Title
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Journal
Amyotrophic lateral sclerosis & frontotemporal degeneration, 21(3-4)

ISSN
2167-8421

Authors
Mitsumoto, Hiroshi
Garofalo, Diana C
Santella, Regina M
et al.

Publication Date
2020-05-01

DOI
10.1080/21678421.2020.1746810

Peer reviewed

HHS Public Access

Author manuscript

Amyotroph Lateral Scler Frontotemporal Degener. Author manuscript; available in PMC 2021 May 01.

Published in final edited form as:

Amyotroph Lateral Scler Frontotemporal Degener. 2020 May ; 21(3-4): 263–272. doi:10.1080/21678421.2020.1746810.

Plasma Creatinine and Oxidative Stress Biomarkers in Amyotrophic Lateral Sclerosis

Hiroshi Mitsumoto, MD, DSc[1,*], **Diana C. Garofalo, MPH**[2,*], **Regina M. Santella, PhD**[3], **Eric J. Sorenson, MD**[4], **Bjorn Oskarsson, MD**[5], **J Americo M. Fernandes Jr, MD**[6], **Howard Andrews, PhD**[7], **Jonathan Hupf, BA**[1], **Madison Gilmore, BA**[1], **Daragh Heitzman, MD**[8], **Richard S. Bedlack, MD, PhD, MS**[9], **Jonathan S. Katz, MD**[10], **Richard J. Barohn, MD, PhD**[11], **Edward J. Kasarskis, MD, PhD**[12], **Catherine Lomen-Hoerth, MD, PhD**[13], **Tahseen Mozaffar, MD**[14], **Sharon P. Nations, MD**[15], **Andrea J. Swenson, MD**[16], **Pam Factor-Litvak, PhD**[2]

[1]Eleanor and Lou Gehrig ALS Center, Department of Neurology, Columbia University Irving Medical Center;

[2]Department of Epidemiology, Mailman School of Public Health, Columbia University Irving Medical Center;

[3]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University Medical Center;

[4]Department of Neurology, Mayo Clinic, Rochester;

[5]Department of Neurology, Mayo Clinic, Jacksonville;

[6]Department of Neurological Sciences, University of Nebraska Medical Center;

[7]Coordinating Center (DCC), Mailman School of Public Health Biostatistics Department, Columbia University Irving Medical Center, New York State Psychiatric Institute & Department of Psychiatry, Columbia University;

[8]Texas Neurology, P.A.;

[9]Department of Neurology, Duke University;

[10]Forbes Norris ALS Center, California Pacific Medical Center;

[11]Department of Neurology, University of Kansas;

[12]Department of Neurology, University of Kentucky;

[13]Department of Neurology, University of California, San Francisco;

[14]Department of Neurology, University of California, Irvine;

[15]Department of Neurology and Neurotherapeutics, University of Texas Southwestern;

Corresponding Author: Hiroshi Mitsumoto, MD, DSc, Eleanor and Lou Gehrig ALS Center, Department of Neurology, Columbia University Irving Medical Center, 710 West 168th St, New York, NY 10032, Tel 212-305-1319, Cell 646-337-7431, Fax 212-305-2750, hm264@cumc.columbia.edu.

*Equal first authors

Supplemental Data: Supplemental Table 1, Supplemental Table 2, Supplemental Table 3

Appendix e-1: The ALS COSMOS Study Group (lists names, locations, roles, and contributions of all authors in a tabular format)

[16]Department of Neurology, University of Iowa

Abstract

Objective: To determine the associations between plasma creatinine (PCr), plasma uric acid (PUA), and urinary oxidative stress (OS) biomarkers with the ALSFRS-R at baseline and survival in a large epidemiological cohort study (ALS COSMOS) with a well-phenotyped patient population (N=355).

Methods: Fasting plasma and first void urine samples were obtained. PCr, PUA, urinary 8-oxo-deoxy guanosine (8-oxodG), and 15-F_{2t}-isoprostane (IsoP) were analyzed at baseline, near the midpoint of follow-up, and at the final blood draw (before death or withdrawal from study). We estimated associations between these biomarkers and the ALSFRS-R at baseline and survival.

Results: At baseline, PCr correlated with ALSFRS-R (Spearman r = 0.30), percent (%) FVC (r = .20), PUA (r = .37), and 8-oxodG (r = −.13, all p<.05). Baseline PCr significantly predicted survival (adjusted hazard ratio 0.28, p<.001). Time to death from baseline was shortest for those in the lowest two PCr quartiles relative to the highest two quartiles. PCr and ALSFRS-R values were significantly correlated at all three time points (baseline: r = 0.29, midpoint: r = .23, final: r = .38, all p <.001). PCr and PUA significantly declined over time, whereas OS biomarkers significantly increased over time.

