Neurol Sci (2017) 38:2177–2182
DOI 10.1007/s10072-017-3138-8

ORIGINAL ARTICLE

Changes in routine laboratory tests and survival in amyotrophic lateral sclerosis

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Received: 22 June 2017 / Accepted: 26 September 2017 / Published online: 4 October 2017

Abstract The aim of this study is to evaluate the association between changes in routinely prescribed laboratory tests and tracheostomy-free survival in amyotrophic lateral sclerosis (ALS). Two hundred seventy-five ALS patients were retrospectively studied. BMI, forced vital capacity, hemoglobin, hematocrit, lymphocytes, cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, proteins, albumin, creatine-phosphokinase, iron, ferritin, transferrin, glucose, urea, uric acid, and creatinine were measured every 6 months from baseline to 24 months, death or study end, together with the probability of death or tracheostomy. Missing data were handled using multiple imputation chained equations. Hemoglobin (OR = 1.71, 95%CI 1.24–2.36 for IQR increase), hematocrit (OR = 1.87, 95%CI 1.34–2.63 for IQR increase), urea (OR = 1.51, 95%CI 1.21–1.89 for IQR increase), and uric acid (OR = 1.98, 95%CI 1.23–3.20 for IQR increase) were directly associated, while triglycerides (OR = 0.69, 0.51 to 0.93 for IQR increase) were inversely associated with the odds of death or tracheostomy. In our cohort, an increase of hemoglobin, hematocrit, urea, and uric acid was directly associated, and an increase of triglycerides was inversely associated with the odds of death or tracheostomy. Should these findings be replicated in an external cohort, they might help to discriminate ALS progression and patients' decisions about procedures and end of life.

Keywords Amyotrophic lateral sclerosis · Routine laboratory tests · Hemoglobin · Hematocrit · Urea · Uric acid

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Introduction

Amyotrophic lateral sclerosis (ALS) has a progressive course characterized by worsening disability and death after a period of time which is highly variable among patients. Such heterogeneity makes it difficult to predict how ALS will progress in a given patient and to evaluate the efficacy of new treatments in clinical trials [1].

The uncertain prognosis of ALS is discomforting to the patients and their families, who have to cope with an increasing number of difficulties in an unpredictable time. Being able to better discriminate the progression of ALS might allow patients and physicians to make better decisions about ventilation, nutritional support, and end of life [1].

Known prognostic factors for ALS include age at onset, phenotype, diagnostic delay, degree of diagnostic certainty according to El Escorial-Revised Criteria (EEC-R), concurrent dementia, and body mass index (BMI) [2, 3]. Many potential prognostic markers have been studied in ALS patients including uric acid [4, 5], albumin, creatinine [6], blood lipids [7, 8], ferritin, transferrin [9, 10], creatine-phosphokinase (CPK) [11], regulatory T cells [12, 13], and neurofilaments [14, 15]. The study of such prognostic markers has generally led to conflicting results. A recent crowd-sourced analysis of data from multiple clinical trials has suggested a prognostic

role for uric acid and creatinine [16]. However, only one study so far has tested the association between repeated measures of prognostic markers and ALS prognosis [17].

An ideal prognostic marker for ALS should be obtained with minimum discomfort to the patient and should be rapidly available. For this reason, we retrospectively evaluated the association between the changes in routinely prescribed laboratory tests and tracheostomy-free survival in the cohort of ALS patients followed at our Center.

Patients and methods

Study design

This was a retrospective cohort study performed on ALS patients diagnosed between 1 January 2000 and 31 December 2013 in Modena (Italy). Starting from 1 January 2000, an ALS multidisciplinary Center is working in Modena and collecting all cases of incident ALS on a registry [18]. The data of the ALS patients diagnosed from 1 January 2009 are also available in the web-based Regional (Emilia Romagna) ALS Registry [19]. ALS was diagnosed in all cases using the EEC-R [20]. ALS patients were followed up from diagnosis until death or last observation date, which was set at 31 December 2014 for the present analysis. The study was approved by the local Ethical Committee.

