Neurology Asia 2026; 31(3) : 981 – 991

Correlation between cystatin C and ALS: A cohort study and meta-analysis

Nan Hu, Huihong Tian, Jianfeng Ding, Dongchao Shen, Xunzhe Yang, Jingwen Niu, Mingsheng Liu, Liying Cui

Department of Neurology, Peking Union Medical College Hospital, Beijing, China.

Abstract

The study aimed to explore the prognostic role of cystatin C (Cys C) in amyotrophic lateral sclerosis (ALS). Patients with sporadic ALS were consecutively recruited and follow-up. Blood tests of Cys C were conducted at the first time of evaluation. Online database was systematically searched to identify studies on Cys C and ALS. Meta-analyses were conducted to provide evidence for clinical application of Cys C in ALS. A total of 143 ALS patients with available data of Cys C were included in our analysis. Correlation analyses revealed serum levels of Cys C were positively correlated with lower motor neuron (LMN) score (rho=0.180, p=0.032). Pooled results suggested that serum level of Cys C was significantly higher in ALS population than that in healthy controls (HCs) (MD 0.50, 95%CI 0.43-0.57). In cerebrospinal fluid (CSF), the level of Cys C was predominantly higher in HCs than that in ALS (MD 0.32, 95%CI 0.12-0.53). There was a positive correlation between serum level of Cys C and progression rate in ALS (rho=0.18, 95%CI 0.10-0.25). Serum level of Cys C was a predictor of death or invasive respiratory support of ALS patients (HR 1.33. 95%CI 1.18-1.51). The level of Cys C was significantly elevated in serum and decreased in CSF among ALS population compared to HCs. Serum level of Cys C was significantly higher in patients with severe LMN involvement, and might act as a potential predictor of ALS progression and death.

Keywords: Amyotrophic lateral sclerosis, cystatin C, prognosis

INTRODUCTION

Amyotrophic lateral sclerosis (ALS), characterized by involvement of both upper motor neuron (UMN) and lower motor neuron (LMN), is a fatal neurodegenerative disorder.[1] As the typical phenotype of motor neuron disease (MND), the relentless progression of paralysis and atrophy troubles numerous ALS patients and their caregivers, causing enormous economic losses, care burden and high prevalence of psychological disorders within these groups.[2,3] The pathogenesis of ALS remains unclear. Around 5-10% ALS patients carry causative gene mutations in *SOD1*, *TARDBP*, *FUS*, etc, which are known as the familial ALS.[4] The majority are sporadic, presenting huge heterogeneity in clinical manifestations, disease progression and survival.[5] Although several drugs can lower the progression rate of ALS, no effective disease-modifying treatment has been found.

One main problem of research on ALS is the lack of reliable biomarkers for disease monitoring. Cystatin C (Cys C) is a non-glycosylated 13-kDa basic protein that is present in all human body fluids, but is most abundant in the cerebrospinal fluid (CSF).[6] Commonly used as a renal function indicator, Cys C abnormally altered in neurodegenerative disease including Alzheimer's disease (AD), Parkinson's disease (PD) and multiple systematic atrophy (MSA), and may play an important role in aging.[7-9] It has been reported that Cys C was stained positive in Bunina bodies, which was the only specific pathological hallmark of ALS.[10] A meta-analysis involving nine studies in 2018 suggested that Cys C levels in CSF were significantly lower in ALS patients than in HCs, but there was no statistical difference in serum.[11] A negative correlation between serum Cys C and ALSFRS-R score was also reported by Ren *et al*.[12], which indicated

Address correspondence to: Dr Mingsheng Liu and Dr Liying Cui, Department of Neurology, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100730, China. EmailsS: MS Liu: liumingsheng_pumch@163.com; YY: Cui: cuily@pumch.cn

Date of Submission: 18 November 2025; Date of Acceptance: 30 March 2026

https://doi.org/10.54029/2026zfp

that Cys C might be a useful indicator for ALS progression.

In the study, we explored the prognostic effects of Cys C on ALS among a Chinese cohort. An updated meta-analysis based on Zhu *et al*. in 2018[11] and Agah E *et al.* in 2018[13] was conducted to further provide evidence for the clinical application of Cys C in ALS in evidence-based medicine (EBM).

METHODS

Subjects

A prospective single-center cohort study of ALS was conducted at the Department of Neurology, Peking Union Medical College Hospital (PUMCH). Patients diagnosed as definite or probable ALS according to the revised El Escorial criteria[14] were consecutively recruited and registered online. All enrolled patients were recorded with their name, gender, age, clinical symptoms and medications with detailed physical examination. All included patients were assessed using the ALSFRS-R score.[15] Muscle strength was measured using the Medical Research Council (MRC) score, including assessment of the following muscle actions: neck flexion and extension, bilateral neck rotation, shoulder abduction, elbow flexion, elbow extension, wrist flexion, wrist extension, finger flexion, finger extension, thumb abduction, little finger abduction, hip flexion, knee flexion, knee extension, ankle dorsal extension, ankle plantar flexion, toe dorsal extension, and toe plantar flexion. The total MRC score was 180.

A follow-up interview was performed every three to six months either by phone call or in our outpatient clinic to collect a follow-up ALSFRS-R score till patients died or needed invasive respiratory support. Patients that could not be followed up at the appointed time or misdiagnosed were excluded from our cohort. Patients with causative gene mutations or familial history of MND were excluded. A total of 1372 patients with sporadic ALS were enrolled and followed-up for at least three months from September 2015 to October 2023 was involved in the study. And only patients with Cys C values were involved in our analyses.

The study was approved by the Ethics Committee of the Peking Union Medical College Hospital (PUMCH) (JS1210). Informed consent was obtained from all participants and/or their legal guardians.

Blood tests for Cys C levels

Samples were collected by venipuncture, performed between 8:00 and 11:00a.m., after fasting from midnight at the time of first evaluation in our institution. The blood specimens were left at room temperature for 30min to clot and centrifuged for 10min at 1,200g. The CysC level was measured by the automated particle-enhanced immunoturbidimetric method, and the measurement were completed in the Olympus AU5400 analyzer (Olympus, Tokyo, Japan) using the manufacturer's reagents. In addition, creatinine (Cr), estimated glomerular filtration rate based on Cys C (eGFR-Cys C)[16] and the ratio of Cr and Cys C (Cr/Cys C)[17] were also measured based on prior studies. Patients with renal dysfunction were excluded from our analysis. All the experiments in the study were conducted in the Department of Laboratory Medicine of PUMCH to ensure homogeneity of the results.

Identification of included indicators

Severity of UMN and LMN damage was evaluated using the Penn UMN score (Penn score)[18], and a modified version of the LMN score.[19] Besides, the neurophysiological burden of LMN damage was evaluated using a semiquantitative score for active and chronic denervation (AD and CD, respectively) in affected muscles which was recently validated by Gentile *et al*.[20] According to the operating procedures of our laboratory of electromyography (EMG), we simplified the relevant items and the numbers of tested muscles, which was provided in Supplementary Table 1. Assessment of respiratory function was based on the respiratory ALSFRS-R score[15] and percentages of forced vital capacity (%FVC). The King's Clinical Staging System for ALS[21] was used to evaluate the changes of Cys C levels in disease course.

The progression rate was calculated by the difference of the ALSFRS-R score divided by the time interval in months (decrease of ALSFRS-R per month).[22] We selected ALSFRS-R score at the first (3-6 months) and second (9-12 months) follow-up visits as the indicators to represent early and late stages of disease condition, respectively. Survival time referred to the time (months) between onset of ALS and death or need for invasive respiratory support.

