Original Science

Evaluation of Multiple Standard Laboratory Parameters in Amyotrophic Lateral Sclerosis

Bandhu Paudyal, MBBS[1]; Nabil Ahmad, MD[1]; James B. Caress, MD[1]; Leah P. Griffin, MS[2]; and Michael S. Cartwright, MD, MS[1]

Abstract

Objectives:

To examine laboratory tests frequently obtained during the evaluation of ALS and determine how often they are abnormal.

Methods:

We performed a retrospective chart review of all patients diagnosed with "probable," "probable — laboratory supported," and "definite" ALS, seen in the Wake Forest ALS Clinic from May 2009-May 2012. The following test results were analyzed in 105 individuals with ALS: anti-nuclear antibody, calcium, creatine kinase (CK), c-reactive protein, copper, erythrocyte sedimentation rate (ESR), free light chain ratio, human immunodeficiency virus, intact parathyroid hormone, methylmalonic acid, mean corpuscle volume, rapid plasminogen reagent, serum protein electrophoresis, thyroid stimulating hormone, urine protein electrophoresis, urine heavy metals, vitamin B12, and vitamin D.

Results:

Results of four tests were abnormal in more than 15% of patients, including CK (58.8%), vitamin D (54.5%), ESR (26.0%), and methylmalonic acid (16.7%). The CK level was significantly higher in limb-onset (303.6 U/l) than bulbar-onset (194.2 U/l) ALS.

Conclusions:

Common laboratory tests obtained during the evaluation of suspected ALS are often abnormal. Knowledge of this may assist in establishing the diagnosis sooner and with more confidence in those with abnormal laboratory results.

From the Department of Neurology,[1] Wake Forest School of Medicine, Winston-Salem, NC 27157 Division of Public Health Sciences,[2] Department of Biostatistical Sciences, Wake Forest School of Medicine, Winston-Salem, NC 27157

Author contributions:
Participated in research design: BP, NA, JBC, LPG, MSC

Participated in writing of the paper: BP, NA, JBC, LPG, MSC

Participated in performance of the research: BP, NA, JBC, LPG, MSC

Participated in data collection and analysis: BP, NA, JBC, LPG, MSC

Contact:
Michael S. Cartwright, MD, MS
Department of Neurology
Wake Forest School of Medicine
Main Floor Reynolds Tower
Winston-Salem, NC 27157
Email:
mcartwri@wakehealth.edu
Phone: 336-716-5177
Fax: 336-716-7794

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease in which motor neurons are progressively lost from the brain and spinal cord, and it results in limb weakness, dysarthria, dysphagia, dyspnea, and eventually respiratory compromise and death.[1] One of the clinical challenges with ALS lies in establishing the diagnosis. This requires a thorough history and detailed physical examination, as there is no single laboratory, imaging, or electrodiagnostic test to confirm the diagnosis of ALS.[2] In addition, electrodiagnostic and laboratory tests are typically used to exclude similar conditions and assist in the diagnosis.[3] Several studies have demonstrated a typical delay in diagnosis of 9 to 16 months, which can slow enrollment in clinic trials and delivery of medications and devices for symptomatic treatment.[4]

While laboratory testing of blood and urine parameters usually yields the expected normal findings in individuals with ALS, some parameters could potentially be affected by the denervation that occurs with ALS, such as creatine kinase (CK). This study was therefore designed to examine the range of laboratory findings in a group of patients with ALS, because knowledge of this information may assist clinicians in establishing the diagnosis of ALS sooner and with more confidence.

Materials & Methods

A retrospective chart review of all patients diagnosed with "probable," "probable—laboratory supported," and "definite" ALS, based on El Escorial Criteria,[5] over 3 years (May 2009 to May 2012) by one physician (MSC) with expertise in ALS was performed. Prior to performing the chart review this study was approved by the Institutional Review Board of Wake Forest School of Medicine. Patient characteristics were recorded from the first clinic visit at the Wake Forest ALS Clinic, including age, sex, race, ALS functional rating scale revised (ALSFRS-R) score,[6] forced vital capacity (FVC), site of onset, and number of months with symptoms prior to the initial visit at our ALS clinic.