Clinical assessment

Each ALS patient underwent a standardized clinical assessment including the evaluation of the variables of interest for the present analysis: (1) ALS diagnosis according to EEC-R; (2) sex; (3) age at diagnosis; (4) age at onset; (5) site of onset; (6) clinical phenotype [21]; (7) presence of percutaneous endoscopic gastrostomy (PEG); (8) treatment with riluzole; and (9) ventilation. Forced vital capacity (FVC) was measured using whole body pletismography. Weight and height were measured following international guidelines, and BMI was calculated as weight (kg) × height $(m)^{-2}$. A functional assessment was performed using the ALS functional rating scale [22, 23]. The clinical information was updated at each follow-up visit. At our ALS Center, we perform follow-up visits every 3 months. Such visits include support from pulmonologists, dietitians, speech pathologist, rehabilitation physicians, psychologists, and other specialists when needed. For the purpose of the present study, we used the data obtained at follow-up visits performed every 6 months (see below).

Laboratory tests

The following laboratory tests were retrieved for each ALS patient at baseline and every 6 months thereafter until death or last observation date, tracheostomy, or study termination: (1) hemoglobin; (2) hematocrit; (3) lymphocytes; (4) cholesterol; (5) LDL-cholesterol; (6) HDL-cholesterol; (7) triglycerides; (8) proteins; (9) albumin; (10) CPK; (11) iron; (12) ferritin; (13) transferrin; (14) glucose; (15) urea; (16) uric acid; and (17) creatinine. All laboratory tests were performed by the same centralized clinical laboratory using standardized methods.

Statistical analysis

Most continuous variables were not Gaussian-distributed, and all are reported as 50th (median), 25th, and 75th percentiles. Discrete variables are reported as the number and proportion of subjects with the characteristic of interest.

The probability of death or tracheostomy at 6, 12, 18, and 24 months of follow-up was estimated from a discrete-time logistic regression model having death or tracheostomy as response variable (0 = no; 1 = yes) and discrete-time as predictor (0 = baseline; 1 = 6 months; 2 = 12 months; 3 = 18 months; 4 = 24 months) [24, 25].

The choice of such modeling strategy was due to the fact that traditional modeling strategies such as Cox regression are unsuitable for use with discrete-time intervals [24, 25].

To test whether the time-varying continuous variables of interest (BMI, FVC, hemoglobin, hematocrit, lymphocytes, cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, proteins, albumin, CPK, iron, ferritin, transferrin, glucose, urea, uric acid, and creatinine) were associated with death or tracheostomy, we added each of them as predictor to the discrete-time logistic regression model together with ALS type (discrete; 0 = spinal; 1 = bulbar) and age at onset (continuous, years/10). Each continuous predictor was divided by its interquartile range (IQR) to allow the comparison of predictors with different units of measurements [26]. ALS type and age at onset were added to the model, because they are known prognostic indexes in ALS, and we aimed at assessing the independent contribution of the variables of interest to death or tracheostomy. Because the discrete-time logistic regression model has the response variable and the predictors specified at each time-point [25], the values of the predictors after death were put equal to those at death when these were available. Missing data were otherwise handled by using multiple imputation chained equations (MICE) [27]. Among the MICE predictors, the outcome (death or tracheostomy), the (discrete) time of the outcome, sex, ALS phenotype, and age at onset were known for all patients, while the time-varying variables of interest were not available at all times for all patients and were estimated by MICE using linear regression in 100 imputation datasets. Statistical analysis was performed using Stata 14.2 (Stata Corporation, College Station, TX, USA).

Table 1 Clinical characteristics of the 275 ALS patients stratified by sex

		Women (*N* = 122)		Men (*N* = 153)		All (*N* = 275)	
		n	%	*n*	%	*n*	%
Age at onset (years)—median (IQR)		68.0	(58.5, 75.9)	63.3	(53.9, 72.3)	65.2	(55.2, 74.1)
ALS onset	Bulbar	49	40.2	34	22.2	83	30.2
	Spinal	73	59.8	119	77.8	192	69.8
Phenotype	Bulbar	52	42.6	35	22.9	87	31.6
	Classic	51	41.8	72	47.1	123	44.7
	Flail arm	3	2.5	10	6.5	13	4.7
	Flail leg	9	7.4	22	14.4	31	11.3
	UMNP	6	4.9	8	5.2	14	5.1
	Respiratory	1	0.8	6	3.9	7	2.5
Diagnostic delay (months)—median (IQR)		11	(6, 18)	9	(5, 14)	10	(6, 16)
Treated with Riluzole[a]		112	91.8	147	96.1	259	94.2
Percutaneous endoscopic gastrostomy[a]		68	55.7	71	46.4	139	50.5
Invasive ventilation[a]		30	24.6	48	31.4	78	28.4
Non-invasive ventilation[a]		67	54.9	89	58.2	156	56.7

[a] These treatments were performed at different times during the 24-month follow-up

Results

Of 312 patients diagnosed with ALS at our Center from 1 January 2000 to 31 December 2013, 13 were excluded because of unconfirmed diagnosis and 24 because of complete lack of laboratory tests at diagnosis.

