Ana Catarina Lopes Elias
Etiology and Treatment of Amyotrophic Lateral Sclerosis – A Systematic Review/Etiologia e Tratamento da Esclerose Lateral Amiotrófica – Uma Revisão Sistemática

março, 2018
Ana Catarina Lopes Elias

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Mestrado Integrado em Medicina

Área: Neurologia
Tipologia: Revisão Sistemática

Trabalho efetuado sob a Orientação de:
Doutora Carolina Garrett

Trabalho organizado de acordo com as normas da revista:
SINAPSE

março, 2018
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Faculdade de Medicina da Universidade do Porto, 13/03/2018

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DESIGNAÇÃO DA ÁREA DO PROJECTO
Neurologia

TÍTULO DISSERTAÇÃO/MONOGRÁFIA (riscar o que não interessa)
Etiology and Treatment of Amyotrophic Lateral Sclerosis – A Systematic Review

ORIENTADOR
Dr. Carolina Garrett

COORIENTADOR (se aplicável)

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- [ ] É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTE TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.

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Faculdade de Medicina da Universidade do Porto, 13/03/2018

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Etiology and Treatment of Amyotrophic Lateral Sclerosis – A Systematic Review

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Keywords: Amyotrophic Lateral Sclerosis, Motor Neuron Disease, Therapeutics, Risk Factors, Genetics

Header: Etiology and Treatment of ALS

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Disclosure of Conflicts of Interest: The authors declare no conflicts of interest.
Abstract

Introduction: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that results from environmental factors and a predisposed genetic environment. Nowadays, the definition of those environmental risk factors is not consensual and despite progresses in the genetic field, there is no available treatment aside Riluzole.

Objectives: This systematic review aimed at compiling recent information regarding the etiology and treatment of ALS to offer a global perspective and a starting point for future investigation in this area.

Material and Methods: The articles were searched in PubMed and through research in the references of primary articles. Selection and analysis of the articles were made by one author only. The evaluation of methodologic quality was performed using Joanna Briggs Institute' grids. Twenty-five final articles were included.

Results: The environmental factors most associated with ALS were “Chronic Exposure to Lead”, “Smoking” and “Exposure to Fertilizers/Pesticides”. In the genetic field, no specific alteration was shown to have a superior impact. Regarding treatment, experimental animal studies have shown promising results. However, the clinical trials included were mostly phase I and II studies and phase III studies failed to show efficacy.

Discussion: These results confirm the current scientific state of art about ELA: the controversial results about environmental exposures and the growing conquest of the genetic field that does not translate into an ability to generate viable therapeutic options.

Conclusion: This review reinforces the need to perform prospective studies to determine environmental risk factors controlling all their confounding variables, as well as rigorous clinical trials with high methodological quality.
Etiologia e Tratamento da Esclerose Lateral Amiotrófica – Uma Revisão Sistemática

Ana Elias, Carolina Garrett

Resumo

Introdução: A Esclerose Lateral Amiotrófica (ELA) é uma doença neurodegenerativa que se desenvolve pela acção de factores ambientais que actuam sobre um ambiente genético propício à sua ocorrência. Actualmente, a definição desses factores de risco ambientais não é consensual e, apesar dos progressos na área da genética, não existe nenhum tratamento, estando apenas aprovado o Riluzole.

Objectivos: Esta revisão sistemática procurou compilar informação recente relativa à etiologia e tratamento da ELA de modo a fornecer uma visão global do estado da arte e um ponto de partida para investigação científica futura nestas duas áreas.

Material e Métodos: Os artigos foram pesquisados na PubMed e com pesquisa nas referências dos artigos primários encontrados. A selecção e a análise dos artigos foram realizadas por um autor, iniciando por análise do título e resumo. A qualidade metodológica foi avaliada com recurso às grelhas do Joanna Briggs Institute. Esta revisão sistemática inclui um total de 26 artigos finais.

Resultados: Os factores ambientais mais associados ao desenvolvimento de ELA foram a “Exposição Crónica a Chumbo”, o “Tabaco” e a “Exposição a Fertilizantes/Pesticidas”. Na área genética nenhuma alteração se mostrou preponderante. Sobre o tratamento, os estudos experimentais em animais apresentam resultados promissores. Contudo, os ensaios clínicos incluídos são sobretudo de fase I e II, e os de fase III falham em demonstrar eficácia.

Discussão: Os resultados reforçam o paradigma do conhecimento actual sobre a ELA: os resultados controversos das exposições ambientais e o crescente domínio da
genética, sem que isso se traduza por uma capacidade de gerar opções terapêuticas viáveis em humanos.

**Conclusão:** Esta revisão reforça a necessidade de realização de estudos prospectivos para determinação de factores de risco ambientais e controlo das variáveis confundidoras, bem como ensaios clínicos rigorosos com elevada qualidade metodológica.

**Introduction**

Amyotrophic Lateral Sclerosis is a neurodegenerative progressive disease that affects both the first and second motor neuron from the cortex, the brainstem and the spinal cord. (1)

This disease presents a familiar pattern in around 5-10% of cases, but the vast majority is sporadic (2) and this form of the disease is the focus of this systematic review. To this day, very few environmental factors have been identified in a scientifically proved manner as participants in the etiology and genetic alterations explain approximately two thirds of familiar ALS cases and about 20% of sporadic cases. (3)

Despite the undeniable progress in the knowledge of the pathophysiology of this disease, the impact this has in its treatment has not been significant.

For this reason, it was viewed as pertinent to review the current state of art about ALS etiology – both environmental and genetic – and new perspectives for its treatment.