Literature search and data extraction

We identified relevant studies published from inception to November 2024 by searching on PubMed. Studies were restricted to human species and only English manuscripts was involved. The keywords "amyotrophic lateral sclerosis" or "motor neuron disease" in combination with "cystatin C" were used for online searching. No filter for study designs was applied. Pertinent studies were also identified during the screen of reviews and reference lists of search results. After the exclusion of duplication, titles and abstracts were screened during the initial selection. Further review was based on the full texts. Two authors (Nan Hu and Huihong Tian) screened all the studies independently. The disagreement was resolved by a senior reviewer (Mingsheng Liu). This work was performed according to the Meta-analysis of Observational Studies in Epidemiology (MOOSE)[23] and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses.[24]

Based on our perspectives, studies that satisfied the following criteria were included in the meta-analysis: (1) Studies that reported levels of Cys C both in ALS patients and healthy controls (HCs); (2) Studies that available correlation coefficient between levels of Cys C and ALS progression rate or hazard ratio (HR) with 95% confidence interval (CI) between Cys C levels and ALS survival time. Studies which focused on specific participants or lack of clear definition of control groups were excluded. Due to the considerable differences in levels of Cys C among different diseases, studies that compared Cys C levels between ALS and disease controls were not included in the meta-analysis, but were systematically discussed in the study.

Data extraction was carried out by two authors (Nan Hu and Huihong Tian) independently. From each eligible study, we collected authors, publication year, geographical region, mean age, gender ratio (female/male), study period, study design, sample size, disease duration, ALSFRS-R score, diagnostic criteria. The values of Cys C levels in serum and CSF in both patients with ALS and HCs were collected for comparisons. Correlation coefficients and HR with 95%CI were collected for the analysis of the impacts of Cys C on ALS progression and survival. If necessary, we tried to contact the corresponding author of relevant studies for more information.

We used Agency for Healthcare Research and Quality (AHRQ) scale as reference for quality assessment for the included cross-sectional studies and Newcastle-Ottawa Quality Assessment Scale (NOS) for included cohort studies. Two reviewers (Nan Hu and Jianfeng Ding) performed the assessment separately and disagreements were resolved by a senior reviewer (Mingsheng Liu).

Statistical analysis

The Shapiro-Wilk test was used to assess whether data exhibited a normal distribution. Normally distributed variables were expressed as means (standard deviation, SD) and non-normally distributed variables were expressed as medians (interquartile range, IQR). Nonparametric test was used for comparisons of non-normally distributed variables between groups. Spearman correlation coefficient was used for the correlation analysis and a Bonferroni correction was applied for multiple testing. Kaplan-Meier curve was used for survival analysis, with ALS onset as zero time and death as the end-point event. Cox proportional hazard models was used to evaluate the prognostic role of blood parameters. Significance was defined as $p<0.05$.

For levels of Cys C in ALS group and HCs, the MD (mean difference) and 95%CI were calculated. Pooled HR with 95%CI was used to reflect the prognostic role of Cys C and related biomarkers in ALS. Heterogeneity was tested with I^2 statistics and data of heterogeneous studies with $I^2>50\%$ would be calculated using random-effects model. Funnel plot was used to detect the publication bias. To examine the stability of the pooled results, each study was omitted at a time and the pooled results of the rest studies was recalculated.

Statistical analyses were performed using SPSS 23.0 and Stata 15.0.

RESULTS

Results of our cohort

A total of one hundred and forty-three ALS patients with available data of Cys C were included in our analysis. The demographic characteristics were presented in Table 1. The median (IQR) serum levels of Cys C were 0.87 (0.79-0.98). No significant difference was revealed in serum levels of Cys C, Cr, Cr/Cys C or eGFR-Cys C among different clinical stages (Supplementary Figure 1). Comparisons in onset age (51.32±18.54, p=0.546), gender ratio

Table 1: Baseline characteristics of involved ALS patients

Items	N	ALS patients
Onset age (years old)	143	50.64±11.55
Gender (F/M)		69/74
BMI (kg/m^2)		23.58±2.89
Bulbar onset (n, %)		23 (16.08%)
Disease duration (months)		15 (9-24)
Total MRC score		163 (148-176)
ALSFRS-R score		42 (39-44)
Riluzole (n, %)		34 (23.78%)
Serum creatinine		61 (51-68)
Serum cystatin C		0.87 (0.79-0.98)
Penn score		15 (11-17)
LMN score		6 (4-7)
AD score	15	9 (8-9)
CD score		5 (3-6)

Abbreviations: AD active denervation, ALS amyotrophic lateral sclerosis, ALSFRS-R the revised ALS functional research scale, BMI body mass index, CD chronic denervation, F female, LMN lower motor neuron, M male, MRC the Medical Research Council.

Note: Normally distributed variables were expressed as means±standard deviation (SD) and non-normally distributed variables were expressed as medians (interquartile range, IQR).

(female/male 598/631, p=0.841), bulbar onset (17.84%, p=0.124), total MRC score (158±6, p=0.311), ALSFRS-R score (41±3, p=0.189) between involved patients and excluded patients showed no significance.

Serum levels of Cys C were positively correlated with LMN score (rho=0.180, p=0.032), but not significantly related to Penn score or ALSFRS-R score at baseline. There were negative correlations between Cr/Cys C and LMN score (rho=-0.177, p=0.034), AD score (rho=-0.645, p=0.009), and CD score (rho=-0.608, p=0.016). In addition, eGFR-Cys C was negatively related to AD score (rho=-0.529, p=0.043). Further analysis showed a remarkable relationship between serum levels of Cr and Cys C (rho=0.380, p<0.001). (Table 2)

At baseline, no prominent effect of Cys C and related biomarkers on progression rate before enrollment [(48-baseline ALSFRS-R)/disease duration in months] was revealed. During the initial 6-month follow-up, serum levels of Cys C were significantly related to ALS progression rate (rho=0.317, p=0.014, N=120). However, the correlation was not significant at the second visits during follow-up (rho=0.248, p=0.336, N=88). No remarkable correlation between Cr, Cr/Cys C, eGFR-Cys C and progression rate during follow-up was revealed. (Table 3)

As of October 2023, a total of 75 (52.45%) patients died or needed invasive respiratory support. As shown in Supplementary Figure 2, Kaplan-Meier curve suggested that the median (95%CI) survival time of included patients was 47.0 (38.5-55.5) months. Table 4 presented the results of survival analysis, and we found no significant relationship between serum levels of Cys C, Cr, Cr/Cys C, eGFR-Cys C and survival time in our cohort.

Literature selection

A total of 108 studies were included in our study, including the results of keywords search (n=116) and additional records identified from reference lists (n=12). After the detailed reading of full texts, eleven articles[12,25-34] were potentially eligible for meta-analysis (Supplementary Figure 3). Five studies[12,25,27,31,32] reported the serum levels of Cys C in ALS and HCs, and six studies[12,29-31,33,34] provided the CSF levels of Cys C in patients with ALS and HCs. Data of Spearman coeffecient between serum levels of Cys C and ALS progression rate was given in two[12,28] online studies, three[25,26,28] previous studies were included in the meta-analysis of survival time.

