

## Original Investigation

# Amyotrophic Lateral Sclerosis Outcome Measures and the Role of Albumin and Creatinine

## A Population-Based Study

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**IMPORTANCE** There is an urgent need to identify reliable biomarkers of amyotrophic lateral sclerosis (ALS) progression for clinical practice and pharmacological trials.

**OBJECTIVES** To correlate several hematological markers evaluated at diagnosis with ALS outcome in a population-based series of patients (discovery cohort) and replicate the findings in an independent validation cohort from an ALS tertiary center.

**DESIGN, SETTING, AND PARTICIPANTS** The discovery cohort included 712 patients with ALS from the Piemonte and Valle d'Aosta Register for Amyotrophic Lateral Sclerosis from January 1, 2007, to December 31, 2011. The validation cohort comprised 122 patients with ALS at different stages of disease consecutively seen at an ALS tertiary center between January 1, 2007, and January 1, 2009.

**MAIN OUTCOMES AND MEASURES** The following hematological factors were investigated and correlated with survival: total leukocytes, neutrophils, lymphocytes, monocytes, glucose, creatinine, uric acid, albumin, bilirubin, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, creatine kinase, thyroid-stimulating hormones, and erythrocyte sedimentation rate; all analyses were performed separately by sex. The patient of the validation cohort also underwent bioelectrical impedance analysis for the calculation of fat-free mass.

**RESULT** Of the 712 patients in the examined period in Piemonte and Valle d'Aosta, 638 (89.6%) were included in the study. Only serum albumin (men:  $\leq 4.3$  vs  $>4.3$  mg/dL,  $P < .001$ ; women:  $\leq 4.3$  vs  $>4.3$  mg/dL,  $P < .001$ ) and creatinine levels (men:  $\leq 0.82$  vs  $>0.82$  mg/dL,  $P = .004$ ; women:  $\leq 0.65$  vs  $>0.65$  mg/dL,  $P = .004$ ) and lymphocyte count (men:  $\leq 1700$  vs  $>1700/\mu\text{L}$ ,  $P = .04$ ; women:  $\leq 1700$  vs  $>1700/\mu\text{L}$ ,  $P = .02$ ) were significantly associated with ALS outcome in both sexes with a dose-response effect (better survival with increasing levels). These findings were confirmed in the validation cohort. Multivariable analysis showed that serum albumin (men: hazard ratio [HR], 1.39; 95% CI, 1.05-1.90;  $P = .02$ ; women: HR, 1.73; 95% CI, 1.35-2.39;  $P = .001$ ) and creatinine (men: HR, 1.47; 95% CI, 1.11-1.95;  $P = .007$ ; women: HR, 1.49; 95% CI, 1.07-2.05;  $P = .02$ ) were independent predictors of survival in both sexes; no other hematological factor was retained in the model. In patients with ALS, serum albumin was correlated with markers of inflammatory state while serum creatinine was correlated with fat-free mass, which is a marker of muscle mass.

**CONCLUSIONS AND RELEVANCE** In ALS, serum albumin and creatinine are independent markers of outcome in both sexes. Creatinine reflects the muscle waste whereas albumin is connected with inflammatory state. Both creatinine and albumin are reliable markers of the severity of clinical status in patients with ALS and can be used in defining prognosis at the time of diagnosis.

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**A**myotrophic lateral sclerosis (ALS) is a neurodegenerative disorder of adult life characterized by the involvement of upper and lower motor neurons and, in about 50% of patients, prefrontal areas. In most cases, ALS appears sporadically in the population; roughly 10% of patients have a positive family history for ALS or frontotemporal dementia.<sup>1</sup>

Amyotrophic lateral sclerosis is almost invariably fatal with a median survival time of 2 to 4 years from onset and 1 to 3 years from diagnosis.<sup>2</sup> The only drug that has been found to be effective in prolonging life is riluzole, which increases life expectancy by about 3 months.<sup>3</sup>

Several clinical prognostic factors have been identified in ALS, namely age, site of onset, functional and respiratory status, cognitive function, noninvasive ventilation, some genetic mutations,<sup>2</sup> and clinical phenotypes.<sup>4</sup> In addition, various biological markers have been proposed as potentially related to a better ALS outcome including dyslipidemia,<sup>5,6</sup> elevated levels of uric acid<sup>7,8</sup> and creatinine, and reduced granulocyte count.<sup>9</sup> However, most of these markers have been evaluated only in small single-center series and have not been confirmed by subsequent studies.

The aim of this study was to assess the correlation of several hematological markers evaluated at diagnosis with ALS outcome in a population-based series of patients living in the Piemonte and Valle d'Aosta regions of Italy between January 1, 2007, and December 31, 2011. The study findings were then replicated in an independent validation cohort from an ALS tertiary center.

## Methods

### Discovery Cohort

The study design was approved by the institutional ethical committees of Azienda Ospedale Università, Città della Salute e della Scienza, and Azienda Ospedale Università Maggiore di Novara. Patients provided written informed consent.

All patients with ALS in the Piemonte and Valle d'Aosta regions of Italy (n = 712), identified through the Piemonte and Valle d'Aosta Register for ALS<sup>10</sup> and diagnosed between January 1, 2007, and December 31, 2011, were eligible for enrollment in the study. All patients met the revised El Escorial diagnostic criteria for definite and probable laboratory-supported ALS.<sup>11</sup> A complete clinical history was collected for each patient. Disease severity was assessed with the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised scale (ALSFERS-R).<sup>12</sup> Pulmonary function tests were performed at diagnosis and forced vital capacity (FVC) percentage of prediction was annotated. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.<sup>2</sup> Body weight was measured with individuals wearing only underwear and no shoes by means of a steelyard scale (precision, ±100 g). Body height was measured by means of a stadiometer (precision, ±0.5 cm). Patients who could not maintain the erect position were weighed in the seated position on an electronic chair scale (Seca) while body height was determined by measuring the knee height.<sup>13</sup> No differences were found be-

tween these methods. Levels of BMI were categorized according to the World Health Organization classification.<sup>14</sup>

In addition, the decline rate for ALSFRS-R score and its 4 subscores (bulbar, fine motor, gross motor, and respiratory) was calculated as the mean monthly number of points lost from symptom onset to the time of diagnosis. The FVC and BMI decline were also calculated.

