerged as a significant predictor of survival, independent of other prognostic indicators. The joint latent class model identified three distinct patient groups based on functional decline, with 'Red blood cell profile' suggesting a lower likelihood of being in the groups with slower progression.

Conclusion Clinical chemistry markers, including NfL, lipids, albumin, leukocyte count, MCV, MCH, CO₂, and the 'Red blood cell profile,' were associated with ALS survival. As these markers represent broader bodily functions, integrating them in ALS patient care could improve disease management.

Keywords ALS · Clinical chemistry · Blood markers · Survival · Prognosis

Background

Amyotrophic lateral sclerosis (ALS) is a heterogeneous neurodegenerative disease characterized by the degeneration of both upper and lower motor neurons, leading to a wide range of clinical symptoms. The disease manifests in different forms, the most common being spinal onset, affecting the limb and trunk muscles, followed by bulbar onset, affecting the oral, facial, and pharyngeal muscles, and respiratory onset, affecting the thoracic muscles [1, 2]. In more than half of patients with ALS, there is some degree of cognitive or behavioral impairment, even if the criteria for dementia are not fulfilled [3–5].

Approximately 10–15% of ALS patients are familial, with the remaining being sporadic [6]. The diagnostic process for ALS is complex and protracted, typically taking around a

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year from the initial symptom onset [7, 8]. Diagnostic delay can be attributed to delay in seeking medical care, wrong referrals, misdiagnoses, and the necessity to rule out other neurological conditions that mimic ALS [8]. Survival in ALS varies substantially, ranging from only a few months to over 10 years, with a median survival of 2–5 years from symptom onset [9]. Only a minority, about 10%, survive beyond 10 years [10]. Factors such as older age at onset, presence of *C9orf72* repeat expansion, presence of cognitive impairment, and bulbar onset have been linked to a more rapid disease progression [11, 12].

Clinical chemistry measures blood and other fluid components like enzymes, hormones, and lipids, aiding in the diagnosis and management of various diseases [13]. While studies exploring the associations of clinical chemistry markers with outcome of patients with ALS are accumulating, prior studies have mainly focused on specific sets of biomarkers, such as lipids and lipoproteins [14, 15], creatinine and albumin [16, 17], and inflammatory markers [18]. Further, these studies often involved short follow-up periods, without evaluating the synergistic predictive value of these biomarkers.

Management of symptoms in the bodily systems outside of the central nervous system has become increasingly important in ALS, e.g., pharmacological interventions have been proposed targeting hypermetabolism and metabolic disturbances [19] and oxidative stress [20]. A previous clinical trial found that a high-calorie diet extended the survival of patients with ALS [21] whereas another clinical trial showed that curcumin (a natural antioxidant compound) supplementation slightly decreased disease progression [22]. As clinical chemistry markers reflect various bodily functions, it is important to understand the prognostic values of these biomarkers in ALS survival, with the goal of improving personalized treatment and management strategies.

Neurofilaments, part of the cytoskeleton, are a marker of axonal degeneration [23]. Levels of neurofilaments, both light and heavy chains, have been shown to markedly increase in ALS, primarily due to rapid deterioration of motor neurons, and remain relatively stable during disease progression [24, 25]. Beyond its diagnostic potential, neurofilament light chain (NfL) has been found to correlate strongly with the progression rate and survival in ALS [26]. However, little research has been done to understand the correlations between clinical chemistry biomarkers and neurofilaments.

In this study, using a cohort of newly diagnosed patients with ALS in Stockholm, Sweden, we aimed to (1) assess the relationship between various biomarkers commonly measured in clinical chemistry and survival in ALS; (2) investigate the synergistic associations of multiple biomarkers with survival, longitudinal functional decline, and level of NfL measured in cerebrospinal fluid (CSF); and (3) assess if biomarkers

measured through clinical chemistry provide additional value to existing prognostic factors.

Methods

Study design

We leveraged data from the ALSrisc Study (“Biomarkers and Risk Factors for Amyotrophic Lateral Sclerosis”), a case–control study including around 80% of newly diagnosed patients with ALS in the greater Stockholm area since 2016 onward [27]. The ALSrisc Study had a pilot phase in 2015, including patients diagnosed during 2014–2015. The present study included all patients with ALS included in the ALSrisc Study who had at least one measurement of clinical chemistry within 90 days before or 90 days after their reported date of diagnosis (i.e., “baseline”), leading to a total of 270 patients. The study period was from April 8th 2014 to May 18th 2021. We obtained clinical chemistry data from medical records among these patients, including biomarkers assessed in either blood (plasma, serum, or whole blood) or CSF. We excluded biomarkers with a high rate of missing values (> 25%), resulting in a set of 29 biomarkers for final analysis. These biomarkers could be classified into five categories: electrolytes, immunological biomarkers, neurological biomarkers, metabolic biomarkers, and hematopoietic biomarkers (Supplementary Table 1). The 25% cutoff for missing data was decided after considering the reliability of techniques dealing with missing values, particularly in the context of a relatively small sample size for a rare and heterogeneous disease like ALS, for which missingness patterns might be less predictable.