Table 1 gives the clinical characteristics of the 275 studied ALS patients stratified by sex.

Table 2 gives the values of BMI, FVC and laboratory tests at baseline and at 6,12,18 and 24 months.

Figure 1 plots the cumulative incidence of death or tracheostomy from diagnosis as estimated by the discrete-time logistic regression model.

The cumulative incidence of death or tracheostomy was 0.08 (95%CI 0.05–0.11) at 6 months, 0.15 (95%CI 0.11–0.19) at 12 months, 0.24 (95%CI 0.19–0.29) at 18 months, and 0.36 (95%CI 0.30–0.41) at 24 months.

Figure 2 plots the odds ratio (OR) of death or tracheostomy associated with an increase of an IQR of each of the continuous predictors of interest.

These OR were obtained from multivariable logistic regression models controlling for ALS type and age at onset and taking missing data into account via MICE (models not shown; see statistical analysis for details).

Hemoglobin (OR = 1.71, 95%CI 1.24–2.36 for IQR increase), hematocrit (OR = 1.87, 95%CI 1.34–2.63 for IQR increase), urea (OR = 1.51, 95%CI 1.21–1.89 for IQR increase), and uric acid (OR = 1.98, 95%CI 1.23–3.20 for IQR increase) were directly associated, while triglycerides (OR = 0.69, 0.51 to 0.93 for IQR increase) were inversely associated with the odds of death or tracheostomy.

Discussion

In this retrospective repeated-measure cohort study, we tested whether changes in routinely performed laboratory tests could serve as prognostic markers in ALS. We found that an increase of hemoglobin, hematocrit, urea, and uric acid was directly associated and that an increase of triglycerides was *inversely* associated with the odds of death or tracheostomy. Also, using such repeated-measure design, we were not able to confirm some previously reported associations between laboratory tests, e.g., albumin, and the odds of death or tracheostomy.

A great strength of this study is the availability of repeated measures. We evaluated the association between laboratory tests and death or tracheostomy using five repeated measures (0, 6, 12, 18, and 24 months). Although we had to take into account the presence of missing data, the availability of repeated measures within the same patient allows to control patient-level heterogeneity much better than the standard approach of associating a single baseline value with a later outcome [25]. The major drawback of the present study is the presence of missing data, which is nonetheless quite common with both retrospective and prospective cohort studies, especially in diseases characterized by rapidly increasing disability such as ALS. There is a general consensus among statisticians that is much better to handle missing data via multiple imputation (or other techniques) than simply discarding them [27]. We used MICE for handling missing data in the present study, which is presently regarded as the most efficient multiple imputation technique [27]. We did not, however, test the effect of time on the outcome-predictors association. This would require (at least) the addition of a timeXpredictor interaction

Table 2 Anthropometry, respiratory function, and blood tests of ALS patients at baseline and 6, 12, 18, and 24 months