Patients underwent hematological examinations as part of the diagnostic workup. Blood sampling was performed after overnight fasting. The following hematological tests were considered for this study: total leukocytes, neutrophils, lymphocytes, monocytes, glucose, creatinine, uric acid, albumin, bilirubin, total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, creatine kinase, thyrotropin, and erythrocyte sedimentation rate (ESR). Five male patients who had a creatinine level in the range from 1.3 to 1.8 mg/dL (to convert to micromoles per liter, multiply by 76.25), indicating a reduction of renal function, were excluded from the study.

A total of 565 patients also underwent an extensive genetic assessment using standard procedures.<sup>15</sup> Thirty-seven patients (6.6%) carried a *C9ORF72* GGGGCC repeat expansion, 17 (3.0%) carried *SOD1* missense mutations, 6 patients (1.2%) carried *TARDBP* missense mutations, 1 patient carried an *FUS* and 1 patient carried an *OPTN* missense mutation, and 16 patients (2.8%) carried polyQ repeat expansions (≥31) in the *ATNX2* gene.

### Validation Cohort

This cohort consisted of 122 patients with ALS at different stages of the disease consecutively seen at an ALS tertiary center between 2007 and 2009. Patients underwent all the same evaluations of the discovery cohort plus fat-free mass (FFM; in kilograms), determined by bioelectrical impedance analysis using a single-frequency tetrapolar technique with an electrical current of 800 mA at 50 kHz (STA-BIA; Akern) according to a method validated for ALS.<sup>16,17</sup>

### Statistical Methods

Comparisons between means were made with the *t* test or analysis of variance, comparison between categorical variables was made with the  $\chi^2$  test, and the equality of variances was confirmed with the Levene test. Correlations were calculated with the Pearson coefficient; because multiple comparisons were performed, *P* values were Bonferroni-adjusted (Table 1 and Table 2).

Survival was calculated from diagnosis to death, tracheostomy, or censoring date (December 31, 2013) using the Kaplan-Meier method and compared with the log-rank test. No patient was lost to follow-up. For survival analysis, each hematological factor was dichotomized according to its median value. Multivariable analysis was performed with the Cox proportional hazards model (stepwise backward) with a retention criterion of *P* < .10. Two separate models were used for men and women; the variables included in the model are listed in Table 2. A level of *P* < .05 was considered significant for multivariable analyses. To assess the prognostic performance of serum albumin, creatinine, and other significant prognostic

Table 1. Demographic and Clinical Characteristics of Patients Included vs Not Included in the Study

Characteristic	Patients Included (n = 638)	Patients Not Included (n = 74)	P Value
Age at onset, mean (SD), y	66.3 (10.7)	66.6 (11.9)	.83
Sex, No. (%)			
Female	286 (44.8)	36 (48.6)	.52
Site of onset, No. (%)			
Bulbar	198 (31.0)	30 (40.5)	.09
Diagnostic delay, mean (SD), y	0.96 (0.61)	0.91 (0.64)	.75
ALSFRS-R score at diagnosis, mean (SD)	37.4 (7.5)	38.3 (9.1)	.64
BMI	24.5 (4.3)	24.7 (4.5)	.83

Abbreviations: ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared).

factors, we calculated test specificity, sensitivity, positive predictive value, and negative predictive value using 1-year mortality after diagnosis as reference. The number needed to treat was also calculated. All tests were 2-tailed. Statistical analyses were carried out using a version 20.0 statistical package (SPSS).

## Results

### Discovery Cohort

Of the 712 patients in the examined period, 638 (89.6%) were included in the study, 352 men and 286 women. The remaining 74 patients were not included because of incomplete hematological data. Patients' mean (SD) age at onset was 66.3 (10.7) years (range, 25.3-91.5 years) and mean (SD) ALSFRS-R score was 37.4 (7.5; range, 5-47). Patients included and not included in the study did not differ in regards to demographic or clinical variables (Table 1).

The results of hematological analyses are reported in eTable 1 in the Supplement. The levels of all hematological parameters except for lymphocytes and albumin significantly differed between sexes. All values were normally distributed within each sex.

Next, we analyzed the influence of hematological factors on patients' survival. The overall median survival from diagnosis of the whole cohort was 1.7 years (interquartile range, 0.8-3.3 years). The results of univariate analyses for each hematological factor are reported in eTable 2 in the Supplement. Only serum albumin and creatinine levels and lymphocyte count were significantly related to ALS outcome in both sexes (Figure 1); patients' survival increased with higher levels of serum albumin and creatinine and higher lymphocyte count. Among the other hematological factors, higher levels of total cholesterol and LDL cholesterol in men as well as lower LDL cholesterol to HDL cholesterol ratio and higher serum thyrotropin levels in women were significantly related to longer survival. To identify a dose-response effect, serum albumin and creatinine were also assessed according to their quartiles; both factors remained highly significant in both sexes, with a better survival with increasing levels (eFigures 1 and 2 and eTable 3 in the Supplement). The presence of genetic mutations did not modify the results (data not shown).