Table 2: Correlation analysis between cystatin C and other variables at baseline

	BMI		Total MRC		ALSFRS-R		Respiratory ALSFRS-R		Penn score		LMN score		AD score		CD score		FVC%	
	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
Cys C	-0.107	0.212	-0.015	0.854	0.083	0.322	0.101	0.230	-0.049	0.563	0.180	**0.032**	0.498	0.059	0.031	0.912	-0.235	0.101
Cr	-0.055	0.513	0.021	0.799	0.041	0.626	0.054	0.522	-0.010	0.906	-0.011	0.893	-0.016	0.956	-0.379	0.163	-0.074	0.609
Cr/Cys C	-0.012	0.886	0.044	0.598	-0.013	0.877	0.029	0.734	0.004	0.958	-0.177	**0.034**	-0.645	**0.009**	-0.608	**0.016**	0.224	0.118
eGFR-Cys C	0.081	0.338	0.026	0.760	-0.046	0.588	-0.068	0.421	0.042	0.620	-0.157	0.061	-0.529	**0.043**	-0.084	0.765	0.205	0.154

Abbreviations: AD active denervation, ALS amyotrophic lateral sclerosis, ALSFRS-R the revised ALS functional research scale, BMI body mass index, CD chronic denervation, Cr creatinine, Cys C cystatin C, eGFR estimated glomerular filtration rate, F female, FVC forced vital capacity, LMN lower motor neuron, M male, MRC the Medical Research Council.

Note: Spearman coefficient was used for analysis, and $p<0.05$ was considered significance, which was bold.

The main information of included studies was given in Supplementary Table 2. We assessed the quality of included studies using AHRQ and NOS checklists. The results were shown in Supplementary Table 3 and 4, which suggested that all included studies were moderate or high quality.

Risk of bias assessment for included studies

Bias from characters of population

The diversity of demographic features including onset age, gender, geographical region, the disease duration and baseline ALSFRS-R score might contribute to the bias of synthesized results. Besides, the proportion of bulbar onset varied, which might result in significant inter-study differences.

Bias from selection of ALS patients and adjustments

Included studies set different restrictions at the enrollment of ALS population, which provided in the Item "Exclusion" in Supplementary Table 2. The adjustments in the survival analysis were also different among included studies.

Bias from study design

There were five cross-sectional studies and six prospective studies included in our analysis. Differences between study designs and the deficiencies of cross-sectional studies might contribute to the bias of our results.

Results of meta-analysis

Pooled results involving 1660 ALS patients and 1820 HCs suggested that serum level of Cys C was significantly higher in ALS population than that in HCs (MD 0.50, 95%CI 0.43-0.57) (Figure 1A). In CSF, the level of Cys C was predominantly higher in HCs than that in ALS (MD 0.32, 95%CI 0.12-0.53) (Figure 1B).

As presented in Figure 1C, a positive correlation was found between serum levels of Cys C and progression rate in ALS (rho=0.18, 95%CI 0.10-0.25). Pooled analysis involving four longitudinal studies indicated that serum level of Cys C was a risk factor of death or invasive respiratory support of ALS patients (HR 1.33. 95%CI 1.18-1.51) (Figure 1D). No significant heterogeneity was revealed in these

Table 3: Correlation analysis between Cyc C and progression rate

	Pre-progression		Post progression (3-6 months)		Post progression (9-12 months)	
	rho	p	rho	p	rho	p
Cys C	-0.100	0.234	0.317	**0.014**	0.248	0.336
Cr	-0.121	0.149	-0.054	0.683	-0.119	0.650
Cr/Cys C	-0.087	0.303	-0.183	0.166	-0.325	0.203
eGFR-Cys C	0.054	0.523	-0.218	0.097	-0.144	0.583

Abbreviations: Cr creatinine, Cys C cystatin C, eGFR estimated glomerular filtration rate.
Note: Spearman coefficient was used for analysis, and $p<0.05$ was considered significance, which was bold.

analyses and Funnel plot suggested no significant publication bias of included studies.

DISCUSSION

There was an urged need to find reliable biomarkers for ALS monitoring. Cys C, a lysosomal protein, was extensively expressed in neurons, astrocytes, endothelial and microglial cells in the brain and was also found in body fluids.[35] Initially used as an indicator of renal function, Cys C was superior to Cr as it was unaffected by age, sex, and muscle mass.[36] Studies have proved the usefulness of Cys C as a biomarker of renal function in ALS patients and proposed that its applicability could be extended to other neuromuscular diseases.[32] Besides, eGFR based on Cys C could be used for disease monitoring in ALS patients who received long-term treatment with edaravone[37] and serum Cr/Cys C have emerged as promising biomarkers for assessing muscle mass.[16] In the

Table 4: Univariate and Multivariate Cox survival analysis regarding Cystatin C

	Model 0		Model 1		Model 2	
	Hazard ratio (95% CI)	p	Hazard ratio (95% CI)	p	Hazard ratio (95% CI)	p
Cys C						
<0.87	1		1		1	
≥0.87	1.402 (0.886-2.220)	0.149	1.219 (0.719-2.067)	0.462	1.393 (0.813-2.386)	0.228
Cr						
<61	1		1			
≥61	0.878 (0.555-1.390)	0.579	0.876 (0.551-1.392)	0.575		
Cr/Cys C						
<69.6	1		1			
≥69.6	0.772 (0.488-1.222)	0.270	0.783 (0.479-1.277)	0.372		
eGFR-Cys C						
<85.5	1		1			
≥85.5	0.643 (0.405-1.021)	0.061	0.700 (0.402-1.221)	0.209		

Abbreviations: ALSFRS-R the revised ALS functional research scale, BMI body mass index, CI confidence interval, Cr creatinine, Cys C cystatin C, eGFR estimated glomerular filtration rate.
Note: Model 0, unadjusted model; Model 1, adjusted by age, gender, onset region, BMI, baseline ALSFRS-R score and intake of Riluzole; Model 2, adjusted by age, gender, onset region, BMI, baseline ALSFRS-R score, intake of Riluzole and Cr. $p<0.05$ was considered significance.

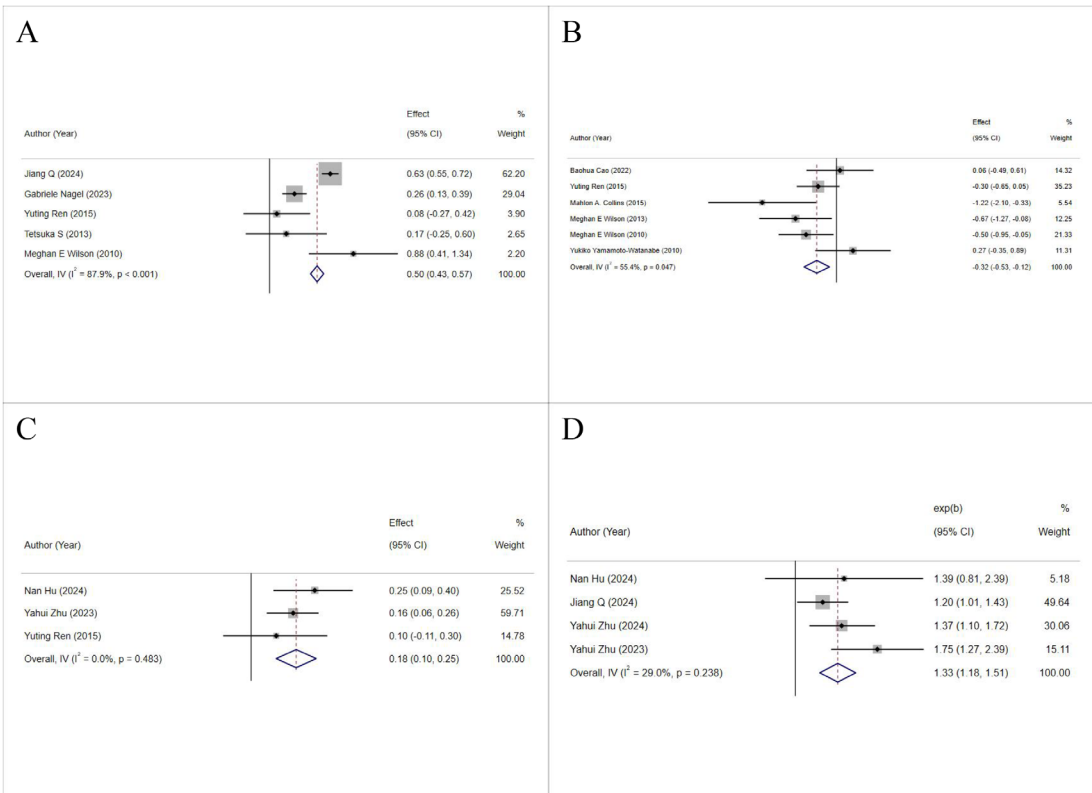


Figure 1. Meta-analysis of involved studies.
A Differences in serum cystatin C between ALS patients and healthy controls, B Differences in CSF cystatin C between ALS patients and healthy controls, C Correlation between serum cystatin C and ALS progression rate, D Impacts of serum cystatin C on ALS survival time.

study, we explored the prognostic roles of Cys C in a prospective ALS cohort and systematically summarized the results of prior studies on Cys C and ALS.