**Methods**

**Type of Study**

To answer the purposed aims, a systematic review was undertaken, because it is a rigorous method that allows the identification and critical evaluation of a group of primary studies in order to obtain the best scientific evidence that enables clinical decision or identifies the state of art for a future new investigation. (4)

Comparing to other methods used to review scientific evidence, the systematic review allows for more reliable results because it is based on systematic procedures that minimize the bias associated with other types of review and it is considered the most adequate to highlight the good practices in the medical field. (4)
In this chapter, we expose the protocol that lead to this review, which incorporated the different phases that should be part of a systematic review.

**Research Strategies**

**Information Sources**

The articles were researched through PubMed and also through references of the first articles found (snowballing) in a period that occurred from 28/09/2016 and 7/02/2017.

**Eligibility Criteria**

It was taken to account selection criteria regarding the type of study. Meta-analysis, experimental studies, case and control studies, clinical trials and observational studies published with available full text online between 2011 and 2016 and that were written in English were accepted.

**Research Protocol**

The research began in PubMed, using the keywords “Amyotrophic Lateral Sclerosis Treatment”, “Amyotrophic Lateral Sclerosis Etiology”, “Amyotrophic Lateral Sclerosis Genetics” and “Clinical Trials in ALS”. From the list of articles that derived from this research, those that had full text available and had a title compatible with content we aimed to review were selected. Through that initial pool of articles, by searching through their references, the remaining articles that compose the basis for this systematic review were found. Some of the articles included motivated a second research in the Cochrane Library to have access to full text article with the results published meanwhile.
Evaluation of Quality, Inclusion and Exclusion Criteria.

During a systematic review, the evaluation of methodological quality is a determinant procedure to evaluate if the methodological options allow for a critical interpretation of the results. (5)

From the initially obtained articles with full text available online (n=46), four were eliminated through abstract reading: one evaluated the efficacy of anti-depressant therapy in ALS, another was a protocol of a study yet to be conducted and its results were published on another article that was included, a third one was a study with a proposal on how to elaborate clinical trials for ALS and the last one was a descriptive study.

Regarding the research on the treatment area, some articles only mentioned symptomatic treatment and so they were excluded (n=2).

The evaluation of the remaining articles (n=40) that included our systematic review sample was made using the tool grids of Joanna Briggs Institution (2014) for each study type. In this work, when applicable, the studies were accepted if they obtained two or more items with the answer “Yes”. In the absence of enough information to evaluate them, an e-mail was sent to the corresponding authors asking for methodological details. Those who did not answer the e-mail were eliminated (n=10).

Articles found that consisted of book chapters (n=2) or compilations of an author's work (n=2) were also eliminated.

The final number of primary studies was 26 and the information regarding those studies was synthetized in tables I (for etiology) and II (for treatment).
## Table I – Final list of etiological studies that integrated this systematic review.

<table>
<thead>
<tr>
<th>Code</th>
<th>Title</th>
<th>Type of Study</th>
<th>Publication Country</th>
<th>Year of Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>Smoking and Risk of Amyotrophic Lateral Sclerosis- A Pooled Analysis of 5 Prospective Cohorts</td>
<td>Prospective Cohort</td>
<td>E.U.A.</td>
<td>2011</td>
</tr>
<tr>
<td>E4</td>
<td>Identification of risk factors associated with onset and progression of amyotrophic lateral sclerosis using systematic review and meta-analysis</td>
<td>Meta-analysis</td>
<td>Canada</td>
<td>2016</td>
</tr>
<tr>
<td>E5</td>
<td>Transglutaminase 2 accelerated neuroinflammation in amyotrophic lateral sclerosis through interaction with misfolded superoxide dismutase 1</td>
<td>Experimental</td>
<td>Japan</td>
<td>2014</td>
</tr>
<tr>
<td>E6</td>
<td>ALS-Causing Mutations Significantly Perturb the Self-Assembly and Interaction with Nucleic Acid of the Intrinsically Disordered Prion-Like Domain of TDP-43</td>
<td>Experimental</td>
<td>Singapore</td>
<td>2016</td>
</tr>
<tr>
<td>E7</td>
<td>The role of D-serine and glycine as co-agonists of NMDA receptors in motor neuron degeneration and amyotrophic lateral sclerosis (ALS)</td>
<td>Experimental</td>
<td>Great Britain</td>
<td>2014</td>
</tr>
<tr>
<td>E8</td>
<td>Gain-of-function profiling 1 mutations linked to familial amyotrophic lateral sclerosis cause seed-dependent intracellular TDP-43 aggregation</td>
<td>Experimental</td>
<td>Japan</td>
<td>2016</td>
</tr>
<tr>
<td>E9</td>
<td>Mutations in the Matrin 3 gene cause familial amyotrophic lateral sclerosis</td>
<td>Experimental</td>
<td>E.U.A.</td>
<td>2014</td>
</tr>
<tr>
<td>E10</td>
<td>Cellular redox Systems impact the Aggregation of Cu-Zn Superoxide Dismutase linked to Familial Amyotrophic Lateral Sclerosis</td>
<td>Experimental</td>
<td>Spain</td>
<td>2016</td>
</tr>
<tr>
<td>E11</td>
<td>A Meta-Analysis of Observational Studies of the Association Between Chronic Occupational Exposure to Lead and Amyotrophic Lateral Sclerosis</td>
<td>Meta-analysis</td>
<td>Canada</td>
<td>2014</td>
</tr>
</tbody>
</table>