Conclusions: To date, PCr predicted survival the best, compared to PUA, 8-oxodG and IsoP. Although PCr represents the degree of muscle mass, it may also represent complex biochemical changes in ALS. Because the field has no reliable prognostic biomarkers, the importance of PCr warrants further investigation through clinical studies in ALS.

Keywords

Amyotrophic Lateral Sclerosis (ALS); Biomarker; Creatinine; Uric Acid; Oxidative Stress

INTRODUCTION

In clinical practice, the diagnosis of amyotrophic lateral sclerosis (ALS) and assessments of disease progression are solely based on clinical findings (1–3). Objective prognostic biomarkers are needed to rigorously assess treatment efficacy in clinical trials, particularly those highly correlated with disease progression (3, 4). Such biomarkers should closely reflect the natural history of the disease (5). Although several biomarkers have been proposed in the past decade (6–9), it is not clear whether these reflect the natural history of ALS.

A recent systematic review and meta-analysis suggested that plasma creatinine (PCr) is a promising biomarker to estimate ALS progression and prognosis (10). However, authors highlighted a range of methodological issues that limit firm conclusions about the use of PCr to monitor ALS progression. These limitations include low participation and high attrition in longitudinal studies, and substantial measurement error of prognostic factors, outcomes, and confounders. Thus, investigators recommended that studies that evaluate the utility of PCr as

a prognostic biomarker use strict methodologies and standardized criteria for ALS progression.

Here, we address the limitations of previous studies by using a large, prospective, multisite, epidemiological cohort study (ALS COSMOS) originally designed to investigate the associations between oxidative stress (OS) and disease progression in 355 patients with ALS (11). Our sample has thorough follow-up data that include dates of death for nearly 90% of participants, as well as serial measures of PCr, plasma uric acid (PUA), urinary 8-oxodeoxy guanosine (8-oxodG), and 15-F_{2t}-isoprostane (IsoP) from baseline assessment to close to death. We report on the utility of PCr, PUA, urinary 8-oxodG, and IsoP as biomarkers of ALS progression.

PATIENTS AND METHODS

IRB Approval

The study protocol, including informed consent procedures and HIPAA compliance, was approved by the Institutional Review Board (IRB) of each participating site as well as Columbia University, the coordinating center.

Clinical and Epidemiological Analyses

The study protocol, including details of study sites, eligibility and enrollment processes, data collection for clinical and survey measures (including cognitive, occupational, environmental, psychological and dietary surveys), and biospecimen collection are described in previous publications (12–14). Briefly, we enrolled newly diagnosed patients with ALS based on the El Escorial ALS diagnostic criteria; symptom onset must have been less than 18 months prior to enrollment. Recruitment occurred over a 3-year period. Patients were followed at 3, 6, 12, 18 and 24 months after enrollment (or until death) with passive follow-up beyond 24 months to assess survival (final ascertainment completed in May 2018). Follow-up visits encompassed examinations that consisted of measures of %FVC, ALSFRS-R, a structured interview that included psychological and lifestyle factors, occupational history, biospecimen collection (blood and urine), and food frequency questionnaires.

Biospecimen Collection and Biomarker Analyses

Blood and urine samples were collected after overnight fasting, before medications were administered (12); patients provided first-void urine specimens at home using a sample kit that was later brought to the clinic visit in an ice cooler. At the clinic, blood samples were obtained and immediately processed. The samples were stored at −80°C until shipped on dry ice to the NIEHS Biomarkers Core Facility (RS) at Columbia University. PCr and PUA were measured using routine assays on an Integra 400 Plus chemistry analyzer (Roche Diagnostics, Indianapolis, IN) in the Biomarkers Core Laboratory of the Irving Institute for Clinical and Translational Research at Columbia University. OS biomarkers (urinary IsoP and 8-oxodG) were measured using well-established immunoassays (15) and corrected for concentration using specific gravity (16). All clinical data were stored and managed by the Columbia Data Management Center.

Biomarkers were analyzed in specimens collected at baseline; the last blood draw/urine collection before withdrawal from study or death (time from baseline, mean ± SD: 12.4±7.8 months, range 1.6 to 33.1 months) and at the "midpoint," i.e., sample drawn closest to the middle point between baseline and the final sample (time from baseline, mean ± SD: 7.2±4.10 months, range 1.1 to 18.6 months).

