Neurology Asia 2024; 29(2) : 385 – 396

Impacts of serum biomarkers regarding glucose, lipid and protein on progression and survival of amyotrophic lateral sclerosis: A Chinese cohort study

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Abstract

Objective: To explore the impacts of nutritional biomarkers regarding glucose, protein and lipid on amyotrophic lateral sclerosis (ALS) progression and survival in a cohort of Chinese ALS patients. *Methods:* A total of 191 ALS patients were included in our analysis. Pearson correlation was employed to analyze the relationships between baseline serological and clinical variables. Uni- and multivariate analysis were performed to analyze the influence of nutritional biomarkers on the progression and survival of ALS. A p-value of less than 0.05 was considered to be statistically significant. *Results:* Hyperglycemia (around 1/6) and hyperlipidemia (1/5-1/3) were common among ALS patients while protein deficiency was not predominant. Serum total cholesterol (TC) (p=0.026) and low-density lipoprotein cholesterol (LDL-C) (p=0.044) was negatively related to baseline ALS functional rating scale-revised (ALSFRS-R) score, while serum PA was positively associated with baseline ALSFRS-R score (p=0.018). Serum levels of TC (p=0.041), apoB (p<0.001), lipoprotein a [Lp (a)] (p=0.002) and free fatty acids (FFA) (p=0.049) were negatively associated with baseline forced vital capacity percentage (FVC%). None of studied biomarkers showed significant relationship with ALS progression or survival time, except for serum level of Lp(a) had a weakly positive correlation to ALS progression rate after backward selection (p=0.048).
Conclusion: **S**erum biomarkers of glucose, lipid or protein might only have a weak relationship with autonomous function and respiratory function status, but have no significant impact on the progression or overall survival of ALS. More studies were needed to provide guidance of nutritional management and diet recommendation for ALS patients.

Keywords: Amyotrophic lateral sclerosis, metabolism, progression, survival

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a heterogeneous, relentless progressive, and fatal neurodegenerative disease characterized by variable loss of upper and lower motor neurons.[1] ALS patients usually develop symptoms at the age of 40-55 years old[2] and present with muscle paralysis, dysarthria, dysphagia, and respiratory dysfunction, which eventually lead to a bed-ridden state and the need for mechanical ventilation.[3] Until recently, the pathogenesis of ALS remains unclear and it is still considered an incurable disorder. Clinical management, including high calorie and protein diet[4], moderate exercise[5], and early medical support [6] might be beneficial to ALS patient in improving the quality of life.

Patients with ALS frequently present with abnormalities in energy metabolism. The mainstream view is that ALS patients are in a state of hypermetabolism[7], prone to show abnormal glucose tolerance[8] and hypolipidemia.[9] Several researchers believed that the energy imbalance might be associated with the pathomechanism of ALS rather than simply an epiphenomenon of neuromuscular degeneration[10], since motor neurons might be more sensitive than other cell types to energy deficits given their high energy demand.[11] However, subsequent studies reached in conflicting results. For instance, a pilot study in 2014 reported that hypercaloric enteral nutrition could improve survival in ALS patients[12] while Mariosa *et al*. (2017) suggested higher serum level of low-density lipoprotein cholesterol (LDL-C),

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Date of Submission: 30 December 2023; Date of Acceptance: 5 March 2024

https://doi.org/10.54029/2024esc

as well as higher apolipoprotein B (apoB) were all associated with a greater future risk of ALS.[13] A meta-analysis in 2020 involving 10 studies indicated that the levels of all lipids were not associated with overall mortality in ALS.[14] Therefore, more studies are still needed unveil the potential energy disturbance among ALS and provide guidance of diet recommendation for ALS patients.

In the study, we performed a cohort study to explore the potential impacts of nutritional biomarkers regarding glucose, protein and lipid on ALS progression and survival. Relevant pathological mechanisms would also be discussed. We hope to provide reference for the nutritional management of ALS patients and render new insights for ALS pathogenesis.

METHODS

Subjects

A prospective single-center cohort study was conducted at the Department of Neurology, Peking Union Medical College Hospital (PUMCH). Patients at the out-patient clinic or hospitalization was recruited if they fulfilled: 1) progressive weakness or atrophy in one limbs or progressive bulbar dysfunction; 2) no electrophysiological evidence for sensory abnormalities that could not be explained by other causes; 3) ancillary examinations excluding other causes. A total of 1078 patients were consecutively assessed in this study between January 2014 and September 2020. All enrolled patients were recorded with their name, gender, age and clinical symptoms with detailed physical examination. Weight before and later than ALS onset were collected to obtain rate of weight loss (current weight-prior weight/month). Body mass index (BMI) was calculated by present weight/height^2 (kg/m^2). Information about disease features at onset and medications was collected retrospectively. All included patients were assessed using the ALS Functional Rating Scale-Revised (ALSFRS-R).[15] Muscle strength was measured using the Medical Research Council (MRC) score, including bilateral assessment of the following limb muscle actions: 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 160.