## Table II – Final list of articles regarding treatment of ALS included in this review.

<table>
<thead>
<tr>
<th>Code</th>
<th>Title</th>
<th>Type of Study</th>
<th>Publication Country</th>
<th>Publication Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Diacetyl bis(N(4)-methylthiosemicarbazonato) Copper(Il) (Cu (atsm)) Protects against Peroxynitrite-induced Nitrosative Damage and Prolongs Survival in Amyotrophic Lateral Sclerosis Mouse Model</td>
<td>Experimental</td>
<td>E.U.A.</td>
<td>2011</td>
</tr>
<tr>
<td>T2</td>
<td>Resveratrol Improves Motoneuron Function and Extends Survival in SOD1&lt;sup&gt;155A&lt;/sup&gt;ALS Mice</td>
<td>Experimental</td>
<td>Spain</td>
<td>2014</td>
</tr>
<tr>
<td>T3</td>
<td>Trehalose delays the progression of amyotrophic lateral sclerosis by enhancing autophagy in motoneurons</td>
<td>Experimental</td>
<td>E.U.A.</td>
<td>2013</td>
</tr>
<tr>
<td>T4</td>
<td>Targeting miR-155 restores abnormal microglia and attenuates disease in SOD1 mice</td>
<td>Experimental</td>
<td>E.U.A.</td>
<td>2015</td>
</tr>
<tr>
<td>T5</td>
<td>An antisense oligonucleotide against SOD1 delivered intrathecally for patients with SOD1 familiar amyotrophic lateral sclerosis: a phase 1, randomized, first-in-man study</td>
<td>Clinical Trial Phase I</td>
<td>E.U.A.</td>
<td>2013</td>
</tr>
<tr>
<td>T6</td>
<td>Hypercaloric enteral nutrition in Amyotrophic Lateral Sclerosis: a randomized, double-blind placebo controlled trial</td>
<td>Clinical Trial Phase II</td>
<td>E.U.A.</td>
<td>2014</td>
</tr>
<tr>
<td>T7</td>
<td>Metabolic Therapy with Deanna Protocol Supplementation Delays Disease Progression and Extends Survival in Amyotrophic Lateral Sclerosis (ALS) Mouse Model</td>
<td>Experimental</td>
<td>E.U.A.</td>
<td>2014</td>
</tr>
<tr>
<td>T8</td>
<td>RNA Toxicity from the ALS/FTD C9ORF72 Expansion is Mitigated by Antisense Intervention</td>
<td>Experimental</td>
<td>E.U.A.</td>
<td>2013</td>
</tr>
<tr>
<td>T9</td>
<td>Efficacy and safety of ceftriaxone for amyotrophic lateral sclerosis: results of a multi-stage, randomized, double-blind, placebo-controlled, phase 3 study</td>
<td>Clinical Trial Phase III</td>
<td>E.U.A.</td>
<td>2014</td>
</tr>
<tr>
<td>T10</td>
<td>Confirmatory double-blind, parallel-group, placebo-controlled study of efficacy and safety of edaravone (MCI-186) in amyotrophic lateral sclerosis patients</td>
<td>Clinical Trial Phase III</td>
<td>Japan</td>
<td>2014</td>
</tr>
</tbody>
</table>
T11  |  Tauroursodeoxycholic acid in the treatment of patients with amyotrophic lateral sclerosis  |  Clinical Trial Phase II  |  Italy  |  2015
T12  |  Efficacy of Stem Cell Therapy in Amyotrophic Lateral Sclerosis: A Systematic Review and Meta-Analysis  |  Meta-Analysis  |  Brazil  |  2016
T14  |  Early-Stage Treatment with Withaferin A Reduces Levels of Misfolded Superoxide Dismutase 1 and Extends Lifespan in a Mouse Model of Amyotrophic Lateral Sclerosis  |  Experimental  |  Canada  |  2014
T15  |  Triheptanoin Protects Motor Neurons and Delays the Onset of Motor Symptoms in a Mouse Model of Amyotrophic Lateral Sclerosis  |  Experimental  |  Australia  |  2016

Results

Etiology

The etiology of ALS is multifactorial and includes a genetic susceptibility component and environmental factors that trigger its development.

   Environmental Factors

   This systematic review suggests three main environmental factors with association with ALS as it is noted in Table III: “Chronic Exposure to Lead” (E3, E4, E11) (6-8), “Smoking” (E1, E4) (6,9), “Exposure to Fertilizers/Pesticides” (E4, E2) (1,9) and a second group of intermediate evidence for “Head Trauma” (E3, E4) (6,7).

   There are, although, peculiarities in these associations: “Chronic Exposure to Lead” is a negative association in other study on this review (1), “Smoking” presents gender differences (as an association only in women) in one study (6) and association with the age on initiation (a younger age being associated with a higher risk of ALS) (9), “Exposure to Fertilizers/Pesticides” in an association specific for organophosphorate compounds in one study (9) and associated only to men with occupational exposure in the last 30 years or who did domestic gardening in the previous 10-30 or 30 years previous to the diagnosis (1) or those who live near and industrial area/agriculture site/water sanitation site in the previous 10 years (1). Regarding “Head Trauma”, one of the studies states an association specifically for traumas that occurred 5 years before the diagnosis of ALS. (7)
The remaining environmental risk factors are less expressive. “Exposure to Electromagnetic Radiation” is associated only in women who were exposed 10-30 years before the diagnosis (E2) (1), “Strenuous Physical Activity/Sports” is found to be associated in E4 (7) but is not associated in E2 (1), “Low Education Level” (E2) (1), “High Level of Fitness” (E4), “History of Electric Shock” (E4) and “Military Service” (E4) (7) are all reported as an association in one study each.