Through the cross-linkage between this study and the Swedish Motor Neuron Disease (MND) Quality Registry [28] using unique Swedish personal identity numbers, we obtained information on the demographic and clinical characteristics of all patients. A full list of the extracted variables can be found in Supplementary Table 2. We followed these patients from the date of diagnosis, until death, initiation of invasive ventilation, withdrawal from the study, or 13th of August 2022, whichever came first. The primary outcome during follow-up was either death or initiation of invasive ventilation, assessed at 6 months, 1 year, or 3 years post-diagnosis. In addition, we used a joint latent class model to combine changes in ALS functional rating scale-revised (ALSFRS-R) score and mortality data as a secondary outcome [29].

Statistical analyses

We first summarized the characteristics of the study participants and compared these characteristics to that of all ALS patients diagnosed during the same period in Stockholm,

according to the Swedish MND Quality Registry, to understand the representativeness of the study sample to its source population.

Multiple imputation

The degree of missingness in the studied biomarkers varied from 2.6% to 24.9%. We employed the method of multiple imputation, using the Multiple Imputation by Chained Equations (MICE) package in R and the Classification and Regression Tree (CART) method, as it is shown to be robust against outliers, skewness, and multicollinearity and accommodate non-linear relationships [30, 31]. All variables (biomarkers, demographic and clinical characteristics) were used as predictors for the imputation process. Based on the average degree of missingness, we concluded that 20 datasets and 5 iterations were sufficient [32]. To assess the quality of the multiple imputed data sets, we plotted the distributions of the biomarkers in each imputed data set against the distributions of the original data set (Supplementary Fig. 1). To deal with non-normal distributions and skewness, we used the Adaptive Box–Cox transformation method to find the best transformation method for each biomarker, based on their distributions and with the utilization of the λ value [33]. After normalization, the biomarkers were standardized.

Analysis 1

Biomarkers were treated as continuous variables and we used Cox proportional hazards model to assess the association of a 1-standard deviation (SD) increase in each biomarker measured at baseline with the risk of death or invasive ventilation use during 6 months, 1 year, or 3 years after ALS diagnosis, using time since diagnosis as the underlying time scale. We used both univariable and multivariable models. For the multivariable model, we controlled for the following variables: sex (male vs female), age at diagnosis (treated as a continuous variable), site of onset (bulbar vs non-bulbar), diagnostic delay (continuous variable), BMI at diagnosis (continuous variable), and the ALSFRS-R score at diagnosis (continuous variable). Diagnostic delay was defined as the time from symptom onset to diagnosis. Results derived from multiple imputed datasets were pooled using Rubin's rules [34].

Analysis 2

To investigate a possible synergistic effect of the biomarkers, we first conducted exploratory factor analysis (EFA), based on the data with multiple imputation, using the mifa package in R [35]. We calculated the average covariance matrix from all the separate covariance matrices among the different imputed data sets. We used the average covariance

matrix to calculate the factor loadings. To assess the adequacy of our data for the factor analysis, we used the Kaiser–Meyer–Olkin (KMO) criterion and variables that had communalities less than 0.5 were excluded from this analysis. We used the maximum likelihood method to determine the efficient number of factors, demonstrated in scree plot and parallel analysis plot. We performed both orthogonal rotation, which assumes that the factors are uncorrelated, and oblique rotation, which allows the factors to be correlated, and the observed patterns were similar in both scenarios. For simplicity, we used the orthogonal rotation (varimax). We then used Cox proportional hazards model to assess the association between 1-SD increase of the identified factors and risk of death or invasive ventilation use during the 6 months, 1 year, or 3 years after ALS diagnosis. As the factors were not correlated, we included them all in the same model, initially without any adjustment for covariates and later with adjustment for the covariates mentioned in Analysis 1. The latter analysis was performed to assess the potential value of using the factors as prognostic indicators in ALS, independently of other known prognostic indicators. In a sensitivity analysis, we additionally adjusted for several other factors potentially related to prognosis, including presence of ALS gene (yes, no, or unknown), presence of dementia (yes or no), and riluzole use at the time of measurement of clinical chemistry markers (yes or no). Finally, we used multiple linear regression to assess the correlation between the identified factors and NfL levels measured in CSF at baseline, with or without multivariable adjustment. The linearity assumption was assessed based on the plotting of residuals.