Statistical Methods

Covariates—All regression analyses were adjusted for age, sex, race, Latin ancestry, region of symptom onset, educational attainment, body mass index (BMI) at baseline, and months between symptom onset and enrollment, which have been associated with ALS function and survival based on our study.[12] Number of months between biospecimen collections was added as an additional covariate in longitudinal models. All analyses were done using SAS (version 9.4; Cary, NC) and R.

Baseline analyses—In cross-sectional analyses using data from the baseline visit only, we calculated Spearman correlations between continuous variables (biomarkers and the three clinical assessments: ALSFRS-R, %FVC, BMI). We compared biomarker values at baseline across clinical characteristics using t-tests and Spearman correlations. Multivariable linear regression was used to estimate associations between the biomarkers and clinical outcomes (ALSFRS-R and %FVC), adjusting for sex, race, region of onset, age, educational attainment, Latin ancestry, and months between symptom onset and the baseline visit.

Longitudinal analyses—Cox proportional hazards models were used to estimate hazard ratios (HRs) between each biomarker and survival time. Separate models were estimated for baseline PCr, PUA, urinary IsoP, urinary 8-oxodG, ALSFRS-R, and %FVC. The proportional hazards assumption was assessed by evaluating the significance of an interaction between exposure and the log of follow-up time. To stratify hazard functions, we defined quartiles of baseline ALSFRS-R, PCr, and PUA to use as predictors in the Cox proportional hazards models. We also used joint models, which can accommodate both longitudinal biomarker measures and time to death data types, to evaluate the role of trajectories of PCr, PUA, and ALSFRS-R measured at three timepoints in a subset of our sample and time to death, using the R package (17).

In a sensitivity analysis, we investigated whether a combination of the baseline ALSFRS-R, %FVC and PCr predicted survival time better than the ALSFRS-R alone using principal component analysis (PCA). We restricted the PCA to three variables only – baseline ALSFRS-R, PCr, and %FVC (scaled using z-scores) – based on our main findings. Spearman correlations were computed between the first principal component (PC1) and clinical variables. We then grouped participants into quartiles of PC1 values and assessed the presence of an association with time to death using adjusted Cox proportional hazards models.

To examine changes in biomarkers between baseline and the final measure, we used paired two-tailed t-tests. We then calculated slopes of change by estimating the best fit line for PCr and ALSFRS-R using values from each of the three time points (baseline, midpoint, and final visit). For simplicity, we assumed a linear decline for both PCr and ALSFRS-R. We

also stratified into the first half of follow-up (baseline to midpoint measures) and the second half of follow-up (midpoint to final measures), as we *a priori* hypothesized a difference in slope with disease progression. We compared the slopes of the PCr and ALSFRS-R trajectories over the three time points using Spearman correlations.

RESULTS

In the ALS COSMOS study, the mean age was 61 years (SD 10.3) and ranged from 27 to 90. Sixty percent of the sample were male. The average (± SD) time between symptom onset and enrollment was 12.7±4.5 months. No participant had renal disease or advanced diabetes; five patients received allopurinol for gout, but PUA levels were within the normal range. At baseline, 346 patients provided blood and urine samples; among these patients, 64 provided only baseline samples, leaving 282 patients who contributed at least 2 samples. These 64 (18.5%) patients were significantly older and sicker indicated by significantly lower ALSFRS-R and %FVC scores at baseline. They also had significantly shorter survival time and were more likely to be female and non-white compared with those who had biospecimens collected at multiple visits (Table 1).

Cross-sectional analyses

Descriptive statistics for baseline and final biomarker values, ALSFRS-R, and %FVC are described in Table 2; all measures showed changes from baseline to the last visit. Overall, PCr (mg/dL) decreased by 23%, from a mean of 0.86 (± 0.20) to 0.66 (± 0.20) in men and from 0.71 (±0.17) to 0.55 (±0.19) in women (normal PCr values: male, 0.7–1.2 and female, 0.5–0.9). PUA (mg/dL) declined approximately 10% in men, from a mean of 5.48 (±1.28) to 4.94 (±1.27), and by 6.5% in women, from a mean of 4.50 (±1.20) to 4.21 (±1.15); laboratory normal PUA values are female: 2.4 to 5.7, and male: 3.4 to 7.0. From baseline to the last visit, we also observed an increase in markers of oxidative stress: urinary IsoP increased 17% and urinary 8-oxodG increased 13%.

Baseline PCr and ALSFRS-R values were correlated (r_s =.30; p<.0001); baseline PCr also correlated with PUA (r_s = .37; p<.0001) and with 8-oxodG (r_s=−.13; p<.02). Baseline ALSFRS-R also correlated with baseline %FVC (r_s=.47, p<.0001). There was a significant correlation between cognition and uric acid levels, where higher levels were found in those with normal cognitive ability (Supplemental Table 1). Interestingly, patients taking Riluzole tended to have higher isoprostane levels. We explored whether this was due to patients on Riluzole having more advanced disease (lower ALSFRS-R scores, longer time since onset, older age), but we found no obvious relationships between these factors and riluzole use.

