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ASPECTOS EPIDEMIOLÓGICOS, PROGNÓSTICOS E TRATAMENTO DA ESCLEROSE LATERAL AMIOTRÓFICA

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Tese apresentada ao Curso de Pós-Graduação em Ciências da Saúde, Faculdade de Ciências da Saúde, Universidade de Brasília, como requisito parcial à obtenção do título de Doutor em Ciências da Saúde.

Orientadores: Prof[a]. Dr[a]. Maria Rita Carvalho Garbi Novaes

Prof. Dr. Luiz Augusto Casulari Roxo da Motta

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Mirian Conceição Moura

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Mirian Conceição Moura

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Tese apresentada ao Curso de Pós-Graduação em Ciências da Saúde da Faculdade de Ciências da Saúde da Universidade de Brasília, para obtenção do título de Doutor em Ciências da Saúde.

Linha de pesquisa: Saúde Coletiva.

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RESUMO

Moura MC. **Aspectos epidemiológicos, prognóstico e tratamento da esclerose lateral amiotrófica.** 2016. 167 folhas. Tese [Doutorado] – Programa de Pós- Graduação em Ciências da Saúde. Universidade de Brasília, Brasil. Orientadores: Profª Drª Maria Rita Carvalho Garbi Novaes e Prof. Dr. Luiz Augusto Casulari Roxo da Motta.

A esclerose lateral amiotrófica é uma síndrome neurodegenerativa, que afeta o primeiro e segundo neurônios motores, causando fraqueza e atrofia muscular progressivas. Os objetivos do estudo foram descrever os aspectos epidemiológicos da doença no Brasil, avaliar os tratamentos existentes e encontrar fatores envolvidos no prognóstico da doença. Sua incidência no Brasil, determinada por dados de mortalidade, é de 0.89/100.00 pessoas-ano na população geral e de 2.3/100.000 pessoas-ano, na população acima de 45 anos. 73,4 % são da raça branca e a média de idade foi de 62,7±13,2 anos. Revisão sistemática com metanálise evidenciou ausência de efetividade de tratamento medicamentoso ou terapia celular em seres humanos com relação à sobrevivência, enquanto que a maior parte dos estudo pré-clínicos demonstraram eficácia. Ensaio clínico realizado com carbonato de lítio associado ao riluzol em 60 pacientes não demonstrou benefício em relação ao grupo controle (log-rank: 0.38), sendo interrompido pela baixa adesão e grande número de efeitos colaterais. No entanto, observou-se que o cuidado multidisciplinar trouxe redução de 75% da frequência e até 90% dos gastos com internações hospitalares. Estudo retrospectivo em 218 pacientes utilizando Regressão de Cox revelou que os fatores relacionados à pior evolução são idade acima de 75 anos, mau estado nutricional (IMC abaixo de 25 Kg/m^2) e forma de início bulbar. Estudo prospectivo em 101 pacientes com a mesma metodologia associada a análise se acurácia através da curva ROC permitiu a criação de modelo preditivo composto por cinco fatores relacionados à menor sobrevivência em 12 meses, com 74% de acurácia: idade acima de 65 anos (RR=2.50 IC 95% 1.23-5.08); envolvimento de segundo segmento corporal em menos de seis meses (RR=2,02 IC 95% 1.04 -3.94); Capacidade Vital Forçada menor que 63% (RR=2.78 IC 95% 1.03-7.48), fraqueza cervical (RR= 2.28 IC 95% 1.03-5.05) e presença de síndrome piramidal (RR= 2.36 IC 95% 1.05- 5.33).

Palavras – chave: epidemiologia; prognóstico; sobrevivência; esclerose lateral amiotrófica; lítio; biomarcador

ABSTRACT

Moura MC. **Epidemiological aspects, prognosis and treatment of amyotrophic lateral sclerosis.** 2016. 167 pages. PhD [Dissertation] – Program in Health Sciences Brasilia University, Brazil. Advisors: Prof[a] Dr[a] Maria Rita Carvalho Garbi Novaes and Prof. Dr. Luiz Augusto Casulari Roxo da Motta.

Amyotrophic lateral sclerosis is a neurodegenerative spectrum that affects upper and lower motor neurons and causes progressive muscle weakness and atrophy. The aims of the study were to describe the epidemiology of the disease in Brazil, to evaluate the possible treatments and to find biomarkers involved in disease prognosis. Its incidence in Brazil using mortality data is 0.89/100,00 person-years in the general population and 2.3/100,000 person-years in the population aged over 45. Seventy-three percent are Caucasians and the mean age of death is 62.7 ± 13.2 years. There is not enough evidence of effectiveness of drug or stem cell therapy on improving survival, whereas many preclinical studies show great efficacy. Clinical trial with lithium carbonate associated to riluzole demonstrated no benefit (log rank: 0.38) and was interrupted by low compliance and the occurrence of many side effects. However, it is observed that the multidisciplinary care brought a 75% reduction in the frequency and up to 90% of costs on hospital admissions. A retrospective analysis of 218 patients using Cox regression showed that the factors related to worse outcomes are age over 75 years, poor nutritional status (BMI below 25 kg/m^2) and bulbar onset. A prospective analysis of 101 patients with Cox regression associated with accuracy analysis by ROC curve created a predictive model with five factors related to lower survival at 12 months, with 74% of accuracy: age over 65 years (RR = 2.50 CI 95% 1.23-5.08); involvement of a second site in less than six months (RR = 2.02 CI 95% 1.04-3.94); Forced Vital Capacity less than 63% (RR = 2.78 CI 95% 1.03-7.48), neck weakness (RR = 2.28 CI 95% 1.03-5.05) and the presence of pyramidal syndrome (RR = 2.36 CI 95% 1.05-5.33).

Key words: epidemiology; prognosis; survival; amyotrophic lateral sclerosis; lithium; biomarker

1 - INTRODUÇÃO

A esclerose lateral amiotrófica (ELA) é a doença do neurônio motor mais comum, mais frequentemente esporádica e caracterizada pela degeneração progressiva dos neurônios motores superiores e inferiores no cérebro, tronco encefálico e medula espinhal(1,2).

O quadro clínico é caracterizado por fraqueza e atrofia muscular progressiva, que usualmente leva à disfagia e insuficiência respiratória (1,2). Há grande variabilidade clínica, o que dificulta o diagnóstico. Entre outras variantes, existem as formas esclerose lateral primária, uma doença restrita ao neurônio motor superior, que compõe 1 a 3%, a atrofia muscular progressiva, limitada ao neurônio motor inferior, que é responsável cerca de 10% dos casos e a paralisia bulbar progressiva, com cerca de 10 a 15% (1). A presença de sinais de acometimento do primeiro neurônio motor ou neurônio motor superior isoladamente confere prognóstico menos sombrio à doença (1,2).

A maioria dos estudos europeus estima a sua incidência em 1,2 a 4,0 por 100.000 pessoas-ano (3-5), sendo cinquenta vezes mais frequente na Ilha de Guam, na Nova Guiné e na Península de Kii do Japão (6), mas no Brasil ela foi estimada em 0,3 a 0,5/ 100.000 pessoas-ano (7). O pico de incidência está entre 70 e 80 anos, afetando principalmente o sexo masculino (6).

Atualmente, pouco conhecimento há sobre a etiologia da ELA em sua forma esporádica, acreditando-se na interação de fatores genéticos e ambientais (8). Os avanços recentes decorrem principalmente da compreensão de formas familiares que levaram a novas hipóteses para gatilhos da doença e mecanismos de propagação (9,10). Acredita-se que os processos patológicos ocorram tanto em neurônios quanto em células da glia, resultando em excitotoxicidade do glutamato (11). Outros eventos importantes são a formação anormal de agregados protéicos, com alteração das proteínas estruturais e a disfunção mitocondrial. A propagação da doença é desconhecida, mas as evidências atuais apontam para propagação através de redes neurais ou do RNA, assemelhando-se à fisiopatologia das doenças por príons (9).

O prognóstico letal e a ausência de tratamento específico para a ELA significam que a maioria dos cuidados comprovadamente eficazes são paliativos (12). Um único

medicamento, o riluzol, aprovado para utilização em 1996 e utilizado com frequência, atrasa a deterioração de cerca de 2 a 4 meses, mas nenhum tratamento pode evitar o curso da doença (13). Recentemente, ensaios clínicos com várias drogas têm sido realizados por terem demonstrado sucesso em modelos experimentais em animais, ainda sem evidências de benefícios em ensaios clínicos (14,15). O cuidado multidisciplinar, a nutrição enteral e a ventilação não-invasiva para os pacientes que desenvolvem insuficiência respiratória melhoram a sobrevida e a qualidade de vida(12).

Para a adoção do cuidado multidisciplinar adequado a cada caso, é necessário estabelecer a velocidade de progressão da doença e o prognóstico. O grande dilema é que a ELA acarreta degeneração progressiva dos neurônios motores que alimentam os músculos estriados de forma clinicamente heterogênea. O efeito clínico é fraqueza muscular progressiva, levando à morte, geralmente por insuficiência respiratória. Em alguns casos, pode haver também alterações cognitivas. A sobrevida é variável, de meses a décadas, sendo a média de 19 a 30 meses do início do quadro (6,13,16,17).

Como a variabilidade na evolução é grande e a maior parte dos estudos é retrospectivo e com número pequeno de pacientes, é difícil prever o tempo de sobrevivência ou o período das intervenções. Em geral, acredita-se que o início em membro inferior, a idade mais jovem, a melhor função motora, a melhor capacidade respiratória, o peso estável e o maior intervalo entre o início dos sintomas e o diagnóstico estejam associados com maior sobrevida (13,16,17).

Há estudos sugerindo que o tempo de progressão da fraqueza ou a difusão dos sintomas motores a partir do local inicialmente afetado possa determinar a velocidade de progressão da doença(18,19). A maior deles se baseia na progressão por avaliação clínica e por medidas subjetivas. No entanto, estima-se ser necessário haver uma perda na ordem de 30% das células do corno anterior da medula antes da fraqueza se tornar aparente (20).

Para melhor compreensão da evolução da doença, é necessário determinar biomarcadores e fatores envolvidos em seu prognóstico. Apesar de a eletromiografia com eletrodo de agulha (EMG) ser rotineiramente usada para detectar o envolvimento subclínico do neurônio motor inferior no diagnóstico, o exame não se presta ao acompanhamento e à quantificação da perda de força muscular. Por outro lado, medidas

como estimativa de número de unidades motoras ou ‘motor unit number estimation’ (MUNE e MUNIX) e o acompanhamento com medidas de capacidade vital forçada (CVF) mostraram-se úteis no acompanhamento da evolução da difusão da fraqueza muscular e do prognóstico da doença (20-22).

Para melhor dimensionar a capacidade de atendimento dos serviços de saúde, foi importante iniciar o estudo determinando a incidência atual e o comportamento epidemiológico da doença na população do Distrito Federal e do Brasil.

O tratamento da doença envolve dois aspectos: uso de medicamentos para redução da progressão da doença e o tratamento multidisciplinar de suas complicações. O surgimento de novas drogas e outras intervenções para reduzir a progressão da doença justificaram a realização de revisão sistematizada da literatura sobre o tema, que posteriormente orientou a escolha de carbonato de lítio para ensaio terapêutico nos pacientes do Centro de Referência para Doenças Neuromusculares do Distrito Federal. Também o impacto do tratamento multidisciplinar, reconhecidamente benéfico em estudos internacionais (12) mereceu avaliação na perspectiva do Sistema Único de Saúde.

2. OBJETIVOS

2.1. Objetivo Geral

Avaliar aspectos clínicos, epidemiológicos e neurofisiológicos e sua correlação com o prognóstico da Esclerose Lateral Amiotrófica.

Avaliar o tratamento da doença nos pacientes acompanhados no Centro de Referência para Doenças Neuromusculares da Secretaria de Estado de Saúde do Distrito Federal.

2.2. Objetivos Específicos

- Correlacionar o tempo de início, a distribuição e difusão da fraqueza muscular determinado pelo exame neurológico com a sobrevivência;
- Estabelecer fatores clínicos e neurofisiológicos associados a sobrevivência no curso da doença;
- Correlacionar o sítio de início, a forma de progressão da doença, as alterações neurofisiológicas e a função respiratória inicial com o desenvolvimento de insuficiência respiratória ou realização de traqueostomia;
- Estabelecer fatores prognósticos preditivos para o desenvolvimento de insuficiência respiratória;
- Estabelecer se há algum tratamento na literatura com potencial para redução da progressão dos sintomas da doença;
- Estabelecer o valor da associação de carbonato de lítio ao tratamento com riluzol na progressão da Esclerose Lateral Amiotrófica, através de escalas funcionais, análise de sobrevivência, avaliação clínica, de função respiratória e neurofisiológica;
- Estabelecer o impacto do tratamento multidisciplinar na frequência e no tempo de internação dos pacientes no Sistema Único de Saúde do Distrito Federal

3. MÉTODO

3.1-Desenho do estudo

O estudo foi realizado em duas etapas retrospectiva e prospectiva, com métodos mistos.

3.1.1 – Etapa retrospectiva:

Realizado estudo descritivo transversal utilizando dados de mortalidade (Declarações de Óbito), para determinação da incidência da doença e descrição de fatores étnicos e demográficos a ela associados no Brasil.

Realizado estudo descritivo transversal utilizando dados secundários das internações por Doença do Neurônio Motor CID 10 G12. 2 no Distrito Federal de 2005 a 2014, avaliando a eficiência do Centro de Referência para Doenças Neuromusculares sobre elas.

Realizado estudo descritivo transversal dos registros dos pacientes avaliados no Centro de Referência para Doenças Neuromusculares e na Gerência de Medicamentos Excepcionais, com avaliação de aspectos clínicos e demográficos em sua sobrevivência.

3.1.2 – Etapa prospectiva:

Foi realizado estudo secundário de revisão sistemática de ensaios clínicos pré-clínicos e clínicos aleatórios sobre tratamentos para reduzir a progressão e aumentar a sobrevivência da Esclerose Lateral Amiotrófica, com posterior metanálise.

