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Serum C-Reactive Protein as a Prognostic Biomarker in Amyotrophic Lateral Sclerosis

Christian Lunetta, MD; Andrea Lizio, MS; Eleonora Maestri, BS; Valeria Ada Sansone, MD; Gabriele Mora, MD; Robert G. Miller, MD; Stanley H. Appel, MD; Adriano Chiò, MD

IMPORTANCE Various factors have been proposed as possible candidates associated with the prognosis of amyotrophic lateral sclerosis (ALS); however, there is still no consensus on which biomarkers are reliable prognostic factors. C-reactive protein (CRP) is a biomarker of the inflammatory response that shows significant prognostic value for several diseases.

OBJECTIVE To examine the prognostic significance of CRP in ALS.

DESIGN, SETTING, AND PARTICIPANTS Patients' serum CRP levels were evaluated from January 1, 2009, to June 30, 2015, in a large cohort of patients with ALS observed by an Italian tertiary multidisciplinary center. Results were replicated in an independent cohort obtained from a population-based registry of patients with ALS. A post hoc analysis was performed of the phase 2 trial of NP001 to determine whether stratification by levels of CRP improves differentiation of responders and nonresponders to the drug.

MAIN OUTCOMES AND MEASURES Serum CRP levels from the first examination were recorded to assess their effect on disease progression and survival.

RESULTS A total of 394 patients with ALS (168 women and 226 men; mean [SD] age at diagnosis, 60.18 [13.60] years) were observed in a tertiary multidisciplinary center, and the analysis was replicated in an independent cohort of 116 patients with ALS (50 women and 66 men; mean [SD] age at diagnosis, 67.00 [10.74] years) identified through a regional population-based registry. Serum CRP levels in the 394 patients with ALS correlated with severity of functional impairment, as measured by total score on the ALS Functional Rating Scale–Revised, at first evaluation (r = −0.14818; P = .004), and with patient survival (hazard ratio, 1.129; 95% CI, 1.033-1.234; P = .007). Similar results were found in the independent cohort (hazard ratio, 1.044; 95% CI, 1.016-1.056; $P \leq .001$). Moreover, a post hoc analysis of the phase 2 trial of NP001 using the same CRP threshold showed that patients with elevated baseline CRP levels receiving the higher dose of NP001 had significantly less functional impairment after the treatment period compared with patients with normal baseline CRP, regardless of whether patients with normal CRP levels received NP001 or placebo (3.00 [3.62] vs −7.31 [6.23]; P = .04).

CONCLUSIONS AND RELEVANCE These findings suggest that patients with ALS and elevated serum CRP levels progress more rapidly than do those with lower CRP levels and that this elevation may reflect a neuroinflammatory state potentially responsive to the immune regulators such as NP001.

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Supplemental content

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Christian Lunetta, MD, NeuroMuscular Omnicentre, Fondazione Serena Onlus, Piazza Ospedale Maggiore, 3, 20162 Milano, Italy (christian.lunetta@centrocliniconemo.it).

Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disorder with a fatal outcome and a mean survival time ranging from 2 to 5 years.[1] No treatment is currently available to modify the disease process of ALS. Age,[2,3] site of onset,[2] functional and respiratory status,[4-6] cognitive dysfunction,[7] clinical phenotypes,[8,9] serum triglyceride and cholesterol levels,[10-12] creatinine level,[3,13] albumin level,[13] and *C9orf72* expansion[14] have been proposed as possible candidates associated with the prognosis of ALS. However, there is still no consensus on which biomarkers are reliable prognostic factors in ALS.[15]

C-reactive protein (CRP) is an acute-phase protein regulated by proinflammatory cytokines and secreted by hepatocytes during the inflammatory response.[16] C-reactive protein is referred to as a pentraxin because of its capacity to aggregate, in a noncovalent fashion, into flat pentameric discs. The pentraxins are presumed to have great survival value and to be intimately associated with innate immune defense.[17,18] C-reactive protein is a biomarker of the inflammatory response with a significant prognostic value for several types of tumors,[19,20] cardiovascular diseases,[21] and rheumatic diseases.[22] Recently, Miller et al[23] presented the results of a randomized phase 2 clinical trial of a novel immune regulator, NP001, in ALS (NCT01281631). Although the results of the trial were negative, they showed that patients treated with NP001 whose baseline CRP levels were above the median for the entire randomized population had a slower progression of ALS than did patients with the same elevated baseline CRP levels who received placebo.

Previously, Keizman et al[24] found in a small group of patients with ALS a significant correlation between the clinical disability and some sensitive biomarkers of inflammation, including CRP. To fully examine the prognostic significance of CRP in ALS, we evaluated its serum levels at first evaluation in a large cohort of patients with ALS observed in an Italian tertiary multidisciplinary center. We replicated the results in an independent cohort from a population-based registry of patients with ALS. Finally, we performed a post hoc analysis of the phase 2 trial of NP001 to evaluate if CRP may contribute to the identification of responders to the drug.

Key Points

Question Is serum C-reactive protein a prognostic biomarker in amyotrophic lateral sclerosis (ALS)?

Findings In this cohort study of 394 patients with ALS, serum C-reactive protein levels at the first examination correlated with the degree of disability and with survival in patients with ALS. Moreover, a post hoc analysis of a phase 2 trial of NP001 showed that patients with elevated baseline serum C-reactive protein levels who received the higher dose of NP001 had significantly less functional impairment compared with other patients.

Meaning Patients with ALS and elevated serum C-reactive protein levels progress more rapidly than do those with lower levels; this elevation may reflect a neuroinflammatory state potentially responsive to immune regulators such as NP001.