To evaluate the ability in predicting 1-year mortality, we calculated the sensitivity, specificity, positive predictive value,

and negative predictive value of serum creatinine and albumin, ALSFRS-R score, FVC, and age (eTable 4 in the Supplement). In both sexes, serum albumin showed sensitivity and specificity similar to FVC, which is the prognostic factor with higher values, while creatinine had values similar to age and ALSFRS-R total score.

Multivariable analysis confirmed that albumin and creatinine levels were independent predictors of survival in both sexes; no other hematological factors were retained in the model (Table 2). As expected, other strong predictors of outcome were patients' age, FVC, ALSFRS-R bulbar score (both sexes), ALSFRS-R respiratory score (men), and ALSFRS-R total score (women). Similar results were obtained when we included the rate of decline of ALSFRS-R score in the multivariable analysis instead of its value at the time of diagnosis (data not shown).

Next, the correlation between hematologic factors and patients' clinical status at diagnosis was explored (eTable 5 in the Supplement). Creatinine was significantly correlated with the ALSFRS-R total score (Figure 2A), its gross motor, fine motor, and respiratory subscores in both sexes, and with FVC (Figure 2B) and BMI, only in men. A significant correlation was found between albumin and ALSFRS-R total score (Figure 2C), all ALSFRS-R subscores, and FVC (Figure 2D) in both sexes.

To better clarify mechanisms underlying the prognostic role of albumin and creatinine, we performed another exploratory analysis assessing whether they were correlated with markers of inflammatory state. In both sexes, serum albumin levels significantly decreased with the increase of total leukocytes, neutrophils, monocytes, and ESR while creatinine levels were not influenced by any marker of inflammatory state (eTable 6 in the Supplement).

### Validation Cohort

To confirm our findings, we considered an independent clinical cohort of patients with ALS. This cohort included 122 patients (54 men, 68 women), with a mean (SD) age at onset of 59.8 (11.0) years (range, 32.7-83.6 years), disease duration of 2.5 (2.1) years (range, 0.5-10.8 years), and ALSFRS-R score of 25.8 (10.0; range, 3-45). Albumin levels were similar in men (mean [SD], 4.0 mg/dL [0.4 mg/dL]) and women (mean [SD], 4.0 mg/dL [0.3 mg/dL]) (to convert to grams per liter, multiply by 10), while serum creatinine levels were higher in men (0.67 mg/dL [0.21 mg/dL]) than in women (0.54 mg/dL [0.19]) ( $P < .001$ ).

Mean (SD) BMI was 23.3 (4.7; range, 14.0-43.1) in men and 22.6 (4.7; range, 11.5-34.6) in women ( $P = .36$ ). Mean (SD) FFM was 47.6 (9.6; range, 14.5-69) in men and 35.7 (7.6; range, 2.3-51.4) in women ( $P < .001$ ). Body mass index and FFM were highly correlated with each other (men,  $r = 0.623$ ,  $P < .001$ ; women,  $r = 0.516$ ,  $P < .001$ ). Serum creatinine levels were correlated with FFM (men:  $r = 0.336$ ,  $P = .02$ ; women:  $r = 0.261$ ,  $P = .04$ ) (eFigure 3 in the Supplement) but not with BMI (men:  $r = 0.191$ ,  $P = .18$ ; women:  $r = 0.067$ ,  $P = .60$ ). Serum albumin was not correlated with BMI (men:  $r = 0.111$ ,  $P = .43$ ; women:  $r = 0.166$ ,  $P = .18$ ) or with FFM (men:  $r = 0.231$ ,  $P = .10$ ; women:  $r = 0.215$ ;  $P = .08$ ). In this cohort, serum albumin and creatinine correlated significantly with survival in both sexes (eFigures 4 and 5 in the Supplement).

## Discussion

In our population-based series of patients diagnosed as having ALS in the regions of Piemonte and Valle d'Aosta from 2007 to 2011, we found that serum albumin and creatinine measured at time of diagnosis were markers of outcome in both sexes, even after correction for known prognostic factors. No other examined hematological factors were significantly related to survival at multivariable analysis. Moreover, a dose-response effect was found for albumin and creatinine in both sexes. These findings were confirmed in an independent validation cohort from a tertiary ALS center.

We also found that lower albumin and creatinine levels were strongly related to worse clinical function at diagnosis (ALSFRS-R score and FVC). Albumin levels were correlated with indices of inflammatory state and not with nutritional parameters. In the validation series, serum creatinine levels (but not albumin levels) were related to patients' FFM, indicating that serum creatinine is a proxy of muscle mass.

Sensitivity and specificity values in predicting 1-year mortality indicated that serum albumin and creatinine have properties similar to the best established prognostic factors of ALS such as FVC, ALSFRS-R score, and age. For all these factors, sensitivity was higher than specificity, indicating that they reliably predict a survival of less than 1 year.

Albumin is a plasma-nonglycosylated protein with various functions including transportation of several substances such as fatty acids, bilirubin, and regulation of colloidal osmotic pressure<sup>18</sup> and has strong antioxidant properties.<sup>19</sup> Albumin is synthesized by the liver and its metabolism is influenced by nutritional intake and inflammatory disorders.<sup>20</sup> It has been reported that albumin is a prognostic marker in geriatric long-term care facility residents,<sup>21</sup> individuals undergoing surgical interventions,<sup>22,23</sup> patients with kidney disorders,<sup>24</sup> internal medicine patients,<sup>25</sup> and patients diagnosed as having cancer.<sup>26</sup> It is unclear whether in these conditions albumin levels are a marker of patients' nutritional status or chronic inflammatory state, which decreases the hepatic synthesis of albumin through the production of proinflammatory cytokines.<sup>26,27</sup>