Realizada coorte descritiva com 101 pacientes com diagnóstico de Esclerose Lateral Amiotrófica acima de 18 anos de idade, definida de acordo com os critérios de El Escorial e Awaji-shima (20-22), de março de 2014 a dezembro de 2015, excluídas outras doenças que cursam com o mesmo quadro clínico, de acordo com o Protocolo de Esclerose Lateral Amiotrófica (23). Os pacientes foram avaliados a cada três meses com exame neurológico e escores de 0 a 5 de força muscular- escala Medical Research Council (MRC) medindo abdução dos ombros, flexão e extensão dos cotovelos, flexão e extensão dos punhos, abdução do polegar e 5º dedo (24), força cervical (0 a 5), CVF em posição supina e prona, saturação de oxihemoglobina em posição supina, amplitude do

PAMC, MUNIX, ALSFRS-R. Foram avaliados ainda, sobrevivência com Curva de Kaplan-Meier com log-rank test em relação ao desfecho morte ou traqueostomia.

Nesse grupo, foi realizada também pesquisa clínica analítica prospectiva na modalidade de ensaio clínico randomizado aberto, envolvendo 60 pacientes acima de 18 anos de idade com diagnóstico de Esclerose Lateral Amiotrófica, definida de acordo com os critérios de El Escorial e Awaji-shima (20-22) de março de 2014 a setembro de 2015, devendo ser excluídas outras doenças que cursem com o mesmo quadro clínico, de acordo com o Protocolo de Esclerose Lateral Amiotrófica (23).

Os medicamentos selecionados para o ensaio clínico após realização de revisão sistematizada e considerando a disponibilidade e o custo foi: associação de riluzol e carbonato de lítio. A dosagem de Riluzol utilizada foi a prevista no Protocolo do Ministério da Saúde – 50 mg de 12/12 horas em jejum(23) e a dosagem de carbonato de lítio inicial foi de 300 mg de 12/12 horas, acompanhada com a litemia mensal até atingir a concentração sérica de 0.4-0.8 mmol/l(14). Os pacientes foram avaliados a cada três meses com exame neurológico, escores de força muscular (MRC), força cervical, CVF em posição supina e prona, saturação de oxihemoglobina em posição supina, amplitude do PAMC, MUNIX, ALSFRS-R. Foram avaliados ainda, sobrevivência por Curva de Kaplan-Meier com *log-rank test* em relação ao desfecho morte ou traqueostomia.

3.2-Fontes de informações

3.2.1: Etapa retrospectiva

1-Sistema de Informação de Mortalidade (SIM) do Departamento de Informática do Sistema Único de Saúde (DATASUS) de 2004 a 2014, utilizando o aplicativo TABWIN, disponível pelo DATASUS. Foi utilizado o CID 10 G12.2 – Doença do Neurônio Motor como causa básica e como causa secundária de morte e executada tabulação de frequência por sexo, idade, raça/cor, grau de instrução, estado civil, local de residência e local de óbito.

2-Sistema de Informação Hospitalar (SIH) do Departamento de Informática dos Sistema Único de Saúde (DATASUS) de 2004 a 2014. Como medidas de eficiência utilizamos a frequência, o tempo de internação e os valores de repasse de Autorização de Internação Hospitalar (AIH) e gasto com Unidade de Terapia Intensiva (UTI).

O universo de estudo foi composto pelas mortes e internações registradas para a doença do neurônio motor, acima de 20 anos de idade ocorridas em todos os municípios do Brasil de janeiro de 2004 a dezembro de 2013. Nos cálculos de incidência anual da doença, foram utilizados dados do Censo de 2010 e projeções anuais a partir do Censo de 2004, feitas pelo Instituto Brasileiro de Geografia e Estatística-IBGE (24). Todos os cálculos de proporções raciais foram ajustados pelo Censo 2010 (24).

3-Prontuários dos pacientes atendidos no Centro de Referência de Doenças Neuromusculares da Secretaria de Estado de Saúde do Distrito Federal de setembro de 2011 a dezembro de 2014, utilizando o aplicativo TRAKCARE da SES-DF, com coleta de dados clínicos e demográficos.

4-Processos para dispensação de riluzol da Gerência de Medicamentos Excepcionais da SES-DF de 2005 a 2014, com coleta manual de dados, referente a dados clínicos e demográficos.

Os dados coletados através dos registros de pacientes e processos foram confrontados com os dados do Sistema de Informação de Mortalidade (SIM), pelo método de captura-recaptura.

Os dados obtidos foram alocados em tabelas Office Excel 2010 e analisados utilizando o SPSS (Statistical Package for the Social Sciences) versão 19.0. e o Epi Info 7.0. Os Intervalos de Confiança foram calculados assumindo uma distribuição de Poisson. As variáveis categóricas foram avaliadas usando o Teste QuiQuadrado two-tailed Z test e as variáveis quantitativas, o Teste t de Student, aceitando-se como significante $p<0,05$.

As Razões de probabilidades (OR) foram calculadas com os grupos étnicos caucasianos, negros, pardos, amarelos e índios, com Intervalo de Confiança de 95% e com base na população acima de 20 anos do Censo 2010 do IBGE (24).

No estudo de fatores prognósticos, as varáveis relacionadas foram submetidas a análise multivariada incluindo todas as variáveis. Inicialmente, análises de regressão de Cox univariadas foram empregadas para variáveis sócio demográficas e clínicas com relação ao tempo de sobrevivência. Variáveis com $p < 0,25$, nas análises univariadas (28) foram selecionadas para serem incluídas na análise de regressão de Cox

multivariada. O modelo de regressão multivariado final foi construído pela exclusão consecutiva de variável a variável a partir do modelo multivariado inicial, empregando-se o teste da razão de verossimilhança para determinar a importância de cada variável excluída. O nível de significância foi fixado em 0,05.

As funções de sobrevivência para os pacientes foram estimadas por Kaplan-Meier e comparadas através do teste de log-rank. As análises foram realizadas empregando-se os programas SAS 9.3.

Foi obtida aprovação por Comitê de Ética em Pesquisa sob o número de Protocolo FEPECS 820.117/2014, com dispensa de Termo de Consentimento Livre e Esclarecido.

3.2.2: Etapa prospectiva

3.2.2.1: Revisão sistematizada:

O método desta pesquisa seguiu as recomendações para realização de revisões sistemáticas propostas pela Colaboração Cochrane.

Critérios de Inclusão:

-Ensaios clínicos aleatórios prospectivos:

Participantes de qualquer faixa etária com diagnóstico possível, provável ou confirmado de Esclerose Lateral Amiotrófica, em qualquer fase evolutiva da doença, exceto com insuficiência respiratória instalada.

Diagnóstico feito através de anamnese e eletroneuromiografia pelos Critérios de El Escorial e Awaji (20-22).

-Ensaios pré-clínicos ‘in vivo’ com avaliação de sobrevida e progressão da doença.

- Revisões sistemáticas com metanálise.

Critérios de exclusão:

Participantes com insuficiência respiratória

Doença do Neurônio Motor Hereditária ou Atrofia Muscular Espinhal

Cartas, Editoriais, Relatos de caso.

Revisões narrativas.

Foram avaliadas as seguintes intervenções: uso de qualquer tipo de substância e/ou intervenção para a redução da progressão dos sintomas motores e/ou sobrevida da doença, comparados ao placebo ou outro tipo de tratamento utilizado por um grupo controle.

A análise dos dados foi realizada, quando possível, entre as seguintes comparações:

a) todos os casos tratados com o tratamento proposto no estudo versus os casos tratados com placebo;

b) comparação dos diferentes tipos de tratamentos entre si;

c) análise da incidência de efeitos adversos dos tratamentos propostos;

d) mortalidade geral ou sobrevida.

e) variação da escala ALSFRS-R

Os estudos foram selecionados com os seguintes critérios: os autores independentemente avaliaram os títulos e resumos de todos os estudos identificados na busca realizada a partir dos descritores nas seguintes bases de dados eletrônicas: Medline, Lilacs, a base de dados de ensaios clínicos controlados da Colaboração Cochrane, Embase, lista de referências dos ensaios clínicos aleatórios encontrados, a comunicação pessoal com os autores. Os estudos que preencheram os critérios para sua inclusão foram obtidos em sua íntegra.

A partir desta ação foi criada uma coleção de estudos para serem avaliados pela revisora.

Seleção dos ensaios clínicos aleatórios:

Inicialmente foi verificado se em cada estudo encontrado se apresentavam os critérios para inclusão: tipo de estudo, tipo de participantes e tipo de intervenções, utilizando-se um formulário padronizado.

A seguir, foi feita uma observação cuidadosa da descrição do processo de sigilo de alocação, determinando-se a classificação do estudo em quatro categorias:

Categoria A: significa que o processo de sigilo da alocação foi adequadamente relatado; Categoria B: significa que o sigilo de alocação não é descrito, mas é mencionado no texto de que o estudo é aleatório; Categoria C: significa que o sigilo de alocação foi inadequado; Categoria D: significa que o estudo não é aleatório.

Os artigos classificados como A ou B foram incluídos para coleta de dados e os artigos classificados como C ou D, excluídos por não serem aleatórios.

Foram coletados os seguintes dados dos estudos:

Métodos: pergunta da pesquisa; sequência do tratamento, sigilo da alocação, seguimento pós-intervenção, avaliação cega do desfecho, medida do desfecho clínico primário, local do estudo, proteção contra contaminação, cálculo do poder estatístico, representatividade da amostra .

Participantes: critérios de inclusão, critérios de exclusão, idade, gênero, gravidade da doença, variantes da doença (anexo 3).

Intervenções: medicamentos e doses ou procedimentos, tempo de seguimento, método de acompanhamento da progressão da doença.

Desfechos: dias de sobrevida e variação da escala ALSFRS-R (Anexo3)

Os resultados dos desfechos primários dos estudos foram coletados segundo o princípio da intenção-de-tratamento: para cada desfecho dicotômico o número de eventos pelo número do total de participantes em cada grupo; para os desfechos contínuos, foram calculados média, desvio-padrão e número de participantes em cada grupo. Os dados de estudos publicados duas ou mais vezes foram extraídos apenas daquele mais completo.

A qualidade de cada estudo incluído nesta revisão sistemática foi avaliada pelo método GRADE(26), e no caso de Revisões sistemáticas, pelos critérios ARMSTAR(27) ou seja, pelas respostas (sim, não, indeterminado, não utilizado para esta revisão) para cada uma das seguintes questões:

Os autores de cada estudo foram contatados para esclarecimento se a resposta para qualquer uma destas perguntas foi “indeterminado”. A autora classificou cada estudo em: A – baixo risco de viés, quando houve “sim” como resposta para todas as questões; B – moderado risco de viés, quando houve “não” como resposta para uma das questões; C – alto risco de viés, quando houve “não” como resposta para duas ou mais questões e D – altíssimo risco de viés .

A análise estatística foi realizada utilizando o programa de computador Review Manager (RevMan5, 2012) produzido pela Colaboração Cochrane.

Para as variáveis contínuas, foi calculada a diferença de médias ponderadas (modelo de efeito randômico) com intervalo de confiança de 95% correspondente.

3.2.2.2: Coorte prospectiva com vistas a estudo descritivo sobre fatores prognósticos da doença e ensaio clínico.

Pacientes atendidos no Centro de Referência para Doenças Neuromusculares do Hospital Regional da Asa Norte (HRAN) por procura espontânea, encaminhados de outros serviços ou da Gerência de Medicamentos Excepcionais da SES-DF.

3.3- Banco de dados e variáveis

1-Critérios de inclusão:

a) diagnóstico de Doença do Neurônio Motor feito por médico neurologista.

b) maior de 18 anos.

c) aceitar participar da pesquisa através de termo de consentimento esclarecido – Termo de Consentimento Livre e Esclarecido.

d) Capacidade Vital Forçada (CVF) maior ou igual a 50 %, exceto na Paralisia Bulbar Progressiva.

d) Preenchimento de critérios El Escorial e Awaji-Shima para ELA Definitiva, Provável e Possível.

2-Critérios de exclusão:

a) Outras formas de doença que acometam o corno anterior da medula.

b) Bloqueios de condução motora à eletroneuromiografia.
c) Insuficiência respiratória, definida por saturação de Oxihemoglobina menor ou igual a 90% e/ou PaO2 menor ou igual a 60 mm Hg.

A randomização ocorreu através do aplicativo iRandomizer. O Grupo controle não recebeu o medicamento carbonato de lítio.

1- Admissão a partir de avaliação clínica por médico neurologista no Centro de Referência para Doenças Neuromusculares do Hospital Regional da Asa Norte (HRAN).
2- As avaliações ocorreram a cada três a quatro meses de intervalo e foram avaliados(anexo4):
    1- Idade e sexo
    2- Etnia/cor por heteroavaliação.
    3- Tempo de início dos sintomas.
    4- Segmento corporal inicialmente afetado pelo deficit motor, pela história clínica e exame neurológico, podendo ser necessária eletroneuromiografia.
    5- Tempo e direção da primeira propagação do deficit: horizontal, rostro-caudal, caudo-rostral ou cruzada, pela história clínica e exame neurológico, podendo ser necessária eletroneuromiografia.
    6- Capacidade Vital Forçada (CVF) e Fluxo de Pico, através de espirometria em equipamento realizada em posição sentada, com bucais descartáveis e de uso individual, com clipe nasal, conforme recomendações das Diretrizes para Testes de Função Pulmonar (Sociedade Brasileira de Pneumologia e Tisiologia: SBPT-2002). O laudo espirométrico foi realizado por pneumologista responsável pelo Laboratório de Pneumologia do HRAN.
    7- Avaliação funcional: ALSFRS-R: ‘Revised ALS Functional Rating Scale’ traduzida e validada (29-30), que avalia os seguintes parâmetros: fala, salivação, deglutição, capacidade de escrita, capacidade de usar garfo e faca para se alimentar, higiene e vestuário, movimentação na cama, marcha, subir escadas e respiração, com escores de 0 a 4, totalizando o valor máximo de 40.

8- Amplitudes dos Potenciais de Ação Musculares Compostos de nervos mediano direito, com estímulo no punho a 7 cm da captação no ponto motor do músculo abdutor do polegar na eminência tenar; e de nervo ulnar direito, com estímulo no punho a 7 cm da captação em músculo abdutor do quinto dedo, com referencia na falange distal.

9- Índex do número de Unidades Motoras funcionantes (MUNIX): razão obtida pela estimulação supramáxima de nervo mediano e captação em músculo abdutor curto do polegar e obtenção do Potencial Composto de Unidades Motora (PAMC) sobre área de de eletromiograma de esforço de superfície no mesmo músculo e do nervo ulnar, com captação no músculo abdutor do quinto dedo (31-32), obtidos por médico neurofisiologista, com equipamento da marca Medtronic 'Keypoint' de dois canais.