A total of 268 patients with laboratory test results regarding carbohydrate, protein, lipid and apolipoprotein metabolisms, gathered during the enrolment period were collected. The laboratory tests were all conducted directly on fresh blood samples in fasting status for at least 8 hours at the same laboratory (Abbott c16000). To avoid the influence of medications in our results, patients with intake of antihyperlipidemic and antidiabetic drugs were excluded. Results of forced vital capacity percentage (%) were also collected. A follow-up interview was performed every three months either by phone call or in our outpatient clinic to collect a follow-up ALSFRS-R score. Two experienced clinicians completed the assessment of ALSFRS-R independently, and disagreements were resolved by consensus. Patients that could not be followed up at the appointed time were excluded from our analysis. In the study, the progression rate was calculated by the difference of the ALSFRS-R score at the first and last visit divided by the time interval between these two visits in months (decrease of ALSFRS-R per month).[16] Finally, a total of 191 patients with baseline ancillary results completed the follow-up for at least one year and diagnosed as definite or probable ALS according to the revised El Escorial criteria.[17] Among them, 115 patients died or needed invasive respiratory support during follow-up. Survival time referred to the time (months) between onset of ALS and death or need for invasive respiratory support. (Figure 1)

This study was approved by the Ethics Committee of the PUMCH. All enrolled patients provided written, informed consent to be included in the study.

Biomarkers of carbohydrate, lipid and apolipoprotein metabolisms

Following biomarkers were selected for our analysis: serum levels of glucose (mmol/L), HbA1c (%), total cholesterol (TC) (mmol/L), triglyceride (TG) (mmol/L), LDL-C (mmol/L), high-density lipoprotein cholesterol (HDL-C) (mmol/L), apoB (g/L), apolipoprotein A-I (apoA-I) (g/L), lipoprotein a [LP(a)] (mg/L), free fatty acids (FFA) (μmol/L), total protein (TP) (g/L), albumin (g/L), prealbumin (PA) (mg/L). Glucose, TC, TG, LDL-C, HDL-C, TP, albumin, PA measures were available for the majority of participants whereas the remaining biomarkers are available for a proportion of the cohort (Table 1). LDL-C/HDL-C ratio and apoB/

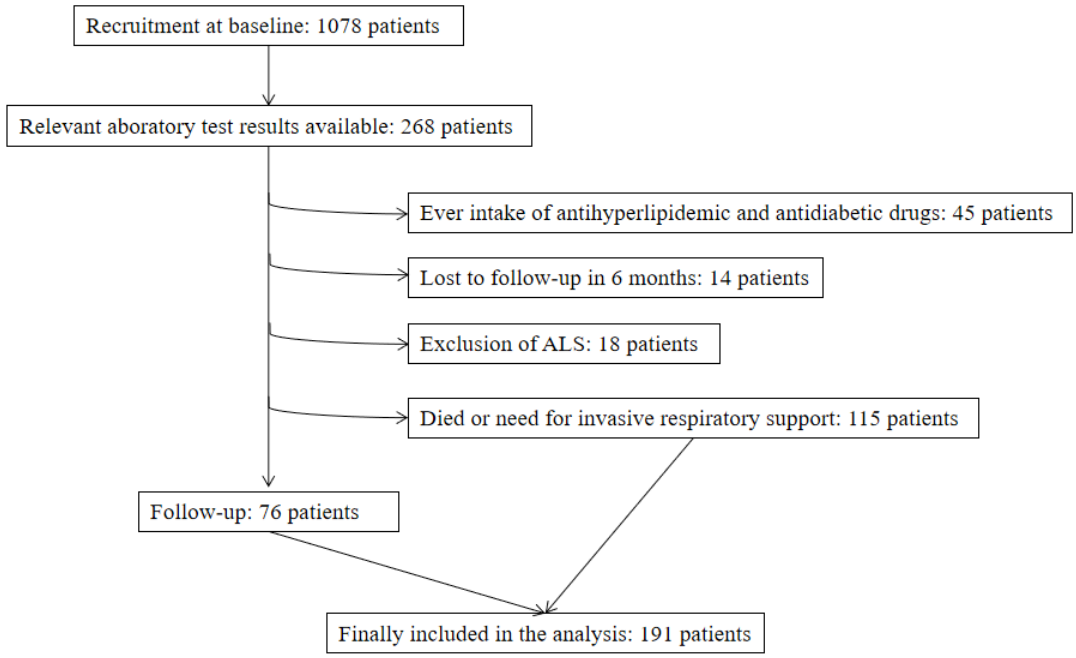


Figure 1. Flowchart of patient screening.