Our results suggested the levels of Cys C were significantly elevated in serum and decreased in CSF among ALS population compared to HCs. Studies on large samples of community population[17,38] and that compared ALS with other diseases[12,31,33,39-41] reported similar results. The epidermis of ALS was immunohistochemically strongly positive for Cys C as compared with that of controls, suggesting that a metabolic alteration of Cys C also took place in the skin of ALS.[42] The observed discrepancy between serum and CSF levels of Cys C might reflect compartment-specific regulatory mechanisms. Cys C was produced by various tissues, and increased serum levels might be driven by systemic inflammation, which was increasingly recognized in ALS. In contrast, CSF levels might be influenced by blood-CSF barrier dysfunction or rapid clearance via impaired glymphatic pathways in neurodegeneration. It was reported that in CSF there might be an increased sequestration of Cys C into Bunina bodies[10], a pathologic hallmark in LMN of ALS.[43] However, the abnormal changes of Cys C were also reported in AD, PD and MSA.[7,9,43] Cao *et al*. reported that CSF cystatin C levels were significantly lower in patients with anti-N-methyl-D-asparate receptor (NMDAR) encephalitis than in patients with ALS, especially among those with poor functional status.[29] Despite these promising associations, several limitations temper the interpretation of Cys C as a specific ALS biomarker. First, elevated serum Cys C is not unique to ALS; it has been reported in other neurodegenerative diseases, chronic kidney disease, and inflammatory disorders. Second, the absence of disease control groups, such as patients with other lower motor neuron syndromes (e.g., spinal muscular atrophy, progressive muscular atrophy), limits our ability to assess diagnostic specificity. Future prospective studies should include such controls to determine whether Cys C could effectively

differentiate ALS from mimicking conditions. All these suggested that it might be a biomarker of neuron degeneration or damage rather than specific to ALS. We noticed the considerable inter-heterogeneity of included studies, which might be attributed to the influences of different protein concentration as reported.[31] Furthermore, there might be a dynamic change within the disease course, although no significant differences among clinical stages were revealed by our results and prior studies.[33] The stability during the ALS course made Cys C might be an ideal biomarker for ALS monitoring.

Patients with severe LMN involvement reported higher levels of serum Cys C and lower levels of Cr/Cys C, indicating that serum Cys C might be related to loss of LMN or subsequent muscle atrophy. Nakane *et al* reported that reduced Cys C in CSF could reflect UMN involvement through high-field magnetic resonance (MR) of ALS, potentially providing a new biomarker for UMN degeneration in ALS.[39] However, we did not find a significant relationship between serum Cys C and UMN score based on clinical manifestations. The inconsistency between imaging and clinical manifestations deserved further exploration. Besides, several studies reported the significant correlation between Cys C and ALSFRS-R score[12,28], suggesting that Cys C might reflect the disease severity in ALS. We did not find remarkable relationship between Cys C and baseline ALSFRS-R score, total MRC score or respiratory function status, which might be contributed to the diversity in demographic characteristics among the studies.

Pooled results suggested that serum levels of Cys C might act as a potential predictor of ALS progression. Confined to our results, Jiang *et al* reported ALS patients with higher Cys C levels had a greater disease progression rate compared to those with lower Cys C levels.[25] In a USA prospective study in 2010, Wilson *et al.* found that slow progressors of ALS exhibited a trend of increasing Cys C levels over time, while fast progressors exhibited a subtle, non-significant decrease in Cys C levels.[33] By longitudinally tracking C9-ALS/FTD in monkeys, Xu *et al*. found that changes in Cys C levels in the CSF corresponded to the phenotypic progression of poly50-induced neurodegenerative disease.[44] The predominant involvement of LMN and loss of muscle mass in patients with high levels of Cys C might contribute to the correlation. It was noteworthy that we found that the significant relationship between serum Cys C and progression rate only existed in the short-term follow-up. In addition to the potential impact of lost data, the treatment and food intake of patients might significantly influence the serum Cys C levels over a long period of time. And the relentless loss of LMN might weaken the correlation with the prolongation of disease duration. More studies were needed to further investigate the interaction between Cys C and progression of ALS in different clinical stages. Beyond single measurements, longitudinal trends of Cys C might offer greater clinical utility. In our cohort, the correlation between serum Cys C and progression rate was significant during the first six months of follow-up but attenuated thereafter, suggesting that the prognostic value of Cys C might vary by disease phase. Collectively, these findings suggest that tracking the dynamic change of Cys C rather than relying on a single measurement might help differentiate fast versus slow disease progression. Future prospective studies with repeated Cys C measurements at standardized intervals were needed to validate this hypothesis and to explore whether longitudinal Cys C trajectories can serve as a surrogate marker for disease severity and prognosis.

As for prognosis, serum levels of Cys C might be a potential risk factor for ALS death. However, we found no significant impact of serum Cys C on ALS survival time in our cohort. The cut-off value of serum Cys C in the multivariate analyses might partly explain the discrepancies. The higher the cut-off value, the more likely the result was to be significant. Besides, the differences in the onset region, exclusion of ALS patients and follow-up time might contribute to the inconsistency. As reported by Ryberg *et al*, Cys C levels examined by mass spectrometry were only correlated with survival in patients with limb-onset.[45] Besides, in Jiang *et al*.[25] the results were no longer significant after adjusting the treatment. It was reported that Cys C levels in CSF but not plasma might be useful as prognostic indicators of patient survival time.[34] Due to the limited data of involved studies, we failed to conduct stratified analysis on site of onset, treatment and follow-up time. Besides, it has been reported that Cr/Cys C and eGFR-Cys C were potential biomarkers of ALS prognosis.[17,27,36] Future studies were needed to further elucidate the prognostic role of Cys C and related biomarkers in ALS.

From a clinical perspective, the identification of a practical cut-off value for serum or CSF Cys C would facilitate its use in routine practice. In our

cohort, the median (IQR) serum Cys C level was 0.87 (0.79-0.98) mg/L, and patients with levels above the upper quartile (0.98 mg/L) exhibited a trend toward more severe LMN involvement. However, due to the absence of a gold standard for defining "severe LMN involvement" and the inherent heterogeneity across studies including differences in assay platforms, population characteristics, and disease stages, a universally applicable cut-off cannot be established from the current data. For CSF Cys C, our meta-analysis confirmed significantly lower levels in ALS patients compared with healthy controls, but the limited availability of individual-level data and variability in pre-analytical handling preclude the definition of a specific threshold. Future large-scale, multicenter studies with standardized protocols were warranted to determine clinically meaningful cut-off values.