10- O Índex MUNIX(N) será calculado através do seguinte modelo matemático (33):

Área do Potencial de Ação Muscular Composto (PAMC) = N x Mr ; onde N = numero de PAUM, Mr = área de um Potencial de Ação de Unidade Motora (PAUM).

PAMC power = N^2 x Mp, onde Mp = poder de um PAUM.

Área do SIP = D x Mr, onde D = número de descargas em 300 milissegundos.

SIP (Potenciais de ação musculares de superfície) power = D x Mp N= PAMC power x área SIP / Area PAMC x SIP power

11- Foi também avaliado e correlacionado o tempo de desenvolvimento de Insuficiência Respiratória, caracterizado por Saturação de O2 abaixo de 90 % à oximetria noturna com oxímetro de Pulso *Moriya* e PaO2 abaixo de 60 mmHg à gasometria arterial ou pela indicação de utilização de Ventilação Não-Invasiva(VNI) por mais de 12 horas/dia ou traqueostomia.

12- Escala MRC – 0 a 5 – valor máximo de 70.

13- Redução da força de musculatura flexora cervical.

14- Índice de Desenvolvimento Humano Municipal (IDHM) do local de residência (34).

15- Peso e altura com cálculo de Índice de Massa Corporal – IMC (35).
16- História familiar .
17- Demência frontotemporal, através da avaliação clínica de sintomas comportamentais e cognitivos e de achados de neuroimagem.
18- Tabagismo.

3.5- Análise dos dados

Os dados obtidos foram alocados em tabelas Office Excel 2010 e analisados utilizando o SPSS (Statistical Package for the Social Sciences) versão 19.0., o Epi Info 7.0 e o SAS 9.3. Os Intervalos de Confiança foram calculados assumindo uma distribuição de Poisson. As variáveis categóricas foram avaliadas usando o Teste QuiQuadrado two-tailed Z test e as variáveis quantitativas, o Teste T de Student, aceitando-se como significante $p<0,05$.

As variáveis relacionadas ao prognóstico - sobrevivência ou traqueostomia - foram submetidas a análise multivariada incluindo todas as variáveis. Inicialmente, análises de regressão de Cox univariadas foram empregadas para variáveis sócio demográficas e clínicas com relação ao tempo de sobrevivência. Variáveis com $p < 0,25$, nas análises univariadas foram selecionadas e incluídas na análise de regressão de Cox multivariada (28). O modelo de regressão multivariado final foi construído pela exclusão consecutiva de variável a variável a partir do modelo multivariado inicial, empregando-se o teste da razão de verossimilhança para determinar a importância de cada variável excluída. O nível de significância foi fixado em 0,05. A seguir, as variáveis significativas criaram um modelo prognóstico, cuja acurácia foi obtida pela análise de sensibilidade e especificidade, através da curva ROC (Receiver Operating Characteristic). As funções de sobrevivência para os pacientes foram estimadas por Kaplan-Meier e comparadas através do teste de log-rank, com nível de significância de p em 0,05. As análises foram realizadas empregando-se os programas SAS 9.3.

Foi obtida aprovação por Comitê de Ética em Pesquisa sob o número de Protocolo FEPECS 525.241 /2014, com necessidade de Termo de Consentimento Livre e Esclarecido assinado. O Ensaio Clínico foi registrado sob o protocolo REBEC RBR-2n5mtq.

4. RESULTADOS

Os experimentos deste trabalho foram conduzidos no período de março de 2014 a dezembro de 2015 e os resultados foram apresentados sob a forma de artigos científicos.

Foram elaborados sete artigos originais.

Foi elaborado artigo original sobre a epidemiologia e os aspectos étnicos da esclerose lateral amiotrófica no Brasil, estudo baseado em dez anos de análise do Sistema de Informação de Mortalidade do Departamento de Informática dos Sistema Único de Saúde (SIH/DATASUS). O artigo intitulado " **Ethnic and demographic incidence of Amyotrophic Lateral Sclerosis in Brazil: a population-based study**" foi publicado no periódico Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, Fator de Impacto = 2.450, correspondente à classificação pelo Programa da CAPES – Qualis Medicina II como B1, em 2014.

Foi elaborado artigo de revisão sistematizada com posterior metanálise sobre os tratamentos farmacológicos empregados na redução da progressão da esclerose lateral amiotrófica em animais e seres humanos nos últimos seis anos e realizada discussão sobre os motivos pelos quais não houve sucesso nos ensaios clínicos, mesmo em face de vários resultados positivos em estudos pré-clínicos. O artigo intitulado "**Efficacy and effectiveness of drug treatments in amyotrophic lateral sclerosis: a systematic review with meta-analysis**" foi publicado no periódico African Journal of Pharmacology and Pharmacy, classificado pelo Programa da CAPES – Qualis Medicina II como B2, 2014.

Foi elaborado artigo de revisão sistematizada com posterior metanálise sobre os tratamentos farmacológicos empregados na redução da progressão da esclerose lateral amiotrófica em animais e seres humanos nos últimos seis anos e realizada discussão sobre os motivos pelos quais não houve sucesso nos ensaios clínicos, mesmo em face de vários resultados positivos em estudos pré-clínicos. O artigo intitulado "**Efficacy of stem cell therapy in amyotrophic lateral sclerosis: a systematic review and meta-analysis**" foi publicado no periódico Journal of Clinical Medicine Research, classificado pelo Programa da CAPES – Qualis Medicina II como B4.

Foi elaborado artigo original evidenciando a eficiência do tratamento multidisciplinar da esclerose lateral amiotrófica no Centro de Referência para Doenças Neuromusculares da Secretaria de Estado de Saúde do Distrito Federal (CRDN-SES-DF), na redução do número de internações e no custo do tratamento para o Sistema Único de Saúde. O estudo "**Impact of multidisciplinary care in amyotrophic lateral sclerosis hospitalizations in the public health system of Brazil**" foi publicado em setembro de 2015 no periódico Journal of Public Health and Epidemiology, 354 artigos publicados, classificação correspondente ao Programa da CAPES – Qualis Medicina II como B3, 2014.

Foi elaborado artigo original descrevendo ensaio clínico randomizado aberto com carbonato de lítio em sessenta pacientes, empregando biomarcadores clínicos e neurofisiológicos. O artigo "**Amyotrophic lateral sclerosis treatment with lithium associated with riluzole versus riluzole alone: an open randomized controlled trial** " foi submetido em janeiro de 2016 ao periódico Journal of Negative Results in BioMedicine, classificado pelo Programa da CAPES – Qualis Medicina II como B2, 2014.

Foi elaborado artigo original identificando três fatores prognósticos em estudo de base populacional retrospectivo de dez anos de duração na população atendida na Secretaria de Saúde do Distrito Federal com a doença. O artigo "**Prognostic factors in amyotrophic lateral sclerosis. A population-based study**" foi publicado no periódico PLoS ONE em outubro de 2015, , classificado pelo Programa da CAPES – Qualis Medicina II como A2, 2014.

Foi elaborado artigo original identificando em estudo prospectivo, conjunto de fatores prognósticos para a doença e sugerindo a criação de modelo preditivo para o prognóstico da doença. O artigo "**Predictors for prognosis in amyotrophic lateral sclerosis: a prospective observational single center study**" foi submetido em fevereiro de 2016 ao periódico PLoS ONE, classificado pelo Programa da CAPES – Qualis Medicina II como A2, 2014.

ARTIGO 1 – ARTIGO ORIGINAL: ETHNIC AND DEMOGRAPHIC INCIDENCE OF AMYOTROPHIC LATERAL SCLEROSIS IN BRAZIL: A POPULATION-BASED STUDY.

Versão aceita para publicação em inglês

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ETHNIC AND DEMOGRAPHIC INCIDENCE OF AMYOTROPHIC LATERAL SCLEROSIS IN BRAZIL: A POPULATION-BASED STUDY

Abstract

Objectives: to examine demographic and ethnic factors associated with amyotrophic lateral sclerosis in Brazil.

Methods: retrospective study of death certificates performed in 2015 June, identifying the incidence of amyotrophic lateral sclerosis in ten years, from January 2004 to December 2013, related to sex, age and race.

Results: 8,942 death certificates with 8,152 as the underlying cause and 790 as a secondary cause. The average age was 62.7±13.2 years, with predominance in males (1·3:1). The adjusted mortality rate over 20 years was 0·61 to 0·89 /100,000 person-years, and over 45 years was 1·77 to 2·3 /100,000 person-years. There was a predominance of amyotrophic lateral sclerosis in Caucasians compared to the general population above 20 years (2010 Census), with an odds ratio (OR) of 2·92 (95% CI: 2·78 to 3·07). The OR in blacks was 0·04 (95% CI: 0·03-0·04), in mestizos was 0.05 (0.04 to 0.07), and in Indians was 0·02 (0·01-0·04). The mean age was lower than in European populations (48.5±12.3 years) ($p<0·0001$).

Conclusion: the incidence of amyotrophic lateral sclerosis in Brazil is close to other Latin American populations, with a lower age at death and clear predominance in Caucasians.

KEY WORDS: Epidemiology, race, Amyotrophic Lateral Sclerosis, Motor Neuron Disease, incidence

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive motor neuron disease and its etiology is attributed to interactions between genetic and environmental factors over time.[1]

The disease is associated with a poor prognosis and high lethality; its average survival time after diagnosis is approximately 24 to 36 months.[2,3] Prospective epidemiological studies based on case records are considered ideal, but are difficult to perform due to the delays and high costs necessary to confirm the diagnosis, which leads researchers to use mortality data to indirectly estimate the incidence of the disease.[4]

Most epidemiological studies have been performed in Europe, where the incidence of ALS varies between 1·2 to 4·0 per 100,000 person-years, and the incidence is higher in males than in females. The age of onset varies from 62·8 to 67·9 years, and its incidence decreases significantly after the age of 80 years.[5] An epidemiological study in Cuba[6] based on mortality data showed a reduction in the frequency of the disease in the mixed populations compared to the Caucasian population. In other continents, particularly South America, there are few studies of ALS based on case records[7,8] and mortality,[9] and they did not address the racial aspect of this disease.

Epidemiological studies may suggest factors related to the etiology of ALS, especially those that examined a prospective registry of incident cases. However, we observed a lack of these studies in Latin America, where they are rare and often unreliable because there is a delay in establishing a disease diagnosis, difficulty of accessing medical records and the lack of a national registration database in most countries. However, Brazil is a country of continental dimensions and great ethnic variability, which justifies conducting an epidemiological study on the incidence of ALS within its borders, and the influence of ethnic and demographic factors on this disease.

Materials and Methods

A retrospective epidemiological study based on secondary observational mortality data obtained from the SIM / DATASUS system (Mortality Information System / Information Technology Department of the Brazilian National Health System) was collected in June 2015. The SIM / DATASUS system is based on Death Certificates (DCs), which are compulsory and validated by the Ministry of Health of Brazil.

The data were collected and analyzed using TABWIN. The data surveyed listed International Classification of Disease 10 (ICD 10) G12.2 - Motor Neuron Disease as the underlying cause of death as well as a secondary cause of death. Data were analyzed for frequency of sex, age and race/color.

The death records in 2,124 cities of Brazil who died from Motor Neuron Diseases between January 2004 to December 2013 were examined. Census data from 2010 and annual projections based on the 2004 Census performed by the Brazilian Institute of Geography and Statistics (IBGE)[10] were used to adjust the calculations for the racial proportions and odds ratio. It was reported crude and adjusted incidence rates over 20 and 45 years old. However, only the incidence rates over 20 years old were used in comparison to the other countries and between races. In this study, race was defined by the IBGE based on self-determination and attested by the Census[10,11]. The attending physician attested the race and cause of death reported in the DCs.

The adjusted incidence rates were considered in reference to the United States population, because Brazilian population over 45 years of age grew up to 26% in the last decade, showing a tendency of approaching the population structure of that country[12].

Data were allocated in Office Excel 2010 charts and analyzed using SPSS (Statistical Package for Social Sciences) version 19.0 and Epi Info 7.0. Confidence intervals were calculated assuming a Poisson distribution. The differences in mortality rates by ethnic group, educational level, marital status and sex were assessed using the chi-square test and a two-tailed Z test. Differences in age were analyzed using a two-tailed student t-test; $P<0.05$ indicates a significant difference. Odds ratios (ORs) were calculated for the ethnic groups with 95% confidence intervals.

The data used were considered as being part of the public domain, but the research was approved by the Research Local Ethics Committee (820.117/2014 protocol).

Results

In Brazil, from 2004 to 2013, 9,779,121 overall deaths were registered by SIM, but 839,445 (8.6%) had ill-defined causes.

A total of 8,942 death statements mentioned ICD 10 G12.2; it was the underlying cause of death in 8,152 statements and a secondary cause of death or a competitor in 790 statements. Regarding gender, 4,803 (53.7%) persons were male and 4,139 (46.3%) persons were female (male: female ratio of 1.3:1); this difference was not statistically significant ($p = 0.27$).

As shown in Figure 1, the average age at the time of death was 62.7±13.2 years. It was observed that women had a significantly lower average age of death than men ($p = .0001$) and that the average age of death in blacks and mixed-race persons was less than that of whites. The average age of death was 59.2±13 years in blacks ($p = 0.01$) and 59.2±13 years in mestizos compared to 63.8±12.8 years in Caucasians ($p = 0.02$). In Asians ($n = 63$), the average age of death was higher than that of the Caucasians (68.6±10.3 years) ($p = 0.003$). The lowest average age of death was observed in the indigenous population ($n = 10$) compared with the other races (48.5±12.3 years) ($p < 0.0001$).