apoA-I ratio were also calculated. Normal levels of the studied biomarkers among adults were determined a priori for glucose (3.9-6.1 mmol/L), HbA1c (<6.3%), TC (2.85-5.70 mmol/L), TG (0.45-1.70 mmol/L), LDL-C (<3.37 mmol/L), HDL-C (0.93-1.81 mmol/L), LDL-C/HDL-C ratio (3.50), apoB (0.55-1.30 g/L), apoA-I (1.05-2.05 g/L), apoB/apoA-I ratio (0.90 in men, 0.80 in women), Lp(a) (<300 mg/L), FFA (129-769 μmol/L), TP (60-85 g/L), albumin (35-52 g/L), PA (200-400 mg/L) according to either previous PUMCH publications or published guidelines in cardiovascular prevention.[18-21]

Data of additional variables including serum levels of creatinine (μmol/L), urea (mmol/L), alanine aminotransferase (ALT) (U/L), aspartate aminotransferase (AST) (U/L), bilirubin (μmol/L) and uric acid (UA) (μmol/L) were also obtained, because they have been suggested as potentially associated with ALS.[22,23]

Statistics

Continuous variables were normally expressed as means (standard deviation, SD). Pearson correlation was employed to analyze the relationships between studied serological and clinical variables. To reveal the dynamic changes of nutritional biomarkers along with disease progression, we divided included patients into 4 clinical stages according to the staging system proposed by Roche et al in 2012 [24]. One-way analysis of variance (ANOVA) was used to compare studied variables among different clinical stages. A p-value of less than 0.05 was considered to be statistically significant.

To analyze the influence of studied biomarkers on ALS progression, we performed linear regression with ALSFRS-R decrease per month as the dependent variables and serological variable as the independent one. We first analyzed each variable in a univariate regression. Afterwards, variables with a low p value ($p<0.2$) were included into a multivariate linear regression. Using backward selection, relevant factors ($p<0.05$) were finally identified.

Kaplan-Meier curve was used for survival analysis, with ALS onset as zero time and death as the end-point event. For the convenience of analysis, we divided the continuous variables into two groups according to the median of data. Log-rank tests were applied to compare survival between the two groups. A multivariate regression analysis (Cox proportional hazard regression model) which included all the variables with $p<0.2$ in the univariate model, was used to assess the independent influence of each variable on survival. It is worth noting that basic variables and confounding factors as age, gender, onset region, and serum levels of creatinine, urea, ALT, AST, bilirubin, UA were included in the multivariate analysis (data were not provided).

Statistical analyses were performed using SPSS 23.0.

RESULTS

A total of 191 ALS patients [49.21% male (n=94), mean age at onset 51.59 years] were finally included in our analysis. The mean follow-up time was 42.50 (SD: 23.89) months. One hundred and thirteen patients died, and two patients needed invasive respiratory support during follow-up. Among them, twenty-three (20.00%) died of respiratory failure. Kaplan-Meier analysis showed that the mean survival time was 60.20 (SD: 3.58) months (Supplementary Figure 1). Further characteristics were presented in Table 1.

Glucose

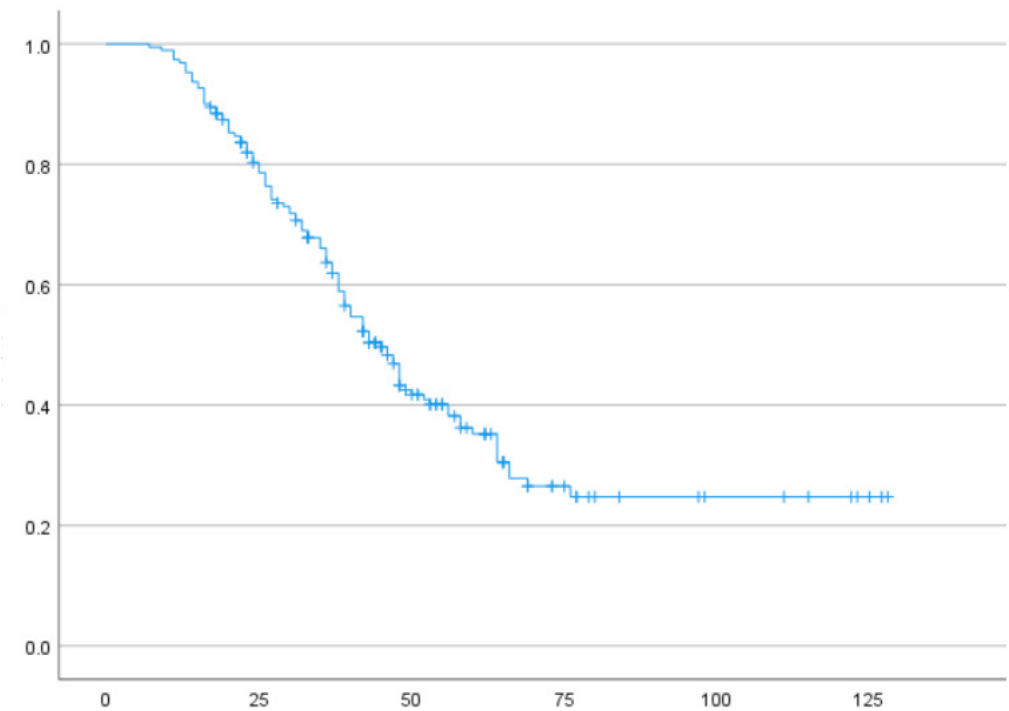
Measures of fasting blood glucose and HbA1c were available in 180 and 58 patients, among which 28 (15.56%), 4 (2.22%), 7 (12.07%) presented hyperglycemia, hypoglycemia, abnormal increase in HbA1c, respectively. (Supplementary Table 1)