Baseline PCr was associated with baseline ALSFRS-R score; for each 0.1 mg/dL increase in PCr, ALSFRS-R increased by 0.77 points (95% confidence interval (CI) 0.40, 1.13) (Table 3), adjusted for sex, race, region of onset, age at case ascertainment, educational attainment, Latin ancestry, and months since onset. Baseline 8-oxo-dG was negatively associated with baseline ALSFRS-R (p=.02); for each unit increase, ALSFRS-R declined by 0.22 (95% CI 0.39, 0.44) points. PUA was also associated with ALSFRS-R (p=.06); for each 0.1 mg/dL increase, ALSFRS-R increased 0.05 (95% CI −0.01, 0.11) points.

Prospective analyses

PCr measured at baseline was associated with ALSFRS-R measured at the last visit; for each 0.1 mg/dL increase in PCr, ALSFRS-R increased by 0.96 (95% CI 0.36, 1.56) points (p<.01) (Table 3). Urinary IsoP measured at baseline was also associated with ALSFRS-R measured at the last visit; for each unit increase in urinary IsoP, ALSFRS-R declined by 1.41 (95% CI 0.36, 2.47) points.

Baseline biomarkers and time to death

Baseline PCr was a significant predictor of longer survival time [hazard ratio (HR) for a 0.1 mg/dL increase in PCr=0.88, 95% CI (0.82, 0.95), p<.001]. ALSFRS-R and %FVC at baseline were also significantly associated with survival time (ALSFRS-R: HR=0.93, 95% CI (0.91, 0.95); %FVC: HR=0.98, 95% CI (0.97, 0.99) for a 1 unit increase, p<.0001 for both) (Table 4).

To visualize the hazard functions between baseline PCr or ALSFRS and time to death, we stratified baseline PCr and ALSFRS-R into four quartiles, ranging from highest to lowest values. Time to death was shortest for those in the lowest two PCr quartiles compared to those in the highest two quartiles (Figure 1). Time to death was shortest for those in the lowest baseline ALSFRS-R quartile, compared to those in the three higher quartiles. Trends in time to death were statistically significant for both when stratified by PCr (p<.05) and ALSFRS-R (p<.0001) at baseline.

The results from the principal component analysis using baseline ALSFRS-R, PCr, and %FVC as inputs revealed that PC1 explained 56% of the combined variance. The weights of ALSFRS-R, %FVC and PCr in PC1 were relatively equivalent (weights = 0.65, 0.60, and 0.47, respectively). PC1 significantly correlated with time since symptom onset, age at diagnosis, baseline uric acid level, last ALSFRS-R score, last PCr measure, last %FVC measure, and total survival time in months. When we analyzed associations between quartiles of PC1 and time to death, results were very similar to our findings above using baseline ALSFRS-R as the main predictor of time to death (Supplemental Table 1).

Longitudinal change in biomarkers

PCr values and ALSFRS-R scores significantly correlated at baseline, midpoint, and final visit for the 220 participants who had all three assessments (baseline: r=.29, midpoint: r=.23, final: r=.38, all p<.001). The average slope over all three points of ALSFRS-R decline was 1.2 points per month and 0.02 mg/dL per month for PCr decline. The magnitude of the correlations between these slopes from baseline to the mid-point visit (r=.16; p=.02) was lower than that from the mid-point visit to the final visit (r=.30; p<.0001).

In separate joint models using trajectories of all three measures of PCr, PUA, and ALSFRS-R to predict time to death, we found strong HRs relating PCr and ALSFRS-R. For a 0.1 mg/dL increase in longitudinal PCr, we estimated a 38% (95% CI 27%, 49%) reduced hazard of death and for a 1 unit (the average monthly change) increase in longitudinal ALSFRS-R score, we estimated a 6% (95% CI 6%, 8%) reduced hazard of death (Supplemental Table 3).

DISCUSSION

Our findings indicate that PCr is strongly associated with ALS clinical progression and can also inform survival time. We confirmed findings from earlier studies that showed PCr to be strongly associated with prognostic clinical measures such as ALSFRS-R and %FVC at baseline and at the visit closest to death. PCr also strongly predicted time to death (18–25). Furthermore, the pattern of disease progression shown by change in PCr in our study resembled that of ALSFRS-R score. When all three repeated measurements of PCr, PUA, and ALSFRS-R were modeled longitudinally, their relationship to time to death became stronger, indicating that trajectories of these biomarkers may indeed be important in understanding disease progression.