Methods

Analysis of Prognostic Value of CRP

We retrospectively collected data from 394 patients who received a diagnosis of ALS based on a detailed history, physical examination, and electrophysiologic evaluation[25] and who were living in the Lombardy region and in other adjacent Italian regions at the time of their initial visit to the NeuroMuscular Omnicentre (NEMO), a tertiary multidisciplinary center in Milan, between January 1, 2009, and December 31, 2014. All patients met the revised El Escorial diagnostic criteria for definite, probable, and probable laboratory-supported ALS.[26] The ALS Functional Rating Scale–Revised (ALSFRS-R) was used to assess disease severity.[27] Assessments were repeated every 3 months until June 30, 2015. Patients with any clinical evidence of acute infection or chronic active inflammatory disease, such as rheumatoid arthritis, were excluded. The study design was approved by the institutional ethical committees of Niguarda Ca' Granda Hospital. Patients provided written informed consent.

To validate the results obtained in the NEMO cohort, we replicated the analysis in a cohort consisting of 122 patients with ALS at different stages of the disease who were identified through the Piemonte and Valle d'Aosta Register for ALS (PARALS)[28] and evaluated at the ALS Center of the "Rita Levi Montalcini" Department of Neuroscience and Azienda Ospedale Università, Città della Salute e della Scienza, Turin, Italy, between January 1, 2009, and December 31, 2009.

To determine the prognostic value of CRP, serum levels were evaluated at enrollment and correlated with the clinical demographics of patients with ALS, such as age at diagnosis, sex, duration of disease at time of evaluation, site of onset, ALSFRS-R total score, body mass index, smoking status, and survival. To standardize the threshold that defines higher and lower CRP levels within the 3 centers, we arbitrarily chose the median value (0.20 mg/dL [to convert to nanomoles per liter, multiply by 9.524]) obtained in the NEMO cohort to subdivide patients into the normal CRP group (CRP, ≤0.20 mg/dL) and the elevated CRP group (CRP, >0.20 mg/dL). This threshold was used for all subsequent evaluations.

Post Hoc Analysis of the Phase 2 Trial of NP001

The phase 2 trial of NP001 was a randomized, double-blind, placebo-controlled clinical trial of patients with probable or definite ALS according to El Escorial criteria conducted by Neuraltus Pharmaceuticals from January 2011 through November 2012 in the United States.[23] Patients were allocated in a 1:1:1 manner to receive 1 mg/kg of NP001, 2 mg/kg of NP001, and placebo. Patients received a total of 20 infusions for 6 cycles during a 25-week double-blind treatment period. For our study, Neuraltus Pharmaceuticals provided clinical data from the trial, including the ALSFRS-R scores measured every 4 weeks during the trial and for a further 3 months after the end of treatment. To determine the value of CRP in detecting responders to the treatment, we evaluated serum CRP levels at enrollment and then subdivided patients into a normal CRP group and elevated CRP group.

Table. Demographics and Clinical Characteristics of Patients With ALS in Both Cohorts at Inclusion

Characteristic	NEMO Cohort (n = 394)	Validation Cohort (n = 116)	*P* Value
Age at diagnosis, mean (SD), y	60.18 (13.60)	67.00 (10.74)	<.001
Sex, No. (%)			.93
Male	226 (57.4)	66 (56.9)	
Female	168 (42.6)	50 (43.1)	
Site of onset, No. (%)			.74
Bulbar	115 (29.2)	35 (30.2)	
Limb	279 (70.8)	81 (69.8)	
ALSFRS-R total score, mean (SD)	31.26 (10.08)	38.84 (8.21)	<.001
Disease duration, mean (SD), mo[a]	11.23 (9.32)	12.00 (11.28)	.46
Survival, mean (SD), mo[b]	21.66 (17.26)	31.72 (21.93)	<.001

Abbreviations: ALS, amyotrophic lateral sclerosis; ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised; NEMO, NeuroMuscular Omnicentre; PARALS, Piemonte and Valle d'Aosta Register for ALS.

[a] Interval between the onset and diagnosis.

[b] Interval between first evaluation of disease and death, tracheostomy, or censoring date.

Statistical Analysis

Statistical analyses were performed with SAS version 9.3 (SAS Institute Inc). Data are reported as mean (SD) values for continuous variables and numbers for noncontinuous variables. For each variable, we used the Shapiro-Wilk test to evaluate the normality of the distribution and the Levene test to evaluate the homogeneity of variance. We used 2-tailed *t* tests and nonparametric Wilcoxon rank-sum tests as appropriate. For dichotomous variables, we used the χ^2 test. $P < .05$ was considered statistically significant with Bonferroni adjustment for all tests. Correlation analysis was performed using the Pearson correlation coefficient for variables with normal distribution, the nonparametric Spearman rank correlation coefficient for the others, and the multivariable regression model adjusted for age, sex, body mass index, and smoking status (with $\alpha < .05$ considered significant).

To determine whether the serum CRP level was independently associated with survival, we used a multivariable Cox proportional hazards regression model and Kaplan-Meier analysis. To validate the prognostic value of the serum CRP level, we applied the same analyses to the validation cohort.

For the post hoc NP001 analysis, the primary end point was the change in ALSFRS-R score from baseline through the end of the treatment period and follow-up. To analyze the effect of NP001 (both the 1- and 2-mg/kg dose) or placebo, each group was divided into normal CRP and elevated CRP subgroups, and the nonparametric Wilcoxon rank-sum test was used.