Cross-sectional analysis suggested no statistically correlation between fasting blood glucose or Hb1Ac and baseline ALSFRS-R score, respiratory ALSFRS-R score, total MRC score, rate of weight loss or FVC% (Table 2). There was no relationship between the levels of fasting blood glucose or Hb1Ac and clinical stages (Supplementary Table 2).

Neither serum level of glucose nor HbA1c showed remarkable influence on ALS progression or survival (Table 3 and 4).

Lipid

Means (SD) of TC, TG, LDL-C were 4.97 (0.99) mmol/L, 1.67 (1.16) mmol/L, 3.01 (0.84) mmol/L, respectively, among 131 patients. Excessive level of TC was detected in 33 (25.19%) patients while no patients had TC that was lower than normal level. There were 2 (1.53%) patients with below normal levels of TG and 43 (32.82%) with above normal levels of TG. There were 39 (29.77%) participants whose levels of LDL-C were higher than normal. Among 128 patients who provided data of HDL-C, the number of patients with below and above levels of HDL-C were 25 (19.53%) and 4 (3.13%), respectively. Besides, the average ratio of LDL-C/HDL-C (2.73) was relatively lower than normal level. Measures of apoA-I and apoB were available in 91 and 89 patients, among which there were 7 (7.69%) and 1 (1.10%), 1 (1.12%) and 7 (7.87%) patients with abnormal increase or decrease in levels of apoA-I and apoB, respectively. The mean ratio



Supplementary Figure 1. Kaplan-Meier curve representing survival of ALS patients.
x-axis: time in months; y-axis: percentage of survived patients

Table 1: Demographic and clinical data

Items	N	ALS patients
Age of onset (years)	191	51.59±11.52
Gender (M/F)		94/97
Disease duration (months)		17.98±19.12
BMI (kg/m^2)		23.34±3.06
Bulbar onset (n, %)		38 (19.90%)
El Escorial (n, %) at baseline		
Definite		43 (22.51%)
Probable		56 (29.32%)
Probable lab supported		53 (27.75%)
Possible		9 (4.71%)
Diagnostic time (months)		17.65±18.93
Clinical stages (n, %) at baseline		
1		69 (36.13%)
2		76 (39.79%)
3		43 (22.51%)
4		3 (1.57%)
Total MRC		136.12±19.37
ALSFRS-r		39.28±5.10
Riluzole (⩾3 months, %)		27 (14.14%)
FVC%	64	91.57±20.21
glucose (mmol/L)	180	5.39±1.20
HbA1c (%)	58	5.62±0.84
TC (mmol/L)	131	4.97±0.99
TG (mmol/L)	131	1.67±1.16
HDL-C (mmol/L)	128	1.16±0.29
LDL-C (mmol/L)	131	3.01±0.84
LDL-C/HDL-C ratio	128	2.73±0.96
apoA-I (g/L)	91	1.32±0.33
apoB (g/L)	89	0.98±0.22
apoB/apoA-I ratio	89	0.75±0.22
LP(a) (mg/L)	96	175.11±191.64
FFA (μmol/L)	89	451.36±214.45
TP (g/L)	170	70.25±6.29
albumin (g/L)	174	43.72±3.29
PA (mg/L)	166	265.93±47.76
Death or invasive respiratory support	115	
Died of respiratory failure (n, %)		23 (20.00%)

Note: Data are presented as mean±SD. Due to missing values, the number corresponding to each factor does not necessarily add up to the total number of 191.

Abbreviations: ALS amyotrophic lateral sclerosis; ALSFRS-r Revised Amyotrophic Lateral Functional Rating Scale; apoA-I apolipoprotein A-I; apoB apolipoprotein B; BMI body mass index; F female; FFA free fatty acids; FVC forced vital capacity; HDL-C high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp(a) lipoprotein a; M male; MRC Medical Research Council; PA prealbumin; TC total cholesterol; TG triglycerides; TP total protein.

of apoB/apoA-1 among 89 patients was 0.75, significantly lower than normal level. Means (SD) of FFA and Lp (a) were 451.36 (214.45) (μmol/L) and 175.11 (191.64) (mg/L). Sixteen (16.67%) and six (6.74%) patients had excessive levels of Lp (a) and FFA, respectively. (Table 1 and Supplementary Table 1)