The mechanisms of the correlation between Cys C and ALS remained unclear. Through analyses of a large-scale online database, Zhang *et al* concluded that Cys C might be related to ALS risk.[46] Another study reported that lower Cr/Cys C was significantly associated with a higher risk of ALS onset.[17] Besides, chronic renal disease evaluated by Cys C might increase ALS risk.[27] All these indicated that Cys C might participate in the pathogenesis of ALS. Two distinct pathways had been proposed: autophagy induction through AMPK-mTOR pathway and inhibition of cathepsin B.[47] Furthermore, exogenously added Cys C was transduced into the cells and aggregated in the cytosol under oxidative stress conditions, implying a relationship between the neuroprotective activity of Cys C and Bunina body formation.[47] Intracerebroventricular administration of Cys C during the early symptomatic SOD1G93A mice could extend their survival times.[48] However, genetic analysis indicated that there might not be a direct genetic link between Cys C and ALS.[49] Whether Cys C alteration was an epiphenomenon or the initial factors of ALS needed further investigations.

We admitted several limitations of the study. Firstly, serum Cys C was only available in a small proportion of our cohort, which might result in considerable selection bias. Secondly, lack of control group disabled us to make the comparisons in serum Cys C between ALS and HCs or disease controls. Thirdly, stratification analyses based on age, gender and onset region were not conducted due to limited data. Fourthly, lack of repeated tests of Cys C disabled us to reveal the dynamic changes of Cys C during follow-up, and identify the potential confounding factors. Fifthly, serum neurofilament light chain (NfL), a well-established neuroaxonal injury marker in ALS, was not available in this cohort. Future studies incorporating NfL alongside Cystatin C will be valuable to determine whether these markers provide complementary or independent prognostic information. Lastly, some confounding factors such as levels of creatine kinase (CK)[50] and lipid metabolism[51] were not included in our analyses. Additionally, the absence of established cut-off values for serum and CSF Cys C limits the direct clinical applicability of our findings, and the lack of repeated measurements precludes assessment of longitudinal Cys C trends in relation to disease progression phenotypes. More well-designed prospective studies and basic studies were needed to further explore the prognostic effects of Cys C in ALS and the relevant mechanisms.

In conclusion, the levels of Cys C were significantly elevated in serum and decreased in CSF among ALS population compared to HCs. Serum levels of Cys C were significantly higher in patients with severe LMN involvement, and might act as a potential predictor of ALS progression and death.

DISCLOSURE

Financial support: CAMS Innovation Fund for Medical Sciences (CIFMS 2021-I2M-1-003), National High Level Hospital Clinical Research Funding (2022-PUMCH-B-017, 2022-PUMCH-D-002), National Science and Technology Major Project (2022ZD0118003), The Strategic Priority Research Program (Pilot study) "Biological basis of aging and therapeutic strategies" of the Chinese Academy of Sciences (XDB39040000).

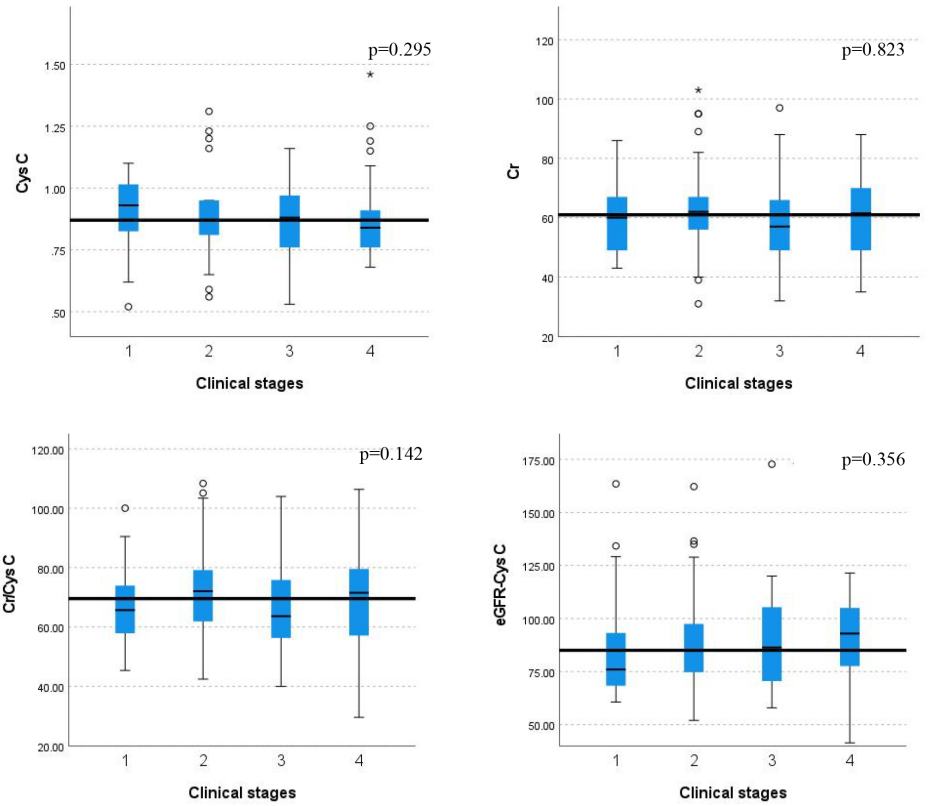
Conflict of interest: None

REFERENCES

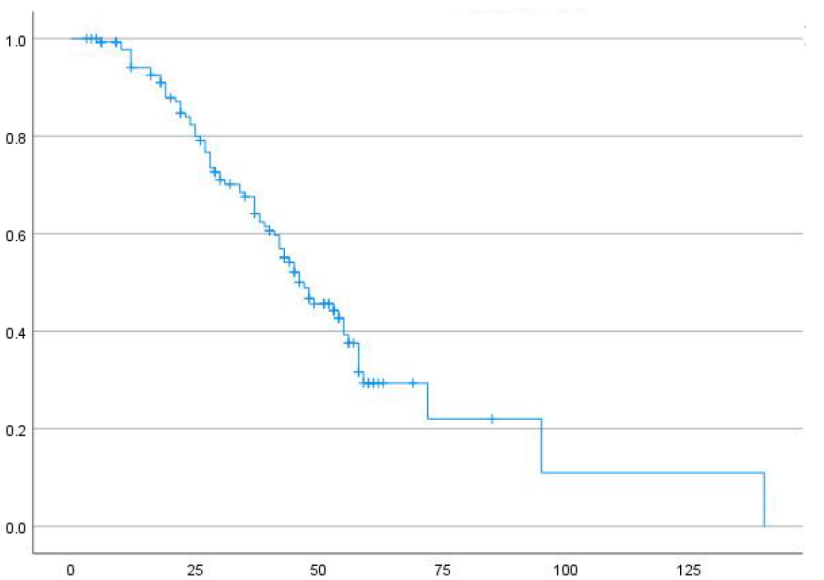
1. Masrori P, Van Damme P. Amyotrophic lateral sclerosis: a clinical review. *Eur J Neurol* 2020; 27(10): 1918-29. doi: 10.1111/ene.14393.
2. Heidari ME, Nadali J, Parouhan A, *et al*. Prevalence of depression among amyotrophic lateral sclerosis (ALS) patients: A systematic review and meta-analysis. *J Affect Disord* 2021; 287:182-90. doi: 10.1016/j.jad.2021.03.015.
3. Grabler MR, Weyen U, Juckel G, Tegenthoff M, Mavrogiorgou-Juckel P. Death anxiety and depression in amyotrophic lateral sclerosis patients and their primary caregivers. *Front Neurol* 2018;