**Table III** – Environmental risk factors and articles that point their association with ALS

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Articles that show association (n)</th>
<th>Statistical Data</th>
</tr>
</thead>
</table>
| Chronic Exposure to Lead                           | 3                                  | E3: \( p<0.001, CI~95\%~excluding~the~null~value. \)  
E4: OR\(^*\)=1.72 (1.33-2.23)  
E11: RR\(^*\)=1.5009, AR=4.9\%, OR=1.87 (1.51-2.33) |
| Smoking                                            | 2                                  | E1: RR=1.11; 95\% CI, (1.01-1.22), \( p<0.3 \), by each 5 years younger \( \)  
E4: RR=1.34 (1.17-1.55) |
| Exposure to Fertilizers/Pesticides                 | 2                                  | E4: OR (CI 95\%)= 1.57 (1.25-1.98) \( )  
E2: OR (CI 95\%)=6.95 (1.23-39.1), \( p<0.05 \)  
E2: OR (CI 95\%)=2.97 (1.01-8.76), \( p<0.05 \)  
E2: OR (CI 95\%)= 2.97 (0.81-10.9), \( p<0.1 \)  
E2: OR= 1.15 (0.40-3.28) |
| Head trauma                                         | 2                                  | E4: OR= 1.40 (1.06-1.86) |
| History of Electric Shock                          | 1                                  | E4: OR=3.27 (1.87-5.73) |
| Exposure to Electromagnetic Radiation              | 1                                  | E2: OR=1.73 (0.37-8.14) |
| Strenuous Physical Activity/Professional Sports    | 1                                  | E4: OR=2.70 (1.97-3.6)/ OR=1.35 (1.11-1.65) |
Low Education Level 1  
High Level of Fitness 1  
Military Service 1  

\( E2: p<0.001 \)  
\( E4: OR=0.24\ (0.34-0.14) \)  
\( E2: RR=1.36\ (1.00-1.71) \)

*CI - Confidence Interval; **OR – Odds Ratio; ***RR – Relative Risk

**Genetic Factors**

The genetic factors are more important for familial ALS, but they act as susceptibility factors in sporadic ALS. The results of this research are summarized in Table IV.

Centering the evidence on sporadic ALS, in this systematic review were included articles about the following alterations: TG2 (tranglutaminase 2) (E5) (10), TARDBP (E6) (11), DAO (D-aminoacid oxidase) (E7) (12), Matrin 3 (E9) (13), C9ORF72 expansion (T8) (14),PFN1 (Profilin 1) (E8) (15), SOD1 (E10) (16), e ATXN2 (Ataxin 2) (E4 – OR: 3.93 (2.49,6.20)) (7).

**Table IV** – Genes with evidence of association with ALS found in this systematic review.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Articles that show association (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG2 (tranglutaminase 2)</td>
<td>1</td>
</tr>
<tr>
<td>TARDBP (TAR DNA Binding Protein)</td>
<td>1</td>
</tr>
<tr>
<td>DAO (D-aminoacid oxidase)</td>
<td>1</td>
</tr>
<tr>
<td>Matrin 3 (Matrin 3)</td>
<td>1</td>
</tr>
<tr>
<td>Expansão C9ORF72</td>
<td>1</td>
</tr>
<tr>
<td>PFN1 (Profilin 1)</td>
<td>1</td>
</tr>
<tr>
<td>SOD1</td>
<td>1</td>
</tr>
<tr>
<td>ATXN2 (Ataxin 2)</td>
<td>1</td>
</tr>
</tbody>
</table>

**Treatment**

**Results from Experimental Studies in Animals**

This review included articles studying treatment with Cu II (atsm) (T1) (17), resveratrol (T2) (18), trehalose (T3) (19), Withaferin A (T14) (3), supplementation according to Deanna Protocol (T7) (20), intrathecal therapy with antisense oligonucleotide (miR-155) (T4) (21), stem cell therapy (T12) (22), tripeptanoin (T15) (23) and Guanabenz (T13) (24).
The clinical and pathophysiological outcomes are listed on Table V.