The ALS COSMOS study is a 16-site prospective cohort study that investigated clinical characteristics, environmental correlates, and biomarkers among individuals with ALS. We have reported that demographics, disease duration, EEC diagnostic groups, clinical trial participation, and use of alternative treatments did not differ between enrolled (355 total) and non-enrolled (yet eligible) patients (477 total), except for the observation that more enrolled patients had insurance than those not enrolled (12). One limitation of the design of ALS COSMOS is a lack of control patients, which limits our ability to investigate etiologic questions (versus survival and progression, addressed here). The sample size is also relatively small for the study of ALS, a heterogeneous disease. Nine years after the onset of the project, we have survival data for most of our participants (316/355). Although there is considerable loss to follow up due to death in our study, this is unavoidable in studies of ALS, and our use of death records allows us to account for the majority of our cases in survival analyses.

One strength of our study is the use of a phenotypically well-characterized ALS patient population, with a depth of clinical information that increases our ability to establish the utility of biomarkers collected throughout the disease course. As described by Mildvan and colleagues,(5) the most important step in developing a prognostic biomarker is ensuring that the biomarker faithfully predicts the natural history of the disease progression.

Nearly all (94%) PCr is derived from healthy muscle (26) and is a metabolic end-product of creatine. Increased urinary creatinine secretion among patients with ALS or spinal muscular atrophy may be a simple indicator of increased muscle catabolism (27). Severe weight loss (ALS cachexia) that develops long before muscle weakness is also well recognized, suggesting that complex systemic metabolic changes occur in ALS (28, 29). In fact, recent epidemiological studies showed that lipid and carbohydrate metabolic changes and weight loss seem to proceed ALS symptoms, such as progressive muscle weakness and atrophy (30, 31). A hypermetabolic state and mitochondrial changes perhaps represent distinct, preceding events (32–34). In a disease such as ALS, changes in PCr may be influenced by complicated metabolic changes.

We found that increased PCr levels significantly correlated with increased PUA levels and negatively correlated with other urinary OS biomarkers. UA is known to be elevated in patients with ALS(35) and is predictive of patient function and survival (21, 23, 36, 37).

PUA has natural antioxidant properties and multiple functions: 1) reacting with peroxynitrite as an antioxidant (38) 2) chelating Fenton reaction transitional metals and antioxidant quenching of superoxide and hydroxyl free radicals (39), 3) increasing glutathione antioxidant defenses as an electron donor (40), and 4) acting against glutamate neurotoxicity (41). Given these properties, it is intriguing to note that the antioxidant edaravone, used for treatment in patients with ALS, increases serum UA (42). In the early stages of the disease, we found that creatine metabolism appears to be well-maintained, indicated by high PCr levels; PUA antioxidative activity was also well-maintained in the early stages. PUA decreased with disease progression, whereas OS biomarkers clearly increased. Evidence is mounting that high levels of OS are present in patients with ALS (43–45). In our sample, however, we noted higher isoprostane levels among patients taking riluzole, a surprising observation that should be explored in future studies. Our results suggest complex biochemical interactions exist in patients and warrant research to better understand the metabolic mechanisms of disease progression in ALS.

Understanding changes in PCr with ALS progression may also be useful in optimizing clinical trial design. Higher PCr levels were helpful in identifying treatment responders in a post-hoc analysis of the previous clinical trial with dexpramiperole (24). Additionally, using PCr as one intermediate outcome in clinical trials may be useful in reducing the sample size, particularly when the study period is long (25).

Our study indicates that PCr may be an important ALS biomarker. We found that among the investigated biomarkers, PCr performed most similarly to ALSFRS-R scores in predicting survival. Measurement of PCr is very inexpensive; thus, PCr could be useful in clinical settings in which the ALSFRS-R is not available. We hypothesize that PCr is not widely used as a biomarker for ALS progression because it is non-specific, influenced by coexisting renal disease, and does not represent neuronal changes in ALS. However, we posit that it is very useful to monitor patients without severe renal disease. Further, PCr has been routinely used for more than 60 years (26), and some may perceive it to lack novelty. Yet, the value of PCr as a biomarker for ALS has been repeatedly shown (10). Novel biomarkers, such as neurofilament antibodies (46–48) and urine neurotrophin receptor p75 extracellular domain ($p75^{ECD}$)(4, 49) must be vigorously developed and validated in ALS. For the time being, however, we should not ignore the utility of PCr as an available biomarker in ALS clinical studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement(s)

