Pre-morbid Laboratory Tests, Diseases, and Medications in Amyotrophic Lateral Sclerosis in Israel

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ABSTRACT **Background:** There is an unmet need for real-world data regarding laboratory results, co-morbidities, and medication use prior to the first symptoms of amyotrophic lateral sclerosis (ALS). Researchers must identify specific subpopulations at risk for developing ALS and understand pathogenic mechanisms preceding the clinical presentation of ALS as well as possible subclinical disease manifestations.

Objectives: To valuate the role of laboratory results, co-morbidities, and medication use prior to the first symptoms of patients with ALS in Israel so that specific subpopulations at risk for developing ALS can be identified and for possible subclinical disease manifestations. To understand pathogenic mechanisms preceding the clinical presentation of ALS.

Methods: At the ALS clinic at Tel Aviv Sourasky Medical Center, 259 ALS patients insured by Maccabi Healthcare Services and seen between January 1998 and December 2017 were included. Comparisons of demographics, co-morbidities, medications taken, history of trauma, and laboratory tests prior to disease onset were performed between patients and 1295 matched controls.

Results: Prior to disease presentation, ALS patients had a higher frequency of hypertension and cardiovascular disease; presented more frequently with trauma and viral infections; more frequently used analgesics, non-steroidal anti-inflammatory drugs, narcotics, antibiotics, and antiviral medications; and had higher creatine kinase levels.

Conclusions: ALS patients showed higher frequency of cardiovascular disease prior to diagnosis, as well as higher frequency of trauma, infections, and pain medication usage.

IMAJ 2023; 25: 617–621

KEY WORDS: amyotrophic lateral sclerosis (ALS), co-morbidities, laboratory tests, risk factors

Conflict of Interest

Vivian E. Drory reports consultancy fees from Biogen and speaker fees from Biogen and Roche, outside the submitted work.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive degeneration of upper and lower motor neurons leading to progressive muscle weakness and atrophy, respiratory failure, and ultimately death. The exact etiology of the disease is currently unknown and assumed to be multifactorial, with both genetic and environmental risk factors [1]. Previous studies have found a lower prevalence of cardiovascular risk factors or diseases in patients with ALS [2-9], suggesting a role for higher metabolic rate in ALS development, although two studies failed to demonstrate lower frequency of hypertension and cardiovascular disease in ALS [7,8]. Furthermore, regular use of anti-hypertensive and anti-diabetes drugs has been suggested to protect against the incidence of ALS [9].

There is an unmet need for real-world data regarding laboratory results, co-morbidities, and medication use prior to the first symptoms of ALS (disease presentation) in order to identify specific subpopulations at risk for developing ALS and to understand pathogenic mechanisms preceding the clinical presentation of ALS as well as possible subclinical disease manifestations. We evaluated the role of potential clinical biomarkers for ALS development and progression in Israel using extensive data from a nationwide health fund [9].

PATIENTS AND METHODS

The study population included patients with a diagnosis of definite or probable ALS according to the revised El Escorial criteria [10], who were seen at the ALS clinic at Tel Aviv Sourasky Medical Center at least once between January 1998 and December 2017. Written informed consent was obtained from all patients. The institutional review board at Tel Aviv Sourasky Medical Center approved the study protocol (approval 0135-15).

The ALS clinic at Tel Aviv Sourasky Medical Center is a national tertiary ALS clinic that receives referrals from the entire country. Therefore, the patients are assumed to be representative of the Israeli ALS population.

Only patients insured by Maccabi Healthcare Services were included in the current study. Maccabi is the second largest healthcare provider in Israel and covers approximately 25% of the population with a relatively uniform distribution of insured

individuals and services throughout the country. It has used a central database of laboratory test results, diagnoses, and medication purchases since 1998.