Analysis 3

We performed an analysis to examine whether the EFA-derived factors based on clinical chemistry markers could be predictive of trajectories of functional decline by time since ALS diagnosis. We first used a joint latent class model to identify distinct latent classes of functional decline trajectories including 242 of the 270 ALS patients who had at least 2 ALSFRS-R measurements. This model comprises three sub-models: (1) a multinomial logistic regression model to identify latent classes, (2) a linear mixed model to assess the temporal trend of ALSFRS-R, and (3) a survival model for time to death [29]. This integrated approach aims to minimize bias from informative censoring, e.g., due to death during follow-up, by combining longitudinal measures of functional status with survival data. The multinomial model, which determines latent classes, was not adjusted for any covariate. The linear mixed model was adjusted for sex, age at diagnosis, onset site, and time since diagnosis, whereas the survival model was adjusted for sex, age at diagnosis, and onset site. Patients were allocated to the class for which

they had the highest probability of belonging. The number of classes was determined based on the Bayesian Information Criterion (BIC) and clinical meaningfulness. We then used multinomial logistic regression model to assess the associations of the EFA-derived factors and NfL in CSF with the membership in different latent classes. Factors were added to the same model with or without multivariable adjustment. To assess the robustness of the results, in a sensitivity analysis, we excluded from the analysis the patients who had a probability of $\leq 70\%$ in belonging to a specific latent class.

All statistical analyses were conducted in R software (R 4.3.1). A two-sided $p < 0.05$ was considered statistically significant.

Results

The mean age of diagnosis was 66 years and the ratio between men and women was almost 1:1 among the study participants (Table 1). Approximately one third of the patients had bulbar onset. By the end of the study, 213 (78.9%) patients had died or initiated the use of invasive ventilation. Patients diagnosed with ALS in Stockholm during the same period who were not included in the study were slightly older, compared to the patients included in the present study, at the time of diagnosis. Otherwise, there was no substantial difference between the two groups.

Analysis 1

During the 6 months after ALS diagnosis, several blood biomarkers exhibited a significant association with the risk of death (or the use of invasive ventilation), including

total cholesterol (HR: 0.62, 95%CI 0.41–0.94), low-density lipoprotein (LDL-C, HR: 0.57, 95%CI 0.38–0.87), apolipoprotein B (HR: 0.64, 95%CI 0.43–0.96), mean corpuscular volume (MCV, HR: 1.53, 95%CI 1.07–2.19), and carbon dioxide (CO_2 , HR: 1.42, 95%CI 1.08–1.87), in addition to NfL in CSF (HR: 2.61, 95%CI 1.47–4.65) (Fig. 1A). During the 1 year after diagnosis, LDL-C (HR: 0.76, 95%CI 0.59–0.99), serum albumin (HR: 0.74, 95%CI 0.58–0.95), and plasma albumin (HR: 0.76, 95%CI 0.60–0.96) were associated with a lower risk of death, whereas count of leukocytes (HR: 1.24, 95%CI 1.02–1.52), mean corpuscular hemoglobin (MCH, HR: 1.35, 95%CI 1.06–1.71), MCV (HR: 1.50, 95%CI 1.18–1.89), CO_2 (HR: 1.35, 95%CI 1.11–1.63), and NfL (HR: 2.27, 95%CI 1.67–3.10) were associated with an increased risk of death (Fig. 1B). During the 3 years after diagnosis, albumin in serum or plasma was still associated with a lower risk of death, whereas MCV, MCH, leukocytes count, CO_2 , and NfL were still associated with a higher risk of death (Fig. 1C).

After multivariable adjustment, hemoglobin was associated with a higher risk of death at all time points, although the effect size declined with increasing time since diagnosis [6 months (HR: 2.07, 95%CI 1.28–3.37), 1 year (HR: 1.45, 95%CI 1.06–1.97), and 3 years (HR: 1.20, 95%CI 0.99–1.46)] (Supplementary Table 3). Erythrocyte volume fraction (EVF) exhibited a similar trend [6 months (HR: 1.70, 95%CI 1.09–2.65), 1 year (HR: 1.39, 95%CI 1.03–1.88), and 3 years (HR: 1.18, 95%CI 0.97–1.42)]. In addition, erythrocytes count was associated with a higher risk of death during the 6 months after diagnosis (HR: 1.53 95%CI 1.01–2.32), although not thereafter.