We are deeply grateful to all the patients and their caregivers for their commitment and participation in this lengthy study. Dr. Annette Kirshner at NIEHS strongly supported us in our endeavor. We also thank Drs. Robert Miller, Jeremy Shefner, Jinsy Andrews, and Boguslawa A. Koczon-Jaremko and Yvonne Rollins, and Ms. Janet Bowen who participated in the early stages of the study. Serge Cramer, PhD, Irving Institute of Clinical Translational Research, Biomarkers Core Laboratory and Departments of Pathology, Cell Biology and Medicine, Columbia University, measured PCr and PUA. David Merle, BA, Coordinating Center (DCC), Mailman School of Public Health Biostatistics Department, Columbia University, developed and managed the clinical database for the project. Irina Gurvich, MS, managed the ALS COSMOS Biorepository. Jess Singleton, BA, Columbia ALS Center,

contributed to the data acquisition, input, and analyses. Georgia Christodoulou, MA, University of Southern California, and Cassandra Talerico-Kaplin, PhD, Cleveland Clinic, reviewed the manuscript.

Study Funding: ALS Ride for Life, Anthony Senerchia Family Foundation, Judith and Jean Pape Adams Charitable Foundation, William Spina Foundation, MDA Wings Over Wall Street, and the NIEHS (R01-ES016348)

Disclosure of Interests

Hiroshi Mitsumoto, MD, DSc: Grants - NINDS, NIEHS, MDA Wings, SPF, ALSA, MNDA, CDC/ATSDR, Cytokinetics, Tsumura; Advisory Board: Mitsubishi-Tanabe, Biohaven, and Dainippon-Sumitomo.

Diana C. Garofalo, MPH: None

Regina M. Santella, PhD: Grants - NIEHS, NCI, Breast Cancer Research Foundation

Eric J. Sorenson, MD: None

Bjorn Oskarsson, MD: Grants-NINDS, MDA, ALSA, Cytokinetics, Biogen, Genentech; Advisory Board -- Mitsubishi-Tanabe, Biohaven

J Americo M. Fernandes, Jr, MD: Clinical trials -- Malliinckrodt Pharmaceuticals

Howard Andrews, PhD: None

Jonathan Hupf, BA: None

Madison Gilmore, BA: None

Daragh Heitzman, MD: Speaker/Consultant for Biohaven Pharmaceuticals; Research Grant Support: Cytokinetics, Malliinckrodt Pharmaceuticals, Orion Pharma, Amylyx Therapeutics, Revance Therapeutics, Muscular Dystrophy Association, ALS Association, and The Neurologix Foundation.

Richard S. Bedlack, MD, PhD, MS: Research Grants -- ALSA, Cytokinetics, MNDA, Orion, Ultragenyx. Consulting Support -- ALSA, Biogen, Biohaven, Brainstorm, ITF Pharma, Mallinkrodt,

Jonathan S. Katz, MD: Grants from Genentech, Biogen, Orion and Brainstorm. Medical advisory board -- MT Pharma

Richard J. Barohn, MD, PhD: Advisory: NuFactor and Momenta Pharmaceutical and research support from PTC Therapeutics, Ra Pharma, Orphazyme, Sanofi Genzyme, FDA OOPD, NIH, and PCORI.

Edward J. Kasarskis, MD, PhD: Clinical support from ALSA; serves on the Data Safety Monitoring Board for a Cytokinetics reldesemtiv *s*tudy

Catherine Lomen-Hoerth, MD, PhD: None

Tahseen Mozaffar, MD: Grants - NIH, MDA, The Myositis Association, Alexion, Amicus, Argenx, aTyr, Bristol-Myers-Squib, Idera, Ionis, Grifols, Momenta, Ra Pharmaceuticals, Sanofi-Genzyme, Spark Therapeutics, UCB, Ultragenyx, Valerion. Advisory Board - Alexion, Amicus, Argenx, aTyr, Bristol-Myers-Squib, Idera, Ionis, Grifols, Momenta, Ra Pharmaceuticals, Sanofi-Genzyme, Spark Therapeutics, UCB, Ultragenyx, Valerion. Data Safety Monitoring Board - Acceleron and Avexis.