9:1035. doi: 10.3389/fneur.2018.01035.
4. Al-Chalabi A, Hardiman O. The epidemiology of ALS: a conspiracy of genes, environment and time. *Nat Rev Neurol* 2013; 9(11): 617-28. doi: 10.1038/nrneurol.2013.203.
5. Foster LA, Salajegheh MK. Motor neuron disease: Pathophysiology, diagnosis, and management. *Am J Med* 2019; 132(1): 32-7. doi: 10.1016/j.amjmed.2018.07.012.
6. George PM, Sheat JM. Cystatin C quantification in CSF. *Clin Chem* 1989; 35(1): 179-80.
7. Mathews PM, Levy E. Cystatin C in aging and in Alzheimer's disease. *Ageing Res Rev* 2016; 32: 38-50. doi: 10.1016/j.arr.2016.06.003.
8. Imarisio A, Pilotto A, Garrafa E, *et al*. Plasma Cystatin C correlates with plasma NfL levels and predicts disease progression in Parkinson's disease. *Neurodegener Dis* 2021; 21(5-6): 109-16. doi: 10.1159/000523982.
9. Urbizu A, Canet-Pons J, Munoz-Marmol AM, *et al.* Cystatin C is differentially involved in multiple system atrophy phenotypes. *Neuropathol Appl Neurobiol* 2015; 41(4): 507-19. doi: 10.1111/nan.12134.
10. Okamoto K, Mizuno Y, Fujita Y. Bunina bodies in amyotrophic lateral sclerosis. *Neuropathology* 2008; 28(2): 109-15. doi: 10.1111/j.1440-1789.2007.00873.x.
11. Zhu Y, Yang M, Li F, *et al.* Aberrant levels of Cystatin C in amyotrophic lateral sclerosis: A systematic review and meta analysis. *Int J Biol Sci* 2018; 14(9): 1041-53. doi: 10.7150/ijbs.25711.
12. Ren Y, Zhu W, Cui F, *et al*. Measurement of cystatin C levels in the cerebrospinal fluid of patients with amyotrophic lateral sclerosis. *Int J Clin Exp Pathol* 2015; 8(5): 5419-26.
13. Agah E, Saleh F, Sanjari Moghaddam H, Saghazadeh A, Tafakhori A, Rezaei N. CSF and blood biomarkers in amyotrophic lateral sclerosis: protocol for a systematic review and meta-analysis. *Syst Rev* 2018; 7(1): 237. doi: 10.1186/s13643-018-0913-4.
14. 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): 293-9. doi: 10.1080/146608200300079536.
15. Cedarbaum JM, Stambler N, Malta E, *et al*. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). *J Neurol Sci* 1999; 169(1-2): 13-21. doi: 10.1016/s0022-510x(99)00210-5.
16. Inker LA, Schmid CH, Tighiouart H, *et al*. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med* 2012; 367(1): 20-9. doi: 10.1056/NEJMoa1114248.
17. Wang Z, Cao W, Deng B, Fan D. Lower creatinine-to-cystatin C ratio associated with increased risk of incident amyotrophic lateral sclerosis in the prospective UK biobank cohort. *Sci Rep* 2024; 14(1): 28289. doi: 10.1038/s41598-024-79910-1.
18. Woo JH, Wang S, Melhem ER, *et al.*, Linear associations between clinically assessed upper motor neuron disease and diffusion tensor imaging metrics in amyotrophic lateral sclerosis. *PLoS One* 2014; 9(8): e105753. doi: 10.1371/journal.pone.0105753.
19. Devine MS, Ballard E, O'Rourke P, Kiernan MC, Mccombe PA, Henderson RD. Targeted assessment of lower motor neuron burden is associated with survival in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener* 2016; 17(3-4): 184-90. doi: 10.3109/21678421.2015.1125502.
20. Colombo E, Doretti A, Scheveger F, *et al.* Correlation between clinical phenotype and electromyographic parameters in amyotrophic lateral sclerosis. *J Neurol* 2023; 270(1): 511-8. doi: 10.1007/s00415-022-11404-4.
21. Balendra R, Al Khleifat A, Fang T, Al-Chalabi A. A standard operating procedure for King's ALS clinical staging. *Amyotroph Lateral Scler Frontotemporal Degener* 2019; 20(3-4): 159-64. doi: 10.1080/21678421.2018.1556696.
22. 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): 265-7. doi: 10.1212/01.wnl.0000194316.91908.8a.
23. Stroup DF, Berlin JA, Morton SC, *et al.* Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; 283(15): 2008-12. doi: 10.1001/jama.283.15.2008.
24. Moher D, Shamseer L, Clarke M, *et al*. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* 2015; 4(1): 1. doi: 10.1186/2046-4053-4-1.
25. Jiang Q, Guo Y, Yang T, *et al*. Cystatin C is associated with poor survival in amyotrophic lateral sclerosis patients. *Front Neurosci* 2023; 17: 1309568. doi: 10.3389/fnins.2023.1309568.
26. Zhu Y, Zhang Y, Li M, *et al.* Prognostic value of systemic inflammation, nutritional status and sarcopenia in patients with amyotrophic lateral sclerosis. *J Cachexia Sarcopenia Muscle* 2024; 15(6): 2743-55. doi: 10.1002/jcsm.13618.
27. Nagel G, Kurz D, Peter RS, *et al*. Cystatin C based estimation of chronic kidney disease and amyotrophic lateral sclerosis in the ALS registry Swabia: associated risk and prognostic value. *Sci Rep* 2023; 13(1): 19594. doi: 10.1038/s41598-023-46179-9.
28. Zhu Y, Huo Y, Bai J, *et al.* Serum Cystatin C is a potential biomarker for predicting amyotrophic lateral sclerosis survival. *Neurol Sci* 2024; 45(1): 197-201. doi: 10.1007/s10072-023-06957-9.
29. Cao B, Luo M, Li J, *et al*. Cerebrospinal fluid cystatin C levels in patients with anti-NMDAR encephalitis and other neurological diseases. *J Neuroimmunol* 2022; 369: 577900. doi: 10.1016/j.jneuroim.2022.577900.
30. Collins MA, An J, Hood BL, Conrads TP, Bowser RP. Label-free LC-MS/MS proteomic analysis of cerebrospinal fluid identifies protein/pathway alterations and candidate biomarkers for amyotrophic lateral sclerosis. *J Proteome Res* 2015; 14(11): 4486-501. doi: 10.1021/acs.jproteome.5b00804.