Table 1 Baseline characteristics of patients with ALS included in the study

Characteristics	Patients included in the study ($N=270$)	Patients diagnosed in Stockholm during the same period who were not part of the study ($N=132$)	p^a
Age at diagnosis, years			0.05
Mean (SD)	65.9 (11.1)	68.4 (12.3)	
Sex, N %			0.50
Male	136 (50.4%)	61 (46.2%)	
Female	134 (49.6%)	71 (53.8%)	
Onset site, N %			0.27
Non-bulbar	171 (63.3%)	86 (65.2%)	
Bulbar	98 (36.9%)	37 (28%)	
Unknown	1 (0.4%)	9 (6.8%)	
Diagnostic delay, months			0.83
Median (IQR)	12.57 (10.55)	12.23 (14.15)	
Death or initiation of invasive ventilation			0.14
N (%)	213 (78.9%)	113 (85.6%)	

^aDerived from Student's t test, Chi-square test, or Mood's median test

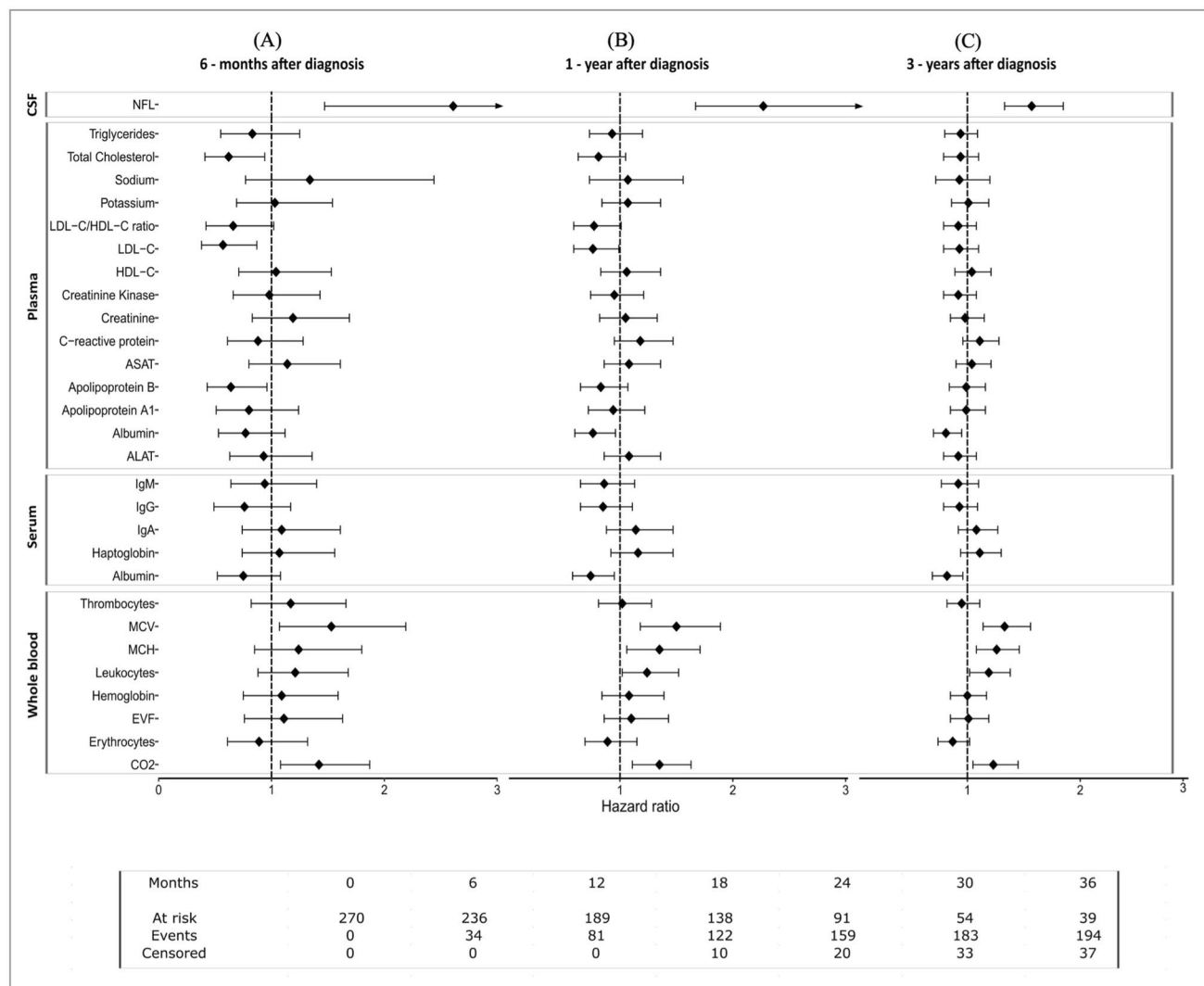


Fig. 1 Associations of clinical chemistry biomarkers (per 1-SD increase) with risk of mortality after ALS diagnosis (6 months, 1 year, and 3 years since diagnosis), using Cox proportional hazard models after multiple imputation, normalization, and standardization

Analysis 2

In EFA, six uncorrelated factors were identified among the clinical chemistry biomarkers studied, based on their factor loadings (Fig. 2).

These factors were named according to their predominant loaded variables. The “LDL profile” factor was associated with a lower risk of death during 6 months after ALS diagnosis, while the “albumin & CRP” factor was associated with a lower risk of death during 1 year or 3 years after ALS diagnosis (Table 2).