An anonymized dataset of ALS patients insured by Maccabi was securely delivered to Maccabi Healthcare Services. Data linkage to Maccabi was performed through re-identification using date of birth and a date of at least one clinic visit. In addition, up to five matched controls were selected from the Maccabi database for each ALS case by individual matching using birth year, sex, and residence area (surrogate for socioeconomic status). Controls with a history of ALS or other neurological disorders were excluded. Only patients and controls older than 18 years of age at index date with at least 12 months of pre-index enrollment in Maccabi were included. The index date for patients was defined as disease onset (self-reported date of first symptom of weakness). For controls, the index date corresponded to their matched ALS patient index date. For cases and controls, data obtained from the Maccabi database included any co-morbidity prior to index date, body mass index (BMI) within the past 5 years closest to the index date, medications purchased up to 5 years prior to the index date, and laboratory test results closest to the index date within a year prior. Injuries from falls were not included as they could reflect an early consequence of ALS and not a pre-morbid factor, in contrast to other injuries (e.g., road accidents).

Last, to ensure the representativeness of our cohort of patients with ALS insured by Maccabi and followed at the Tel Aviv Medical Center, we also compared our cohort with patients with ALS not insured by Maccabi but followed at our center.

Table 1. Comparison of demographics between ALS patients and controls

	ALS (n=259)	Controls (n=1292)	*P*-value
Demographics			
Age at diagnosis, years	61.1 ± 12.0	61.1 ± 12.0	1
Sex: Female	107 (41.3%)	107 (41.3%)	1
Smoker	26 (10.0%)	167 (12.9%)	0.29
BMI (kg/m²) (median [IQR])	26.1 (22.9–30)]	27.1 (24.3–30.7	0.28
Co-morbidities			
Hypertension	115 (44.4%)	488 (37.8%)	**0.046**
Diabetes	39 (15.1%)	176 (13.6%)	0.54
Autoimmune disease	30 (11.6%)	115 (8.9%)	0.18
Cancer	24 (9.3%)	123 (9.5%)	0.90
Chronic kidney disease	21 (8.1%)	137 (10.6%)	0.23
Liver disease	20 (7.7%)	87 (6.7%)	0.57
Cardiovascular disease	13 (5.0%)	25 (1.9%)	**0.003**
Inflammatory gastrointestinal tract disorder	4 (1.5%)	10 (0.8%)	0.23

ALS = amyotrophic lateral sclerosis, BMI = body mass index, IQR = interquartile range

Data are presented as n (%), unless indicated otherwise

Bold indicates significance

Table 2. Comparison of history of trauma, infections and medications between ALS patients and controls

	ALS (n=259)	Controls (n=1292)	*P*-value
Trauma			
Any fracture*	42 (16.2%)	164 (12.7%)	0.13
Any injury*	130 (50.2%)	525 (40.6%)	**0.004**
Head injury/fracture*	7 (2.7%)	20 (1.5%)	0.20
Upper arm/shoulder injury/fracture*	18 (6.9%)	34 (2.6%)	**< 0.001**
Foot/ankle injury/ fracture*	26 (10.0%)	118 (9.1%)	0.65
Nerve or spinal cord injury*	2 (0.8%)	6 (0.5%)	0.53
Infections			
Viral infection	151 (58.3%)	616 (47.7%)	**0.002**
Other infections	14 (5.4%)	56 (4.3%)	1
Drugs			
Analgesic/antipyretic	158 (61.0%)	680 (52.6%)	**0.01**
Anti-inflammatory	178 (68.7%)	780 (60.4%)	**0.01**
Narcotic	14 (5.4%)	28 (2.2%)	**0.003**
Antihypertensive	156 (60.2%)	720 (55.7%)	0.18
Lipid lowering	96 (37.1%)	494 (38.2%)	0.72
Anti-diabetic	31 (12.0%)	141 (10.9%)	0.62
Antibiotics	193 (74.5%)	860 (66.6%)	**0.01**
Antivirals	38 (14.7%)	122 (9.4%)	**0.01**
Antineoplastic	10 (3.9%)	40 (3.1%)	0.52
Immunosuppressant	1 (0.4%)	3 (0.2%)	0.66