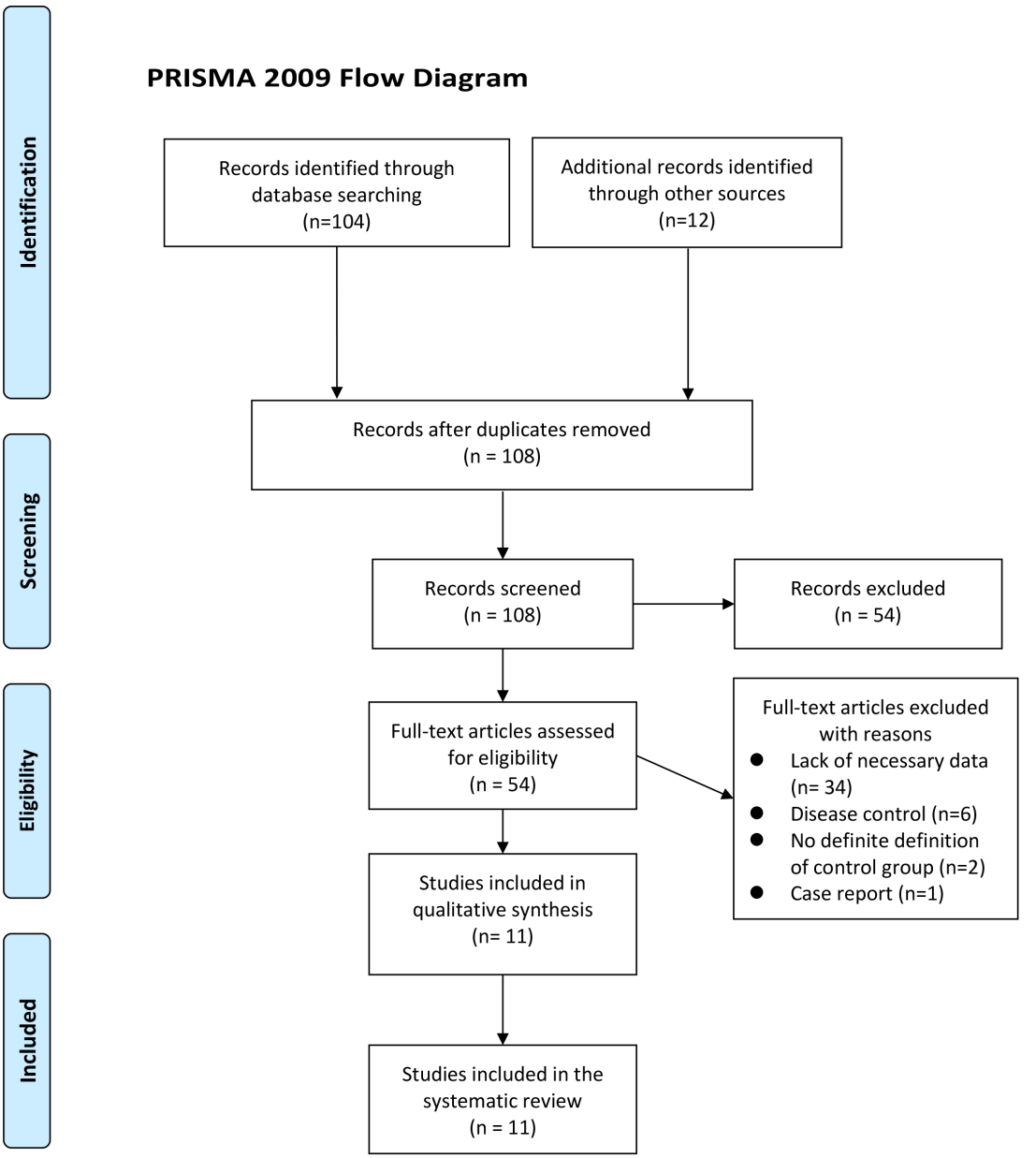
31. Wilson ME, Boumaza I, Bowser R. Measurement of cystatin C functional activity in the cerebrospinal fluid of amyotrophic lateral sclerosis and control subjects. *Fluids Barriers CNS* 2013; 10(1): 15. doi: 10.1186/2045-8118-10-15.
32. Tetsuka S, Morita M, Ikeguchi K, Nakano I. Utility of cystatin C for renal function in amyotrophic lateral sclerosis. *Acta Neurol Scand* 2013; 128(6): 386-90. doi: 10.1111/ane.12134.
33. Wilson ME, Boumaza I, Lacomis D, Bowser R. Cystatin C: a candidate biomarker for amyotrophic lateral sclerosis. *PLoS One* 2010; 5(12): e15133. doi: 10.1371/journal.pone.0015133.
34. Yamamoto-Watanabe Y, Watanabe M, Jackson M, *et al*., Quantification of cystatin C in cerebrospinal fluid from various neurological disorders and correlation with G73A polymorphism in CST3. *Brain Res* 2010; 1361: 140-5. doi: 10.1016/j.brainres.2010.09.033.
35. Gauthier S, Kaur G, Mi W, Tizon B, Levy E. Protective mechanisms by cystatin C in neurodegenerative diseases. *Front Biosci* (Schol Ed) 2011; 3(2): 541-54. doi: 10.2741/s170.
36. Newman DJ. Cystatin C. *Ann Clin Biochem* 2002; 39(Pt 2): 89-104. doi: 10.1258/0004563021901847.
37. Kakimoto A, Ishizaki M, Ueyama H, Maeda Y, Ueda M. Renal function in amyotrophic lateral sclerosis patients on long-term treatment with edaravone. *Medicine* (Baltimore) 2021; 100(21): e26127. doi: 10.1097/MD.0000000000026127.
38. Lehnert S, Costa J, de Carvalho M, *et al*. Multicentre quality control evaluation of different biomarker candidates for amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener* 2014; 15(5-6): 344-50. doi: 10.3109/21678421.2014.884592.
39. Nakane S, Fujita K, Azuma S, *et al.* CSF cystatin C and diffusion tensor imaging parameters as biomarkers of upper motor neuron degeneration in amyotrophic lateral sclerosis. *Clin Neurol Neurosurg* 2018; 172: 162-8. doi: 10.1016/j.clineuro.2018.07.008.
40. Tsuji-Akimoto S, Yabe I, Niino M, Kikuchi S, Sasaki H. Cystatin C in cerebrospinal fluid as a biomarker of ALS. *Neurosci Lett* 2009; 452(1): 52-5. doi: 10.1016/j.neulet.2009.01.026.
41. Chen X, Chen Y, Wei Q, *et al*. Assessment of a multiple biomarker panel for diagnosis of amyotrophic lateral sclerosis. *BMC Neurol* 2016; 16: 173. doi: 10.1186/s12883-016-0689-x.
42. Ono S, Shimizu N, Imai T, Mihori A, Nagao K. Increased cystatin C immunoreactivity in the skin in amyotrophic lateral sclerosis. *Acta Neurol Scand* 2000; 102(1): 47-52. doi: 10.1034/j.1600-0404.2000.102001047.x.
43. Okamoto K, Hirai S, Amari M, Watanabe M, Sakurai A. Bunina bodies in amyotrophic lateral sclerosis immunostained with rabbit anti-cystatin C serum. *Neurosci Lett* 1993; 162(1-2): 125-8. doi: 10.1016/0304-3940(93)90576-7.
44. Xu L, Wang D, Zhao L, *et al.* C9orf72 poly(PR) aggregation in nucleus induces ALS/FTD-related neurodegeneration in cynomolgus monkeys. *Neurobiol Dis* 2023; 184: 106197. doi: 10.1016/j.nbd.2023.106197.
45. Ryberg H, An J, Darko S, *et al.* Discovery and verification of amyotrophic lateral sclerosis biomarkers by proteomics. *Muscle Nerve* 2010; 42(1): 104-11. doi: 10.1002/mus.21683.
46. Zhang J, Cao W, Xie J, *et al.* Metabolic syndrome and risk of amyotrophic lateral sclerosis: Insights from a large-scale prospective study. *Ann Neurol* 2024; 96(4): 788-801. doi: 10.1002/ana.27019.
47. Watanabe S, Hayakawa T, Wakasugi K, Yamanaka K. Cystatin C protects neuronal cells against mutant copper-zinc superoxide dismutase-mediated toxicity. *Cell Death Dis* 2014; 5(10): e1497. doi: 10.1038/cddis.2014.459.
48. Watanabe S, Komine O, Endo F, Wakasugi K, Yamanaka K. Intracerebroventricular administration of Cystatin C ameliorates disease in SOD1-linked amyotrophic lateral sclerosis mice. *J Neurochem* 2018; 145(1): 80-9. doi: 10.1111/jnc.14285.
49. Watanabe M, Jackson M, Ikeda M, *et al*. Genetic analysis of the cystatin C gene in familial and sporadic ALS patients. *Brain Res* 2006; 1073-1074: 20-4. doi: 10.1016/j.brainres.2005.12.046.
50. Gao J, Dharmadasa T, Malaspina A, *et al*. Creatine kinase and prognosis in amyotrophic lateral sclerosis: a literature review and multi-centre cohort analysis. *J Neurol* 2022; 269(10): 5395-404. doi: 10.1007/s00415-022-11195-8.
51. Vaage AM, Benth JŠ, Meyer HE, Holmøy T, Nakken O. Premorbid lipid levels and long-term risk of ALS-a



Supplementary Figure 1. Serum levels of blood parameters in different clinical stages. Abbreviations: Cr creatinine, Cys C cystatin C, eGFR estimated glomerular filtration rate. Note: Nonparametric tests were used for comparisons, $p<0.05$ was considered significance.