### Table V – Pathophysiological and clinical outcomes of experimental studies in animals.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pathophysiological Outcomes</th>
<th>Clinical Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Withaferin A (T14)</td>
<td><strong>Reduction of 39% of levels of SOD1 misfolded</strong>&lt;br&gt;Upregulation of heat-shock proteins and their transcription factor Hsf-1. Decrease of reactivity of Iba-1 and TLR-2. Increase in IL-6, IL-10 (anti-inflammatory) and decrease of GM-CSF (pro-inflammatory).</td>
<td><strong>Survival increase (difference of 8 days, p&lt;0.05).</strong>&lt;br&gt;Delay in loss of motor neurons. Prevention of weight loss. Increase of 30% of motor neuron survival (neuroprotective effect). Suppression of inflammation and decrease in microglia activity. No benefit if late initiation.</td>
</tr>
<tr>
<td>Trehalose (T3)</td>
<td>Decrease in SOD1 oligomers and monomers and its aggregation&lt;br&gt;Increase in LC3 and LC3-II and decrease in SQSTM (autophagy substrate)&lt;br&gt;Activation of FOXO-1 (autophagy regulator).</td>
<td>Survival increase.&lt;br&gt;Delay in disease progression&lt;br&gt;Decrease in glial activity.&lt;br&gt;Increase in motor neuron survival.</td>
</tr>
<tr>
<td>Resveratrol (T2)</td>
<td>Induction of expression and activation of Sirtuin 1 in motor neurons.&lt;br&gt;Decrease in acetylation of p53 (Sirtuin 1 substrate)&lt;br&gt;Normal levels of LC3-II and Beclin 1.&lt;br&gt;Increase in Fis-1 (marker of mitochondrial biogenesis).</td>
<td>Survival increase.&lt;br&gt;Better performance in rotarod test.&lt;br&gt;Motor neuron function preservation. And decrease in microglia activity (even with late initiation of treatment). Normal autophagy. Restoration of mitochondrial function and biogenesis.</td>
</tr>
<tr>
<td>Cu II (atsm) (T1)</td>
<td>Pre-symptomatic treatment: decrease in oxidative stress biomarkers.&lt;br&gt;Decrease in TDP-43 in cytosol.</td>
<td>Pre-symptomatic treatment:&lt;br&gt;Survival increase (14% more)&lt;br&gt;Delay in manifestations of motor deficit and weight loss. Delay of 70% of time between motor deficit and death and 30% between weight loss and death.&lt;br&gt;No adverse effects.&lt;br&gt;Post-symptomatic treatment:&lt;br&gt;Survival increase (10% more).&lt;br&gt;Delay in motor neuron deficit.</td>
</tr>
<tr>
<td>Guanabenz (T13)</td>
<td>Decrease in the ammount of mutant SOD1&lt;br&gt;Upregulation of peIF2α and Bcl-2 in final stages.</td>
<td>Survival increase.&lt;br&gt;Delay on symptomatic manifestations.&lt;br&gt;Increase in time of initial phase of the disease.&lt;br&gt;Less motor neuron loss and less gliosis.&lt;br&gt;Later stages of disease with no difference comparing to controls.</td>
</tr>
<tr>
<td>Supplementati on by Deanna Protocol (T7)</td>
<td>Better performance in rotarod test, PaGE test and grip test (KD*) Better neurological scores with delay in progression (SD+DP**).&lt;br&gt;Survival increase for KD+DP*** and SD+DP groups.</td>
<td></td>
</tr>
<tr>
<td>Interference RNA miR-155 (T4)</td>
<td>Decrease in expression of pro-inflammatory genes.&lt;br&gt;Decrease in expression of APOE.</td>
<td>Survival increase.&lt;br&gt;Better performance in rotarod test. Decrease in weight loss.&lt;br&gt;Decrease in symptomatic manifestations in female gender.</td>
</tr>
<tr>
<td>Stem cell Therapy (T12)</td>
<td></td>
<td>Survival increase.&lt;br&gt;Motor neuron survival increase.&lt;br&gt;Decrease in gliosis.</td>
</tr>
<tr>
<td>Triheptanoin (T15)</td>
<td>Decrease in expression of piruvate desidrogenase, succinate desidrogenase and propionil-carboxilase.&lt;br&gt;Increase in plasmatic β-hidroxibutirate</td>
<td>33% more preservation of motor neurons.&lt;br&gt;No effect on survival.&lt;br&gt;Delay in weight loss and loss of strenght in grip test and balance.</td>
</tr>
</tbody>
</table>

*KD – Ketogenic Diet; **SD+DP – Standard Diet + Deanna Protocol; ***KD+DP – Ketogenic Diet + Deanna Protocol

### Results of Clinical Trials

This review included the following clinical trials: a pilot study using Tauroursodesoxycolic Acid (TUDCA) (T11) that aimed to demonstrate tolerability and...
proof of principle (25), a study that was conducted to confirm the efficacy and safety of Edaravone (T10) (despite not having an impact on the primary endpoint (ALSFRS-R score), it showed positive results on a secondary endpoint – pinch strength ($p=0.038$)) (Table 5) (26), a phase III clinical trial with ceftriaxone (T9) (27), one study with an hypercaloric diet (T6) to evaluate safety and tolerability which was confirmed (28), a phase I clinical trial with intrathecal injection of and antisense oligonucleotide (T5) conducted in patients with familial ALS related to SOD1 mutations but generalizable to sporadic ALS cases (29) and a meta-analysis about the safety and efficacy of stem cell therapy in humans (T12) with a high heterogeneity in the designs and results of the evaluated studies that make the evaluation of results of this therapy very challenging (22).

Therefore, this review has one phase I trial to evaluate tolerability and efficacy two phase II studies, two phase III studies – both ineffective in the primary endpoints and one with positive results in a secondary endpoint – and one meta-analysis about stem cell therapy, in a total of six articles relative to clinical trials in humans found in this research.

The main results of each clinical trial are listed in Table VI.