ALS = amyotrophic lateral sclerosis

*Injuries from falls were not included

Data are presented as n (%)

Bold indicates significance

STATISTICAL ANALYSIS

Statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics software, version 24 (SPSS, IBM Corp, Armonk, NY, USA). Descriptive statistics were presented as n (%), mean ± standard deviation (SD), or median (interquartile range [IQR]) as appropriate. Differences between groups were assessed using a chi-square test (proportions), *t*-test (means), or non-parametric Mann-Whitney U Tests where appropriate. Patient characteristics (including laboratory results, co-morbidities, and medication use) were compared between ALS patients and controls. Since this was an exploratory study, correction for multiple comparisons was not performed. *P*-value < 0.05 was considered statistically significant.

Table 3. Comparison of laboratory tests between ALS patients and controls

Laboratory tests	ALS (n=259)	Controls (n=1295)	*P*-value
Albumin (g/L)	4.4 (4.2–4.5)	4.3 (4.2–4.5)	0.26
CRP (mg/L)	0.5 (0.5–0.5)	0.5 (0.5–0.5)	0.94
CK (U/L)	122 (78.5–186)	93 (65–132)	**< 0.001**
Creatinine (mg/dl)	0.9 (0.8–1)	0.9 (0.8–1.1)	0.09
Glucose (mg/dl)	100 (92–109)	99 (92–11)	0.26
HbA1C (%)	5.8 (5.6–6.2)	5.9 (5.5–6.4)	0.10
HDL (mg/dl)	49.6 (41.4–60.2)	48.3 (41.2–58.2)	0.46
LDL (mg/dl)	123.8 (99.7–142)	118.2 (96.5–143.5)	0.25
Triglycerides (mg/dl)	124 (92–164)	125.5 (94–175)	0.65
TSH (mu/L)	1.96 (1.4–2.7)	1.9 (1.3–2.8)	0.37
Uric acid (mg/dl)	5.4 (4.6–6.4)	5.4 (4.5–6.4)	0.89
Vitamin B12 (pg/ml)	383 (300–488)	372.5 (296–468.50)	0.33
ALT (U/L)	27 (20–15)	20 (16–27)	0.44
AST (U/L)	26 (22–18)	21.7 (18–26)	0.51
ESR (mm/hour)	14 (8–24)	16 (9–24)	0.16
Iron (mg/dl)	86 (68–110)	84 (65–109)	0.57
Folic Acid (ng/ml)	9 (6.5–11)	8.9 (6.9–11)	0.51
Hemoglobin (g/dl)	14 (13.1–15)	14.05 (13.2–15)	0.96
Hematocrit (%)	44.7 (42.1–40.1)	42.5 (39.9–44.7)	0.83
Platelets (× 10^3/µl)	232 (193–270)	234.5 (203.5–273)	0.13
RBC (× 10^6/µl)	4.8 (4.5–5.1)	4.8 (4.5–5.1)	0.93
WBC (× 10^3/µl)	6.8 (5.7–8.0)	6.8 (5.6–7.9)	0.61

Data are presented as mean (range)

ALS = amyotrophic lateral sclerosis, ALT = alanine transaminase, AST = aspartate transaminase, CK = creatine kinase, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, HbA1C = hemoglobin A1c, HDL = high-density lipoprotein, LDL = low-density lipoprotein, RBC = red blood cells, TSH = thyroid stimulating factor, WBC = white blood cells

Bold indicates significance

RESULTS

The mean age of ALS patients at disease onset in our cohort was 61.1 ± 12 years, with male predominance (58.7%). Age and sex was identical to the matched group of non-ALS controls [Table 1]. The most frequent co-morbidities of both groups included hypertension (37.8–44.4%) and diabetes (13.6–15.1%). Compared with controls, ALS patients had a higher frequency of hypertension and cardiovascular disease prior to disease presentation [Table 1]. In addition, medications used more frequently by ALS patients included analgesics, antipyretics, anti-inflammatory, narcotics, antibiotics, and antivirals [Table 2]. After excluding injuries from falls, ALS patients had a higher incidence of injuries, especially upper arm or shoulder injuries and fractures prior to disease presentation [Table 2]. Last, ALS patients presented more often with viral infections prior to disease presentation [Table 2]. Table 3 shows comparisons of laboratory test results between controls and ALS patients. ALS patients had higher creatine kinase (CK) levels prior to disease presentation. Otherwise, both groups had similar laboratory results values including blood counts, blood chemistry, thyroid stimulating factor (TSH), vitamin B12, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP).