Results

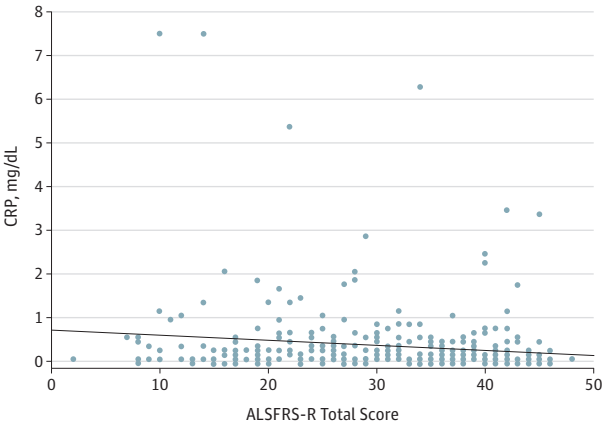
Analysis of CRP Prognostic Value

Baseline characteristics of the NEMO cohort and the PARALS cohort are summarized in the **Table**. Patients in the NEMO cohort were younger (mean [SD] age at diagnosis, 60.18 [13.60] years vs 67.00 [10.74] years; $P < .001$), with a lower mean (SD) ALSFRS-R score at baseline (31.26 [10.08] vs 38.84 [8.21]) and a shorter mean (SD) survival time (21.66 [17.26] months vs 31.72 [21.93] months) compared with those in the PARALS cohort.

In the NEMO cohort, CRP was not correlated with age at diagnosis ($r = 0.06812$; $P = .19$), sex ($r = 0.00794$; $P = .65$), disease duration at time of evaluation ($r = 0.01154$; $P = .97$), or site of onset ($r = 0.04776$; $P = .19$). The CRP level was inversely correlated with the ALSFRS-R total score ($r = -0.14818$; $P = .004$), and this correlation remained significant even when adjusted for age, sex, body mass index, and smoking status (standardized β, -0.13499; $P = .04$) (**Figure 1**). In the multivariable Cox proportional hazards regression model and Kaplan-Meier analysis, the elevated CRP group had a significantly shorter survival compared with the normal CRP group (hazard ratio, 1.129; 95% CI, 1.033-1.234; $P = .007$; 22.94 [16.97] vs 19.79 [17.57] months; $P = .02$) (eTable 1 in the Supplement; **Figure 2**). The PARALS cohort confirmed that patients with ALS and elevated CRP levels had shorter survival compared with other patients (hazard ratio, 1.044; 95% CI, 1.016-1.056; $P \leq .001$; 37.03 [22.89] vs 29.52 [21.27] months; $P = .04$) (eTable 1 in the Supplement; Figure 2).

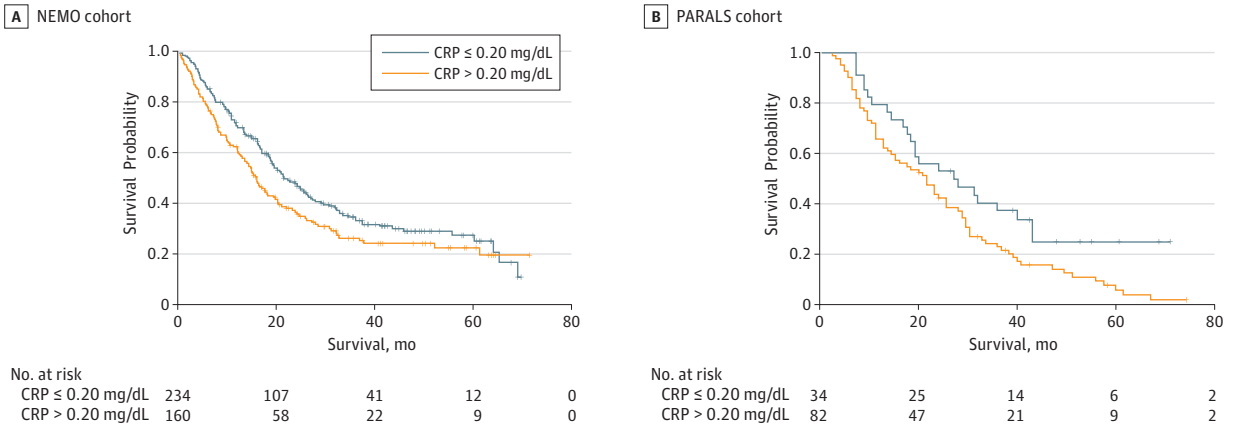
To evaluate whether the changes in CRP level over time were correlated with the changes in the ALSFRS-R score, we reviewed the available records of a group of 50 patients in the NEMO cohort who were evaluated in 3 consecutive follow-up visits during 1 year of observation. We correlated the slope of the ALSFRS-R score with the slope of serum CRP levels and found that this correlation was significant ($r = -0.37811$; $P < .001$) even when adjusted for age, sex, body mass index, and smoking status (eFigure in the Supplement).

Figure 1. Serum C-Reactive Protein (CRP) Levels and Disease Progression



Correlation between Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R) score and serum CRP levels ($r = 0.14818$; $P = .04$) (to convert to nanomoles per liter, multiply by 9.524).

Figure 2. Serum C-Reactive Protein (CRP) Levels and Survival



A, Survival in the NeuroMuscular Omnicentre (NEMO) cohort in the normal CRP group (CRP, ≤0.20 mg/dL [to convert to nanomoles per liter, multiply by 9.524]) and elevated CRP group (CRP, >0.20 mg/dL) at first evaluation (P = .03). B, Survival in the Piemonte and Valle d'Aosta Register for ALS (PARALS) cohort in the normal CRP group and elevated CRP group at first evaluation (P = .03).