Table 2. Discovery Cohort Multivariable Model in Men and Women<sup>a</sup>

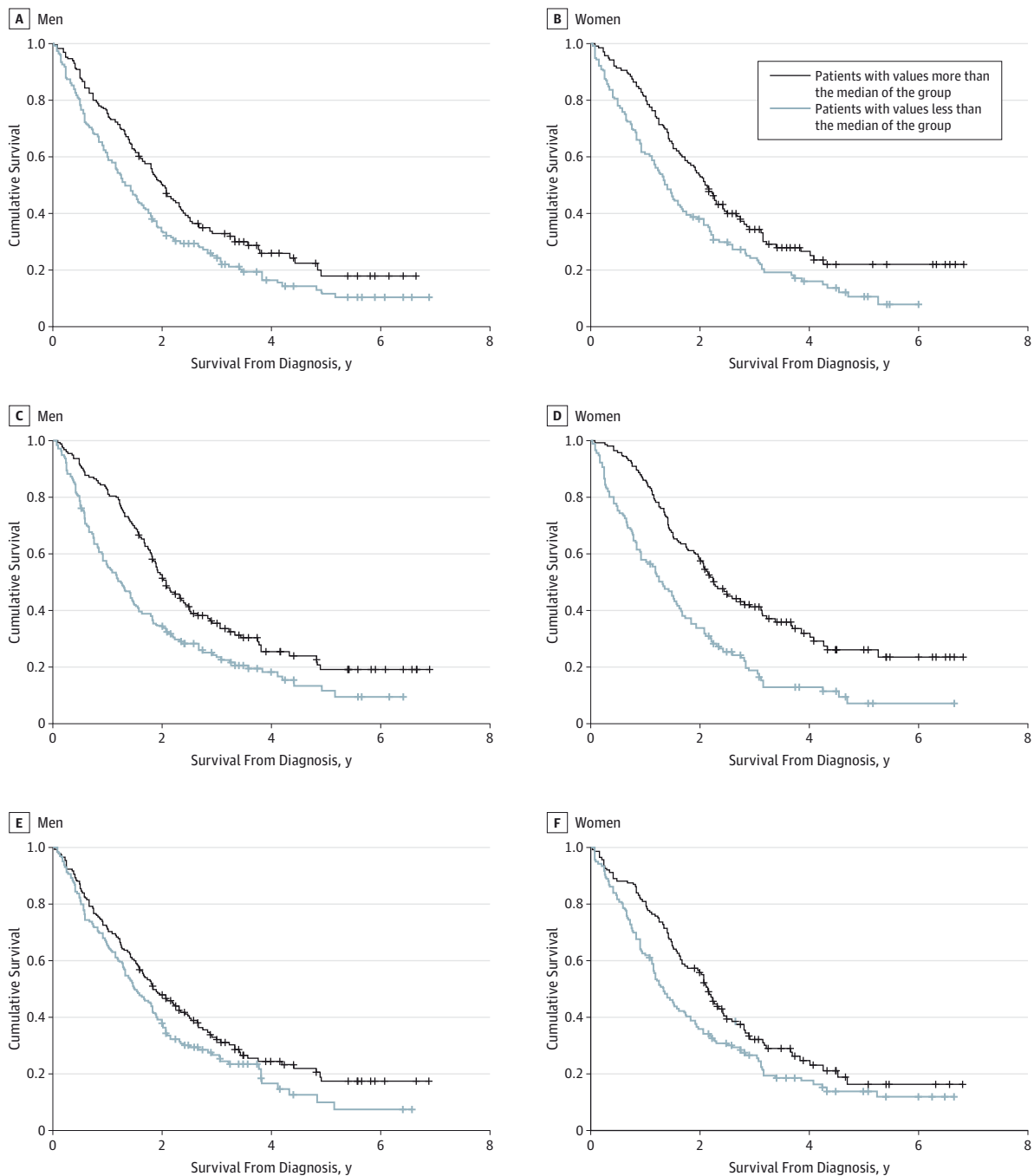
Variable	Hazard Ratio (95% CI)	P Value
<b>Men</b>		
Age, y		
<50	1 [Reference]	
50-59	1.49 (1.15-2.58)	
60-69	2.14 (1.22-3.75)	<.001
70-79	3.08 (1.69-5.61)	
≥80	4.57 (2.08-10.0)	
FVC		
>85	1 [Reference]	
≤85	1.91 (1.33-2.73)	<.001
ALSFRS-R bulbar score		
12	1 [Reference]	
<12	1.63 (1.23-2.15)	.001
ALSFRS-R respiratory score		
12	1 [Reference]	
<12	1.53 (1.15-2.05)	.004
Creatinine, mg/dL		
>0.82	1 [Reference]	
≤0.82	1.47 (1.11-1.95)	.007
Albumin, g/dL		
>4.3	1 [Reference]	
≤4.3	1.39 (1.05-1.90)	.02
<b>Women</b>		
Age, y		
<50	1 [Reference]	
50-59	1.30 (1.07-2.40)	
60-69	1.56 (1.15-2.86)	.01
70-79	2.13 (1.22-4.18)	
≥80	3.50 (1.53-8.00)	
FVC		
>0.85	1 [Reference]	
≤85	1.77 (1.25-2.49)	.001
ALSFRS R bulbar score		
12	1 [Reference]	
<12	1.75 (1.24-2.46)	.001
Creatinine, mg/dL		
>0.65	1 [Reference]	
≤0.65	1.49 (1.07-2.05)	.02
Albumin, mg/dL		
>4.3	1 [Reference]	
≤4.3	1.73 (1.25-2.39)	.001
ALSFRS-R total score		
≥39	1 [Reference]	
<39	1.40 (1.01-1.94)	.04

Abbreviations: ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; FVC, forced vital capacity.

SI conversion factors: To convert albumin to grams per liter, multiply by 10; to convert creatinine to micromoles per liter, multiply by 76.25.