**Table VI** – Pathophysiological and clinical outcomes of the clinical outcomes of the clinical trials in humans included in this systematic review.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Clinical Trial Specificities</th>
<th>Clinical Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tauroursodesoxicolic Acid (TUDCA) (T11)</td>
<td>34 participants: 17 controls (3 left the study, 14 included in the primary analysis) and 17 treated with TUDCA (2 left the study, 15 included in the primary analysis. Participants in initial stages of ELA, without great incapacity. 54 weeks of TUDCA after 12 previous weeks on Riluzole.</td>
<td>ALSFRS-R score at the end, better in the treated group (23.3 (19.9-26.6) vs 16.3 (12.9-19.7), $p=0.007$). Better bulbar ALSFRS-R score in TUDCA group. Delay in loss of function (slower decline of pulmonary function and less muscular strength – non-significant). Better survival in treated group (65.7 (65.2-66.3) weeks vs 61.1 (55.3-66.9)). Good tolerability. Adverse effects: light diarrhea. No difference in secondary outcomes.</td>
</tr>
<tr>
<td>Edaravone (MCI-186) (T10)</td>
<td>205 patients (23 left treatment). 12 weeks of pre-observation followed by 24 weeks of treatment. Differences between controls and treated regarding the duration of disease ($p=0.104$), ALSFRS-R score prior to pre-observation</td>
<td>No statistically significant differences in ALSFRS-R score. Secondary endpoint achievement in treated group – pinch strength ($p=0.038$). No adverse reactions.</td>
</tr>
<tr>
<td>Treatment</td>
<td>Participants</td>
<td>Outcome</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Ceftriaxone (T9)</td>
<td>513 participants: 340 treated through central venous catheter (46% remained until the end of the trial or until reaching an endpoint), 173 controls with placebo (41% until the end of trial or achieving and endpoint).</td>
<td>In stages 1 and 2 of the disease: lower decline in ALSFRS-R score in treated group ($p=0.0416$). Not present in stage 3 ($p=0.237$). No efficacy and no dose-dependent effect. Adverse effects: Gastrointestinal, hepatobiliary (coelelithiasis) and in the bone marrow.</td>
</tr>
<tr>
<td>Hypercaloric Diet (T6)</td>
<td>24 participants: 7 controls, HC/HC diet (High carbon hydrates), 8 HC/HF (high carbon hydrates and lipids). Participants with advanced disease and malnutrition.</td>
<td>Controls gained, on average 0.11 kg per month; HC/HC gained 0.39 kg per month; HC/HF lost 0.46 kg per month. Less adverse effects on treated group. HC/HC with higher tolerability over HF/HC. Adverse effects: gastrointestinal.</td>
</tr>
<tr>
<td>Intrathecal antisense oligonucleotide (T5)</td>
<td>21 participants treated with growing doses of ISIS333611 through intrathecal infusion.</td>
<td>Good tolerance with no dose-dependent toxicity. Adverse effects (84%): Post lumbar puncture syndrome, back pain (both related with technic and nausea.</td>
</tr>
<tr>
<td>Stem Cell Therapy (T12)</td>
<td>Endpoint was to investigate adverse effects in most studies analysed. Average number of participants was 11.</td>
<td>The majority states the safety of the procedure. Two studies with increase in survival, both of low methodological quality.</td>
</tr>
</tbody>
</table>

**Discussion**

**Etiology**

**Environmental Factors**

The fact that ALS is a rare disease turns the study of environmental risk factors involved in its etiology a difficult task. Besides that, this disease’ possible relation with many physical and chemical agents during the whole life of an individual (and not only during a restricted period) acts as a confounding factor and limits the conclusions extracted from the studies that were conducted. (30) On top of this, there is a high economical demand to conduct these studies, they are very long studies in terms of time and the absence of a guaranteed result lowers the drive to conduct them. (30)

According to this review, the environmental factors most associated with ALS are “Chronic Exposure to Lead”, “Smoking” and “Exposure to Fertilizers/Pesticides”. For all of them it is possible to find biological plausibility: lead can act as a trigger in SOD1 misfolding (8), tobacco can be responsible to direct neuronal damage (9), increasing oxidative stress (9,31), inhibiting VEGF (31) and aberrant methylation of
DNA (9) and fertilizers/pesticides can have a role in increasing excitotoxicity and producing neurotoxic metabolites (32).

Despite being supported by various studies (33, 34), “Chronic Exposure to Lead” has also some contradictory evidence (35), which by itself shows the heterogeneity of results in this field. There is also a paradoxical result in literature that shows that higher levels of lead in circulation are associated with a longer survival (36), which can be justified by the production of antioxidant substances in response to this exposure that fight the oxidative damage inherent to ALS.

Regarding tobacco, we highlight the association with the female gender only (possibly because of a greater male exposure to other confounding factors as exposure to pesticides (7)) that is corroborated by the available research (37,38) and also the association with the younger age at initiation (7,39) possibly explained by the selection of genetically susceptible individuals, relevance only in susceptible individuals or relevance in the growth period when motor neurons are physiologically under a greater stress (9). Despite these peculiarities, this risk factor is practically established. (7,9, 37-39).

The male preponderance of “Exposure to Fertilizers/Pesticides” is supported by external literature (40, 41) as well as its association with occupational activity (40). The gender difference in this exposure is explained by a greater prevalence of males conducting the activities associated with it (1, 40) and possibly by gender differences associated with the metabolism of these products (40). The majority of studies does not specify the class of fertilizers/pesticides studied, but the association this review found specifically with organophosphorolate compounds is in line with external evidence. (41)

The remaining environmental factors found in this review are more controversial. “Head Trauma” might be a contributive factor to neuroinflammation of microglia (42), however, there are studies that do not evidence this association (43,44)
and those that do are heterogeneous in the time where this event is relevant, its frequency, localization and age of greater impact and many results were produced from a non-representative population (45,46).

The “Exposure to Electromagnetic Radiation” might create an imbalance in the reactive oxygen species (ROS) (47) or lower the levels of nitric oxide (NO) (48) which are mechanisms that support the association found in this review (1). However, despite this plausible mechanism, many studies failed to associate this factor with ALS (49, 50) and report the possibility of a coexistent confounding factor, namely a “History of Electric Shock” – a factor also found in this review (7). In spite of this hypothesis, “History of Electric Shock” presents contradictory results in literature and its sustenance as a possible risk factor lies mostly in case reports (51,52).