Post Hoc Analysis of the Phase 2 Trial of NP001

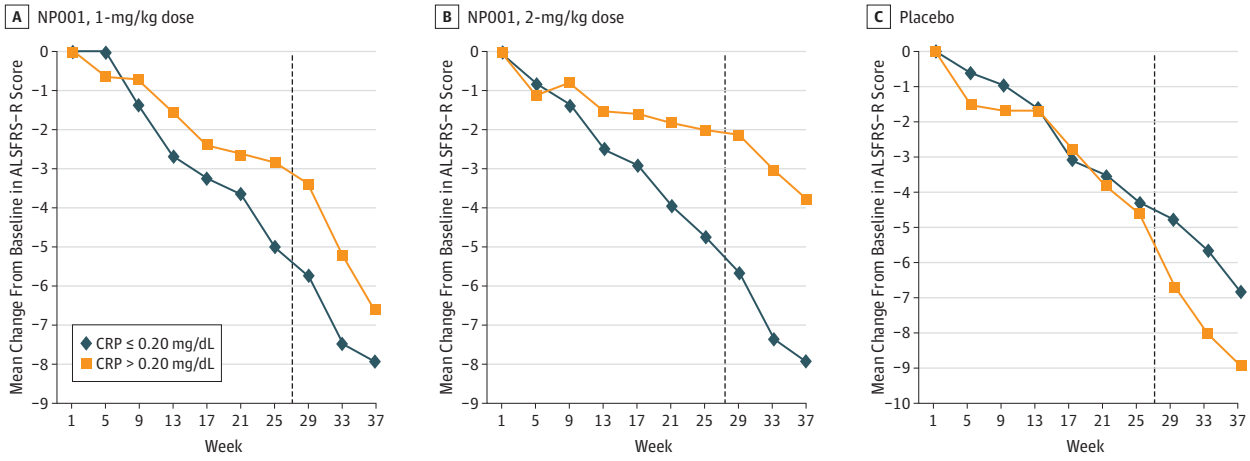
The post hoc analysis included data from 113 patients, excluding 23 patients with missing information about disease progression after the end of the treatment period. eTable 2 in the Supplement summarizes the demographic and clinical characteristics at baseline of patients in each treatment group (1 mg/kg of NP001, 2 mg/kg of NP001, and placebo). Baseline median CRP levels were similar between groups (1.41 mg/L for the patients who received 1 mg/kg of NP001, 0.96 mg/L for the patients who received 2 mg/kg of NP001, and 1.03 mg/dL for the patients who received placebo). There were no significant clinical differences at baseline between groups, except for mean (SD) disease duration, which was higher in the group receiving 1 mg/kg of NP001 than in the other 2 groups (22.52 [9.40] months for the patients who received 1 mg/kg of NP001, 17.23 [8.36] months for the patients who received 2 mg/kg of NP001, and 16.85 [8.26] months for the patients who received placebo; P = .007). In line with previous analyses, NP001 in both dose groups did not have a statistically significant effect on reducing ALS progression compared with placebo. However, when we subdivided each treatment group according to CRP level at enrollment (normal CRP group and elevated CRP group), we found that for patients treated with the higher dose of NP001 (2 mg/kg), the decrease in the mean ALSFRS-R score in the elevated CRP group was less than half the decrease in the normal CRP group for all periods following the end of treatment (weeks 25, 29, 33, and 37) (eTable 3 in the Supplement; **Figure 3**). One evaluation point, week 33, showed a statistically significant difference in the decrease in mean ALSFRS-R score among the group receiving 2 mg/kg of NP001 (normal CRP level, -7.31 mg/L; elevated CRP level, -3.00 mg/L; P = .045). Moreover, using the same subgroups to compare the NP001 arms with placebo, we found that patients in the elevated CRP group receiving the higher dose of NP001 had significantly less functional impairment (eTable 4 in the Supplement; **Figure 4**). Again, after the end of treatment, patients with elevated serum CRP levels who were receiving 2 mg/kg of NP001 had statistically significant decreases in ALSFRS-R score of less than half the decrease of patients receiving placebo at weeks 29 (-2.1 vs -6.7; P = .01), 33 (-3.0 vs -8.0; P = .03), and 37 (-3.7 vs -9.0; P = .03). More important, there appeared to be a dose-response association in patients with elevated serum CRP levels and no discernible difference between the NP001 and placebo arms in patients with normal baseline serum CRP levels (Figure 4).

Discussion

We have analyzed the prognostic significance of CRP in ALS, evaluating its serum levels at first evaluation in a large cohort of patients with ALS in an early phase of the disease (as expressed by a disease duration <20 months from first onset of symptoms) without an active inflammatory process and observed in an Italian tertiary multidisciplinary center. In our analysis, CRP level was not correlated with age at diagnosis, sex, disease duration, or site of onset of ALS. Serum CRP levels were correlated with the severity of functional impairment, as measured by ALSFRS-R total score; this correlation was independent from patients' age, sex, body mass index, and smoking status. Moreover, evaluating a group of 50 patients with ALS in a year of follow-up visits, we found a significant negative correlation between the slope of the ALSFRS-R score and the slope of serum CRP levels, emphasizing the significance of the serum CRP level as a useful, feasible, and potentially prognostic factor in patients with ALS.