The following laboratory values were recorded for each patient: anti-nuclear antibody (ANA), calcium, CK, c-reactive protein (CRP), copper, erythrocyte sedimentation rate (ESR), free light chain ratio, human immunodeficiency virus (HIV), intact parathyroid hormone (PTH), methylmalonic acid, mean corpuscle volume (MCV), rapid plasminogen reagent (RPR), serum protein electrophoresis (SPEP), thyroid stimulating hormone (TSH), urine protein electrophoresis (UPEP), urine heavy metals, vitamin B12, and vitamin D. These specific parameters were chosen because they are often tested in the evaluation of suspected ALS to help exclude ALS mimics.[3] If multiple values for a parameter were available, the value obtained closest to the initial visit was used. When necessary, outside records were also reviewed to obtain values for the parameters of interest; again, the value obtained closest to the initial visit was used. Most individuals did not have every parameter checked, and if the parameter was not checked within 6 months of the first visit to our clinic, it was excluded from analyses.

All individuals in this study with abnormal laboratory values were further assessed clinically to ensure that the abnormal laboratory value did not result from another underlying condition. For example, those with low vitamin B12 or high methylmalonic acid underwent aggressive vitamin B12 replacement with no change in their disease. Similarly, for those with abnormal free light chain ratios or protein electrophoreses, further testing found either normal values or monoclonal gammopathy of undetermined significance (MGUS) that did not contribute to their motor neuron disease. In all cases, a diagnosis of ALS was confirmed and other conditions potentially associated with the abnormal laboratory value were excluded.

For each laboratory parameter, the percentage of abnormal results was calculated. In addition, the mean value and reference range were calculated, if applicable. Table 1 includes the reference range for each parameter. For those tests in which different reference ranges are present, based on factors such as age and sex, we used the most conservative and inclusive reference values from our laboratory. In addition, if the parameter included one or more values from an outside laboratory, the outside reference range was incorporated into the range (presented in Table 1), again to create the most conservative and inclusive reference range. Finally, Pearson product-moment correlation coefficients were calculated to examine for an association between months after ALS onset and all continuous parameters.

Parameter (n)	Percent Abnormal	Mean Value	Range	Reference Values
ANA (44)	11.4	NA	NA	Negative
Calcium (75)	4.0	9.6	8.1-10.6	8.5-10.5 mg/dl
CK (80)	58.8	275.9	23-1470	50-180 U/l
CRP (59)	6.8	4.9	0.1-72.8	0-10 mg/l
Copper (64)	12.5	113.7	56-228	75-175 µg/dl
ESR (50)	26.0	16.7	1-55	0-20 mm/hr
Free light chain ratio (74)	9.5	1.12	0.31-2.59	0.26-1.65
HIV (29)	0	NA	NA	Negative
Intact PTH (74)	4.1	40.6	14-97	12-72 pg/ml
Methylmalonic acid (66)	16.7	194.3	60-633	87-318 nm/l
MCV (69)	5.8	92.7	80.2-130	80-99 fl
RPR (40)	0	NA	NA	Non Reactive
SPEP/IFE (103)	1.9	NA	NA	No M Spike
TSH (58)	5.2	2.0	0.4-6.8	0.4-5.5 uIU/ml
UPEP/IFE (20)	0	NA	NA	No M Spike
Urine heavy metals (20)	15.0	NA	NA	Negative
Vitamin B12 (74)	13.5	497.3	142-1500	>200 pg/ml
Vitamin D (11)	54.5	28.9	15-45	>30 ng/ml

Table 1. Laboratory Findings in 105 Individuals with ALS

ANA = anti-nuclear antibody, CK = creatine kinase, CRP = c-reactive protein, ESR = erythrocyte sedimentation rate, HIV = human immunodeficiency virus, IFE = immunofixation, NA = not applicable, PTH = parathyroid hormone, MCV = mean corpuscle volume, RPR = rapid plasminogen reagent, SPEP = serum protein electrophoresis, TSH = thyroid stimulating hormone, UPEP = urine protein electrophoresis.

Further investigation was performed for CK values to explore whether the site of onset might influence this parameter. The mean CK in those with bulbar-onset ALS was compared to those with limb-onset using linear regression and controlling for age, sex, and months since onset. In addition, since the distribution of CK values was skewed, a log transformation of CK values was used to normalize the data.