While these associations diminished after multivariable adjustment, the ‘Red blood cell profile’ factor was still associated with an increased mortality risk at 6 months and 1 year post-diagnosis. In the sensitivity analysis with additional adjustment for genetic mutation, dementia, and

riluzole use, 37 patients were found to have a genetic mutation, 23 to have dementia at the time of diagnosis, and 150 to be using riluzole. This additional adjustment did not lead to largely changed results (Supplementary Table 4). Finally, although none of the factors reached statistically significant results in relation to NfL levels in CSF, there was a notable trend for the “Liver enzymes & CK” factor in both models.

Analysis 3

For the joint model, we selected the model with three trajectory classes, showing distinct trajectories of ALSFRS-R decline over time (Fig. 3A). Specifically, 49 patients were assigned to the slow, 107 to the intermediate, and 86 to the fast progression classes. The survival plot of the three classes presents consistent trend as ALSFRS-R decline

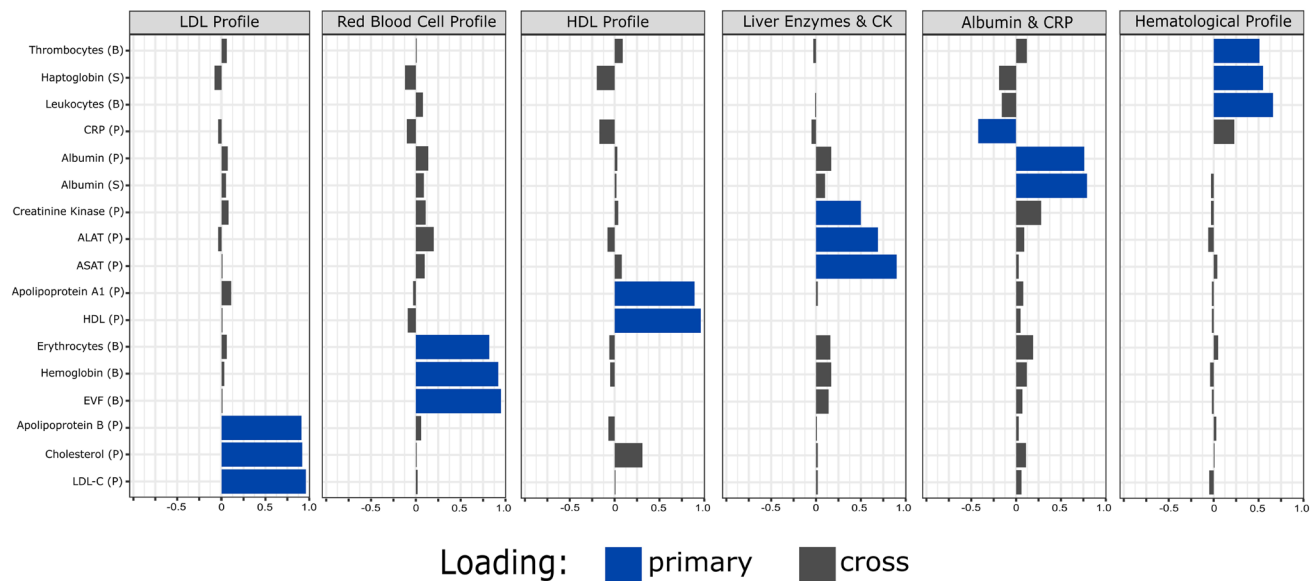


Fig. 2 Exploratory factor analysis showing the six factors that explained 68% of the total variance in the clinical chemistry data, with blue color indicating the primary variable loadings included in each factor. Weights were calculated using the absolute value of the loadings

(Fig. 3B). Although the associations between the six factors and trajectory classes did not reach statistical significance, patients with higher values in the 'Red blood cell profile' factor were less likely to be classified into the slow or intermediate classes, regardless of multivariable adjustment (Fig. 3C). NfL in CSF showed significant association with class membership. Specifically, patients with higher level of NfL were less likely to be classified into slow or intermediate classes, compared to fast progression class. When we changed the probability threshold for class membership to 70%, we did not notice significant change in the results (Supplementary Tables 5 and 6).