There were no differences between patients insured by Maccabi who were followed at the Tel Aviv Medical Center and who were not followed at the ALS clinic, aside from a higher rate of viral infections (58.3% vs. 46/8% respectively; $P = 0.01$) [Supplemental Table 1 and Supplemental Table 2, available on the online version only). In addition, there were no differences between patients followed by the ALS clinic at Tel Aviv Medical Center who were insured by Maccabi with those who were not insured by Maccabi [Supplemental Table 3, available on the online version only] except a higher score of the Amyotrophic Lateral Sclerosis Functional Rating Scale Revised (ALSFRS-R) (33.2 ± 12.8 vs. 31.7 ± 12.8 respectively; $P = 0.005$).

DISCUSSION

In contrast to previous research [2-9], our study results showed that ALS patients had a higher frequency of hypertension and cardiovascular disease prior to disease presentation. However, these findings correspond with findings by Diekmann et al. [8], who reported a higher risk of stroke in patients with ALS shown by a univariate analysis (more than fivefold, $P = 0.007$), although not with multivariate analysis ($P = 0.06$) as stroke and cardiovascular risk factors and disease are commonly associated. These findings are surprising as they contrast with previous studies showing a lower prevalence of cardiovascular risk factors or diseases in patients with ALS [2-9]. In addition, two of these studies did not demonstrate an association between hypertension and/or cardiovascular disease and ALS [7,8]. We have not found a plausible explanation for this discrepancy; therefore, additional studies are required to confirm these results.

In addition, our study results showed that, compared with controls, ALS patients more frequently used analgesics and antipyretics, anti-inflammatory medications, and narcotic drugs before symptom onset. The most prominent difference was more than double-fold usage of narcotics in ALS patients. These findings are in line with those reported by Diekmann and colleagues [8], which showed a higher frequency of anti-inflammatory drug usage in ALS patients. Pain is common in ALS patients, even in early disease stages and affects more than 50% of patients [11,12]. Furthermore, it has been suggested that pain can even precede motor dysfunction [13]. We therefore suppose that the higher usage of pain medications in ALS patients prior to disease presentation might reflect, at least in some cases, an unrecognized presenting symptom.

A previous study by Sun et al. [14] showed an association between antibiotic use, especially repeated, and higher risk for ALS development. Those authors suggested a role of altered gut microbiome as a possible mechanism. Similarly, we showed that, compared with controls, ALS patients more frequently used antibiotic medications prior to symptom onset. However, we also found an association between antiviral medications and a higher risk for ALS development, broadening the association between ALS and anti-microbial agents, which does not support the microbiome alteration theory as a sole explanation.

Our findings may be explained by two main hypotheses. First, ALS patients are more prone to infections than controls, possibly due to alterations in the immune system [15,16], or other causes such as increased risk for respiratory infections due to respiratory compromise and aspirations. Consequently, the higher usage of antibiotics and antiviral medications prior to disease presentation may be related to subclinical disease manifestation. Alternatively, as previously suggested [14], antibiotic usage might increase the risk for ALS development by altering the gut microbiome.