Results

Data from 105 patients were reviewed. The mean age was 62.4 years (range 20 to 88 years), 53.3% were male, the mean forced vital capacity was 66.5% of predicted (range 16 to 124%), and the mean ALSFRS-R score was 32.9 (range 13 to 48). The mean time between onset of symptoms and the lab collection was 17.3 months (range 3 to 60 months).

Of the 18 laboratory parameters evaluated, the four that were most often abnormal were CK (58.8% abnormal, checked in 80 patients), vitamin D (54.5% abnormal, checked in 11 patients), ESR (26.0% abnormal, checked in 50 patients), and methylmalonic acid (16.7% abnormal, checked in 66 patients) (Table 1). Those with limb-onset ALS had higher CK values than those with bulbar-onset ALS (Table 2). No significant correlations were found between time since ALS onset and the continuous parameters recorded.

Discussion

Although various laboratory parameters have been analyzed independently in the past, this study is the first collective analysis involving many different, and commonly obtained, laboratory parameters relevant to ALS. Of the 18 parameters assessed, eight were abnormal in at least 10% of individuals with ALS, which indicates that routine laboratory tests may be abnormal in those with ALS. Whether this proportion is higher than would be expected in an age- and sex-matched population, or in individuals with other neurologic diseases, is not definitively known. However, reference ranges for laboratory parameters are typically calculated so that only

Parameter (n)	Bulbar-Onset n = 21	Non Bulbar-Onset n = 59	P-Value
Mean Age	67.3	62.2	0.144
Percent Female	65.0	44.1	0.106
Months Since Onset	11.5	18.1	0.008
Mean CK (Range)	194.2 (23-651)	303.6 (45-1470)	0.011*

Table 2. Creatine Kinase (CK) Levels in 80 Individuals with Bulbar and Non Bulbar-Onset ALS

* This regression model controlled for age, sex, and months since onset, and used the log transformed creatine kinase level because of the skewed distribution.

5% of results from control samples are outside the reported range (normal is defined as the middle 2.5 to 97.5 quantiles). Thus, it is expected that no more than 5% of the values for any given parameter would be outside the reference range.[7]

The parameter most often abnormal in this study was CK (elevated in 58.8% of patients); this result has been demonstrated in previous studies. In agreement with this study, a previous report also found that CK levels are higher in those with limb-onset (148.52 U/l) compared to bulbar-onset ALS (58.18 U/l). This information could improve diagnostic accuracy of both conditions.[8]

In one study, spinal bulbar muscular atrophy, which may have a similar presentation to bulbar-onset ALS, was associated with a mean CK level of 939 U/l, significantly higher than the level in those with bulbar-onset ALS 304 U/l).9 The cause of CK elevation in ALS is not entirely clear. CK levels per se do not correlate with the degree of fasciculations or the duration of disease, and it is a poor marker of disease progression.[10-12]

One parameter only minimally investigated previously in ALS patients is the free light chain ratio. A study done in 1979 found similar mean free light chain ratios between 11 patients with ALS versus healthy controls.[13] The current study detected abnormal ratios in 9.5% of those tested. In addition, 1.9% had an M spike on serum protein electrophoresis testing, and none had an abnormal results on urine protein electrophoresis. Evidence of paraproteinemias has previously been reported in those with ALS, but treatment to lower paraprotein levels did not affect the course of ALS.[14]

The only other laboratory test with abnormal results in most of our patients was vitamin D level, although this was only tested in 11 individuals. Hypovitaminosis D has previously been demonstrated in those with ALS (mean of 14.0 ng/ml, lower than the mean in the current study), and those with ALS have an increased rate of fractures.[15, 16]

These results could be related to decreased sunlight exposure, which can occur in chronically ill individuals. Alternatively, those with ALS often have poor nutrition because of dysphagia, which also could result in low vitamin D levels. Recently it has been postulated that vitamin D supplementation could slow the progression of ALS through promotion of axonal regeneration,17 but this hypothesis requires further investigation. Vitamin D levels are not always checked in the evaluation of suspected ALS. However, since low vitamin D levels can cause osteomalacia and resultant lower extremity weakness, it is sometimes evaluated in patients whose symptoms start in the legs.