Pearson analysis regarding baseline data revealed that serum level of TC (p=0.026, 95%CI of Pearson r: -0.361, -0.019) and LDL-C (p=0.044, 95%CI of Pearson r: -0.343, 0.001) were negatively related to ALSFRS-R score. Serum levels of TC (p=0.041, 95%CI of Pearson r: -0.537, -0.004), apoB (p<0.001, 95%CI of Pearson r: -0.782, -0.292), apoB/apoA-I (p=0.016, 95%CI of Pearson r: -0.671, 0.076), Lp(a) (p=0.002, 95%CI of Pearson r: -0.725, -0.202) and FFA (p=0.049, 95%CI of Pearson r: -0.630, 0.008) were negatively associated with FVC% (Table 2). There were no relationship between the levels of lipid-related biomarkers and clinical stages (Supplementary Table 2).

For ALS progression, apoB/apoA-I ratio (p=0.083), Lp (a) (p<0.001) and rate of weight loss (p<0.001) were further included in multivariate analysis, which showed that only serum level of Lp(a) was positively correlated to ALS progression rate after backward selection (p=0.048). (Table 3)

None of studied variables regarding lipid showed significant relationship with survival time in Cox regression analysis (Table 4).

Protein

Data of albumin and TP were available in 174 and 170 patients, among which 3 (1.72%), 1 (0.57%), 4 (2.35%) and 1 (0.59%) presented hypoalbuminemia, hyperalbuminemia, abnormal decrease or increase in levels of TP, respectively. There were 15 (9.04%) patients whose levels of PA were lower than normal value. (Supplementary Table 1)

Cross-sectional analysis revealed that serum PA was positively related to baseline ALSFRS-R score (p=0.018, 95%CI of Pearson r: 0.028, 0.332). There was no statistically remarkable relationship between the levels of TP, albumin or PA and clinical stages (Supplementary Table 2).

No significance was revealed in the analysis of the relationship between levels of TP, albumin or PA and ALS progression or survival (Table 3 and 4).

DISCUSSION

In recent years, the management of ALS has shifted from an attitude of nihilism to treatments that prolonged survival and offered hope. Nutritional status played a pivotal role in the clinical management of ALS patients, and received increasing attention. Due to the high prevalence of malnutrition and progressive muscular atrophy, high calorie and protein diet was the most frequent dietary advice for ALS patients[25], and

Supplementary Table 1: Proportion of ALS patients with abnormalities of studied biomarkers

Items	N	Above (n, %)	Below (n, %)	Normal values
glucose (mmol/L)	180	28 (15.56%)	4 (2.22%)	3.9-6.1
HbA1c (%)	58	7 (12.07%)	-	<6.3
TC (mmol/L)	131	33 (25.19%)	0	2.85-5.70
TG (mmol/L)	131	43 (32.82%)	2 (1.53%)	0.45-1.70
HDL-C (mmol/L)	128	4 (3.13%)	25 (19.53%)	0.93-1.81
LDL-C (mmol/L)	131	39 (29.77%)	-	<3.37
apoA-I (g/L)	91	7 (7.69%)	1 (1.10%)	1.05-2.05
apoB (g/L)	89	1 (1.12%)	7 (7.87%)	0.55-1.30
LP(a) (mg/L)	96	16 (16.67%)	-	<300
FFA (μmol/L)	89	6 (6.74%)	1 (1.12%)	129-769
TP (g/L)	170	1 (0.59%)	4 (2.35%)	60-85
albumin (g/L)	174	1 (0.57%)	3 (1.72%)	35-52
PA (mg/L)	166	0	15 (9.04%)	200-400

Note: Due to missing values, the number corresponding to each factor was not 191.
Abbreviations: ALS amyotrophic lateral sclerosis; apoA-I apolipoprotein A-I; apoB apolipoprotein B; FFA free fatty acids; FVC forced vital capacity; HDL-C high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp(a) lipoprotein a; PA prealbumin; TC total cholesterol; TG triglycerides; TP total protein.

Table 2. Correlations of serological and clinical variables with Pearson analysis

	ALSFRS-R	Respiratory ALSFRS-R	total MRC	Rate of weight loss	FVC%
Glucose (mmol/L)	0.903	0.311	0.447	0.311	0.256
HbA1c (%)	0.424	0.630	0.185	0.340	0.803
TC (mmol/L)	**0.026**	0.132	0.147	0.171	**0.041**
TG (mmol/L)	0.487	0.620	0.197	0.280	0.165
HDL-C (mmol/L)	0.067	0.650	0.416	0.323	0.950
LDL-C (mmol/L)	**0.044**	0.081	0.065	0.105	0.051
LDL-C/HDL-C ratio	0.935	0.271	0.237	0.388	0.284
apoA-I (g/L)	0.736	0.478	0.994	0.662	0.468
apoB (g/L)	0.287	0.073	0.583	0.532	**<0.001**
apoB/apoA-I ratio	0.414	0.156	0.268	0.990	**0.016**
LP(a) (mg/L)	0.076	0.428	0.865	0.434	**0.002**
FFA (μmol/L)	0.721	0.601	0.312	0.666	**0.049**
TP (g/L)	0.539	0.209	0.308	0.988	0.209
albumin (g/L)	0.456	0.905	0.604	0.244	0.373
PA (mg/L)	**0.018**	0.068	0.533	0.881	0.355
BMI (kg/m^2)	0.772	0.693	0.999	**0.009**	0.935