Moreover, the serum CRP level was significantly correlated with patient survival, showing that patients with ALS and elevated serum CRP levels at first evaluation had a significantly shorter survival compared with those with normal serum CRP levels. Similar results were found in an independent cohort obtained from a population-based registry of patients with ALS. In particular, according to the hazard ratio evaluated in discovery and validation cohorts, for every 1-point in-

Figure 3. Role of Serum C-Reactive Protein (CRP) Levels as Therapeutic Biomarker



A, Mean change from baseline in Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R) score in patients receiving NP001, 1-mg/kg dose, in the normal CRP group (CRP, ≤0.20 mg/dL [to convert to nanomoles per liter, multiply by 9.524]) and elevated CRP group (CRP, >0.20 mg/dL) at first evaluation (P = .19). B, Mean change from baseline in ALSFRS-R score in patients receiving NP001, 2-mg/kg dose, in the normal CRP group and elevated CRP group at first evaluation (P = .045). C, Mean change from baseline in ALSFRS-R score in patients receiving placebo in the normal CRP group and elevated CRP group at first evaluation (P = .70). The vertical line indicates the end of the treatment period.

crease in the serum CRP level, we detected an effect of 13% (hazard ratio, 1.129; 95% CI, 1.033-1.234) and 4% (hazard ratio, 1.044; 95% CI, 1.016-1.056), respectively, on survival time in the patients with ALS evaluated in our study (eTable 1 in the Supplement). These data suggest that serum CRP levels may be useful as a prognostic biomarker in patients with ALS.

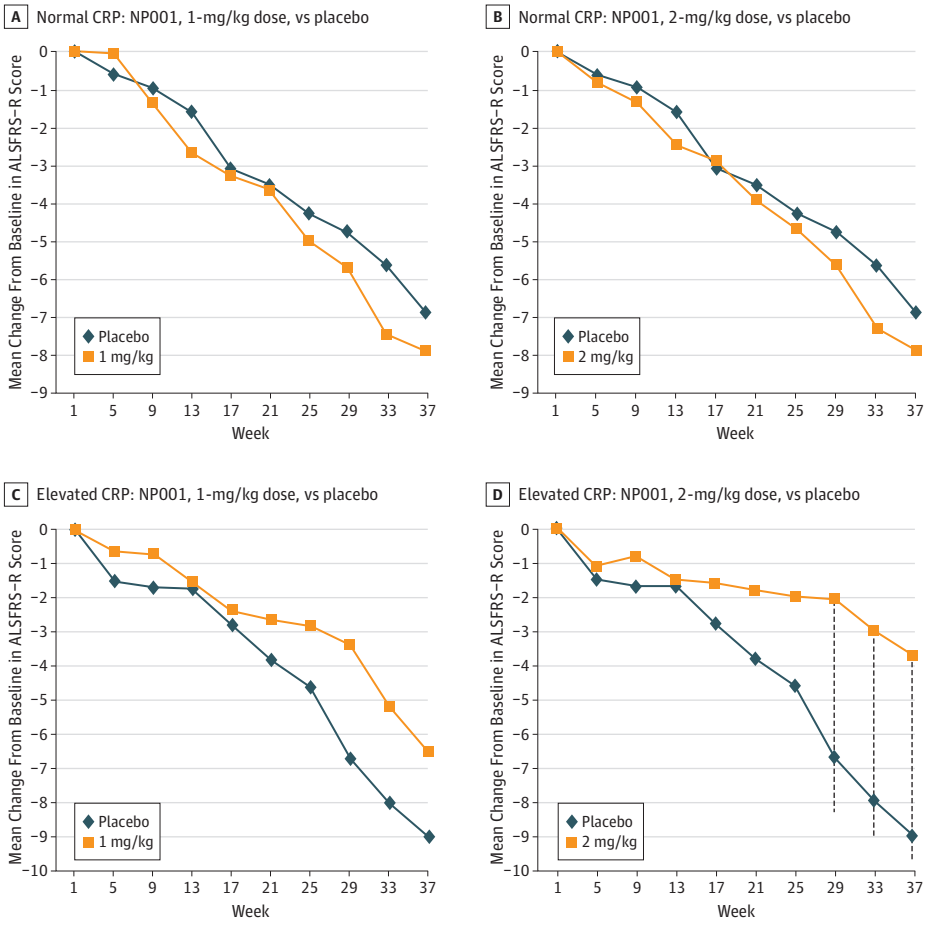
Both acute and chronic inflammation stimulates the release of proinflammatory cytokines, mainly IL-6, into the bloodstream. The liver responds to this release by producing acute-phase reactants such as CRP, which is the most commonly used marker of an acute-phase reaction and was first discovered in the serum of patients with pneumococcal pneumonia.[29] Thus, our study supports the importance of inflammation in ALS and that CRP may represent a simple biomarker obtainable from blood samples from each patient independent of his or her clinical condition. Some studies also showed an increment of CRP in the cerebrospinal fluid of patients with ALS, emphasizing the significance of neuroinflammation in the disease.[30-32]

C-reactive protein is an in vivo activator of complement.[33] The complement pathway has been postulated to contribute to motor neuron disease, and levels of complement proteins, including C3, have been shown to be increased in the cerebrospinal fluid and spinal cord tissue of patients with ALS.[34-36] Therefore, CRP may contribute to the activation of the complement pathway in motor neuron disease. Activation of inflammatory and complement pathways is not specific to ALS or other neurodegenerative diseases. However, in cerebrospinal fluid, the phosphorylated neurofilament heavy chain to CRP ratio showed significant differences in ALS compared with both disease controls and healthy control groups, suggesting that inclusion of general inflammatory responses allows more specificity in identifying ALS.[37] High CRP levels increase permeability of the blood-brain barrier[38] and elicit microglial activation in the brain.[39] Results of animal studies showed that systemic inflammation contributes to the neurodegenerative process through microglial activation.[32,40,41]