The divergent results in this review regarding “Strenuous Physical Activity/Professional Sports” are a picture of the state of art on this matter. This exposure might be a trigger for glutamate excitotoxicity, generate oxidative stress or derive from an interaction from the physiological response to exercise with the genetic environment of the individual. (53, 54) Despite this, the current evidence is diverse: some studies do not report association (55, 56), some imply a protective role (57) and some identify association in very heterogeneous populations in terms of duration of exercise (58), age (59) and type of exercise (professional or leisure) (60). A recent work reveals that physical fitness (a definition related to genotype and exogenous associated factors) would be the real underlying factor for this association and not physical exercise by itself. (60)

The “High Fitness Level” might be a risk factor associated with the previous one, given that individuals who exercise have a tendency to a lower BMI and the BMI is consistently associated with a higher risk of ALS. (62). In fact, a lower BMI might be part of a group of metabolic alterations that underlie this disease. (63)
A “Low Level of Education” as well as “Military Service” are probably confounding variables: individuals with a lower instruction tend to work in jobs related to other risk exposures (“Strenuous Physical Activity”, “Exposure to Electromagnetic Radiation” and “Exposure to Fertilizers/Pesticides” and “Chronic Exposure to Lead”). (1, 7) There are reported associations of these factors but never independently of others (7, 64, 65), like the exposure to lead, trauma and strenuous physical activity (65).

Generally speaking, the study of environmental factors is highly challenging and has various limitations: it is technically difficult to access the exposure to various factors by non-subjective means (for example quantifying physical activity) (1) and the results obtained by interviews (retrospective studies) are associated with memory bias and difficulties in defining concepts like “Physical Activity”. Besides that, some risk factors are potential confounders for others: “Exposure to Fertilizers/Pesticides” and “Head Trauma” can easily be confounders for the association found with “Military Service” or “Strenuous Physical Activity/Professional Sports” in grass fields; and there is also the inverse causality relation between “Strenuous Physical Activity/Professional Sports” with “Head Trauma” and “High Level of Fitness” which challenges the interpretation of these results. (7)

**Genetic Factors**

The genetic basis for ALS is widely known and can be organized by its influence in big fields of cellular and non-cellular processes – excitotoxicity, autophagy, neuroinflammation and protein aggregation with neurotoxicity- which highlights the convergent mechanisms inherent to different mutations.
Mutations in DAO (D-aminocid oxidase), responsible for degrading D-serine, cause and elevation of the latter with its extracellular diffusion and action on the NMDA receptor, initiating autophagy processes that can be responsible by the neuronal apoptosis in ALS. (12) The impact of excitotoxicity (66), and autophagy (67) in ALS is largely supported by the current scientific view.

On the neuroinflammation field, this review found an article about the role of TG2 (Transglutaminase 2), which is inactive under physiologic conditions and is activated upon tissue damage and inflammation. (68) TG2 is the mediator between the cellular stress inherent to SOD1 deposition – an hallmarker of ALS (69) – and the microglia activation. This protein might be involved in a cross talk with NF-kB factor (an inflammation regulator), activating it in a sustained manner. (70)

The remaining genetic alterations found in this review fall of the field of protein aggregation and its neurotoxicity. The protein TDP-43 has functions that include biogenesis and stabilization of RNA, apoptosis and cellular division (70) and is capable of associating with RNA, ssDNA and other proteins. In line with the results of this review, most mutations reported in this protein occur in its prion-like domain (11, 71) and act by a mix phenotype of gain and loss of function: its precipitation when it interacts with ssDNA causes a loss of function (11) but the interaction of the aggregates with membrane proteins and posterior fragmentation of the membrane is associated with neurotoxicity (11).

The C9ORF72 expansion is one of the most prevalent genetic alterations and involves mechanisms of haploinsufficiency (11, 72), gain of toxic function with sequestration of RNA and RNA binding proteins (14, 73) and translation of RAN (repeat associated non-AUG) with prejudice of RNA biogenesis and splicing processes (14, 73). The results in this review suggest a greater vulnerability of cells with the
repeat to excitotoxicity (14), which is evidence of interconnection of impact of a single mutation in various degenerative processes.

PFN1 (Profilin 1) in a protein responsible for the dynamics of the cytoskeleton and its mutations are associated with the formation of aggregates that sequester TDP-43, inhibiting its function. (15) The scientific knowledge has also identified a role of this protein in the formation of stress granules, with a plausible convergence with mutations in TDP-43 and its role in neurotoxicity. (74)

Evidence from the study of mutations in SOD1 protein points to the relevance of an alteration of its conformation to form neurotoxic aggregates. (75) This hypothesis is in line with the results of this review that showed that mutant SOD1 is more prone to the action of glutathione and thyoredoxin systems, with alterations in the redox homeostasis of cytosol triggering aggregation of SOD1. (16)

The nuclear protein Matrin 3 is responsible to connect to RNA and DNA and interact with TDP-43. (13,76) A new mutation affecting the RNA dependent interaction with TDP-43 supports the growing evidence of the role of RNA alterations in ALS and also the central role of TDP-43 in various mutations. (13, 15)

Lastly, Ataxin 2 (ATXN2) has a role in the formation of stress granules and interacts with genetic products of the C9ORF72 and TDP-43 genes. (77) The intermediate repeats (27-33) are the most relevant for the development of ALS, which agrees with this review (7) and with scientific literature (78) and explained by a possible greater affinity of ATXN2 to TDP-43 in this context.

Summing up, the genetic field in ALS is widely known by now, and the main goal now is to understand the underlying mechanisms and translate them in therapeutic options.