<sup>a</sup> Hematological factors were included in the Cox model, stratified according to their median value. Enteral nutrition and noninvasive ventilation were included as time-dependent variables. A second model was performed replacing ALSFRS-R total score and its subscores with their decline rates (data not shown).

Figure 1. Discovery Cohort Kaplan-Meier Curves



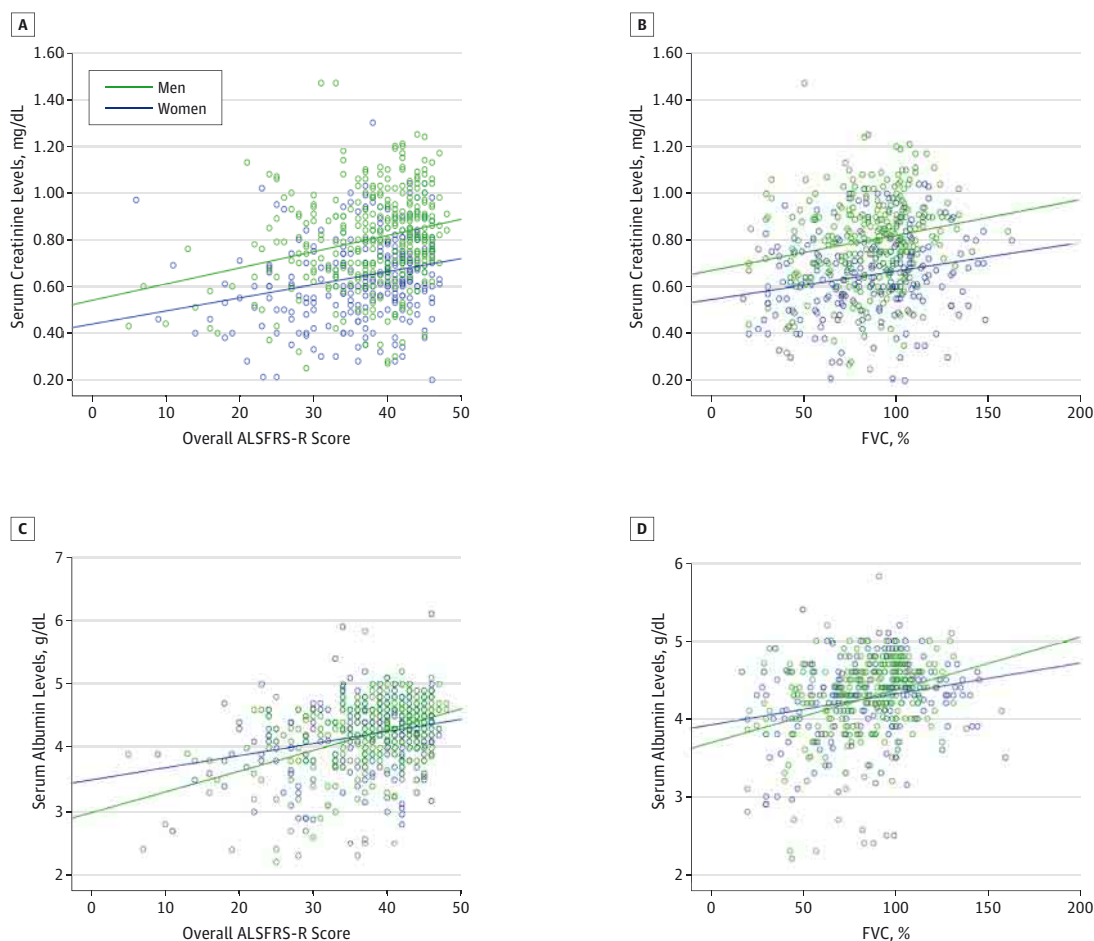
Survival was stratified by serum creatinine levels at diagnosis. A, Men:  $\leq 0.82$  vs  $> 0.82$  mg/dL ( $P = .004$ ). B, Women:  $\leq 0.65$  vs  $> 0.65$  mg/dL ( $P = .004$ ). Survival was stratified by serum albumin levels at diagnosis. C, Men:  $\leq 4.3$  vs  $> 4.3$  g/dL ( $P < .001$ ). D, Women:  $\leq 4.3$  vs  $> 4.3$  g/dL ( $P < .001$ ). Survival was stratified by

serum lymphocytes count at diagnosis. E, Men:  $\leq 1700$  vs  $> 1700$  / $\mu$ L ( $P = .04$ ). F, Women:  $\leq 1700$  vs  $> 1700$  / $\mu$ L ( $P = .02$ ). To convert lymphocytes to  $\times 10^9$ /L, multiply by 0.001; albumin to grams per liter, multiply by 10; and creatinine to micromoles per liter, multiply by 76.25.

In 2 studies, serum albumin decreased in patients with ALS compared with healthy control participants.<sup>28,29</sup> However, to our knowledge, this is the first study demonstrating that albumin levels, evaluated at the time of diagnosis, are a strong and independent marker of ALS outcome and strictly correlated with patients' clinical status. The lack of correlation of

serum albumin with BMI in both cohorts indicates that in ALS, albumin levels are poorly influenced by nutritional status. On the other hand, the significant correlation of serum albumin with leukocytes, granulocytes, and ESR indicates that the lowering of albumin levels in patients with ALS is probably caused by their inflammatory state. In both our cohorts, the increase