Supplementary Figure 2. Kaplan-Meier curve representing survival of ALS patients. x-axis: time in months; y-axis: percentage of survived patients.



Supplementary Figure 3 . Results of systematic literature search

Supplemental Table 1. Simplified criteria used to calculate active (AD) and chronic denervation (CD) scores in needle electromyography (EMG).
For the bulbar region (sternocleidomastoid muscle), cervical region (first dorsal interosseus), thoracic region (thoracic paraspinal muscles), lumbosacral region (tibialis anterior). In case of different scores between bilateral muscles, the higher score was considered.

Active denervation **(spontaneus activity)**	***Chronic denervation*** **(MUAPs characteristics)**	***Score***
absent	Normal amplitude and duration	0
Fibrillation potentials and positive sharp waves + *or* insertional activity + *or* Presence of high frequency discharge *or* fibrillation potentials +/-	Increased duration, normal amplitude *or* normal duration, increased amplitude *or* decreased amplitude, increased/ decreased/ normal duration *or* decreased duration, normal amplitude	1
Fibrillation potentials and positive sharp waves ++	Increased duration, increased amplitude *or* very increased duration and amplitude	2
Fibrillation potentials and positive sharp waves +++ *or* ++++ *or* Fibrillation potentials and positive sharp waves in every site	No activity during voluntary muscle activation	3

Abbreviations: MUAPS Motor Unit Action Potentials.

Supplementary Table 2: Characteristics of involved studies in meta-analysis

Author	Year	Region	Study design	Time period	Sample of ALS	Onset age (years)	Gender (F/M)	Disease duration (months)	Bulbar onset (N, %)	ALSFRS-R score	Diagnostic criteria	Sample of HC	Exclusion	Adjustment
Nan Hu (present study)	2024	China	Prospective	Sep 2015-Oct 2023	143	Mean 50.64, SD 11.55	69/74	Median 15, IQR 9-24	24%	Median 42, IQR 39-44	the revised El Escorial	/	renal dysfunction, familial ALS	age, gender, onset region, BMI, baseline ALSFRS-R score, intake of Riluzole, Cr
Jiang Q [25]	2024	China	Prospective	Aug 2012-Jun 2021	1086	Mean 53.1, SD 11.2	412/674	Mean 15.4, SD 15.1	NR	Mean 40.7, SD 4.6	the revised El Escorial	1026	juvenile ALS, renal dysfunction	age, gender, ALSFRS-R score
Yahui Zhu [26]	2024	China	Prospective	Jan 2014-Dec 2019	506	Mean 54.2, SD 10.5	218/288	Median 11.0, IQR 6.0–18.0	17%	Median 40, IQR 36–44	the revised El Escorial	/	malignant tumour, rheumatoid arthritis and hepatic or renal dysfunction, recently (≤3 months) received glucocorticoid therapy or had acute cardiovascular events	age, gender, BMI, onset region, diagnostic delay, ALSFRS-R score
Gabriele Nagel [27]	2023	Germany	Prospective	Oct 2010-Jun 2014	362	Mean 65.7, SD 10.6	147/215	NR	32%	NR	the revised El Escorial	681	NR	age, gender, BMI, diabetes mellitus, smoking
Yahui Zhu [28]	2023	China	Prospective	Jan 2016-Dec 2019	356	Mean 53.3, SD 10.7	103/203	Mean 13.8, SD 11.9	16%	Mean 39.1, SD 5.9	the revised El Escorial	/	malignant tumor, rheumatoid arthritis, liver and renal function disfunction, recently (≤3 months) received glucocorticoid therapy or had acute cardiovascular events, or had any other known major health events affecting Cys C levels	age, gender, onset region, diagnostic delay, Cr
Baohua Cao [29]	2022	China	Cross-sectional	NR	14	Median 47.5, Range 23-74	4/10	NR	NR	NR	the revised El Escorial	136	NR	NR
Mahlon A. Collins [30]	2015	USA	Cross-sectional	NR	20	NR	NR	NR	NR	NR	the revised El Escorial	8	NR	NR
Yuting Ren [12]	2015	China	Retrospective	Mar 2012-May 2014	92	Mean 50.43, SD 10.02	33/59	Mean 19.30, SD 15.48	15%	Mean 37.95, SD 5.97	the revised El Escorial	48	NR	age
Meghan E Wilson [31]	2013	USA	Cross-sectional	NR	23	Mean 48.7, SD 13.9	12/11	NR	NR	NR	the revised El Escorial	23	NR	NR
Tetsuka S [32]	2013	Japan	Cross-sectional	NR	76	Mean 62.7, SD 10.2	32/44	Mean 34.8, SD 42	24%	Mean 29.4, SD 14.9	the revised El Escorial	30	NR	NR
Meghan E Wilson [33]	2010	USA	Prospective	NR	44	Mean 54.8, SD 13.5	13/31	NR	11%	NR	the revised El Escorial	30	NR	NR
Yukiko Yamamoto-Watanabe [34]	2010	Japan	Cross-sectional	NR	31	Mean 67.6, SD 8.9	12/19	NR	NR	NR	the revised El Escorial	15	NR	NR

Abbreviations: ALSFRS-R the revised ALS functional research scale, BMI body mass index, Cr creatinine, Cys C cystatin C, F female, HC healthy control, IQR interquartile range, M male, NR not reported, SD standard deviation, USA the United States.

Supplementary Table 3. Quality assessment of cross-sectional studies based on Agency for Healthcare Research and Quality (AHRQ) scale

Study	1. Define the source of information (survey, record review)	2. List inclusion and exclusion criteria for exposed and unexposed subjects (cases and controls) or refer to previous publications	3. Indicate time period used for identifying patients	4. Indicate whether or not subjects were consecutive if not population-based	5. Indicate if evaluators of subjective components of study were masked to other aspects of the status of the participants	6. Describe any assessments undertaken for quality assurance purposes (e.g., test/retest of primary outcome measurements)	7. Explain any patient exclusions from analysis	8. Describe how confounding was assessed and/or controlled.	9. If applicable, explain how missing data were handled in the analysis	10. Summarize patient response rates and completeness of data collection	11. Clarify what follow-up, if any, was expected and the percentage of patients for which incomplete data or follow-up was obtained
Baohua Cao 2022	Yes	Yes	Yes	Yes	Yes	No	No	Yes	No	No	No
Mahlon A. Collins 2015	Yes	Yes	Yes	Yes	Yes	No	Np	Yes	Yes	No	No
Meghan E Wilson 2013	Yes	Yes	Yes	No	Yes	No	Yes	Yes	No	No	No
Tetsuka S 2013	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes
Yukiko Yamamoto-Watanabe 2013	Yes	Yes	Yes	Yes	No	No	No	No	No	Yes	Yes

Supplementary Table 4. Newcastle-Ottawa scale (NOS) for assessing the quality of included studies

Author, year	Selection	Comparability	Outcome	Overall quality score (Max=9)
Nan Hu 2024	4	2	2	8
Jiang Q 2024	4	3	2	9
Yahui Zhu 2024	4	2	2	8
Gabriele Nagel 2023	3	3	2	8
Yahui Zhu 2023	4	2	2	8
Yuting Ren 2015	3	3	2	8
Meghan E Wilson 2010	3	3	2	8