	Baseline				6 months				12 months				18 months				24 months			
	N	P_{50}	P_{25}	P_{75}	*N*	P_{50}	P_{25}	P_{75}	*N*	P_{50}	P_{25}	P_{75}	*N*	P_{50}	P_{25}	P_{75}	*N*	P_{50}	P_{25}	P_{75}
BMI (kg m^{-2})	129	24.5	22.5	27.1	113	24.8	22.5	27.6	135	23.9	22.1	27.1	87	23.9	22.3	26.9	68	23.9	22.2	27.0
FVC (%)	175	86	66	101	164	81	58	98	141	74	54	94	97	71	51	94	74	74	45	93
Hemoglobin (g dl^{-1})	266	13.9	12.9	14.9	192	13.8	12.8	14.8	148	13.9	13.0	14.8	118	13.8	12.6	15.0	96	14.2	13.2	15.2
Hematocrit (%)	254	41	38	44	184	42	38	44	139	42	40	45	115	42	38	45	93	43	40	45
Lymphocytes (cells mm^{-3})	237	1790	1340	2200	174	1800	1440	2250	130	1800	1370	2260	110	1860	1430	2220	90	1790	1520	2300
Cholesterol (mg dl^{-1})	245	198	172	232	163	207	187	233	133	208	182	228	106	204	181	229	81	204	175	223
LDL (mg dl^{-1})	222	130	102	152	137	137	115	154	107	131	106	157	88	130	107	156	64	129	104	154
HDL (mg dl^{-1})	225	50	41	58	138	51	44	63	102	51	43	60	88	51	42	61	60	47	40	63
Triglycerides (mg dl^{-1})	240	100	74	132	160	110	76	150	131	111	81	149	103	116	80	153	79	108	79	143
Proteins (g dl^{-1})	233	6.5	6.2	7.0	140	7.0	6.5	7.3	123	6.9	6.4	7.2	93	6.9	6.4	7.2	73	6.9	6.4	7.2
Albumin (g dl^{-1})	198	3.9	3.5	4.2	100	4.0	3.6	4.4	85	4.0	3.7	4.5	73	3.8	3.5	4.4	45	4.0	3.7	4.4
CPK (U l^{-1})	213	150	88	282	146	190	87	303	121	172	112	303	87	173	110	326	71	191	96	346
Iron (μg dl^{-1})	149	93	72	118	112	90	67	108	95	90	75	105	77	90	73	116	57	96	81	115
Ferritin (ng ml^{-1})	104	138	59	214	103	149	58	259	86	139	56	235	72	120	73	218	54	148	84	255
Transferrin (mg dl^{-1})	127	250	213	302	99	234	202	291	84	225	201	270	72	221	200	268	49	240	204	265
Glucose (mg dl^{-1})	249	91	83	102	165	91	85	100	132	94	84	104	100	90	80	104	79	94	81	104
Urea (mg dl^{-1})	244	34.0	29.0	42.0	145	35.0	27.0	43.0	115	36.0	29.0	43.0	87	37.0	30.0	45.0	67	35.0	28.0	42.0
Uric acid (mg dl^{-1})	185	5.0	3.9	6.0	109	4.8	3.6	5.6	85	4.8	4.0	5.8	78	4.6	3.7	5.8	57	4.7	3.7	5.7
Creatinine (mg dl^{-1})	257	0.8	0.6	0.9	187	0.7	0.6	0.9	152	0.7	0.5	0.9	119	0.7	0.5	0.9	94	0.7	0.5	0.8

N, number of subjects; P_x, X^{th} percentile; *BMI*, body mass index; *FVC*, forced vital capacity; *LDL*, low-density lipoprotein cholesterol; *HDL*, high-density lipoprotein cholesterol; *CPK*, creatine-phosphokinase

term to the logistic regression model, something which was not possible here, because the missing data and the intrinsically discrete nature of time did not allow to have enough points to estimate a time-related effect with acceptable precision [25]. We plan to perform such analysis on a future version of our ALS database offering with the availability of more time-points.

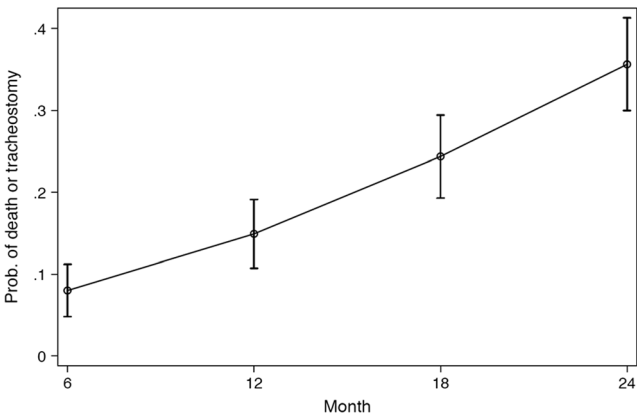

Fig. 1 Point estimates and 95% confidence intervals of the cumulative probability of death or tracheostomy at 6, 12, 18, and 24 months. The values are obtained from a discrete-time logistic regression model (see statistical analysis for details)

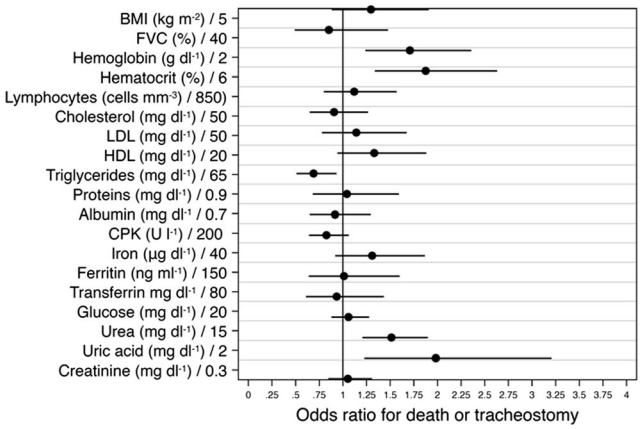

Fig. 2 Point estimates and 95% confidence intervals of the odds ratio of death or tracheostomy associated with an increase of an interquartile range of the continuous predictor of interest. The values are obtained from 19 multivariable logistic regression models controlling for ALS type (discrete, bulbar vs. spinal) and age at onset (continuous) and taking missing data into using multiple imputation chained equations (see statistical analysis for details)