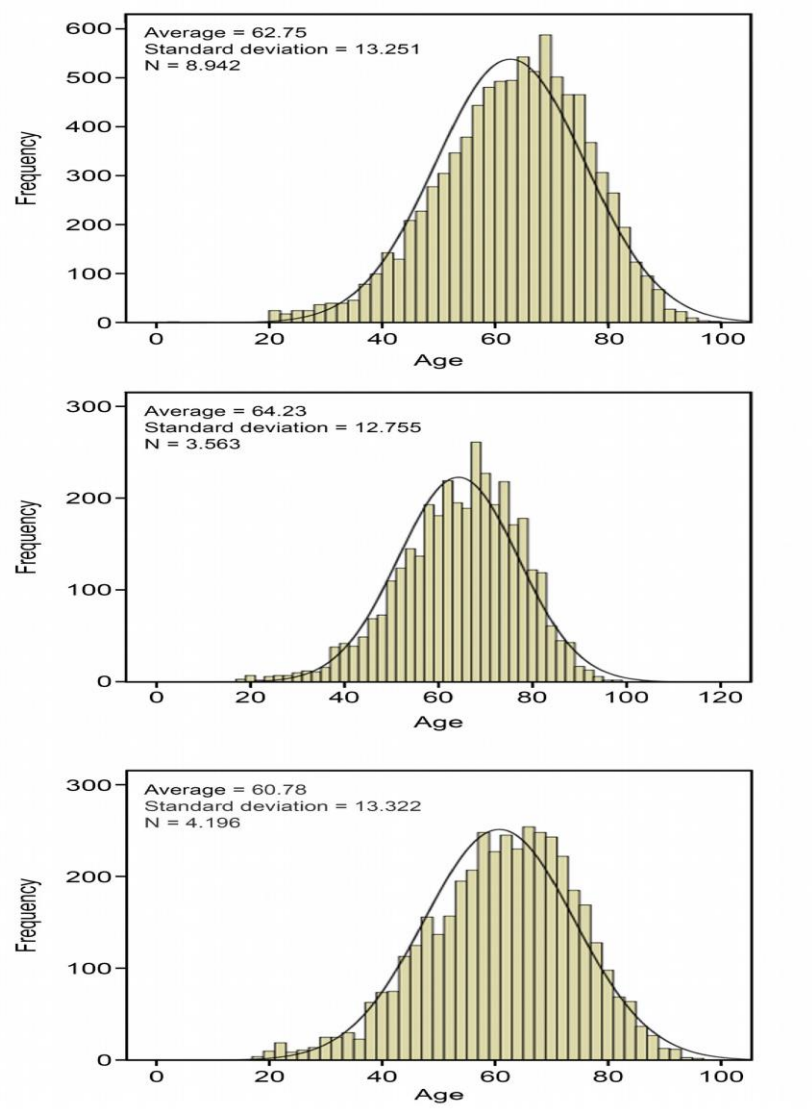

Figure 1: Age distribution and its average of amyotrophic lateral sclerosis in Brazil. First chart in both gender, second in females and third in males. Source: SIM. Period: 2004-2013.

Crude and adjusted annual incidences of ALS per 100,000 person-years, from 2004 to 2013, are shown in Table 1. According to the IBGE Census data of the Brazilian population,[10] the number of persons over 45 years of age was expected to increase approximately 26% during the study period, while the total population was expected to increase by 17%. The crude death rate from the disease ranged from 0.36 / 100,000 person-years in 2004, to 0.58 / 100,000 person-years in 2013. Adjusted mortality rates show that the incidence of ALS in persons over 20 years of age was 0.61 to 0.89 /100,000 person-years and that in persons above 45 years of age was 1.77 to 2.3 /100,000 person-years.

Table1: Crude and adjusted annual incidence of amyotrophic lateral sclerosis per 100,000, from 2004 to 2013, Brazil

Year	Male	%	Fem	%	Total	Crude incidence	Incidence >20 years	Incidence 45-80 years
2004	362	53.3	306	46.7	668	0.36	0.61	1.77
2005	364	53.1	316	46.9	680	0.37	0.6	1.72
2006	400	52.2	352	47.8	752	0.37	0.66	1.86
2007	398	51.6	372	48.4	770	0.41	0.64	1.81
2008	477	58.2	343	41.8	820	0.43	0.67	1.77
2009	506	53.3	444	46.7	950	0.49	0.76	2.09
2010	490	54.2	414	45.8	904	0.46	0.71	1.92
2011	594	55.5	476	44.5	1,070	0.54	0.82	2.2
2012	603	53	535	47	1,138	0.6	0.86	2.27
2013	615	51.7	575	48.3	1,190	0.58	0.89	2.3
Total	4,809	53.7	4,133	46.3	8,942			

By examining the distribution of ALS by race, 73.4% of deaths occurred in Caucasians, most of whom lived in the South and Southeast regions of the country, which are the most populous regions. Of all the ALS deaths, mixed people accounted for 16.8%, the black race accounted for 3.8%, Asians accounted for 0.8% and Indians accounted for 0.1%. In 455 people, the race was undefined (5.1%).

Odds ratio and total relative risk of ALS and proportion of deaths from motor neuron disease with respect to ethnic groups in the Brazilian regions are shown in Table 2.

Table 2: Odds ratio (OR) and total relative risk (RR) of amyotrophic lateral sclerosis, and proportion of deaths from motor neuron disease with respect to races in the Brazilian regions, from 2004 to 2013, Brazil

Region	Caucasian	Black	Asian	Mestizo	Indian	% (*)
	OR [95%CI]	OR [95%CI]	OR [95%CI]	OR [95%CI]	OR [95%CI]	
Northern	2.26 [1.71-2.98]	0.18 [0.05-0.56]	0.40 [0.05-2.90]	0.55 [0.42-0.72]	0.32 [0.04-2.32]	2.6
Northeast	2.41 [2.13-2.71]	0.34 [0.25-0.47]	0.15 [0.03-0.60]	0.42 [0.37-0.47]	0.51 [0.12-2.04]	13.7
Midwest	2.13 [1.74-2.61]	0.38 [0.20-0.71]	0.32 [0.08-1.29]	0.45 [0.36-0.56]	0.31 [0.04-2.25]	8.1
Southeast	2.56 [2.39-2.75]	0.49 [0.42-0.57]	0.69 [0.50-0.95]	0.27 [0.25-0.30]	0.34 [0.08-1.37]	55.7
Southern	2.65 [2.22-3.16]	0.49 [0.34-0.70]	0.71 [0.35-1.42]	0.17 [0.13-0.23]	-	18.8
Brasilia	2.85 [2.04-3.07]	0.28 [0.10-0.76]	-	0.44 [0.31-0.62]	-	1.1
Total (RR)	2,92 [2,78-3,07]	0,04 [0,03-0,04]	0,05 [0,04-0,07]	0,27 [0,26-0,29]	0,02 [0,01-0,04]	100

(*) Proportion of deaths from motor neuron disease in the Brazilian regions;

Odds ratio reference categories: general population above 20 years (2010 Census) [10]

We obtained odds ratios by assessing the proportions of the different races based on the 2010 Census (IBGE).[10] In all regions, the odds ratio was higher than 2 (2.92, 95% CI: 2.78 to 3.07), showing that the odds ratio of death due to ALS is almost three or 292% higher in Caucasians than in all other races. The odds ratio of the indigenous race was the lowest: 0.02 (95% CI: 0.01-0.04). The proportion of deaths from motor neuron disease was higher in the Southeast (55.7%) and Southern (18.8%) regions.

Figure 2 correlates the geographical distribution to the race distribution in Brazil and shows that the highest concentration of cases occurs in areas with a high population density and a higher proportion of white race persons. It also presented in states of the North, Northeast and Midwest, where there are predominantly black individuals even though the disease primarily affects the white race.

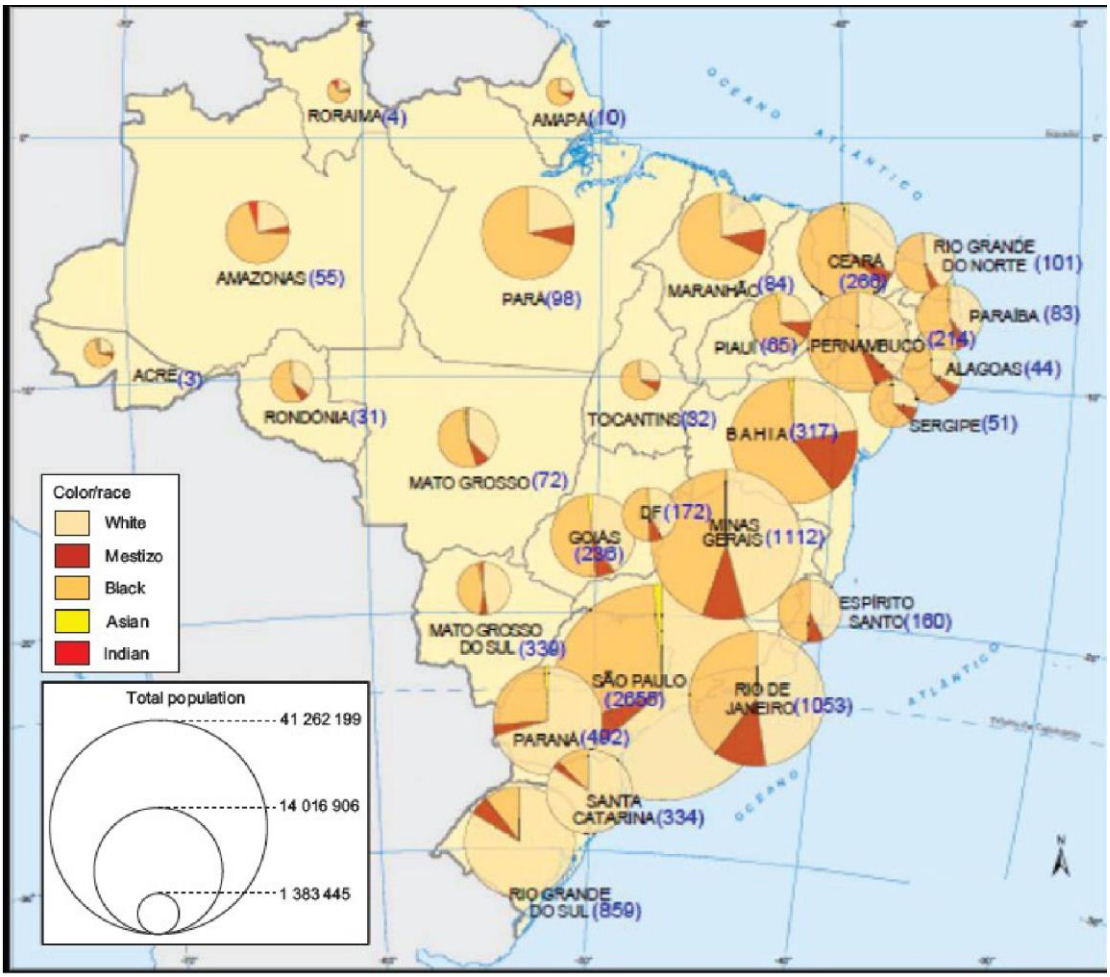

Figure 2 – Absolute distribution of cases of amyotrophic lateral sclerosis and its correlation with the proportion of the races in each Brazilian state. Period: 2004-2013. Source: 2010 IBGE Census and Mortality Information System (SIM).

In Table 3, the basic cause of death of patients with motor neuron disease is shown. In 8,149 persons (91%), the underlying cause of death was ALS or its complications, such as respiratory failure, pneumonia and septicemia. In 790 statements, other underlying causes of death were reported, and there was a predominance of vascular disease accounting for 511 deaths (64.7%). In deaths due to external causes, no suicides were noted.

Table 3: Basic causes of death in patients with motor neuron disease, from 2004 to 2013, Brazil

BASIC CAUSE OF DEATH	Frequency	%
G122- ALS and its complications	8,149	91.1
Other basic causes of death	790	8.9
I64- Stroke	283	35.8
I21-Myocardial infarction	113	14.3
N - Kidney disease	76	9.6
C - Malignancy	60	7.6
K- Digestive bleeding	52	6.6
I10- Hypertension	51	6.4
I67- Cerebral aneurism	48	6.1
E- Diabetes Mellitus	41	5.2
B - Infections	21	2.6
I802- Pulmonary thromboembolism	11	1.4
Y- External cause – poisoning/intoxication	11	1.4
W- External cause - fall	8	1
L - Skin Diseases	5	0.6
I42- Cardiomyopathies	3	0.4
O - Pregnancy, Delivery and puerperium	2	2.8
I330 - Bacterial endocarditis	1	0.1
I47 - Cardiac arrhythmia	1	0.1
Q- Congenital Malformations	1	0.1
T- Polytrauma	1	0.1
X- External cause – fire gun	1	0.1
Total	**8,942**	**100**

Discussion

The accuracy of the study can be estimated in 76% of the Brazilian regions. A sample of case records over a three-year period from a tertiary Neuromuscular Reference Center in which 145 patients were compared to 99 DCs in the Federal

District reported a sensitivity of 94% and a specificity of 99%. Additionally, in these ten years, only 8.6% of the overall DCs had ill-defined causes.

The most recent studies on the incidence of ALS in Europe estimate the incidence of ALS from 1·9 to 2·9 per 100,000 person-years, depending on the geographical location.[5,13-16]

An increased adjusted incidence rate was observed over a decade, which ranged from 1.77 to 2.3 for every 100,000 person-years in people over 45 years of age. This increase possibly occurred due to the aging of the population, as the number of people over 45 years of age increased by 26% over the study period according to IBGE data.[10,11] Additionally, there was improvement in the socio-economic indicators in Brazil, which increased access to health services and diagnosis.

An American study[16] between 2004 and 2007 reported a mortality rate of 2·9 per 100,000 person-years.

Studies in Hispanic, Latin American and Asian populations showed a lower incidence of the disease than in the present study.[6,8,9,18-20] A systematic review of 61 articles[18] found a uniform incidence of ALS in the Caucasian populations of Europe and North America, but a lower incidence in black and Asian populations; however, the quality levels of these studies are considered low. Several of these studies were performed in individuals of African descent in Europe,[21] and an estimated gross incidence of approximately 0.87 / 100,000 person-years and an age adjusted incidence of 4.6 / 100,000 person-years were found.

Table 4 summarizes several incidence studies of ALS involving non-Caucasian populations. Such studies, as well as the current study, tend to value the incidence of ALS in persons over 45 years of age because the African population is younger than the European population. As Kazamel[22] also noted, the present study similarly observed an average age of death that was lower in blacks than in whites.

Table 4: Incidence of amyotrophic lateral sclerosis in Asia and Americas. * :/100,000 person-years; DC: Death Certificates; P: prospective; R: retrospective.