Note: A p-value of less than 0.05 was considered to be statistically significant, which was in bold.
Abbreviations: ALS amyotrophic lateral sclerosis; ALSFRS-R Revised Amyotrophic Lateral Functional Rating Scale; apoA-I apolipoprotein A-I; apoB apolipoprotein B; BMI body mass index; FFA free fatty acids; FVC forced vital capacity; HDL-C high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp(a) lipoprotein a; MRC Medical Research Council; PA prealbumin; TC total cholesterol; TG triglycerides; TP total protein.

Supplementary Table 2: Comparison of levels of serological variables according to disease stages (p values are calculated by ANOVA across four groups)

	Stage 1	Stage 2	Stage 3	Stage 4	p-value
glucose (mmol/L)	5.269±1.015	5.609±1.313	5.264±1.292	4.800±0.458	0.251
HbA1c (%)	5.779±1.091	5.565±0.732	5.464±0.667	5.600	0.774
TC (mmol/L)	4.905±0.868	4.924±1.060	5.063±1.068	6.065±1.138	0.400
TG (mmol/L)	1.813±1.191	1.638±1.323	1.628±1.321	1.486±0.807	0.689
HDL-C (mmol/L)	1.122±0.270	1.168±0.331	1.210±0.233	1.215±0.247	0.635
LDL-C (mmol/L)	2.960±0.811	2.950±0.912	3.139±0.742	4.045±0.757	0.249
LDL-C/HDL-C ratio	2.785±1.081	2.691±0.981	2.686±0.745	3.334±0.057	0.787
apoA-I (g/L)	1.315±0.192	1.355±0.479	1.258±1.157	1.370±0.141	0.774
apoB (g/L)	0.984±0.236	0.952±0.209	0.982±0.200	1.230±0.141	0.370
apoB/apoA-I ratio	0.739±0.253	0.732±0.216	0.790±0.178	0.897±0.011	0.619
LP(a) (mg/L)	146.031±141.504	156.260±179.294	260.253±273.585	190.501±89.803	0.166
FFA (μmol/L)	458.822±168.194	440.323±230.428	467.211±275.078	365.500±45.982	0.911
TP (g/L)	71.070±5.989	69.462±6.808	69.884±5.889	75.000±6.557	0.281
albumin (g/L)	43.844±3.456	43.587±3.128	43.806±3.376	43.333±4.041	0.964
PA (mg/L)	271.282±41.170	263.766±53.376	264.791±48.294	216.677±37.554	0.254
BMI (kg/m^2)	23.773±2.947	23.255±2.851	22.796±3.613	23.539±2.593	0.426

Note: A p-value of less than 0.05 was considered to be statistically significant.
Abbreviations: ALS amyotrophic lateral sclerosis; apoA-I apolipoprotein A-I; apoB apolipoprotein B; BMI body mass index; FFA free fatty acids; FVC forced vital capacity; HDL-C high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp(a) lipoprotein a; PA prealbumin; TC total cholesterol; TG triglycerides; TP total protein.

Table 3: Influence of serological and clinical variables on progression rate of ALS

Items	Univariate analysis		Multivariate analysis		Multivariate analysis backward selection	
	p value	95%CI	*p* value	95%CI	*p* value	95%CI
glucose (mmol/L)	0.575	(-0.096, 0.054)				
HbA1c (%)	0.475	(-0.304, 0.143)				
TC (mmol/L)	0.269	(-0.047, 0.168)				
TG (mmol/L)	0.986	(-0.092, 0.093)				
HDL-C (mmol/L)	0.941	(-0.368, 0.396)				
LDL-C (mmol/L)	0.208	(-0.046, 0.208)				
LDL-C/HDL-C ratio	0.644	(-0.087, 0.141)				
apoA-I (g/L)	0.311	(-0.635. 0.204)				
apoB (g/L)	0.287	(-0.298, 0.995)				
apoB/apoA-I ratio	**0.083**	(-0.072, 1.162)	0.505	(-0.507, 1.011)	0.580	(-0.548, 1.192)
LP(a) (mg/L)	**<0.001**	(0.001, 0.002)	**<0.001**	(0.001, 0.002)	**0.048**	(0.000, 0.002)
FFA (μmol/L)	0.495	(-0.001, -0.236)				
TP (g/L)	0.897	(-0.016, 0.014)				
albumin (g/L)	0.710	(-0.034, 0.023)				
PA (mg/L)	0.942	(-0.002, 0.002)				
BMI (kg/m^2)	0.548	(-0.019, 0.036)				
Rate of weight loss (kg/month)	**<0.001**	(0.180, 0.519)	**<0.001**	(0.347, 0.911)	0.151	(0.072, 1.114)