Sharon P. Nations, MD: None

Andrea J. Swenson, MD: None

Pam Factor-Litvak, PhD: CDC, MDA, NIH (NIEHS, NICHD)

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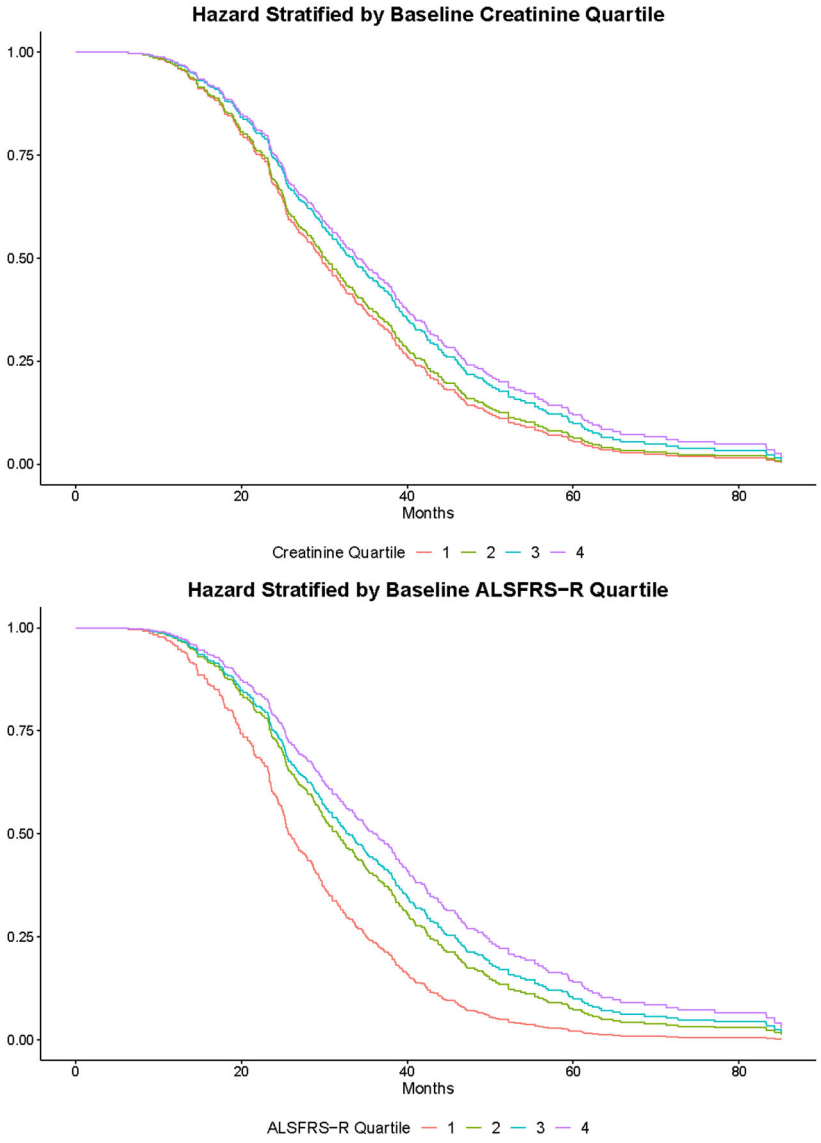

Figure 1.

Adjusted Hazard of Death Stratified by Baseline Plasma Creatinine (PCr) and ALSFRS-R Quartiles

Quartile 4 refers to highest PCr and ALSFRS-R values, while quartile 1 is lowest in both.

Table 1.

Comparison of baseline sample characteristics between patients who provided a biospecimen at baseline only and those who provided specimens at additional time points

Variable		> 1 Specimen (n=282)	Baseline specimen only (n=64)	p-value
Continuous Variables		**Mean (SD)**	**Mean (SD)**	
Age at case ascertainment		60.2 (10.4)	64.8 (9.3)	0.001
Months from onset to enrollment		12.8 (4.5)	12.3 (4.6)	0.39
Total survival months since onset		34.8 (13.7)	24.0 (15.4)	<0.0001
Months from enrollment to death		21.9 (12.6)	11.7 (14.1)	<0.0001
ALSFRS-R		36.9 (6.0)	31.7 (7.8)	<0.0001
% FVC		82.0 (21.5)	66.9 (24.2)	<0.0001
BMI		26.7 (4.5)	26.4 (5.2)	0.62
PCr		0.8 (0.2)	0.7 (0.2)	0.01
PUA		5.2 (1.3)	4.8 (1.4)	0.05
8-oxodG		4.7 (3.9)	5.4 (3.2)	0.18
IsoP		1.7 (1.0)	1.5 (0.7)	0.35
Categorical Variables		**N (%)**	**N (%)**	**p-value**
Education	Less than BA/BS	151 (53.6)	38 (65.5)	0.09
	BA/BS or higher	131 (46.5)	20 (34.5)	
Region of onset	Bulbar	87 (30.9)	18 (28.1)	0.67
	Upper limb	195 (69.2)	46 (71.9)	
Race	White	252 (89.4)	51 (79.7)	0.03
	Black/other *	30 (10.6)	13 (20.3)	
Sex	Male	177 (62.8)	30 (46.9)	0.02
	Female	105 (37.2)	34 (53.1)	
Employment	Working	105 (38.0)	13 (25.0)	0.07
	Not working	171 (61.9)	39 (75.0)	

* Among non-white, 50.0% were black (n=22), 33.3% Asian (n=14), and 16.7% Hispanic, native Americans (n=7).