Author (ref)	Period	N	Country	Incidence*	Age (SD)	Case records	DC	P/R
Zaldivar (6)	2001-2006	432	Cuba	0.67	63.7		X	R
Dietrich-Neto(7)	1998	443	Brazil	0.3-0.5	52 (13)	X		R
Vázquez(8)	2002-2003	89	Uruguay	1·4	58·7	X		P
Valenzuela(9)	1997-2010	1,671	Chile	1·13	-		X	R
Moriwaka(17)	1980-1989	389	Japan	0.86	58	X		R
Fong(18)	1989-1992	84	China/HongKong	0.31	55	X	X	R
Kazamel(20)	1998-2011	236	African American	-	59·2 (13)	X		R
This Study	2004-2013	8,942	Brazil	0·61 - 0·89	62.7(13.2)		X	R

According to the ALS incidence rates reported by Cronin,[18] a prospective study in Japan,[19] and a retrospective study in China and Hong Kong,[20] the ALS incidence rates are lower in Asian populations than in the European population. In the present study, it was observed that ALS is quite rare in Asians, and that the average age at death was higher in Asians compared to Caucasians.

Lower incidence rates have also been reported in Latin American populations, even considering the migration of European peoples to these countries. A prospective study conducted in Uruguay[8] and a retrospective mortality data-based study in Chile[9] have found ALS incidence rates of 1.13 to 1.4 / 100,000 person-years and in a previous retrospective study with patient records in Brazil[7], the incidence rates ranged from 0·5 to 0·9 / 100,000 person-years.

An American study[17] observed a lower average age of onset in males compared to females, which was also observed in other retrospective studies in Brazil[7] and Chile[9], but not in the present study.

A previous study in Cuba[6] assessed the ethnical aspects in ALS mortality, showing a smaller incidence in the mixed population than in Caucasians and blacks. However, it is difficult to distinguish mestizo from Hispanic persons in Latin America because the area has a mixture of races.

Figure 2 shows the racial distribution of the disease in the country. In the southern region of Brazil, where the relative risk of dying from ALS in Caucasians is higher (2·65), it is estimated that 64·5% of the resident population is descended from Germans and Italians. In the southeast region (RR=2·56), 44·3% of residents are descendants of Italian, Spanish and Portuguese persons. In the northern region, there remains a higher incidence in Caucasians, although 38% of the population are descendants of indigenous people.[10,23]

Compared to other studies of ALS in America,[6,17] we observed a lower overall age of incidence of death due to ALS in mixed, Indian and black populations ($p<0·0001$). However, the lowest average age of death was observed in native Indians compared to the other races (48.5 ± 12.3 years) ($p <0.0001$).

The lower average age of the disease in non-Caucasian persons may be associated with new genetic mutations or a new susceptibility to environmental factors that remains unclear. Recent studies[24-26] have shown that the repeat expansion in C9orf72 hexanucleotide was found in approximately 6% of sporadic ALS and 44% of familiar ALS patients of European ancestry; several of them experienced a younger age of onset, shorter disease duration and higher frequency of Frontotemporal Dementia and relation to different ethnic populations[26].

No other studies have been conducted on ALS in populations of native Indians. In the present study, only a small number of native Indians were included (N=10), which was possibly due to the mixture of races in Brazil and poor access to health care services; furthermore, ALS may have been underreported in native Indians.

In diseases with a high mortality and short duration such as ALS, studies based on mortality rates can estimate the actual incidence of the disease, but they do have the limitation of being retrospective. Several authors[16,27] suggest that the best ALS incidence studies are those that conjugate multiple data sources. Previous epidemiologic studies[16,27] have shown that incidence varies according to their design; the incidence is generally higher in prospective studies. The prospective database registry is the most accurate method in terms of the identification of new cases, but retrospective and administrative studies have the possibility of accessing numerous data sources, although they also have the possibility of using inadequate recorded information.[27]

Additionally, administrative data has the advantage of investigating changes over a period of time within the same country.[4]

In conclusion, the incidence of ALS in Brazil is near that of Latin America countries, with clear predominance in Caucasians but with a lower age of onset. These data may suggest the presence of new genetic mutations or environmental factors.

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References

1 Al-Chalabi A, Hardiman O. The epidemiology of ALS: a conspiracy of genes, environment and time. Nat Rev Neurol 2013; 9: 617–28.

2 Turner MR, Bowser R, Bruijn L, Dupuis L, Ludolph A, McGrath M, et al. Mechanisms, models and biomarkers in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2013; 14(Suppl 1):19–32.

3 Forbes RB, Colville S, Cran GW, Swingler RJ. Unexpected decline in survival from amyotrophic lateral sclerosis/motor neurone disease. J Neurol Neurosurg Psychiatry 2004; 75: 1753–5.

4 Marin B, Couratier P, Preux P-M, Logroscino G. Can mortality data be used to estimate amyotrophic lateral sclerosis incidence? Neuroepidemiology 2011; 36: 29–38.

5 Logroscino G, Traynor BJ, Hardiman O, Chiò A, Mitchell D, Swingler RJ, et al; EURALS.Incidence of amyotrophic lateral sclerosis in Europe. J Neurol Neurosurg Psychiatry 2010;81(4):385-90.

6 Zaldivar T, Gutierrez J, Lara G, Carbonara M, Logroscino G, Hardiman O. Reduced frequency of ALS in an ethnically mixed population: a population-based mortality study. Neurology 2009; 72: 1640–5.

7 Dietrich-Neto F, Callegaro D, Dias-Tosta E, Silva HA, Ferraz ME, Lima JM, et al. Amyotrophic lateral sclerosis in Brazil: 1998 national survey. Arq Neuropsiquiatr 2000; 58: 607–15.

8 Vázquez MC, Ketzoián C, Legnani C, Rega I, Sánchez N, Perna A, et al. Incidence and prevalence of amyotrophic lateral sclerosis in Uruguay: a population-based study. Neuroepidemiology 2008; 30: 105–11.

9 Valenzuela D, Zitko P, Lillo P. Amyotrophic lateral sclerosis mortality rates in Chile: a population based study (1994–2010). Amyotroph Lateral Scler Frontotemporal Degener 2015.

10 Instituto Brasileiro de Geografia e Estatística (IBGE) (2014). Censo Demográfico. Available at: http://www.ibge.gov.br/estadosat/perfil.php?sigla=DF Last Accessed(07-10-2015).

11 Belchior M. Características Étnico-Raciais da População. Um Estudo das Categorias da Classificação de Cor ou Raça. Rio de Janeiro: Instituto Brasileiro de Geografia e Estatística – IBGE, 2008.

12 United States Census Bureau. 2010. In: http://www.census.gov/prod/cen2010/briefs/c2010br-03.pdf (accessed in 11/17/15).

13 Fang F, Valdimarsdóttir U, Bellocco R, Ronnevi LO, Sparén P, Fall K, et al. Amyotrophic lateral sclerosis in Sweden, 1991-2005. Arch Neurol 2009; 66: 515–9

14 Uenal H, Rosenbohm A, Kufeldt J, Weydt P, Goder K, Ludolph A, et al. Incidence and geographical variation of amyotrophic lateral sclerosis (ALS) in southern Germany – completeness of the ALS Registry Swabia. PLoS One 2014; 9: e93932.

15 Chiò A, Mora G, Calvo A, Mazzini L, Bottacchi E, Mutani R. Epidemiology of ALS in Italy: A 10-year prospective population-based study. Neurology 2009; 72: 725–31.

16 Chiò A, Logroscino G, Traynor BJ, Collins J, Simeone JC, Goldstein LA, et al. Global epidemiology of amyotrophic lateral sclerosis: a systematic review of the published literature. Neuroepidemiology 2013; 41: 118–30.

17 Caller TA, Andrews A, Field NC, Henegan PL, Stommel EW. The epidemiology of amyotrophic lateral sclerosis in New Hampshire, USA, 2004-2007. Neurodegener Dis 2015.

18 Cronin S, Hardiman O, Traynor BJ. Ethnic variation in the incidence of ALS: a systematic review. Neurology 2007; 68: 1002–7.

19 Moriwaka F, Okumura H, Tashiro K, Hamada T, Matsumoto a., Matsumoto H, et al. Motor neuron disease and past poliomyelitis. J Neurol 1993;240(1):13–6.

20 Fong KY, Yu YL, Chan YW, Kay R, Chan J, Yang Z, et al. Motor neuron disease in Hong Kong Chinese: epidemiology and clinical picture. Neuroepidemiology 1996;15: 239–245.

21 Marin B, Kacem I, Diagana M, Boulesteix M, Gouider R, Preux PM, et al. Juvenile and adult-onset ALS/MND among Africans: incidence, phenotype, survival: a review. Amyotroph Lateral Scler 2012; 13: 276–83.

22 Kazamel M, Cutter G, Claussen G, Alsharabati M, Oh SJ, Lu L, et al. Epidemiological features of amyotrophic lateral sclerosis in a large clinic-based African American population. Amyotroph Lateral Scler Frontotemporal Degener 2013; 14: 334–7.

23 Seyferth G. Colonização, imigração e a questão racial no Brasil. Revista USP 2002; 53: 117–49.

24 Cooper-Knock J, Hewitt C, Highley JR, Brockington A,Milano A,Man S, et al. Clinico-pathological features in amyotrophic lateral sclerosis with expansions in C9ORF72. Brain 2012;135:751–764.

25 Byrne S,Elamin M, Bede P, Shatunov A, Walsh C, Corr B, et al. Cognitive and clinical characteristics of patients with amyotrophic lateral sclerosis carrying a C9orf72 repeat expansion:a population-based cohort study. Lancet Neurol 2012;11:232–40.

26 García-Redondo A, Dols-Icardo O, Rojas-García R, Esteban-Pérez J, Cordero-Vázquez P, Muñoz-Blanco JL, et al. Analysis of the C9orf72 Gene in Patients with Amyotrophic Lateral Sclerosis in Spain and Different Populations Worldwide. Hum Mutat 2013;34(1):79–82.

27 Chancellor AM, Warlow CP. Adult onset motor neuron disease: worldwide mortality, incidence and distribution since 1950. J Neurol Neurosurg Psychiatry. 1992;55(12):1106-15.

ARTIGO 2 – ARTIGO ORIGINAL - EFFICACY AND EFFECTIVENESS OF DRUG TREATMENTS IN AMYOTROPHIC LATERAL SCLEROSIS: A SYSTEMATIC REVIEW WITH META-ANALYSIS

Versão em inglês

Efficacy and effectiveness of drug treatments in amyotrophic lateral sclerosis: a systematic review with meta-analysis, aceito pelo periódico científico African Journal of Pharmacology and Pharmacy, 2016, in press.

EFFICACY AND EFFECTIVENESS OF DRUG TREATMENTS IN AMYOTROPHIC LATERAL SCLEROSIS: A SYSTEMATIC REVIEW WITH META-ANALYSIS

Abstract

The results of published studies with various neuroprotectors seeking to preserve motor neuron function and improve survival in amyotrophic lateral sclerosis patients have poor evidence in humans, although there are several studies in animal models with positive results. A systematic review and meta-analysis of studies on drug treatment options and survival times in animal models and patients with amyotrophic lateral sclerosis from March 2009 to March 2015 was conducted. Four hundred eighty-nine (489) articles were found, and from these, we selected 30 preclinical 'in vivo' studies, 18 randomized controlled trials, and four systematic reviews. A meta-analysis confirmed the effectiveness of various drugs in improving the life span in preclinical trials, in particular Resveratrol, which had a mean difference of 10.8 days (95% CI 9.57 to 12.02), whereas no drug showed efficacy in clinical trials. The positive results of preclinical studies should be interpreted with caution because there is a mismatch between those results and the negative results in clinical trials.

KEY WORDS: ALS, Amyotrophic Lateral Sclerosis, motor neuron disease, drug, treatment.

Introduction

Amyotrophic Lateral Sclerosis (ALS) is the most common motor neuron disease. It is sporadic and characterized by the progressive degeneration of both upper and lower motor neurons in the brain, brainstem and spinal cord [1]. Its incidence may vary between 1.2 and 4.0 per 100,000 individuals per year [3-5]. It is more predominant in males (3.0 per 100,000 individuals per year, 95% CI 2.8 - 3.3) than females (2.4 per 100,000 individuals per year, 95% CI 2.2 - 2.6). Its onset occurs between 58 and 63 years of age, and its incidence decreases considerably after the age of 80 [6].

The few advances in knowledge about the mechanisms of development of ALS are primarily due to the understanding of familial forms, which correspond to 5 to 10% of cases [7,8]. The pathophysiology of the disease is still poorly understood, but it is believed that the disease's injury mechanisms involve both glial cells and neurons [8]. The main known mechanisms are oxidative stress with damage to RNA species, mitochondrial dysfunction, impairment of axonal transport, glutamate excitotoxicity as a mechanism contributing to motor neuron injury, protein aggregation, endoplasmic reticulum stress, abnormal RNA processing, neuroinflammation, and excitability of peripheral axons [9].

Death occurs on average between 2 and 4 years after onset due to respiratory complications, but patients given multidisciplinary care and undergoing enteral nutrition and noninvasive ventilation have an improved quality of life [10]. A single drug, Riluzole, cleared in 1996 by the Food and Drug Administration (FDA), slows the progression of the disease by approximately 2 to 4 months, but it does not prevent the disease's fatal outcome [10].

Recently, the results of studies on the use of various neuroprotectors seeking to preserve motor neuron function and reduce the toxic levels of glutamate have been considered without any evidence in humans, although their use has been fairly efficacious in experimental animal models [11].

Considering the need to identify new alternatives to treat ALS, the aim of this study was to conduct a systematic review to access the efficacy and effectiveness of

drug treatments and procedures in clinical and preclinical trials described in the literature

Material and methods

Strategies to search for and select studies

In May 2015, we investigated primary preclinical in vivo studies, clinical trials and systematic reviews with subsequent meta-analyses published between March 2009 and March 2015 in the following electronic databases: Medline, Embase, Cochrane Library and Lilacs. The following Medical Subject Headings (MeSH) and Health Science Descriptors (HScDe) were used: ‘Amyotrophic Lateral Sclerosis' AND 'Motor Neuron Disease' AND 'Treatment' AND ‘drug’ AND ‘survival’.

Two authors independently evaluated the titles and abstracts of all studies identified in the search in the aforementioned electronic databases based on the descriptors.