It had been generally believed for many years that CRP was produced only in the liver and carried in the circulation to other organs. Molecular genetic techniques have demonstrated that CRP can be produced locally in the brain and that its production of CRP is sharply upregulated in areas damaged by neurodegenerative processes, as in Alzheimer disease.[42] Neurons are the most prominent generators of CRP in the central nervous system.[42,43] In Alzheimer disease, CRP is associated with damaged fibers within senile plaques.[44] In our study, the significant correlation of serum CRP level with neurologic functional impairment and survival in patients with ALS in the early phase of the disease and without an active inflammatory process supports the hypothesis that the increment of CRP in the peripheral blood may be the mirror of the upregulation of the production of CRP in the central nervous system. Recently, Lu et al[45] showed that the cytokine IL-6 was strongly associated with CRP levels and was the only marker showing increasing expression toward end-stage disease in the longitudinal analysis. In a retrospective analysis, patients with Parkinson disease and elevated CRP levels at baseline had a significantly shortened survival compared with those with normal CRP levels.[46] Moreover, CRP has been associated with severity of functional impairment in other neuromuscular disorders. In particular, in patients with Duchenne muscular dystrophy, high levels of CRP (mean, 3.94 md/dL) were associated with poor functional impairment and obesity.[47] In another study, CRP was significantly higher (mean, 0.99 mg/dL) in patients with type 2 diabetes and peripheral neuropathy compared with those with type 2 diabetes without peripheral neuropathy (mean, 0.25 mg/dL).[48]

In our study, we also used the serum CRP level as a biomarker to better stratify responders or nonresponders to treat-

Figure 4. Role of Serum C-Reactive Protein (CRP) Levels to Detect ALS Patients Possibly Responsive to Treatment



A, Mean change from baseline in Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R) score in patients in the normal CRP group (CRP, ≤0.20 mg/dL [to convert to nanomoles per liter, multiply by 9.524]) receiving NP001, 1-mg/kg dose, vs placebo. B, Mean change from baseline in ALSFRS-R score in patients in the normal CRP group receiving NP001, 2-mg/kg dose, vs placebo. C, Mean change from baseline in ALSFRS-R score in patients in the elevated CRP group (CRP, >0.20 mg/dL) receiving NP001, 1-mg/kg dose, vs placebo. D, Mean change from baseline in ALSFRS-R score in patients in the elevated CRP group receiving NP001, 2 mg/kg/dose, vs placebo. Patients in the elevated CRP group receiving the higher dose of NP001 had significantly less functional impairment at weeks 29 (P = .01), 33 (P = .03), and 37 (P = .03).

ment with NP001, a pH-adjusted intravenous formulation of purified sodium chlorite that regulates inflammation in vitro and in vivo.[49] NP001 is a novel regulator of inflammatory macrophages and monocytes that downregulates nuclear factor kB expression and inhibits production of the proinflammatory cytokine IL-1b.[24,50-53] These mechanisms of downregulation transform monocytes and macrophages from a proinflammatory state to a basal phagocytic state. The randomized phase 2 clinical trial showed that NP001 did not significantly slow disease progression in patients with ALS. However, the results of the study suggested a slowing of progression in the high-dose group among patients with elevated levels of inflammation. In our study, we performed a post hoc analysis of the phase 2 trial of NP001, which showed that, in the group of patients treated with the higher dose of NP001, the worsening of functional impairment after the end of the treatment was significantly less in patients with elevated CRP levels compared with those with normal CRP levels. When compared with the patients in the placebo group, patients with elevated CRP levels at baseline showed a significant NP001 dose-dependent slowing in loss of function as measured by change from baseline ALSFRS-R scores.

Moreover, our study emphasizes the importance of developing treatments to control disease processes that increase levels of CRP or pentraxin activity. Because pentraxin activity is associated with activation of the complement cascade, immunomodulatory agents such as NP001 might mitigate complications of an autotoxic attack thought to be associated with neurodegenerative diseases.[42]

Limitations

Although our results are encouraging, there are some limitations to the study. First, the number of patients included was limited, in particular, in the group of patients with consecutive follow-up visits. Second, we did not correlate the serum CRP level with other peripheral neuroinflammatory biomarkers or with the cerebrospinal fluid CRP levels.

Conclusions

Our findings confirm that CRP may be used both as a prognostic factor and a biomarker to stratify patients with ALS who have a more prominent neuroinflammatory process that may re-

spond to targeted treatments. However, further analyses in larger cohorts of patients with ALS, as well as studies using longitudinal samples to detect how CRP levels really reflect the rate of progression and disease states, are needed to justify its use as a prognostic peripheral biomarker for patients with ALS with a significant central nervous system inflammatory process. In this context, these studies may be useful to detect how the CRP levels change over time in patients with ALS who have higher initial CRP levels and more rapid progression, or in patients with lower initial CRP levels.

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Author Affiliations: NeuroMuscular Omnicentre, Fondazione Serena Onlus, Milano, Italy (Lunetta, Lizio, Maestri, Sansone); Department of Biomedical Sciences for Health, University of Milan, Milan, Italy (Sansone); Department of Neurological Rehabilitation, Fondazione Salvatore Maugeri, Istituto di Ricovero e Cura a Carattere Scientifico, Istituto Scientifico di Milano, Milano, Italy (Mora); Forbes Norris MDA/ALS Research and Treatment Center, California Pacific Medical Center, San Francisco, California (Miller); Peggy and Gary Edwards ALS Laboratory, Department of Neurology, Houston Methodist Neurological Institute, Houston, Texas (Appel); Houston Methodist Research Institute, Houston, Texas (Appel); Department of Neurology, Methodist Neurological Institute, Houston Methodist Hospital, Houston, Texas (Appel); Amyotrophic Lateral Sclerosis Center, "Rita Levi Montalcini" Department of Neuroscience, Neurology II, University of Torino, Turin, Italy (Chiò); Azienda Ospedaliero-Universitaria Città della Salute e della Scienza, Torino, Italy (Chiò).