In our study, ALS patients presented with more injuries prior to disease presentation compared with controls, even after excluding injuries from falls. Interestingly, upper arm or shoulder, but not head injury/fracture, were more common in ALS patients in our study. These findings contrast with the results of previous studies, suggesting an increased risk for ALS after head injury [9] but not after injury to other body parts [17]. However in a case control study comprising 377 patients and 370 controls, Pupillo and co-authors [18] found that the most consistent site of trauma preceding ALS was the arm, followed by the head and abdomen. We did not include injuries from falls, as these events might be related to early unrecognized ALS presentation; therefore, our findings might more accurately detect injuries that do not merely reflect early disease. A possible mechanism that has been suggested to explain the relationship between trauma and ALS is motor axonal injury, triggering molecular pathways leading to neurodegeneration [18]. These pathways might include oxidative stress [19], glutamate excitotoxicity, and inflammatory pathways [20,21].

We found that ALS patients had higher CK levels prior to disease presentation compared with controls, although mean values were within the normal range (122 I/U [78.50–186]). These results are in line with those reported by Ito at al. [22], who showed elevated CK levels at early ALS stages and suggested that CK elevation reflects muscle denervation at an early stage preceding muscular atrophy. We therefore suppose that the higher CK levels in ALS patients reflect subclinical disease preceding the clinical manifestations. However, as CK values were in the normal range, with significant overlap with controls, CK values, when normal, cannot be used to discriminate between the two groups.

Last, both groups had similar values including blood count, blood chemistry, TSH, vitamin B12, ESR, CRP, and uric acid. Interestingly, previous studies showed lower uric acid levels in patients with ALS [23], while no differences were found in our study. A possible explanation for this discrepancy might be related to the higher frequency of hypertension and cardiovascular disease in patients with ALS in our cohort, which are known to be associated with higher uric acid levels [24,25].

A significant limitation of our study is its design as an observational study, which is more vulnerable to confounding biases. However, it provides real-world results in a rare disease that cannot be addressed by randomized controlled trials. The strength of our study is the usage of data from a large national ALS clinic linked to extensive data from a nationwide health fund [9], including multiple parameters such as co-morbidities, medications, trauma, and laboratory tests [24,25].

CONCLUSIONS

In contrast with previous studies [2-9], we found that prior to disease presentation, ALS patients had higher frequency of hypertension and cardiovascular disease. In addition, ALS patients used analgesics, antibiotics, and antiviral medications more often; presented more frequently with upper arm or shoulder injury or fracture; and had higher CK levels. These findings may shed light on potential risk factors and early disease manifestations, as well as subclinical disease manifestations, warranting additional studies.

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The man who has begun to live more seriously within begins to live more simply without.

Ernest Hemingway (1899–1961), American novelist, short-story writer, and journalist; Nobel laureate

Love is like quicksilver in the hand. Leave the fingers open and it stays. Clutch it, and it darts away.

Dorothy Parker (1893–1967), American author, poet, critic, screenwriter

Capsule

Evolutionary histories of breast cancer and related clones

Recent studies have documented frequent evolution of clones carrying common cancer mutations in apparently normal tissues, which are implicated in cancer development. However, knowledge is still missing regarding what additional driver events take place in what order before one or more of these clones in normal tissues ultimately evolve to cancer. Using phylogenetic analyses of multiple microdissected samples from both cancer and non-cancer lesions, **Nishumura** and colleagues showed unique evolutionary histories of breast cancers harboring der(1;16), a common driver alteration found in roughly 20% of breast cancers. The approximate timing of early evolutionary events was estimated from the mutation rate measured in normal epithelial cells. In der(1;16)(+) cancers, the derivative chromosome was acquired from early puberty to late adolescence, followed by the emergence of a common ancestor by the patient's early 30s, from which both cancer and non-cancer clones evolved. Replacing the pre-existing mammary epithelium in the following years, these clones occupied a large area within the premenopausal breast tissues by the time of cancer diagnosis. Evolution of multiple independent cancer founders from the non-cancer ancestors was common, contributing to intratumor heterogeneity. The number of driver events did not correlate with histology, suggesting the role of local microenvironments and/or epigenetic driver events. A similar evolutionary pattern was also observed in another case evolving from an *AKT1*-mutated founder. Taken together, these findings provide new insight into how breast cancer evolves.

Nature 2023; 620: 607

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