Discussion

In this study, we performed a comprehensive analysis using clinical chemistry data from 270 patients with ALS to examine their association with survival and disease progression, individually and collectively. We observed that increased levels of total cholesterol, LDL-C, apolipoprotein B, and albumin at diagnosis were protective factors for survival. However, the strength of the associations diminished over time and, among these biomarkers, only albumin was associated with survival throughout the 3 years post-diagnosis. Conversely, increased levels of blood leukocyte counts, MCV, MCH, CO₂, and NfL in CSF were associated with a higher risk of mortality during all the 3 years after diagnosis. In factor analysis, we identified six uncorrelated factors among the studied clinical chemistry biomarkers, among which the composite 'LDL profile' and 'albumin

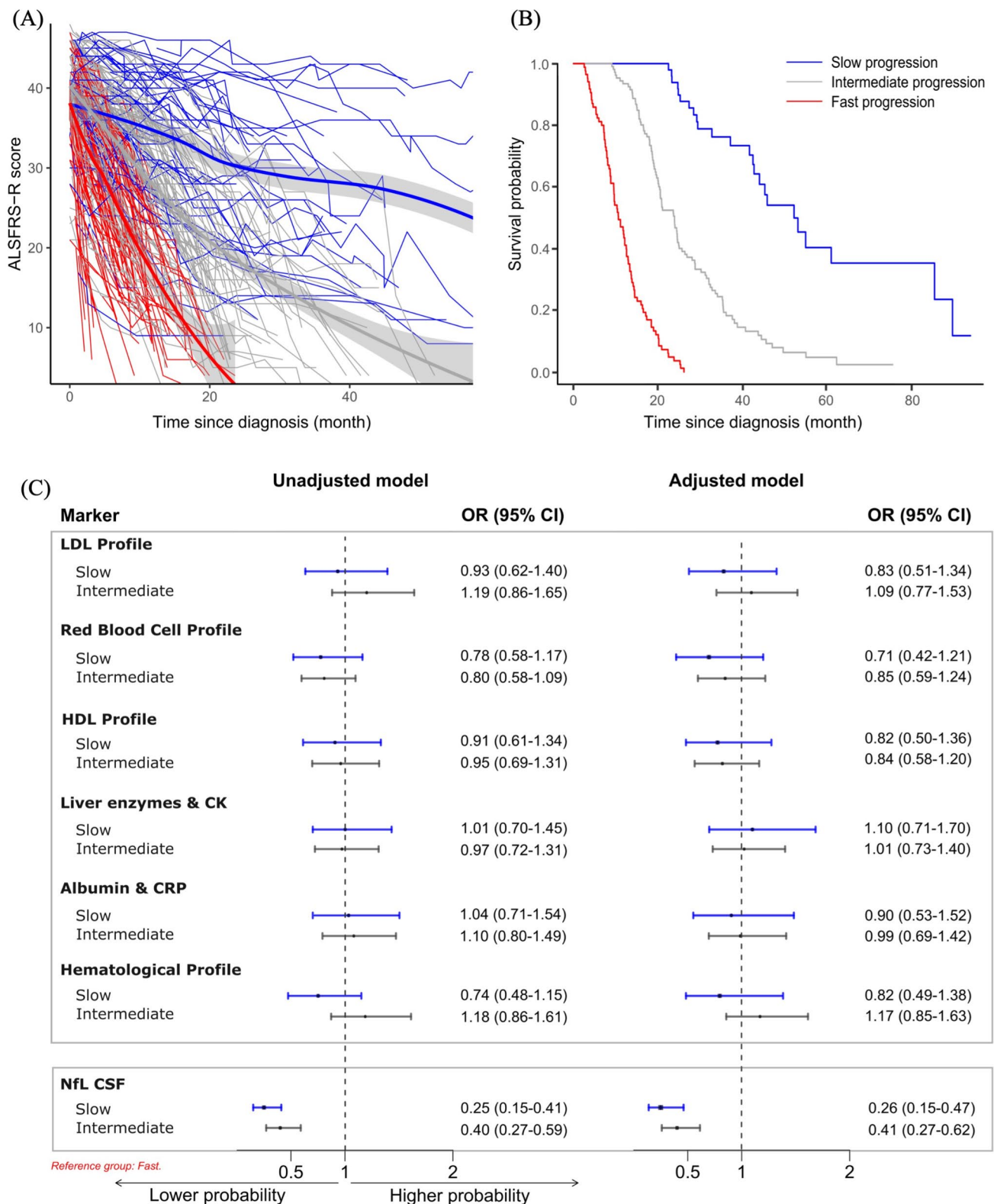
& CRP' factors were associated with a decreased risk of mortality after ALS diagnosis. After adjusting for other prognostic indicators, however, only the 'Red blood cell profile' factor was associated with increased risk of mortality across 3 years after ALS diagnosis. Interestingly, we did not observe an association between the 'Red blood cell profile' factor and NfL level in CSF measured at the time of diagnosis.