Author Contributions: Dr Lunetta had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Lunetta, Miller, Chiò.
Acquisition, analysis, or interpretation of data: Lunetta, Lizio, Maestri, Sansone, Mora, Appel, Chiò.
Drafting of the manuscript: Lunetta.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Lizio.
Administrative, technical, or material support: Lizio, Maestri.
Study supervision: Lunetta, Chiò.

Conflict of Interest Disclosures: Dr Lunetta reported serving on a scientific advisory board for Neuraltus and Italfarmaco and receiving research support from Agenzia Italiana per la Ricerca sulla SLA (ARISLA). Dr Mora reported receiving research support from the Italian Ministry of Health (Ricerca Finalizzata) and ARISLA. Dr Miller reported receiving research support as a principal investigator on a clinical trial from Neuraltus. Dr Appel reported serving as a member of the Neuraltus Scientific Advisory Board. Dr Chiò reported serving on scientific advisory boards for Biogen Idec, Cytokinetcs, Neuraltus, Italfarmaco, and Mitsubishi Tanabe. No other disclosures were reported.

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REFERENCES

1. Qureshi M, Schoenfeld DA, Paliwal Y, Shui A, Cudkowicz ME. The natural history of ALS is changing: improved survival. *Amyotroph Lateral Scler*. 2009;10(5-6):324-331.

2. 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.

3. Lunetta C, Lizio A, Melazzini MG, Maestri E, Sansone VA. Amyotrophic Lateral Sclerosis Survival Score (ALS-SS): a simple scoring system for early prediction of patient survival. *Amyotroph Lateral Scler Frontotemporal Degener*. 2015;17(1-2):93-100.

4. Watanabe H, Atsuta N, Nakamura R, et al. Factors affecting longitudinal functional decline and survival in amyotrophic lateral sclerosis patients. *Amyotroph Lateral Scler Frontotemporal Degener*. 2015;16(3-4):230-236.

5. Kollewe K, Mauss U, Krampfl K, Petri S, Dengler R, Mohammadi B. ALSFRS-R score and its ratio: a useful predictor for ALS-progression. *J Neurol Sci*. 2008;275(1-2):69-73.

6. Gordon PH, Salachas F, Lacomblez L, et al. Predicting survival of patients with amyotrophic lateral sclerosis at presentation: a 15-year experience. *Neurodegener Dis*. 2013;12(2):81-90.

7. Elamin M, Bede P, Montuschi A, Pender N, Chio A, Hardiman O. Predicting prognosis in amyotrophic lateral sclerosis: a simple algorithm. *J Neurol*. 2015;262(6):1447-1454.

8. Wolf J, Safer A, Wöhrle JC, et al. Variability and prognostic relevance of different phenotypes in amyotrophic lateral sclerosis—data from a population-based registry. *J Neurol Sci*. 2014;345(1-2):164-167.

9. 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.

10. 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.

11. Dupuis L, Corcia P, Fergani A, et al. Dyslipidemia is a protective factor in amyotrophic lateral sclerosis. *Neurology*. 2008;70(13):1004-1009.

12. 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.

13. Chiò A, Calvo A, Bovio G, et al; Piemonte and Valle d'Aosta Register for Amyotrophic Lateral Sclerosis. Amyotrophic lateral sclerosis outcome measures and the role of albumin and creatinine: a population-based study. *JAMA Neurol*. 2014;71(9):1134-1142.

14. Sabatelli M, Conforti FL, Zollino M, et al; ITALSGEN Consortium. *C9ORF72* hexanucleotide repeat expansions in the Italian sporadic ALS population. *Neurobiol Aging*. 2012;33(8):1848.e15-1848.e20.

15. Creemers H, Grupstra H, Nollet F, van den Berg LH, Beelen A. Prognostic factors for the course of functional status of patients with ALS: a systematic review. *J Neurol*. 2015;262(6):1407-1423.

16. Castell JV, Gómez-Lechón MJ, David M, Fabra R, Trullenque R, Heinrich PC. Acute-phase response of human hepatocytes: regulation of acute-phase protein synthesis by interleukin-6. *Hepatology*. 1990;12(5):1179-1186.

17. Gewurz H, Zhang XH, Lint TF. Structure and function of the pentraxins. *Curr Opin Immunol*. 1995;7(1):54-64.

18. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med*. 1999;340(6):448-454.

19. Li YJ, Li ZM, Xia Y, et al. Serum C-reactive protein (CRP) as a simple and independent prognostic factor in extranodal natural killer/T-cell lymphoma, nasal type. *PLoS One*. 2013;8(5):e64158.

20. de Martino M, Klatte T, Seemann C, et al. Validation of serum C-reactive protein (CRP) as an independent prognostic factor for disease-free survival in patients with localised renal cell carcinoma (RCC). *BJU Int*. 2013;111(8):E348-E353.

21. Roubille F, Cayla G, Picot MC, et al. C-reactive protein (CRP) after revascularized STEMI: is CRP a prognostic factor? [in French] *Rev Med Interne*. 2008;29(11):868-874.

22. Schreiber S, Buyse M. The CRP initial response to treatment as prognostic factor in patients with polymyalgia rheumatica. *Clin Rheumatol*. 1995;14(3):315-318.

23. Miller RG, Block G, Katz JS, et al; Phase 2 Trial NP001 Investigators. Randomized phase 2 trial of NP001—a novel immune regulator: safety and early efficacy in ALS. *Neurol Neuroimmunol Neuroinflamm*. 2015;2(3):e100.

24. 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.

25. Andersen PM, Abrahams S, Borasio GD, et al; EFNS Task Force on Diagnosis and Management of Amyotrophic Lateral Sclerosis. EFNS guidelines on the clinical management of amyotrophic lateral sclerosis (MALS)—revised report of an EFNS task force. *Eur J Neurol*. 2012;19(3):360-375.