Note: Biomarkers with p-value of less than 0.20 was included in multivariate analysis, which was in bold.
Abbreviations: ALS amyotrophic lateral sclerosis; apoA-I apolipoprotein A-I; apoB apolipoprotein B; BMI body mass index; FFA free fatty acids; FVC forced vital capacity; HDL-C high-density lipoprotein cholesterol; LDL-C, low-density

Table 4: Influence of serological and clinical variables on survival time of ALS

Items	Mean survival (±SD) (months)		Log rank test	Cox regression analysis
Glucose [≥5.1/<5.1] (mmol/L)	54.957±4.397	68.947±5.861	**0.162**	0.264
HbA1c [≥5.4/<5.4] (%)	59.377±8.936	47.122±8.936	0.966	
TC [≥4.87/<4.87] (mmol/L)	60.860±6.000	62.078±6.294	0.807	
TG [≥1.37/<1.37] (mmol/L)	56.233±5.916	66.798±6.306	0.257	
HDL-C [≥1.14/<1.14] (mmol/L)	65.692±6.132	56.471±6.698	0.207	
LDL-C [≥3.0/<3.0] (mmol/L)	59.764±5.924	64.156±6.225	0.820	
LDL-C/HDL-C ratio [≥2.67/<2.67]	53.559±6.190	67.505±6.217	**0.057**	0.663
apoA-I [≥1.29/<1.29] (g/L)	65.436±6.927	58.484±8.418	0.351	
apoB [≥1.00/<1.00] (g/L)	59.676±6.845	69.635±8.383	0.617	
apoB/apoA-I ratio [≥0.74/<0.74]	61.537±7.675	63.313±7.306	0.549	
LP(a) [≥114/<114] (mg/L)	52.899±6.382	67.727±7.484	**0.128**	0.469
FFA [≥408/<408] (μmol/L)	63.012±7.038	62.426±8.253	0.845	
TP [≥70/<70] (g/L)	53.391±6.034	70.941±6.554	0.236	
Albumin [40≥/<40] (g/L)	55.663±4.185	65.243±5.937	0.991	
PA [≥272/<272] (mg/L)	53.410±5.237	65.737±5.795	**0.159**	0.270
BMI [≥23.42/<23.42] (kg/m^2)	63.153±5.052	57.103±5.031	0.231	

Note: Biomarkers with p-value of less than 0.20 was included in multivariate analysis, which was in bold.
Abbreviations: ALS amyotrophic lateral sclerosis; apoA-I apolipoprotein A-I; apoB apolipoprotein B; BMI body mass index; FFA free fatty acids; FVC forced vital capacity; HDL-C high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp(a) lipoprotein a; PA prealbumin; SD standard deviation; TC total cholesterol; TG triglycerides; TP total protein.

has been reported to be a prognostic factor of long survival.[26]

In the study, we selected indicators commonly used in clinical practice to objectively observe the influences of serum levels of nutrients in ALS progression and survival. In general, mean BMI of included patients was within the normal range in all clinical stages and we found no prominent relationship between BMI and baseline ALSFRS-R score, total MRC score, FVC% or subsequent progression rate. Compared to patients with high BMI, those with low BMI tended to have shorter lives while no significant difference was reached. However, our results showed that rate of weight loss was a predictor of rapid progression (Table 2), which coincided with prior studies.[27,28] This supported the necessity of weight monitoring and external nutritional supplements among ALS patients.

Carbohydrates are the main energy source in the human body. Fasting blood glucose is a clinically available indicator that reflects the absorption and utilization of glucose, and HbA1c can reflect the average blood sugar level over the past 8-12 weeks. Our results showed a considerable proportion of participants tended to have persistent hyperglycemia, indicating the abnormalities of glucose clearance and uptake in ALS. Consistent with our results, epidemiological studies showed that prevalence of impaired glucose tolerance (IGT) in ALS patients was relatively higher than that in healthy control.[8] In addition, diabetes mellitus (DM) seemed to reduce the risk of developing ALS[29], indicating the potential role of glucose metabolism disorder in the etiology of ALS. McDonald *et al*. found that exogenous glucose uptake was increased in the $SOD1^{G93A}$ mouse model of ALS, possibly through an insulin-independent mechanism.[30] *TARDBP* and *TBK1* were considered among ALS-related genes, were showed to be involved in the phosphorylation of the insulin receptor and in vivo insulin resistance.[31,32] All these might contribute to hyperglycemia in ALS. Also, higher serum glucose could compensate ALS patients' so-called hypermetabolism[7], thus might be a subsequent phenomenon to motor neuron loss. We failed to find a significant impact of serum glucose level or HbA1c on the progression or survival time among included patients, conformed to previous studies[33], possibly weakening the potential causal relationship between the two disorders. Whether ALS patients could take antidiabetic drugs also deserved further discussion.