Our results of individual biomarkers are generally consistent with prior research on albumin, lipids, apolipoproteins, and neurofilaments [14, 16, 36–38]. We showed further that the predictive value of these biomarkers declined over time in general, and that some biomarkers were predictive of survival only during the first year (e.g., total cholesterol, LDL-C, and apolipoprotein B), whereas others during the entire 3 years (e.g., albumin, MCV, CO₂, and NfL) after ALS diagnosis. Albumin, for instance, is associated with a lower risk of death throughout the 3 years after ALS diagnosis. Albumin regulates plasma oncotic pressure and transports physiological metabolites [39] and lower levels of blood albumin (hypoalbuminemia) have been associated with poor nutrition or inflammation [40, 41]. This finding supports further the importance of nutritional support and inflammation control for patients with ALS [21, 42]. In addition, we found that elevated CO₂ levels in venous blood served as a prognostic indicator in ALS, both in the short (within 6 months) and long (within 3 years) run. Elevated CO₂ levels in blood can indicate impaired respiratory function and hypercapnia, which are common complications in ALS due to respiratory muscles atrophy and insufficient removal of CO₂ [43,

Table 2 Associations of EFA-derived factors (per 1-SD increase) with risk of mortality after ALS diagnosis (6 months, 1 year, and 3 years after diagnosis) or baseline level of neurofilament light chain (NFL) in CSF

Factor	6 months after diagnosis ⁱ (N = 270)		1 year after diagnosis ⁱ (N = 270)	
	HR (95% CI) Unadjusted ^a	HR (95% CI) Adjusted ^b	HR (95% CI) Unadjusted ^a	HR (95% CI) Adjusted ^b
LDL profile	0.58 (0.38–0.88)	0.84 (0.55–1.28)	0.76 (0.59–0.99)	0.96 (0.75–1.23)
Red blood cell profile	1.16 (0.82–1.65)	1.65 (1.07–2.65)	1.20 (0.94–1.54)	1.37 (1.03–1.83)
HDL profile	1.07 (0.74–1.55)	1.14 (0.72–1.80)	1.10 (0.87–1.40)	1.21 (0.92–1.59)
Liver enzymes and CK	1.05 (0.75–1.49)	1.24 (0.85–1.80)	1.04 (0.84–1.30)	1.11 (0.88–1.39)
Albumin and CRP	0.78 (0.55–1.10)	1.27 (0.84–1.93)	0.70 (0.56–0.88)	0.94 (0.72–1.22)
Hematological profile	1.12 (0.81–1.55)	0.99 (0.70–1.38)	1.13 (0.93–1.37)	1.08 (0.88–1.32)
Factor	3 years after diagnosis ⁱ (N = 270)		Baseline NFL (CSF) ⁱⁱ (N = 270)	
	HR (95% CI) Unadjusted ^a	HR (95% CI) Adjusted ^b	β (95% CI) Unadjusted ^a	β (95% CI) Adjusted ^b
LDL profile	0.91 (0.90–1.24)	1.03 (0.86–1.22)	0.02 (–0.11 ~ 0.16)	0.07 (–0.06–0.19)
Red blood cell profile	1.06 (0.90–1.24)	1.16 (0.97–1.39)	0.001 (–0.13 ~ 0.13)	–0.01 (–0.14–0.12)
HDL profile	1.04 (0.89–1.21)	1.05 (0.88–1.25)	–0.03 (–0.17 ~ 0.11)	0.03 (–0.11–0.18)
Liver enzymes and CK	0.96 (0.83–1.10)	1.05 (0.91–1.21)	0.12 (–0.02 ~ 0.25)	0.08 (–0.06–0.22)
Albumin and CRP	0.77 (0.66–0.89)	0.96 (0.80–1.14)	–0.01 (–0.13 ~ 0.12)	0.04 (–0.09–0.17)
Hematological profile	1.09 (0.95–1.24)	1.03 (0.89–1.19)	0.05 (–0.08 ~ 0.18)	0.02 (–0.11–0.15)

ⁱDerived from multivariable Cox proportional hazards modelⁱⁱDerived from multiple linear regression^aUnadjusted for other covariates^bAdjusted for sex, age at diagnosis, onset site, ALSFRS-R score at diagnosis, BMI at diagnosis, and diagnostic delay



44]. Similarly, serum chloride, which plays a critical role in maintaining hydration, muscle function, and pH balance, has been found to be associated with respiratory failure and overall survival in patients with ALS [45].

Hypercapnia is one of the indication criteria for the initiation of non-invasive ventilation (NIV) [46, 47]. As shown in a randomized clinical trial, early intervention with NIV slows the decline of respiratory function and minimizes

Fig. 3 **A** Spaghetti plot showing three trajectories of ALSFRS-R score by time since ALS diagnosis. Derived from joint trajectory model for latent classes. Linear regression model was adjusted for sex, age at diagnosis, onset site, and time since diagnosis. Survival model was adjusted for sex, age at diagnosis, and onset site. **B** Survival curves for patients with the three trajectories of ALSFRS-R score by time since ALS diagnosis. **C** Forest plot displaying odds ratio (OR) and 95% confidence interval (CI) of belonging to different trajectories of ALSFRS-R score in relation to the six factors and neurofilament light chain (NfL) in CSF, derived from multinomial logistic regression. All factors were included in the same multivariable model, after adjustment for sex, age at diagnosis, onset site, ALSFRS-R score at diagnosis, BMI at diagnosis, and diagnostic delay

complications [48]. Therefore, early assessment of the presence of respiratory failure or hypercapnia is essential in the management of patients with ALS.