26. 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.

27. 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.

28. 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.

29. Tillett WS, Francis T. Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus. *J Exp Med*. 1930;52(4):561-571.

30. Ryberg H, An J, Darko S, et al. Discovery and verification of amyotrophic lateral sclerosis biomarkers by proteomics. *Muscle Nerve*. 2010;42 (1):104-111.

31. Mizwicki MT, Fiala M, Magpantay L, et al. Tocilizumab attenuates inflammation in ALS patients through inhibition of IL6 receptor signaling. *Am J Neurodegener Dis*. 2012;1(3): 305-315.

32. Hooten KG, Beers DR, Zhao W, Appel SH. Protective and toxic neuroinflammation in amyotrophic lateral sclerosis. *Neurotherapeutics*. 2015;12(2):364-375.

33. Wolbink GJ, Brouwer MC, Buysmann S, ten Berge IJ, Hack CE. CRP-mediated activation of complement in vivo: assessment by measuring circulating complement–C-reactive protein complexes. *J Immunol*. 1996;157(1):473-479.

34. Annunziata P, Volpi N. High levels of C3c in the cerebrospinal fluid from amyotrophic lateral sclerosis patients. *Acta Neurol Scand*. 1985;72(1): 61-64.

35. Goldknopf IL, Sheta EA, Bryson J, et al. Complement C3c and related protein biomarkers in amyotrophic lateral sclerosis and Parkinson's disease. *Biochem Biophys Res Commun*. 2006;342 (4):1034-1039.

36. Woodruff TM, Costantini KJ, Crane JW, et al. The complement factor C5a contributes to pathology in a rat model of amyotrophic lateral sclerosis. *J Immunol*. 2008;181(12):8727-8734.

37. Ganesalingam J, An J, Shaw CE, Shaw G, Lacomis D, Bowser R. Combination of neurofilament heavy chain and complement C3 as CSF biomarkers for ALS. *J Neurochem*. 2011;117 (3):528-537.

38. Closhen D, Bender B, Luhmann HJ, Kuhlmann CR. CRP-induced levels of oxidative stress are higher in brain than aortic endothelial cells. *Cytokine*. 2010;50(2):117-120.

39. Hsuchou H, Kastin AJ, Mishra PK, Pan W. C-reactive protein increases BBB permeability: implications for obesity and neuroinflammation. *Cell Physiol Biochem*. 2012;30(5):1109-1119.

40. Cerejeira J, Firmino H, Vaz-Serra A, Mukaetova-Ladinska EB. The neuroinflammatory hypothesis of delirium. *Acta Neuropathol*. 2010;119 (6):737-754.

41. Lunnon K, Teeling JL, Tutt AL, Cragg MS, Glennie MJ, Perry VH. Systemic inflammation modulates Fc receptor expression on microglia during chronic neurodegeneration. *J Immunol*. 2011; 186(12):7215-7224.

42. Yasojima K, Schwab C, McGeer EG, McGeer PL. Human neurons generate C-reactive protein and amyloid P: upregulation in Alzheimer's disease. *Brain Res*. 2000;887(1):80-89.

43. Mancinella A, Mancinella M, Carpinteri G, et al. Is there a relationship between high C-reactive protein (CRP) levels and dementia? *Arch Gerontol Geriatr*. 2009;49(suppl 1):185-194.

44. Akiyama H, Yamada T, Kawamata T, McGeer PL. Association of amyloid P component with complement proteins in neurologically diseased brain tissue. *Brain Res*. 1991;548(1-2): 349-352.

45. Lu CH, Allen K, Oei F, et al. Systemic inflammatory response and neuromuscular involvement in amyotrophic lateral sclerosis. *Neurol Neuroimmunol Neuroinflamm*. 2016;3(4):e244.

46. Sawada H, Oeda T, Umemura A, et al. Baseline C-reactive protein levels and life prognosis in Parkinson disease. *PLoS One*. 2015;10(7):e0134118.

47. Cruz-Guzmán OdelR, Rodríguez-Cruz M, Escobar Cedillo RE. Systemic inflammation in Duchenne muscular dystrophy: association with muscle function and nutritional status. *Biomed Res Int*. 2015;2015:891972.

48. Papanas N, Katsiki N, Papatheodorou K, et al. Peripheral neuropathy is associated with increased serum levels of uric acid in type 2 diabetes mellitus. *Angiology*. 2011;62(4):291-295.

49. Miller RG, Zhang R, Block G, et al. NP001 regulation of macrophage activation markers in ALS: a phase I clinical and biomarker study. *Amyotroph Lateral Scler Frontotemporal Degener*. 2014;15(7-8):601-609.

50. Marcinkiewicz J, Grabowska A, Bereta J, Stelmaszynska T. Taurine chloramine, a product of activated neutrophils, inhibits in vitro the generation of nitric oxide and other macrophage inflammatory mediators. *J Leukoc Biol*. 1995;58(6): 667-674.

51. McGrath MS, Kahn JO, Herndier BG. Development of WF10, a novel macrophage-regulating agent. *Curr Opin Investig Drugs*. 2002;3(3):365-373.

52. Giese T, McGrath MS, Stumm S, Schempp H, Elstner E, Meuer SC. Differential effects on innate versus adaptive immune responses by WF10. *Cell Immunol*. 2004;229(2):149-158.

53. Joo K, Lee Y, Choi D, et al. An anti-inflammatory mechanism of taurine conjugated 5-aminosalicylic acid against experimental colitis: taurine chloramine potentiates inhibitory effect of 5-aminosalicylic acid on IL-1β-mediated NFκB activation. *Eur J Pharmacol*. 2009;618(1-3):91-97.