The following inclusion criteria were adopted:

i) In clinical studies, including prospective randomized trials and meta-analytical systematic reviews, patients diagnosed with a motor neuron disease by means of anamnesis and electromyography according to the El Escorial and Awaji criteria [12];

ii) in preclinical studies, ‘in vivo’ studies with assessment of survival compared to control group and studies of treatment after the onset of weakness; and

iii) studies based on the use of any drug to increase survival time compared to placebo or other treatments used by the control group.

The exclusion criteria were studies in which participants presented with respiratory failure or spinal muscular atrophy; studies in which the treatment was administered only prior to disease; or narrative reviews, letters, editorials, case reports, duplicate publications or those without objective data to be evaluated.

Articles published in all languages were included.

The studies that met the inclusion criteria were obtained in full. References were also considered, and communication with the authors was established in cases of doubt.

Disagreements were resolved by consensus, and when this was impossible, there was subsequent analysis by two additional reviewers.

Data Extraction

Data were obtained from each study using a review form with the following content: author, place where the work was conducted, year of publication, intervention, study design, number of participants, age, analysis by intention to treat, declaration of conflict of interest, evaluation by a research ethics committee, and animal species used if the study was preclinical. The following outcomes were assessed:

i) comparison between different treatments;

ii) analysis of mean survival and absolute days of survival;

iii) mean duration of the disease until the start of intervention;

iv) alteration of the Revised ALS Functional Rating Scale - ALSFRS-R [13] between the start and end of the study; and

v) incidence of reactions and adverse effects of proposed treatments.

Assessing the quality of the studies

Quality was assessed by two independent reviewers, and in cases of disagreement, the situation was resolved by consensus among all reviewers. The GRADE (Grading of Recommendations, Assessment, Development and Evaluation) model [14] was used for primary studies and/or the AMSTAR (Assessment of Multiple Systematic Reviews) criteria were used for systematic reviews [15]. The following data were observed in the studies:

Methods: research question, treatment sequence, allocation confidentiality, post-intervention follow-up, blinded outcome assessment, primary clinical outcome measures, location of study, protection against contamination, calculation of statistical power, sample representativeness, conflict of interest, and ethical aspects.

Participants: inclusion criteria, exclusion criteria, age, gender, disease severity, and disease variants.

Interventions: medications and doses or procedures, follow-up time, and method for monitoring disease progression.

Outcomes assessed in the review: disease duration before intervention, survival time, and/or alteration of the ALSFRS-R.

The results of the primary outcomes were obtained based on the intention-to-treat principle: for each dichotomous outcome, the number of events was divided by the total number of participants in each group; for continuous outcomes, the following variables were calculated: mean, standard deviation and number of participants in each group. Data from work published more than once were obtained from the more thorough study.

The reviewers rated each primary study according to the overall quality of evidence: A-high; B-moderate; C-low and D-very low, assigning scores of 1-5 according to the number of biases. The total AMSTAR score was used for systematic reviews.

For analytical purposes, the studies were grouped as i) interventions in animal models and ii) clinical studies.

Statistical Analysis

Statistical analysis was performed in preclinical trials using RevMan software, version 5.3. All p values < 0.05 were considered to be statistically significant.

For dichotomous variables, such as patients who were alive at the end of the period analyzed, the absolute risk reduction method with a confidence interval of 95% (random effects model) was used.

For continuous variables, such as animal survival in days, the weighted mean difference (random effects model) was calculated based on the DerSimonian and Laird method, with a corresponding confidence interval of 95%.

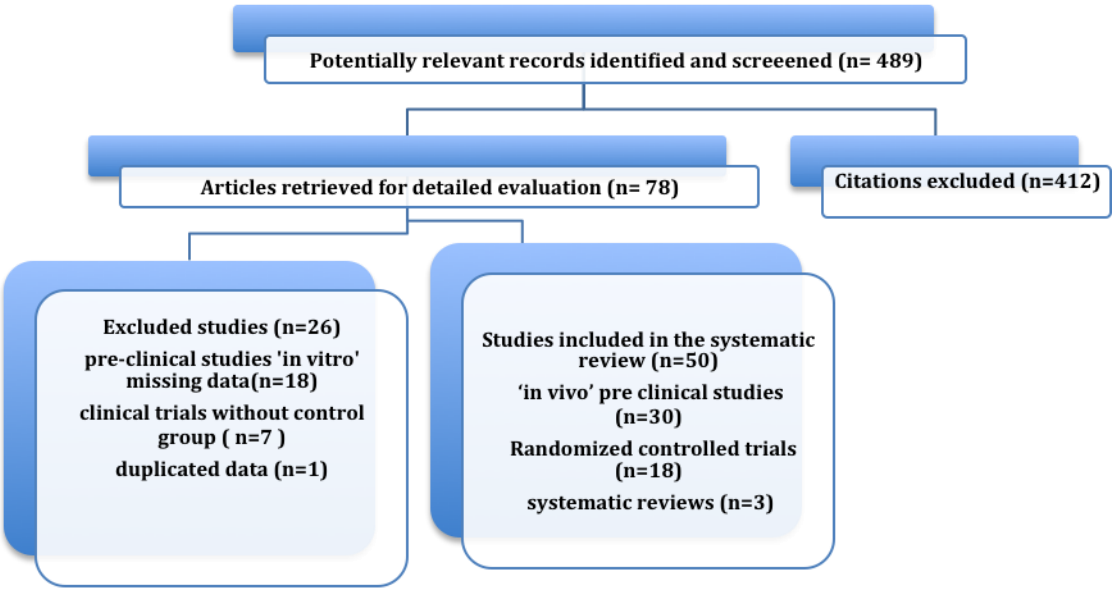
To evaluate heterogeneity among studies, a heterogeneity test was performed by calculating both the Q-test of heterogeneity and the I^2 test of inconsistency. Heterogeneity was considered significant when $p < 0.10$. In addition, a sensitivity

analysis was conducted using the funnel plot to quantify the presence of publication bias.

Results

Initially, 489 articles were obtained. Based on their abstracts, the following were selected: 23 prospective clinical trials, 48 preclinical trials, and 3 systematic reviews.

Figure 1 – Systematic Review Flowchart



After each original document was reviewed and data were obtained, 18 preclinical trials were excluded; of these, 2 were 'in vitro' [16-17], 6 did not include a quantitative evaluation of survival [18-23], one had duplicate data [24], 7 only reported survival ratios and proportions [25-31], and 2 involved interventions that occurred only prior to disease onset [32-33]. The authors of 16 studies were contacted via email for completion of missing data, without any success.

Five clinical trials were excluded. One contained duplicate data [34], and 4 were not controlled [35-38]. Finally, 30 preclinical 'in vivo' studies, 18 randomized and controlled clinical trials, and 3 systematic reviews of the Cochrane Collaboration were included. A flowchart illustrates the selection process adopted in the systematic review (Figure 1).

Clinical studies

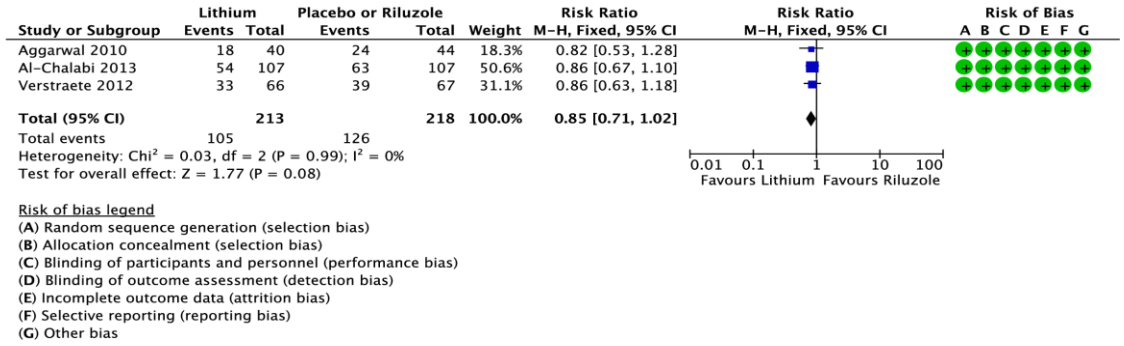
Table 1 lists the selected primary randomized clinical trials and systematic reviews of the Cochrane Collaboration, classifying them according to year, intervention, mean disease duration at baseline, follow-up time, number of participants, completion and quality.

INTERVENTION (REF)	N	DISEASE(MONTHS)	FOLLOW-UP(MONTHS)	SURVIVAL	ALSFRS-R SLOPE	GRADE
TALAMPANEL 50 MG (47)	59	12	9	NOT RELATED	E=(-7,1);C=(-10,2)(P=0,081)	1B
URSODEOXYCHOLIC ACID(48)	64	12.5	8	NOT RELATED	E=1,04±0,28; C= 1,61±0,28 (P= 0.16)	1B
DEXPRAMIPEXOLE 300MG(39)	102	15.4	9	68% HR: 0,32(95%CI 0,086 to 1,18) log rank p=0,07	31% REDUCTION:0,40 (95%: CI 0,38 to 1,18)	1A
DEXPRAMIPEXOLE 300 MG(40)	943	12	12	HR 1-03 (95%CI 0-75 to 1-43] p=0-84	E=13-34;C=-13-42(p=0-90)	1A
COQ10 1.800 OU 2.700 MG (49)	185	27	18	NOT RELATED	E=8.4± 7.3;C= 9,0±8,2(P=)	1A
BCAA/THREONIN (50)	476/86	NOT RELATED	6	BCAA HR 1,57 P=0,209 (95%CI 0,78 to 3,19); L-TR HR=2,76 P=0,151 (95%CI :0,71 to 9,27)	NOT RELATED	31*
CILIARY NEUROTROPHIC FACTOR –CTNF (51)	1300	NOT RELATED	6	RR 1.07(95% CI 0.81 to 1.41)	RR 1.07(95% CI 0.81 to 1.41)(P= 0.85)	31*
CREATINE (52)	386	NOT RELATED	18	DOSING ESCALATION: 83%, 96% and 96%	DIFFERENCE IN SLOPE 0.09(P= 0,76)	30*
GROWTH HORMONE- 2UI (53)	40	15.6	18	RR: 1,03± 0,15	E=-45,2±6,1 C= 33,1±7,8(P=0,61)	1B
ACETYL-L-CARNITINE (54)	82	NOT RELATED	12	HR 0,72(95%CI 0,45 to 1,16) P=0,1804	MONTHLY E=-0,97± 1,09; C=1,60± 1,39	1A
CEFTRIAXONE 2G 4G IV/DAY (55)	66	18	3	NOT RELATED	E=36,8±6,0; C=35,2±5,7	1A
LITHIUM TG 0,4-0,8 mEq/l X STG 0,2-0,4 mEq/l(59)	117	24	15	NR; DID NOT DIFFER P= 0,94	TG=1,26±1,43; STG=1,15±1,03(P= 0,60)	1B
LITHIUM + RILUZOLE (58)	84	20.3	5.4	HR 1,13(95%CI=0,61 to 2,07)	0,15(95%CI -2,58 to 0,13) p=0.08	1A
LITHIUM + RILUZOLE (61)	214	20.5	18	HR 1,35(95%CI 0,90 A 2,02)	9,50(95% CI -10,31 to-8,70) slope 0,19(95% CI -1,28 to 0,90)	1A
LITHIUM X PLACEBO (60)	133	13	16	HR 1,03 (95%CI 0,66 A 1,63)	E= 40- 22; C= 40- 24 (P =0,74)	1A
G-CSF X PLACEBO(46)	39	22.4	12	NOT RELATED	E=-4,66±3,37; C= -6,56±5,3 (P=0.289)	1B
G-CSF (45)	10	13.2	0.9	NOT RELATED	E=35,3±9,4;)C=34,4± 8,2	1B
OLESOXIME (57)	512	17.5	18	E= 69.4%(95%CI 63.0 to 74.9); HR:1.21 (95% CI: 0.71-2.07, p = 0.48).	HR 0.997 (95%CI 0.958 to 1.04)(p= 0.87)	1A
PIOGLITAZONE (56)	219	18.9	15	C=67.5 %(95%CI 61.0 to 73.1)(P=0.71)	NOT RELATED	1A
LITHIUM + VALPROIC ACID (58)	49	46.5	17	HR 0.72± 0.6(12 months); 0.59± 0.07(16 months)(P= 0.016)	BETTER IN EXPERIMENTAL GROUP(p<0.05)	2B
CEFTRIAXONE 4 G IV X PLACEBO (59)	340	18	72	NO DIFFERENCE (P=0.5972)	HR 0.9 (95%CI 0.71 to 1.15) P= 0.4146	1A

Table 1- Selected clinical trials including ALS patients with randomic allocation, according to intervention, mean time of disease , follow-up time, outcomes and quality (GRADE/AMSTAR) 2009 - 2015. E: experimental group; C: control group; TG: therapeutic group; STG: subtherapeutic group.

Only quality A and B primary studies were selected. Great heterogeneity among the studies was observed. Heterogeneity in relation to the method, time of patient follow-up and intervention prevented us from performing a meta-analysis with respect to the proposed outcomes with the exception of three studies using Lithium, in which it was possible to conduct a meta-analysis based on the number of survivors at the end of 15 to 16 months (Figure 2).

Figure 2 - Forest plot - Use of Lithium versus Placebo in ALS patients. Risk ratio analysis related to the endpoint in 15 to 16 months of follow-up. Period: 2009 to 2015.



The mean disease duration at the time of randomization ranged from 12 to 72 months, with a weighted average of 16.2 months and a median of 15.6 months. The follow-up period after intervention ranged from 25 days to 18 months and was performed in a variable manner relative to the ALSFRS-R, survival, Forced Vital Capacity (FVC) and occurrence of adverse events.

Lithium, Dexpramipexole and G-CSF (Granulocyte-colony stimulating factor) had greater consideration in the review because they were the most studied and a larger number of patients were enrolled in the trials.

A phase II clinical trial using Dexpramipexole [39] in 102 patients conducted in two phases with duration of nine months and dose escalation moderately slowed disease progression and increased survival time (HR: 0.32 95% CI; 0.086 to 1.18). With the dosage increase to 300 mg/day, in relation to the ALSFRS-R, a reduced functional decline of 31% was observed in the first stage compared to placebo, particularly in the subdomain of the scale relative to fine motor control, where the difference was greater (-1.4 SD 0.30 versus -0.60 SD 0.24), favoring larger studies with the drug. However, a phase III, multicenter, placebo-controlled clinical trial of 943 patients involving a 12-month follow-up was conducted and found no changes in survival time or ALSFRS-R scores [40].