Table 2.

Biomarker values at baseline and final blood draw

Marker	Baseline		Final Draw		Change (95% CI)	N	p-value
	Mean (SD)	Range	Mean (SD)	Range			
PCr (mg/dL)	0.8 (0.2)	0.4–1.4	0.6 (0.2)	0.2–1.3	−0.2 (−0.22, −0.17)	282	<0.0001
PUA (mg/dL)	5.2 (1.3)	2.3–9.1	4.7 (1.3)	1.9–10.0	−0.5 (−0.61,−0.36)	282	<0.0001
IsoP (ng/mL)	1.7 (1.0)	0.2–8.6	1.9 (1.5)	0.2–11.7	0.3 (0.09,0.48)	276	<0.001
8-oxodG (ng/mL)	4.7 (3.9)	1.0–54.9	5.4 (3.3)	0.4–23.3	0.6 (0.06,1.20)	276	<0.03
ALSFRS-R	36.1 (6.7)	7.0–47.0	24.9 (9.5)	2.0–47.0	−11.1 (−12.05,−10.21)	355	<0.0001
%FVC	79.1 (22.6)	20.0–138.0	57.1 (24.6)	5.0–133.0	−22.0 (−24.46, −19.52)	355	<0.0001

Table 3.

Cross-sectional and longitudinal associations between biomarkers measured at baseline and functional outcomes*

Baseline Biomarker	First ALSFRS-R**		First %FVC**	
	Beta (95% CI)	p-value	Beta (95% CI)	p-value
PCr (0.1 mg/dL)	0.77 (0.40, 1.13)	<0.0001	2.44 (1.20, 3.68)	0.0001
PUA (0.1 mg/dL)	0.05 (−0.01, 0.11)	0.06	0.06 (−0.13, 0.25)	0.53
Urine IsoP (1 ng/mL)	−0.29 (−0.98, 0.40)	0.40	−0.70 (−3.07, 1.67)	0.56
Urine 8-oxodG (1 ng/mL)	−0.22 (−0.39, −0.04)	0.02	−0.41 (−1.03, 0.20)	0.18
	Last ALSFRS-R***		Last %FVC***	
	Beta (95% CI)	p-value	Beta (95% CI)	p-value
PCr (0.1 mg/dL)	0.96 (0.36, 1.56)	<0.01	1.90 (0.30, 3.50)	0.02
PUA (0.1 mg/dL)	0.06 (−0.03, 0.15)	0.19	0.13 (−0.11, 0.37)	0.27
Urine IsoP (1 ng/mL)	−1.41 (−2.47, −0.36)	<0.01	−0.65 (−3.49, 2.19)	0.65
Urine 8-oxodG (1 ng/mL)	−0.06 (−0.51, 0.39)	0.79	−0.71 (−1.89, 0.48)	0.24
Baseline ALSFRS-R	0.91 (0.73, 1.08)	<0.0001	1.03 (0.50, 1.55)	0.0001
Baseline %FVC	0.11 (0.05, 0.17)	0.0001	0.55 (0.41, 0.68)	<0.0001

*Associations were determined by linear regression with adjustment for sex, race, region of onset, age at case ascertainment, educational attainment, Latin ancestry, months since onset, and time between baseline and last measure for longitudinal models.

**n=340 patients,

***n=276 patients

Table 4.

Association of baseline biomarkers and clinical markers with survival time*

Marker	Hazard Ratio (95% CI)	*P*	N
PCr (0.1 mg/dL)	0.88 (0.82, 0.95)	<0.001	340
PUA (0.1 mg/dL)	0.99 (0.98, 1.01)	0.38	340
Urine IsoP (1 ng/mL)	1.07 (0.96, 1.19)	0.23	342
Urine 8-oxodG (1 ng/mL)	1.01 (0.98, 1.04)	0.69	343
ALSFRS-R	0.93 (0.91, 0.95)	<0.0001	348
% FVC	0.98 (0.97, 0.99)	<0.0001	348

*Adjusted for sex, race, region of onset, age at case ascertainment, educational attainment, Latin ancestry, months between onset and baseline, and BMI at baseline