Another limitation of the present study is its observational nature. Whether a modifiable risk factor can increase survival in ALS can only be tested by performing a randomized controlled trial aiming at modifying that factor. Confounding is the main problem of all observational epidemiology, and its effect may be even greater than usual in a disease such as ALS which requires different treatments and is characterized by substantial patient-level heterogeneity.

This is the first study showing that change during time of hemoglobin and hematocrit could serve as prognostic markers in ALS. A rising hematocrit in ALS patients may be a surrogate measure of the dehydration associated with drink dysphagia and/or respiratory insufficiency [28]. In the present repeated-measure study, however, we found no association between the changes of FVC and those of hemoglobin and hematocrit (data not shown). Also, we found no association between the changes of BMI and those of hemoglobin and hematocrit (data not shown), but this was not unexpected because of the nutritional support received by our patients. On the other hand, a rising hemoglobin level may signal an increased risk of vascular access thrombosis and adverse cardiovascular events.

The association between a rising urea and the odds of death or tracheostomy has never been reported before, to the best of our knowledge. Instead, in a large Italian cohort, creatinine was found to be a prognostic marker of ALS [6], but blood urea was not measured in that study. It should nonetheless be noted that, contrarily to that study [6], we found no association between the changes in creatinine and the odds of death or tracheostomy in our patients. Both urea and creatinine can increase because of dehydration so that, as hypothesized above for hemoglobin and hematocrit, the increase of urea may signal dehydration. Another explanation of the urea-survival association may come from the fact that urea is the product of the catabolism of ammonia and oxidation of amino acids. The ubiquitous enzyme glutamine synthetase (GS) catalyzes the conversion of ammonia and glutamate into glutamine. GS is present in the brain, mostly in astrocytes, and plays a central role in brain detoxification and glutamate metabolism regulation [29, 30]. Whether the association between a rising urea and the odds of death or tracheostomy found in our ALS patients signals a dysregulated brain metabolism is highly speculative but warrants further exploration.

Uric acid, the product of purine metabolism, has immunological and pro-inflammatory functions and has been extensively studied in ALS [31]. Low levels of uric acid have been reported not only in ALS but also in neurodegenerative disorders such as Alzheimer's disease, Huntington's disease, Parkinson's disease, and multiple system atrophy [31]. A meta-analysis of ALS studies [4, 5, 31, 32] has reported an inverse association between baseline uric acid levels and mortality. Our finding of a *direct* association between an increase of uric acid and death or tracheostomy is not necessarily at odds with these findings, because our study focused on changes and not on static values of laboratory tests. This interpretation is supported by the finding of a recent study [33], which measured uric acid at two time-points and showed that the association of uric acid with survival was present at one but not at the other time-point. As for urea, if and how the association between uric acid and ALS survival reflect changes occurring inside astrocytes is highly speculative but warrants further exploration [31].

In our study, there was an *inverse* association between the changes of triglycerides and the odds of death and tracheostomy in ALS. On the other hand, we observed no association between the changes in total, HDL and LDL cholesterol, and the odds of death and tracheostomy. Blood lipids have been studied more than other laboratory tests in ALS with contradictory findings [7, 8, 34]. The precision of the effect size attributable to an increase of an IQR (i.e., 65 mg × dl-1) of triglycerides is wide enough (OR = 0.69, 0.51 to 0.93) to suggest that larger studies are needed before one can reliably infer the existence of a clinically relevant relationship between triglycerides and survival in ALS.

In conclusion, in the present retrospective cohort study of ALS patients, an increase of hemoglobin, hematocrit, urea, and uric acid was positively associated, and an increase of triglycerides was inversely associated with the odds of death or tracheostomy. These findings need to be replicated in external cohorts before they can be translated into clinical practice.

Compliance with Ethical Standards We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Conflict of Interest The authors declare that they have no conflict of interest.

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