Treatment
Experimental Studies in Animals

All of the included experimental studies in animals had positive results and only one did not confirm the achievement of the survival outcome. (23) This can represent a publication bias and, because of that, it was recently published guidelines regarding the pre-clinical investigation on animal models in the field of ALS (79) which now obligatory includes force analysis, therapeutic testing in a pre-symptomatic and symptomatic phase and a high number of animals population studied to determine the effect on raising survival.

Despite acting through different mechanisms, experimental treatments present similar outcomes: a positive impact in the degree of gliosis (2, 7-19, 22, 24), in the loss of weight (2, 17, 21, 23), in the survival of motor neurons (2, 17-19, 22-24), in the disease progression (2, 17, 19-21) and in the beginning of its symptomatic manifestations (17, 21, 24). However there are some points to take in account: the experimental study about Cull (atsm) was the only one to report an increase in survival even when initiated after the symptomatic phase (17), which is a main goal of the therapy, since in humans this is the point where we diagnose the cases; and the positive results regarding neuronal survival and microglia reduction obtained with resveratrol were maintained even with a late initiation of treatment and was the only therapy with impact on the restauration of mitochondrial function and biogenesis (18).

The studies of this review rely on some etiological premises: metabolic therapy relies on the dysfunction and glycolytic metabolism and transportation that occur in ALS (80), bypassing the limiting steps of those processes (20,23); studies with interference RNA (miR-155), Whitaferin A and stem cells act in the microglia neuroinflammation; treatment with trehalose relies on the dysfunctional cellular autophagy (67); resveratrol has diverse actions – normalizes autophagy, microglia activity and mitochondrial biogenesis (67,81,82) and promotes neuroprotection (18);
Cull (atsm) lowers the oxidative stress (17) and Guanabenz acts on the misfolded proteins pathway (24).

In general, these results agree with the etiological mechanisms found in this review. However each of these compounds needs a more solid basis for its use in humans.

**Clinical Trials in Humans**

Of the six clinical trials included in this review, only two are phase III clinical trials: The clinical trial of Edaravone (MCI-186) (26), and the one with Ceftriaxone (27).

Edaravone acts as a scavenger of free radicals, therefore it could lower the levels of oxidative stress and contribute to the delay in progression of the symptoms and neurodegeneration, as it was showed in experimental animal studies. (83). However, and despite the results from the phase II trial (84), this clinical trial failed to show efficacy in its primary endpoint (amelioration of ALSFRS-R score), despite showing efficacy on a secondary endpoint (pinch strength). It is suggested that these results were negative because Edaravone was used in a population that was not the most adequate to benefit from it (it would be more beneficial in patients with rapidly progressive ALS and a quick change of ALSFRS-R score). On the other hand, the first clinical trials were applied to populations with ALS during a smaller amount of time and that might have impacted the initial results. (26)

In spite of these explanations, it is relevant to highlight this constant pattern of clinical trials in ALS, where phase III fails to show efficacy in humans, contrary to its benefit in animals. This can translate a different behavior of the disease across species (79), but also low quality methods of the clinical trials.

Ceftriaxone is capable in enhancing the activity of EAAT2 (responsible by the clearance of glutamate from the synapsis) and its genetic promotor, possibly lowering
the excitotoxicity. (27,85). But the phase III clinical trial failed to prove efficacy and the lack of a biomarker that shows the induction of EAAT2 does not allow the generation of conclusions regarding its activity. This could be the next step pursued about this therapy and would allow a better comprehension of its plausibility.

The meta-analysis about the efficacy of the stem cell treatment evaluates mostly trials that aimed at confirming safety and tolerability of the transplantation process and only two studies evaluated reported a greater survival and both are described as low quality trials. (22) This study allows the conclusion that the heterogeneity of studies conducted limits their interpretation and many of them were conducted in populations with advanced disease that would not benefit of this treatment.

The remaining three clinical trials are from earlier phases: the phase I intrathecal injection of and antisense oligonucleotide (which induces RNAse H and degrades SOD1) has limited conclusions due to its small sample and low dosage used (29); and two phase II trials using an hypercaloric diet and tauroursodesoxicolic acid (TUDCA). The latter, is based the anti-apoptotic properties of TUDCA (86) but its conclusions are limited by the small sample where it was tested (25). The hypercaloric diet is based on the fact that a high BMI has a protective effect on the progression and risk of death by ALS (69). This study included people with advanced disease and with severe malnutrition which might have affected the secondary endpoints regarding the efficacy that were not achieved. (28)

All in all, in terms of clinical trials, the results of this review are quite alarming.

This systematic review has some limitations: the research used only one database (PubMed), only articles available in full online text were included and the selection of
the articles was only done by one author and not by and independent pair. Also the clinical trials included in this review are mainly of low methodological quality and of initial phases, with a small representation of efficacy studies.

Conclusion

This systematic review allows the conclusion that the difficulties inherent to the conduction of prospective studies and clinical trials in this field limit the number of studies done about this entity and those that are conducted frequently use populations that are not representative or wouldn’t benefit of the treatment, which limits the power of its results and conclusions.

On the other hand, we highlight a lack of phase III clinical trials in the period of publication researched that have confirmed efficacy, which can evidence both methodological failures and also a possibility that the conclusions from experimental studies in animals might not be applicable in humans with a subsequent need to research new approaches on the animal experimentation field.

ALS is, therefore, an entity with many shadow areas both in the etiological field (mainly regarding environmental factors) and the treatment field and although the genetic progress has contributed to the knowledge of many mechanisms that might be important to create therapeutic options, that last step has not been taken and Riluzole is still the only available option for these patients, even though currently their quality of life can be upgraded though symptomatic treatment and treatment of the disease’ complications.
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