Figure 2. Discovery Cohort Associations



A, Correlation between Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) score and serum creatinine levels (men:  $r = 0.165$ ,  $y = 0.006x + 0.591$ ,  $P = .003$ ; women:  $r = 0.256$ ,  $y = 0.005x + 0.416$ ,  $P < .001$ ). B, Correlation between forced vital capacity (FVC) and serum creatinine levels (men:  $r = 0.24$ ,  $y = 0.002x + 0.622$ ,  $P = .003$ ; women:  $r = 0.151$ ,  $y = 0.001x + 0.544$ ,  $P < .001$ ). C, Correlation between ALSFRS-R score and serum albumin

levels (men:  $r = 0.465$ ,  $y = 0.033x + 2.961$ ,  $P < .001$ ; women:  $r = 0.324$ ,  $y = 0.019x + 3.505$ ,  $P < .001$ ). D, Correlation between FVC of predicted percentage and serum albumin levels (men:  $r = 0.379$ ,  $y = 0.007x + 3.633$ ,  $P < .001$ ; women:  $r = 0.268$ ,  $y = 0.004x + 3.902$ ,  $P < .001$ ). To convert albumin to grams per liter, multiply by 10 and creatinine to micromoles per liter, multiply by 76.25.

of leukocytes and granulocytes was significantly related to a low ALSFRS-R respiratory subscore and reduced FVC, suggesting that respiratory failure could be one of the causes of inflammatory state in ALS.

In a previous study, chronic inflammatory state was identified in 80 patients with ALS in whom wide-range C-reactive protein, ESR, and fibrinogen levels were significantly higher than in matched control participants; these parameters showed a significant correlation with ALSFRS-R scores, which persisted on sequential examinations.<sup>30</sup> In line with this observation, ALSFRS-R score was correlated with ESR and neutrophils count.

Serum creatinine is a product of nonenzymatic catabolism of creatine phosphate in muscles, produced at a fairly constant rate by the body (each day, 1%-2% of muscle creatine is converted to creatinine),<sup>31</sup> and is transported from muscle through the circulation to the kidneys.<sup>32</sup> Its levels depend on muscle mass; men tend to have a higher level than women be-

cause they generally have a greater muscle mass. In contrast to serum creatine kinase, creatinine levels are not modified by physical activity.<sup>33</sup>

It has been shown that creatinine levels are correlated with lean body mass in healthy individuals<sup>34</sup> and in adults and children with various diseases.<sup>31,33,35</sup> Serum creatinine has also been found to be a predictive factor of survival in patients with spinal and bulbar muscular atrophy.<sup>36</sup>

In both our cohorts, creatinine levels, which were independently related to ALS outcome, showed a significant correlation with ALSFRS-R score and with BMI, which are predictors of ALS prognosis.<sup>37-40</sup> Malnutrition, causing loss of weight and, in particular, muscle mass, represents a relevant event in the course of ALS. However, in our discovery cohort, which included patients enrolled at time of diagnosis, BMI was not a prognostic factor. On the other hand, in our validation cohort, serum creatinine was more strictly correlated with FFM than BMI. Fat-free mass consists of all tissues that are not body

fat and therefore is more representative of the loss of muscle mass in ALS.<sup>41</sup>

We did not find any other hematological factor to be independently related to ALS outcome at multivariable analysis. One of the most studied hematological factors in ALS with contrasting results is lipid status.<sup>5,6,8,29,42-44</sup> A pivotal study<sup>5</sup> found that higher LDL cholesterol to HDL cholesterol ratio was correlated with a better ALS prognosis. Conversely, in our discovery cohort, a lower LDL cholesterol to HDL cholesterol ratio was significantly related to better survival in women but was not retained in the multivariable analysis. Two studies have shown that both patients with ALS and their family members have a beneficial vascular risk profile (ie, a lower frequency of cardiovascular disorders) and that LDL cholesterol to HDL cholesterol ratio is not a significant prognostic factor when adjusted for known confounders.<sup>44,45</sup> In addition, cholesterol levels have been found to be inversely related to respiratory function, suggesting the increased use of cholesterol as an energetic nutrient in respiratory failure<sup>42</sup>; this finding is confirmed in eTable 5 in the Supplement.

The protective role of high levels of serum uric acid in neurodegenerative disorders is another area that has been explored following the demonstration that uric acid can have natural antioxidant properties in humans.<sup>46,47</sup> There are indications that high levels of serum uric acid are related to a better prognosis in Parkinson disease,<sup>48,49</sup> Huntington disease,<sup>50</sup> multiple system atrophy,<sup>51</sup> and mild cognitive impairment.<sup>52</sup> In 2 small series, uric acid levels were found to be lower in patients with ALS than in control participants and were correlated with ALSFRS-R score decline rate<sup>7</sup> or disease duration.<sup>53</sup> In other studies, serum uric acid was independently correlated with the decline of ALSFRS-R score and of FVC in both sexes<sup>8</sup> or only in male patients.<sup>43</sup> In both our series, serum uric acid was not prognostic of ALS outcome and showed no correlation with clinical status in either sex.

A strength of our discovery cohort is that it is highly representative of the general ALS population. Our cohort includes roughly 90% of the patients who were diagnosed as having ALS in the study period in Piemonte and Valle d'Aosta regions. Captured cases did not differ for any significant demographic or clinical parameters from noncaptured patients. Our validation cohort consisted of a consecutive series of patients at different clinical stages from a tertiary center, recruited for a study on lean body mass; these patients had longer disease duration and more severe disease than those of the discovery cohort; and as predicted from our findings, in the discovery cohort, patients had lower serum albumin and creatinine levels, suggesting the progressive decrease of these hematological factors during the course of the disease.

## Conclusions

In this study, we have shown that serum creatinine measured at diagnosis is an independent marker of ALS outcome because it reflects the state of muscle mass of the individual and is correlated with both the functional decline measured with ALSFRS-R score and FFM. Albumin levels are also independently related to ALS outcome, likely representing a marker of the inflammatory state rather than a marker of nutritional status. None of the other hematological factors examined were predictive of ALS outcome.