Our study has some limitations, including its retrospective nature. While 105 individuals were included in the analyses, not all laboratory tests were performed in all individuals; for some parameters, the number analyzed was lower than 30. Therefore, it is important to interpret with caution the parameters tested in fewer than 30% of individuals (HIV, status, UPEP/IFE ratio, urine heavy metals, and vitamin D). Some lab results may have been collected at outside offices and not captured in this analysis, although we obtained as much data as possible from physicians outside our medical center. In addition, different laboratories use different reference values. When multiple reference ranges were available, we used the broadest reference range to define an abnormal result.

Practice patterns may vary based upon patient demographics and geographic location, which is important to consider when interpreting these results. Finally, this study did not compare

the results in ALS patients to age-matched or neurologic disease controls, so we cannot say whether the proportion of abnormal tests is more than would be expected in those comparison populations. Further evaluation, perhaps through a case-control design, is warranted.

Nonetheless, these results may help clinicians to evaluate suspected cases of ALS. All laboratory values, and particularly abnormal results, require interpretation in the context of the clinical setting. It is also important to recognize that many of the typical tests performed in the evaluation of ALS can be abnormal, but do not necessarily change the diagnosis of ALS.

Authors

Sources of Funding: There was no funding for this project.

Conflicts of Interest: The authors have no conflicts of interest to report.

Reference List

1. Mitsumoto H, Chad DA, Pioro EP. Amyotrophic Lateral Sclerosis. Contemporary Neurology Series. Philadelphia: Davis Company; 1988.
2. Li TM, Day SJ, Alberman E, Swash M. Differential diagnosis of motoneurone disease from other neurological conditions. Lancet 1986; 2:731-733.
3. Shook SJ, Pioro EP. Racing against the clock: recognizing, differentiating, diagnosing, and referring the amyotrophic lateral sclerosis patient. Ann Neurol 2009; 65 Suppl 1:S10-S16.
4. Kraemer M, Buerger M, Berlit P. Diagnostic problems and delay of diagnosis in amyotrophic lateral sclerosis. Clin Neurol Neurosurg 2010; 112:103-105.
5. Brooks BR, Miller RG, Swash M, et al. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 2000; 1:293-299.
6. 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; 169:13-21.
7. Henny J, Petitclerc C, Fuentes-Arderiu X, et al. Need for revisiting the concept of reference values. Clin Chem Lab Med 2000; 38:589-595.
8. Ilzecka J, Stelmasiak Z. Creatine kinase activity in amyotrophic lateral sclerosis patients. Neurol Sci 2003; 24:286-287.
9. Chahin N, Sorenson EJ. Serum creatine kinase levels in spinobulbar muscular atrophy and amyotrophic lateral sclerosis. Muscle Nerve 2009; 40:126-129.
10. Lima AF, Evangelista T, de CM. Increased creatine kinase and spontaneous activity on electromyography, in amyotrophic lateral sclerosis. Electromyogr Clin Neurophysiol 2003; 43:189-192.
11. Felice KJ, North WA. Creatine kinase values in amyotrophic lateral sclerosis. J Neurol Sci 1998; 160 Suppl 1:S30-S32.
12. Sinaki M, Mulder DW. Amyotrophic lateral sclerosis: relationship between serum creatine kinase level and patient survival. Arch Phys Med Rehabil 1986; 67:169-171.
13. Bollengier F. Bound and free light chains in serum from patients affected with various neurological diseases. J Clin Chem Clin Biochem 1979; 17:45-49.
14. Rudnicki S, Chad DA, Drachman DA, et al. Motor neuron disease and paraproteinemia. Neurology 1987; 37:335-337.
15. Sato Y, Honda Y, Asoh T, et al. Hypovitaminosis D and decreased bone mineral density in amyotrophic lateral sclerosis. Eur Neurol 1997; 37:225-229.
16. Gresham LS, Molgaard CA, Golbeck AL, et al. Amyotrophic lateral sclerosis and history of skeletal fracture: a case-control study. Neurology 1987; 37:717-719.
17. Karam C, Scelsa SN. Can vitamin D delay the progression of ALS? Med Hypotheses 2011; 76:643-645.