Another interesting finding in this study pertains to red blood cells. MCV, which measures average volume of a red blood cell, demonstrated predictive ability during the entire 3 years after ALS diagnosis. In the analysis after adjustment for known prognostic indicators of ALS, hemoglobin, EVF, and count of erythrocytes, which are major components of the ‘Red blood cell profile’ factor, were all associated with risk of death after ALS diagnosis, highlighting the potential utility of these biomarkers as additional biomarkers in the prognostic prediction of ALS patients. To the best of our knowledge, only one previous study has examined hemoglobin in relation to ALS survival, showing that an increased level of hemoglobin was associated with increased odds of death in ALS patients [38]. The lack of correlation between the ‘Red blood cell profile’ factor and CSF NfL at diagnosis suggests that the ‘Red blood cell profile’ factor may not be indicative of the severity of neurodegeneration in ALS but rather supports a role of the bodily systems outside of the central nervous system. For instance, a higher level of chronic hypoxia in ALS patients might lead to a higher level of red blood cells, as a compensating mechanism [49]. Similarly, as hemoglobin is a transporter of oxygen [50], a higher level of hemoglobin can indicate a higher level of oxidative stress [51]. Oxidative stress is caused by an overabundance of reactive oxygen species (ROS) or a failure in the antioxidant defense system, leading to various changes like alteration of the redox state of vital proteins and their function [52].

NfL is a known biomarker for ALS [53, 54]. In line with this, the present study found a significant association of NfL measured in CSF at diagnosis with both mortality and functional decline after ALS diagnosis. The ‘Liver enzyme & CK’ factor showed a positive association with NfL at baseline, both before and after multivariable adjustment, although the results were not statistically significant. Research in mice has indicated that liver damage may play a role in the progression of ALS by influencing the liver’s production of proteins linked to inflammation and oxidative

stress as well as proteins that cause liver fibrosis [55]. However, future research is required to validate this finding.

The strength of our study lies in the comprehensive approach to examine the association of a diverse array of clinical chemistry biomarkers, both individually and jointly, with survival outcomes in different time windows after ALS diagnosis, as well as with functional decline and NfL measured in CSF. Another strength is the complete follow-up of all patients, as well as the combined application of EFA and Cox model, which mitigated potential multicollinearity and allowed the assessment of multiple factors within the same model without inflating Type 1 error. Our study also has limitations. The wide confidence intervals in some of the results suggest that we might have failed to identify associations with small effect sizes, due to limited statistical power, in some of the analyses. The number of measurements of biomarkers was not identical among patients with ALS. Although multiple imputation was applied under the assumption that data were missing at random, which is less stringent than the assumption of missing completely at random in complete case analysis, patients with different disease severities might have received different number of biomarker measurements. Further, given the exploratory nature of Analysis 1 and the high correlations between many of the clinical chemistry markers, we did not apply correction of multiple testing in Analysis 1. Subsequently, the results of this analysis should be interpreted with caution. Finally, although findings of this study are likely generalizable to all ALS patients in Stockholm, given the similar clinical characteristics noted between the study sample and its source population (i.e., all ALS patients diagnosed during the study period in Stockholm), validation from future studies with ALS patients of other areas is needed.

Conclusion

Our study showed the potential of routine clinical chemistry markers in predicting survival and prognosis of ALS. Several biomarkers, including lipids, albumin, leukocytes count, MCV, MCH, and CO₂, were associated with patient survival. The ‘Red blood cell profile’ factor showed additional prognostic value on top of other established prognostic indicators. As these blood markers provide insights into bodily functions outside of the central nervous system, integrating them in the care of ALS patients might lead to a more holistic management strategy for the disease.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00415-024-12774-7>.

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Authors' contributions All authors contributed to the conception and design of the study and data interpretation. Christina Seitz, Fang Fang, Caroline Ingre, and Emily E. Joyce contributed to the acquisition of the data. Anikó Lovik provided statistical advice and both Ioannis Psychogios and Yihan Hu contributed to the statistical analysis. Ioannis Psychogios wrote the draft of the manuscript, and Yihan Hu and Fang Fang revised the final article. All authors read and approved the final manuscript.

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Availability of data and materials Data used in the present study are not possible to share publicly due to Swedish and European regulations. Please contact the corresponding author for more information.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval and consent to participate This study was approved by the Swedish Ethical Review Authority (DNRs: 2014-1815-31-4, 2018-1605-31, and 2021-06397-02). All patients provided informed consent before study participation.

Consent for publication Not applicable.

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