The use of Lithium associated with Riluzole in ALS patients was evaluated in four randomized clinical trials (RCTs) [41-44]. In 2010, a multicenter study was conducted on the use of Riluzole and Lithium combined [41] at a dose of 150-1050 mg per day in 84 patients while maintaining serum concentration between 0.4-0.8 meq/l. The hazard ratio for final outcome (a drop of 6 points in the ALSFRS-R or death) was 1.13 (95% CI; 0.61 to 2.07). Patients were monitored for 5.4 months, and the study was

interrupted because most patients in the experimental group presented with the final outcome. The mean difference in decline in the ALSFRS-R between the group that used lithium and the placebo group was 0.15 (95% CI; -0.43 to 0.73, p = 0.61).

In that same year, in the USA and Canada [42], the drug was administered in two dosages-subtherapeutic (serum concentration of 0.2-0.4 mEq/l) and therapeutic (serum concentration of 0.4-0.8 mEq/l)-to a group of 171 patients with a 15-month follow-up, but 85% had discontinued the drug by the end of this period due to adverse effects or lack of efficacy. No statistically significant difference was observed in the decline of the ALSFRS-R and FVC among the groups.

The results of two randomized trials using a similar method were published in the Netherlands in 2012 [43] and in Great Britain in 2013 [44]. Both studies evaluated Lithium and Riluzole versus placebo. The studies included 133 and 214 patients, respectively, with a 16- to 18-month follow-up. Hazard ratios (HR) of 1.13 (95% CI; 0.61 to 2.07) and 1.35 (95% CI; 0.90 to 2.02), respectively, was observed for survival, with no evidence of better performance of treated patients in relation to the ALSFRS-R or FVC.

It was conducted a meta-analysis assessing survival, including the number of patients who survived 15 to 16 months after treatment with Lithium at a variable dose with a serum level of 0.4-0.8 mEq/l, in the three studies that used Riluzole or a placebo as control [44,46,47]. The study that used subtherapeutic Lithium concentrations as a control was excluded. Two hundred thirty-one events were observed in 431 patients (Figure 2). The risk ratio obtained was 0.85 (95% CI; 0.71 to 1.02). The funnel graph showed that the studies presented similar findings.

A clinical trial conducted in Germany [45] used recombinant human Granulocyte-colony Stimulating Factor (G-CSF) to slow the progression of ALS symptoms in 10 patients at a dose of 10 mcg/kg/day versus placebo. Patients were monitored for 100 days and showed no differences in the percentage of decline in ALSFRS-R scores. The results of a previous pilot study conducted in Israel, in which half the suggested dose of the same drug was administered to 39 patients, suggested smaller declines in the ALSFRS-R variation (p: 0.289) and FVC (p: 0.854) in the experimental group [46]. The studies did not assess survival.

The remaining interventions evaluated in single studies [47-59] were not significantly successful in slowing disease progression or reducing ALSFRS-R variation compared to the control group.

Table 2 lists the adverse events (AEs) related to the use of drugs in clinical trials. It was observed that most of the studies reported weakness, gastrointestinal intolerance, and dizziness as adverse drug events, but they rarely reported severe events. Adverse events caused by Lithium are controversial because more adverse effects are known—126 total—but with little discontinuation except for a clinical trial that was interrupted due to an excessive number of adverse events [43].

AUTHOR(REF)	DRUG	ADVERSE EVENTS (AE)	N (EXPOSED)	N (AE)	N(DISCONTINUED)
NEFUSSY(46)	G-CSF	BONE AND MUSCLE PAIN AFTER THE INJECTIONS	19	2	0
PASCUZZI(47)	TALAMPANEL	DIZZINESS, DROWSINESS, ASTHENIA, DEPRESSION,	40	24	2
MIN(48)	ORAL SOLUBILIZED URSODEOXYCHOLIC ACID500MG	ABDOMINAL PAIN, ANOREXIA, DYSPHAGIA, DYSPEPSIA, NAUSEA, VOMITING	40	25	5
CUDKOWICZ(39,40)	DEXPRAMIPEXOLE 50 MG, 150 MG E 300 MG	DIZZINESS, HEADACHE, ADBOMINAL PAIN, ANOREXIA, DYSPHAGIA, DIARRHEA, NEUTROPENIA	123 + 474	NR + 459	5 +35
KAUFMANN(48)	COQ10 1.800 OU 2.700 MG	RESPIRATORY AND GASTROINTESTINAL EVENTS, FALL, PAIN, NAUSEA,.	110	63	0
PARTON(50)	AMINOACID - BCAA OR THREONIN	HEADACHE AND GASTROINTESTINAL UPSET, GOUT	90	0.58 X control	1.35 X CONTROL
BONGIOANNI(51)	CILIARY NEUROTROPHIC FACTOR(CNTF) -0,5 A 30 MCG/KG	WEIGHT LOSS, ANOREXIA, ASTHENIA, COUGH	914	RR 1.55	NR
PASTULA(52)	CREATINE - 5 A 10 G DIA VO OU PLACEBO	NO SIGNIFICANT	173	NR	NR
SACCÀ(53)			20	15	NR

	GROWTH HORMONE	INCREASE OF HEPATIC ENZYMES, JOINT SWELLING, HYPERTENSION, WEAKNESS , GLUCOSE INCR AND INCR LOCAL REACTIONS			
BEGHI(54)	ACETYL-L-CARNITINE 500 MG	MAI, PNEUMONIA, URINARY, DIZZINESS RETINAL HAEMORRHAGE, GASTRIC INTOLERANCE	42	27	4
BERRY(55)	CEFTRIAXONE 2G AND 4 G IV	PSEUDOMEMBRANOUS COLITIS, CHOLELITHIASIS, CATHETER RELATED	21	5	4
AGGARWAL(41)	LITHIUM CONC: 0.4-0.8 MEQ/L	FATIGUE, SEDATION, RAISED TSH, ANOREXIA , NAUSEA, MUSCLE WEAKNESS	40	20	3
UKMND –AlChalabi (44)	LITHIUM 295 MG CONC 0,4-0,8 MMOL/L	NAUSEA, VOMITING	107	61 HR=1.14	2
VERSTRAETE(43)	LITHIUM CONC 0,4-0,8 MMOL/L	NAUSEA, VOMITING, POLYDIPSIA	66	47	16
DUPUIS(56)	PIOGLITAZONE - 45 MG VO	DYSPHAGIA, DYSPNEA, DEPRESSION, OEDEMA, WEIGHT LOSS,	109	35	NR
DUNING(45)	G-CSF	LEUKOCYTOSIS, NERVOUS SYSTEM DISORDERS, SKIN, MUSCULOSKELETAL AND CONNECTIVE TISSUE	5	11	0
CHIÒ(42)	LITHIUM CONC 0,4-0,8 MMOL/L OR 0,2-0,4 MMOL/L	CARDIAC, CYSTITIS, DEEP VEIN TROMBOSIS, EDEMA, RETINAL, DEHYDRATION	171	38	117 (68.4%)

Table 2- Adverse events in clinical trials with ALS patients. 2009-2015. NR: Not Related

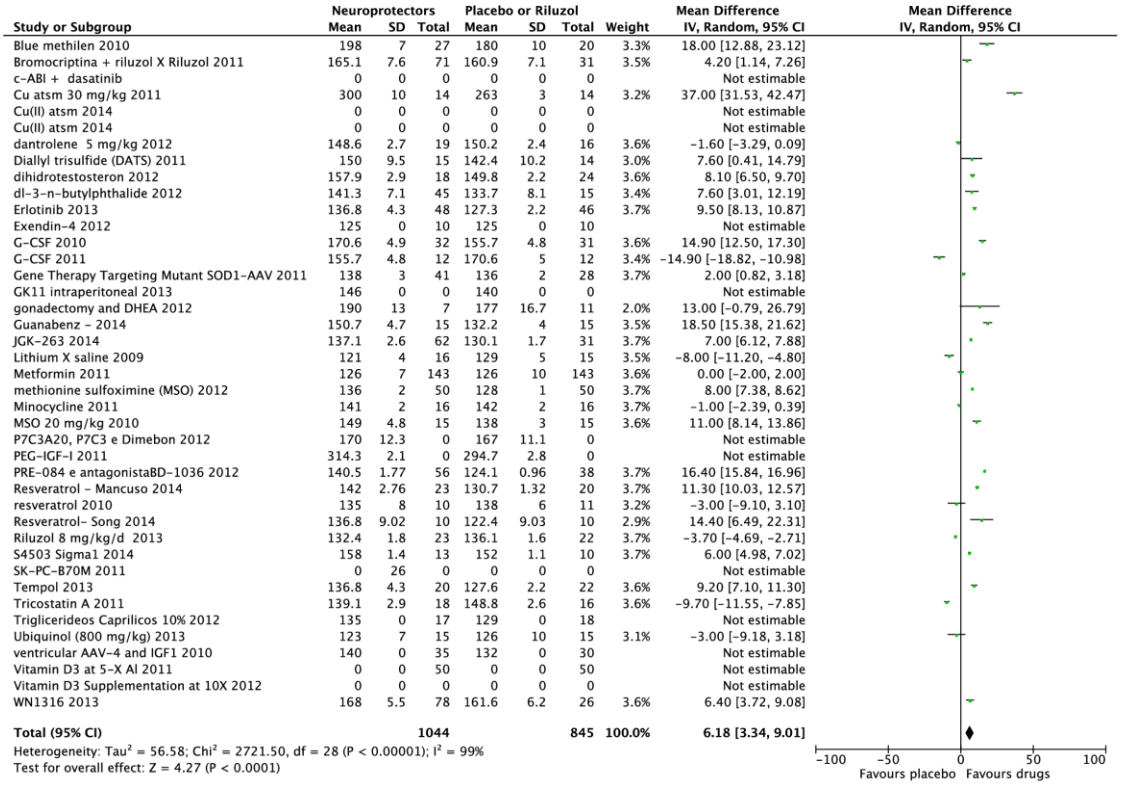
In summary, none of the drugs demonstrated unequivocal effectiveness in controlling the progression of the disease in humans.

Preclinical studies

Preclinical ‘in vivo’ trials were conducted in transgenic mice expressing human mutated superoxide dismutase 1 and G93A ($SOD1^{G93A}$) and present experimental ALS treatments using neuroprotective therapies. The studies were considered homogeneous with respect to the method and the evaluation of outcomes in animals, making the comparison by meta-analytical methods possible. All included studies analyzed survival by the Kaplan-Meier method with the log-rank test, but only those studies that included disease prior to treatment and evaluated the survival of animals with a mean in days and standard deviation were included in our meta-analysis. The authors of all the studies that did not include such data were contacted via email.

Figure 3 describes the global effect of various drugs on the survival of animal models with ALS compared to placebo by means of network meta-analysis conducted from 2009 to 2015. It was observed that there are several effective medications.

Figure 3 - Forest Plot – network meta-analysis including days of survival (life span) in pre clinical studies with drugs. Period: 2009 to 2015.



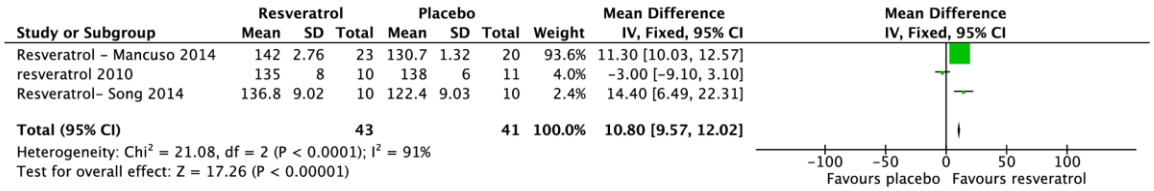
Studies on Vascular Endothelial Growth Factor (VEGF) [19], Olesoxime [21], Exendin-4 [23], and SK-PC-B70M [25] were excluded from the meta-analysis for not including results about survival, although they did report improvements in motor performance and a neuroprotective effect of motor neurons in the spinal cord.

A study that investigated the effects of Dasatinib at a dose of 25 mg/kg/day [60] reported improvement in animal survival associated with weight gain (log rank test, p <0.01). Studies on PEGylated insulin-like growth factor [24,61] showed prolonged survival (p <0 .05) associated with its use in the initial stage of the disease [61], while a study of Gacyclidine [62] showed a 4.3% increase in survival (p = 0.034).

It was also observed that dietary therapy with caprylic triglyceride [22] and vitamin D3 [29] improved motor performance without influencing animal survival.

The most effective drugs in prolonging survival are CuII(atsm) [63], dihydrotestosterone [64], Granulocyte Colony-Stimulating Factor (G-CSF) [65], Methionine Sulfoximine (MSO) [66,67], PRE-084 [68], Tempol [69], WN1316 [70], n-butylphthalide [71], Guanabenz [72,73] and Resveratrol [74-76], with a total mean meta-analytical difference of 6.18 (95% CI 3.34 to 9.01) days of survival, favoring the group of drugs tested when compared to placebo. It was observed that Resveratrol showed major positive effect in three studies, and a meta-analysis (figure 4) with this drug showed a mean difference of 10.8 (95% CI 9.57 to 12.02) days of survival, favoring the Resveratrol group [74-76].

Figure 4 – Forest plot - meta-analysis including days of survival (life span) in pre clinical studies with Resveratrol. Period: 2009 to 2015



The authors analyzed the positive studies as a group to demonstrate that more than one drug could potentially be effective in disease control. The other drugs

evaluated [77-93], including Riluzole [92] and Lithium [93], showed little or no impact on the survival of animal models.

Discussion

Motor neuron disease is a group of progressive neurodegenerative disorders with different etiologies and clinical spectra that have a loss of lower and/or upper motor neurons in common [9]. Although the heterogeneous and complex nature of ALS has been studied extensively, the absence of early detection biomarkers has not allowed the identification of patients at different stages or those developing the disease.

With regard to treatments aimed at slowing the progression of ALS, an analysis of the results of preclinical trials in animal models and clinical trials shows that there is great disparity between the findings of animal trials, which are often positive, and their replication in humans, which almost always yield negative results. In clinical studies, Riluzole [94] remains the single drug that has successfully slowed disease progression in a systematic review with a meta-analysis involving 1,477 patients; a 2- to 3-month increase in survival time was observed with a relative risk of 0.78 (95% CI 0.65 to 0.92) at 18 months.