Both creatinine and albumin are reliable and easily detectable blood markers of the severity of motor dysfunction in ALS and could be used in defining patients' prognosis at the time of diagnosis. Longitudinal studies on the variations of serum albumin and creatinine levels and their relationships to clinical status will help determine whether and how these hematological factors vary during the progression of the disease.

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## REFERENCES

- Renton AE, Chiò A, Traynor BJ. State of play in amyotrophic lateral sclerosis genetics. *Nat Neurosci*. 2014;17(1):17-23.
- Chiò A, Logroscino G, Hardiman O, et al; Eurals Consortium. Prognostic factors in ALS: a critical review. *Amyotroph Lateral Scler*. 2009;10(5-6):310-323.
- Beghi E, Chiò A, Couratier P, et al; Eurals Consortium. The epidemiology and treatment of ALS: focus on the heterogeneity of the disease and critical appraisal of therapeutic trials. *Amyotroph Lateral Scler*. 2011;12(1):1-10.
- Chiò A, Calvo A, Moglia C, Mazzini L, Mora G; PARALS study group. Phenotypic heterogeneity of amyotrophic lateral sclerosis: a population based study. *J Neurol Neurosurg Psychiatry*. 2011;82(7):740-746.
- Dupuis L, Corcia P, Fergani A, et al. Dyslipidemia is a protective factor in amyotrophic lateral sclerosis. *Neurology*. 2008;70(13):1004-1009.
- Dorst J, Kühnlein P, Hendrich C, Kassubek J, Sperfeld AD, Ludolph AC. Patients with elevated triglyceride and cholesterol serum levels have a prolonged survival in amyotrophic lateral sclerosis. *J Neurol*. 2011;258(4):613-617.
- Keizman D, Ish-Shalom M, Berliner S, et al. Low uric acid levels in serum of patients with ALS: further evidence for oxidative stress? *J Neurol Sci*. 2009;285(1-2):95-99.
- Ikeda K, Hirayama T, Takazawa T, Kawabe K, Iwasaki Y. Relationships between disease progression and serum levels of lipid, urate, creatinine and ferritin in Japanese patients with amyotrophic lateral sclerosis: a cross-sectional study. *Intern Med*. 2012;51(12):1501-1508.
- Paillisse C, Lacomblez L, Dib M, Bensimon G, Garcia-Acosta S, Meininger V. Prognostic factors for survival in amyotrophic lateral sclerosis patients treated with riluzole. *Amyotroph Lateral Scler Other Motor Neuron Disord*. 2005;6(1):37-44.
- Chiò A, Mora G, Calvo A, Mazzini L, Bottacchi E, Mutani R; PARALS. Epidemiology of ALS in Italy: a 10-year prospective population-based study. *Neurology*. 2009;72(8):725-731.
- Brooks BR, Miller RG, Swash M, Munsat TL; World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord*. 2000;1(5):293-299.
- Cedarbaum JM, Stambler N, Malta E, et al; BDNF ALS Study Group (Phase III). The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. *J Neurol Sci*. 1999;169(1-2):13-21.
- Chumlea WC, Roche AF, Steinbaugh ML. Estimating stature from knee height for persons 60 to 90 years of age. *J Am Geriatr Soc*. 1985;33(2):116-120.
- World Health Organization. Global Database on Body Mass Index. <http://apps.who.int/bmi/index.jsp>. Published 2013. Accessed January 6, 2014.
- Chiò A, Calvo A, Mazzini L, et al; PARALS. Extensive genetics of ALS: a population-based study in Italy. *Neurology*. 2012;79(19):1983-1989.
- Desport JC, Preux PM, Bouteloup-Demange C, et al. Validation of bioelectrical impedance analysis in patients with amyotrophic lateral sclerosis. *Am J Clin Nutr*. 2003;77(5):1179-1185.
- Desport JC, Marin B, Funalot B, Preux PM, Couratier P. Phase angle is a prognostic factor for survival in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler*. 2008;9(5):273-278.
- Ha CE, Bhagavan NV. Novel insights into the pleiotropic effects of human serum albumin in health and disease. *Biochim Biophys Acta*. 2013;1830(12):5486-5493.
- Anraku M, Chuang VT, Maruyama T, Ottagiri M. Redox properties of serum albumin. *Biochim Biophys Acta*. 2013;1830(12):5465-5472.
- Friedman AN, Fadem SZ. Reassessment of albumin as a nutritional marker in kidney disease. *J Am Soc Nephrol*. 2010;21(2):223-230.
- Sacks GS, Dearman K, Replogle WH, Cora VL, Meeks M, Canada T. Use of subjective global assessment to identify nutrition-associated complications and death in geriatric long-term care facility residents. *J Am Coll Nutr*. 2000;19(5):570-577.
- Engelman DT, Adams DH, Byrne JG, et al. Impact of body mass index and albumin on morbidity and mortality after cardiac surgery. *J Thorac Cardiovasc Surg*. 1999;118(5):866-873.
- Gibbs J, Cull W, Henderson W, Daley J, Hur K, Khuri SF. Preoperative serum albumin level as a predictor of operative mortality and morbidity: results from the National VA Surgical Risk Study. *Arch Surg*. 1999;134(1):36-42.
- Obialo CI, Okonofua EC, Nzerue MC, Tayade AS, Riley LJ. Role of hypoalbuminemia and hypocholesterolemia as copredictors of mortality in acute renal failure. *Kidney Int*. 1999;56(3):1058-1063.
- Chima CS, Barco K, Dewitt ML, Maeda M, Teran JC, Mullen KD. Relationship of nutritional status to length of stay, hospital costs, and discharge status of patients hospitalized in the medicine service. *J Am Diet Assoc*. 1997;97(9):975-978, quiz 979-980.
- Gupta D, Lis CG. Pretreatment serum albumin as a predictor of cancer survival: a systematic review of the epidemiological literature. *Nutr J*. 2010;9:69.
- Barbosa-Silva MC. Subjective and objective nutritional assessment methods: what do they really assess? *Curr Opin Clin Nutr Metab Care*. 2008;11(3):248-254.
- Ghasemzadeh N, Nyberg F, Hjertén S. Highly selective artificial gel antibodies for detection and quantification of biomarkers in clinical samples: albumin in body fluids of patients with neurological disorders. *J Sep Sci*. 2008;31(22):3954-3958.
- Yang JW, Kim SM, Kim HJ, et al. Hypolipidemia in patients with amyotrophic lateral sclerosis: a possible gender difference? *J Clin Neurol*. 2013;9(2):125-129.
- Keizman D, Rogowski O, Berliner S, et al. Low-grade systemic inflammation in patients with amyotrophic lateral sclerosis. *Acta Neurol Scand*. 2009;119(6):383-389.
- Patel SS, Molnar MZ, Tayek JA, et al. Serum creatinine as a marker of muscle mass in chronic kidney disease: results of a cross-sectional study and review of literature. *J Cachexia Sarcopenia Muscle*. 2013;4(1):19-29.
- Viollet L, Gailey S, Thornton DJ, et al. Utility of cystatin C to monitor renal function in Duchenne muscular dystrophy. *Muscle Nerve*. 2009;40(3):438-442.
- Baxmann AC, Ahmed MS, Marques NC, et al. Influence of muscle mass and physical activity on