Dyslipidemia was common among ALS patients, making up 1/4 to 1/3 of studied participants. Among them, hyperlipidemia including hypercholesterolemia, hypertriglyceridemia, low HDL-C and high LDL-C levels accounted for the majority. Besides, abnormally elevated Lp(a) was found in 1/6 of studied patients, while abnormal serum levels of apoB, apoA-I and FFA were not common. This was interesting since prior studies seemed more supportive of hypolipidemia in ALS.[13,34] The divergence might stem from diversity in dietary habits[14], gender ratio[9] and age of studied patients among studies. We noticed that serum level of TC presented a continued increase related to clinical stages while TG showed a sustained decrease, although no significance reached (Supplementary Table 2). ALS is a neurodegenerative disease and as neurons die, cholesterol is released from cells.[35] Thus, TC might represent a biomarker of neurodegeneration, which could also explain the negative relationship between serum levels of TC or LDL-C and ALSFRS-R score. The main destination of triglycerides in the human body is for decomposition and energy supply, therefore increased energy expenditure in ALS might lead to a depletion of serum TG. The negative correlation between serum levels of apoB, Lp(a) or FFA and FVC% might be the manifestation of decreased diaphragm function caused by insufficient energy supply in ALS patients. The high levels of apoB[36] and some metabolites of cholesterol[10] were shown to lead to neuronal degeneration in transgenic mouse models, and might be a trigger to ALS onset. Therefore, a relatively high level of triglycerides and low level of cholesterol might be beneficial to ALS patients.

However, we found no statistically remarkable association between serum levels of TC, TG, LDL-C, HDL-C, apoB, apoB or relevant ratios and ALS progression or survival. Only Lp(a) was weakly related to progression rate among ALS patients. Consistent with our results, two recent meta-analyses reported that the levels of all lipids were not associated with overall mortality in ALS.[14,37] This indicated that serum biomarkers of glucose, lipid or protein might only have a weak relationship with cross-sectional autonomous function and respiratory function status, and that lipid disorders might just be the epiphenomena of ALS pathogenesis.

To avoid to effects of statines and other lipid-lowering drugs on our results, we excluded patients with intake of these drugs. To the best of knowledge, there was still no evidence-based study that supported detrimental influences of

statines in ALS.[38] Therefore, ALS was not a contraindication for statine use.

Protein deficiency in ALS patients has been reported by Yang *et al*.[39] Among our patients, this problem seemed not prominent. Besides, there was no statistically significant relationship between serum level of TP, albumin and baseline ALSFRS-R score, total MRC score or FVC%. We noticed that serum level of PA was positively related to baseline ALSFRS-R score. In clinical practice, PA is commonly considered an indicator of liver function, but it is also a sensitive indicator of early malnutrition of protein. Given the normal levels of both ALT and AST among our patients, our results indicated that protein supplementation for ALS patients in early stages might be beneficial, especially in delaying the decline in functional scores and improving quality of life. Chiò *et al*. reported that serum albumin was independent predictor of survival in ALS patients[40], while no similar result was revealed by our analysis. The involvement of routine blood tests in the multivariate analysis might be one of the causes, as they stated that serum albumin was correlated with markers of inflammatory state. More studies remained to be done to further elucidate the relationship between serum level of protein and ALS.

Through follow-up of 191 Chinese ALS patients, our study provided new evidence for the relationship between serum biomarkers regarding glucose, lipid or protein and ALS progression or survival. We admitted limitations in the following aspects. Firstly, the bias of selection should not be ignored as only a small proportion of ALS patients in our cohort have tested for nutritional biomarkers and included in our analysis. Besides, some patients might start to take specific nutritional supplements or drugs when they realized the abnormalities in blood test, which could significantly affect our results.

In conclusion, our results suggested that serum glucose, lipid or protein might only have a weak relationship with the autonomous function and respiratory function status, but have no significant impact on the progression or overall survival of ALS. More studies on the dietary status of ALS, especially clinical trials, were needed to provide more basis for dietary guidelines for ALS patients.

DISCLOSURE

Availability of date: Data in the manuscript might be available by contacting the corresponding author.

Financial support: The Strategic Priority Research Program (Pilot study) "Biological basis of aging and therapeutic strategies" of the Chinese Academy of Sciences (XDB39040000), CAMS Innovation Fund for Medical Sciences (CIFMS 2021-I2M-1-003), National High Level Hospital Clinical Research Funding (2022-PUMCH-B-017) and Beijing Natural Science Foundation (7202158).

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