- serum and urinary creatinine and serum cystatin C. *Clin J Am Soc Nephrol*. 2008;3(2):348-354.
34. Swaminathan R, Ho CS, Chu LM, Donnan S. Relation between plasma creatinine and body size. *Clin Chem*. 1986;32(2):371-373.
35. Sala A, Tarnopolsky M, Webber C, Norman G, Barr R. Serum creatinine: a surrogate measurement of lean body mass in children with acute lymphoblastic leukemia. *Pediatr Blood Cancer*. 2005;45(1):16-19.
36. Hashizume A, Katsuno M, Banno H, et al. Longitudinal changes of outcome measures in spinal and bulbar muscular atrophy. *Brain*. 2012;135 (pt 9):2838-2848.
37. Desport JC, Preux PM, Truong TC, Vallat JM, Sautereau D, Couratier P. Nutritional status is a prognostic factor for survival in ALS patients. *Neurology*. 1999;53(5):1059-1063.
38. Chiò A, Mora G, Leone M, et al; Piemonte and Valle d'Aosta Register for ALS (PARALS). Early symptom progression rate is related to ALS outcome: a prospective population-based study. *Neurology*. 2002;59(1):99-103.
39. Kimura F, Fujimura C, Ishida S, et al. Progression rate of ALSFRS-R at time of diagnosis predicts survival time in ALS. *Neurology*. 2006;66 (2):265-267.
40. Reich-Slotky R, Andrews J, Cheng B, et al. Body mass index (BMI) as predictor of ALSFRS-R score decline in ALS patients. *Amyotroph Lateral Scler Frontotemporal Degener*. 2013;14(3):212-216.
41. Marin B, Desport JC, Kajeu P, et al. Alteration of nutritional status at diagnosis is a prognostic factor for survival of amyotrophic lateral sclerosis patients. *J Neurol Neurosurg Psychiatry*. 2011;82(6): 628-634.
42. Chiò A, Calvo A, Ilardi A, et al. Lower serum lipid levels are related to respiratory impairment in patients with ALS. *Neurology*. 2009;73(20):1681-1685.
43. Paganoni S, Zhang M, Quiroz Zárate A, et al. Uric acid levels predict survival in men with amyotrophic lateral sclerosis. *J Neurol*. 2012;259 (9):1923-1928.
44. Sutedja NA, van der Schouw YT, Fischer K, et al. Beneficial vascular risk profile is associated with amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry*. 2011;82(6):638-642.
45. Huisman MH, de Jong SW, Verwijs MC, et al. Family history of neurodegenerative and vascular diseases in ALS: a population-based study. *Neurology*. 2011;77(14):1363-1369.
46. Ames BN, Cathcart R, Schwiers E, Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. *Proc Natl Acad Sci U S A*. 1981;78(11):6858-6862.
47. Bowman GL, Shannon J, Frei B, Kaye JA, Quinn JF. Uric acid as a CNS antioxidant. *J Alzheimers Dis*. 2010;19(4):1331-1336.
48. Schwarzschild MA, Schwid SR, Marek K, et al; Parkinson Study Group PRECEPT Investigators. Serum urate as a predictor of clinical and radiographic progression in Parkinson disease. *Arch Neurol*. 2008;65(6):716-723.
49. Ascherio A, LeWitt PA, Xu K, et al; Parkinson Study Group DATATOP Investigators. Urate as a predictor of the rate of clinical decline in Parkinson disease. *Arch Neurol*. 2009;66(12):1460-1468.
50. Auinger P, Kiebertz K, McDermott MP. The relationship between uric acid levels and Huntington's disease progression. *Mov Disord*. 2010;25(2):224-228.
51. Lee JE, Song SK, Sohn YH, Lee PH. Uric acid as a potential disease modifier in patients with multiple system atrophy. *Mov Disord*. 2011;26(8):1533-1536.
52. Irizarry MC, Jin S, He F, et al. Incidence of new-onset seizures in mild to moderate Alzheimer disease. *Arch Neurol*. 2012;69(3):368-372.
53. Zoccollella S, Simone IL, Capozzo R, et al. An exploratory study of serum urate levels in patients with amyotrophic lateral sclerosis. *J Neurol*. 2011; 258(2):238-243.