According to some authors [9,95], there are several possible explanations for the failure of translation from preclinical studies to effective human treatments. In preclinical studies, SOD1 animal models represented familial ALS more than sporadic ALS. In addition, pathophysiology of ALS spectra is poor understood and it is possible that familial and sporadic ALS differ in some fundamental mechanisms that determine the effectiveness of treatments [9]. On the other hand, some drugs used in animal models are used prior to symptom onset, which cannot be replicated in patients. It is also difficult to estimate the optimal target dose of an experimental drug in humans with the absence of the biomarkers.

The accessed preclinical trials used relatively young, mutant SOD1 ^{G93A} mice in homogeneous groups and a controlled environment, in which they showed a similar clinical condition. The drugs were used in the pre-symptomatic and early symptomatic stages, and many studies reported success in slowing the progression of symptoms and prolonging the survival of the animals, as shown in Figure 1.

Most animal studies used the G93A SOD1 mice, but others used a low copy number G93A SOD1 strain or different familiar ALS mouse models with different onset and survival times. These studies have suggested that direct injury on the superoxide dismutase (SOD1) protein in neuronal tissues is crucial for the onset of motor neuron disease but not for the clarification of its progression, which is largely determined by microglia and astrocyte responses [96].

The main mechanisms leading to neuronal death after onset of the disease include mitochondrial dysfunction, formation of free radicals and protein aggregates, glutamate excitotoxicity, axonal transport disruption, apoptosis, and inflammatory processes. There is evidence that protein aggregates can actively spread in the cerebral cortex and spinal cord via communication between cells, a process known as prion-like spread [97].

The role of autophagy in the injury mechanisms of the disease has also been discussed. Autophagy and the ubiquitin-proteasome (UP) system are two ways in which cells can degrade intracellular components. The UP system degrades short-lived proteins, whereas autophagy is responsible for the degradation of long-lasting proteins and damaged mitochondria, but when present in excess, it can lead to self-digestion and cell death [98].

Despite the biases present in preclinical studies, negative results (95%) obtained in the randomized clinical trials are also influenced by several factors, among which are clinical heterogeneity, little knowledge about the disease prognosis, the highly variable course of the disease, the small number of participants, the inclusion of patients who had had the disease for a long time and not just incidental cases, withdrawal due to the adverse effects or lack of efficacy of drugs, and the use of different outcome measures [99], as shown in Tables 1 and 2.

The mean duration of the disease at the beginning of the studies ranged from 12 to 31.8 months, with a weighted average of 16.2 months and a median of 15.6 months. There was great variability among groups, which compromises the assessment of treatment effectiveness because it directly interferes with the scores of functional scales and survival time (Table 1).

Another factor that may be associated with inadequate group set up in clinical trials is the delay in establishing the diagnosis. A recent study conducted in the United States [100], in which logistic regression was used to assess 103 patients, showed that the total median time for diagnosis is 11.5 months. In this study, it was shown that 52% of patients had previously received another diagnosis; on average, evaluation by three doctors was necessary for a conclusive diagnosis.

According to some authors [100,101], dose escalation is important in phase II clinical trials before conducting phase III trials, in which efficacy and safety are determined for a greater number of patients. This did not occur in most of the studies selected and could explain why so many phase II trials are positive and phase III trials proved to be negative. Moreover, the ALSFRS-R, the only widely validated clinical scale, and survival are clinical outcomes that should be used to establish the efficacy of the tested compounds.

The effectiveness of various drugs in slowing the progression of motor neuron disease was tested in Cochrane systematic reviews, including Riluzole, Creatine, amino acids, and ciliary neurotrophic factor (CNTF), and only Riluzole yielded positive results. The systematic review of Riluzole included three clinical trials (Riluzole 876, placebo 406) and one of those included patients of advanced age [9]. Daily treatment with 100 mg of Riluzole increased survival by two to three months in two studies ($p = 0.039$, hazard ratio (HR) 0.80, 95% CI 0.64-0.99), but in patients presenting with advanced disease, the result was not significant ($p = 0.056$, HR 0.84, 95% CI 0.70-1.01).

Since then, various drugs have been used in clinical trials in an attempt to slow disease progression without success. Lithium was used in four clinical trials for this purpose, motivated by positive results in multiple cell culture and animal assays [103], but to date, the same results have not been observed in clinical trials [41-44].

The most significant multicenter clinical trial was conducted in the UK and used Lithium carbonate to treat ALS - LiCALS [44] in 214 patients over 18 months. Although the number of adverse events observed was not significant (hazard ratio for serious adverse events 1.14, 95% CI 0.79-1.65), the drug was not beneficial (Mantel-Cox log-rank $\chi 2$ on 1 df = 1.64, $p = 0.20$). Three published articles that were included in

the review reported negative results in terms of disease progression and many adverse events (Table 2) with the use of Lithium. However, these studies used a non-traditional method, included few patients and did not exclude the possibility of a minor drug effect on survival and disease progression [41-43]. A meta-analysis of these studies that considered the dichotomous variable survivors in the experimental and control groups at 15 to 16 months yielded a hazard ratio of 0.85 (95% CI: 0.71 to 1.02), without statistical significance.

In preclinical trials, a significant improvement in survival with the use of recombinant human Granulocyte-colony Stimulating Factor (G-CSF) was observed in a selected study [65]. The efficacy and safety of the drug in humans with ALS was evaluated based on two articles that had a total of 49 patients and a maximum follow-up time of 12 months [45,46]. Subcutaneous injections of G-CSF or saline solution (5-10 mg/kg/ day of G-CSF for 4 days every three months for 1 year or for 10 days and from day 20 to 25) were administered. Survival was not assessed, and the primary outcome was disease progression. The ALSFRS-R score was evaluated, and it was observed that the drug was well tolerated, with no significant evidence of efficacy.

In addition, some researchers have found that hypermetabolism is present in ALS patients and that there is a correlation between dyslipidemia, good nutrition status and a better prognosis [104]. However, studies with caprylic triglyceride in animal models [22] and hypercaloric enteral nutrition in patients with ALS with a short-term follow up that assessed safety and tolerability found that survival was not modified, [105].

To standardize the results of clinical trials and make them more reliable, approximating the results obtained in animal models, some authors [101,102] have suggested using samples of a representative number of patients, having a shorter diagnosis time; avoiding prevalent cases; stratifying patients into groups with a homogeneous clinical condition; and having as an endpoint validated functional scales such as the ALSFRS-R, death or survival, or mechanical ventilation use. It is also important to conduct studies of different populations to compare patients with different genetic susceptibility and exposure to various environmental risk factors.

Guidelines have been introduced [106] that should reduce the number of false positives in preclinical studies and therefore prevent unnecessary clinical trials. These recommendations include rigorously assessing animals' physical and biochemical traits in terms of human disease; characterizing when disease symptoms and death occur and being alert to unexpected variations; and creating a mathematical model to aid experimental design, including how many mice must be included in a study. Perrin [95] also suggested excluding irrelevant animals; balancing for gender; avoiding put siblings into the same treatment group; and tracking genes that induce non-inherited disease.

Conclusion

Amyotrophic Lateral Sclerosis (ALS) is a rare, heterogeneous disease that is still poorly understood in its pathophysiology and is difficult to manage from a clinical point of view. It was observed great efficacy of preclinical studies, whereas the clinical ones showed no effectiveness in improving survival.

The positive results of preclinical studies should be interpreted with caution. To approximate the results of the two types of studies, it is necessary to standardize preclinical studies and control the biases of clinical trials to allow a greater potential of generalizing the findings. Additionally, interventions should be tested in patients who have been more recently diagnosed, and samples should be stratified into more homogeneous groups.

The most promising drugs observed in preclinical studies were Resveratrol, CuII(atsm), Dihydrotestosterone, Erlotinib, Granulocyte Colony-Stimulating Factor (G-CSF), Methionine Sulfoximine (MSO), PRE-084, Tempol, WN1316, and n-butylphthalide. The new experimental drugs that demonstrate success in slowing the progression of the disease could be used alone compared to placebos but also in combination.

References

1- Gordon PH. Amyotrophic Lateral Sclerosis: An update for 2013 Clinical Features, Pathophysiology, Management and Therapeutic Trials. Aging dis 2013; 4(5): 295-310.

2- Logroscino G, Traynor BJ, Hardiman O, Chio A, Mitchell D, Swingler RJ, Millul A, Benn E, Beghi E. Incidence of amyotrophic lateral sclerosis in Europe. J Neurol Neurosurg Psychiatry 2010; 81:385-90.

3- Marin B, Gil J, Preux PM, Funalot B, Couratier P. Incidence of amyotrophic lateral sclerosis in the Limousin region of France, 1997-2007. Amyotroph Lateral Scler 2009;10: 216-20.

4- Ragonese P, Filippini G, Salemi G, Beghi E, Citterio A, D'Alessandro R, et al. Accuracy of death certificates for amyotrophic lateral sclerosis varies significantly from north to south of Italy: implications for mortality studies. Neuroepidemiology 2004; 23:73-7.

5- Logroscino G, Traynor BJ, Hardiman O, Chio' A, Couratier P, Mitchell JD, et al; EURALS. Descriptive epidemiology of amyotrophic lateral sclerosis: new evidence and unsolved issues. J Neurol Neurosurg Psychiatry 2008; 79:6-11.

6- Chiò A, Logroscino G, Traynor BJ, Collins J, Simeone JC, Goldstein LA, et al. Global epidemiology of amyotrophic lateral sclerosis: a systematic review of the published literature. Neuroepidemiology 2013; 41: 118–30.

7- Ravits J, Appel S, Baloh RH, Barohn R, Brooks BR, Elman L, et al. Deciphering amyotrophic lateral sclerosis: what phenotype, neuropathology and genetics are telling us about pathogenesis. Amyotroph Lateral Scler Frontotemporal Degener 2013; 14 Suppl 1:5-18.

8- Strong MJ. The evidence for altered RNA metabolism in amyotrophic lateral sclerosis (ALS). J Neurol Sci 2010; 288(1–2): 1–12.

9- Mancuso R, Navarro X. Amyotrophic lateral sclerosis: Current perspectives from basic research to the clinic. Prog Neurobiol. 2015;133:1-26

10- Miller RG, Jackson CE, Kasarskis EJ, England JD, Forshew D, Johnston W, et al. Practice parameter update: The care of the patient with amyotrophic lateral sclerosis: drug, nutritional, and respiratory therapies (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 2009; 73:1218-26.

11- Orrell RW. Motor neuron disease: systematic reviews of treatment for ALS and SMA.Br Med Bull 2010; 93:145-59

12- Costa J, Swash M, de Carvalho M. Awaji criteria for the diagnosis of amyotrophic lateral sclerosis: a systematic review. Arch Neurol 2012; 69(11): 1410-6.

13- The Amyotrophic Lateral Sclerosis Functional Rating Scale. Assessment of activities of daily living in patients with amyotrophic lateral sclerosis. The ALS CNTF treatment study (ACTS) phase I-II Study Group. Arch Neurol 1996; 53:141-147.

14- Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE Working Group. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ 2008; 336; 924-6.

15- Kung J, Chiappelli F, Cajulis OO, Avezova R, Kossan G, Chew L, et al. From systematic reviews to clinical recommendations for evidence-based health care: validation of revised assessment of multiple systematic reviews (R-AMSTAR) for grading of clinical relevance. Open Dent J 2010; 4:84-91.

16- Calderó J, Brunet N, Tarabal O, Piedrafita L, Hereu M, Ayala V, et al. Lithium prevents excitotoxic cell death of motoneurons in organotypic slice cultures of spinal cord. Neurosci 2010; 165: 1353-69.

17- Schuster JE, Fu R, Siddique T, Heckman CJ. Effect of prolonged riluzole on cultured motoneurons in a mouse model of ALS. J Neurophysiol 2012; 107 (1): 484-92.

18- Gu R, Hou X, Pang R, Li L, Chen F, Geng J, et al. Human adipose-derived stem cells enhance the glutamate uptake function of GLT1 in SOD1G93A-bearing astrocytes. Biochemical and Biophysical Res Com 2010; 393: 481-6.

19- Tovar-y-Romo LB, Tapia R. Delayed administration of VEGF rescues spinal motor neurons from death with a short effective time frame in excitotoxic experimental models in vivo. ASN NEURO 2012; 4(2): art: e00081.

20- Cappello V, Vezzoli E, Righi M, Fosati M. Analysis of neuromuscular junctions and effects of anabolic steroid administration in the SOD1G93A mouse model f ALS. Mol Cell Neurosci 2012; 51: 12-21.

21- Sunyach C, Michaud M, Arnoux T, Bernard-marrisal N. Olesoxime delays muscle denervation, astrogliosis, microglial activation and motoneuron death in na als mouse model. Neuropharmacology 2012;62: 2346-53.

22- Zhao W, Varghese M, Vempati P, Dzhun A, Cheng A, Wang J, et al.Caprylic Triglyceride as a novel therapeutic approach to effectively improve the performance and attenuate the symptoms due to the motor neuron loss in ALS disease. PloS ONE 2012; 7(11): e49191.

23- Yazhou L, Srinivaslu H, Mohamed W. Exendin-4 Ameliorates Motor Neuron Degeneration in Cellular and Animal Models of Amyotrophic Lateral Sclerosis. PLoS ONE 2012; 7(2): E 32008.

24- Jablonka S, Holtmann B, Sendtner M. Therapeutic effects of PEGylated insulin-like growth factor I in the pmn mouse model of motoneuron disease. Exp neurol 2011; 232: 261-9.

25- Seo JS, Baek IS, Leem YH, Kim TK, Cho Y, Lee SM, et al. SK-PC-B70M alleviates neurologic symptoms in G93A-SOD1 amyotrophic lateral sclerosis mice. Brain res 2011; 1368: 299-307.

26- Ferrucci M, Spalloni A, Bartalucci A. A systematic study of brainstem motor nuclei in a mouse model of ALS, the effects of lithium. Neurobiol dis 2010; 37:370-83.

27- Katsumata R, Ishigaki S, Katsuno M, Kawai K, Sone J, Huang Z, et al. c-Abl inhibition delays motor neuron degeneration in the G93A mouse, an animal model of amyotrophic lateral sclerosis. PLoS ONE 2012; 7(9): e46185.

28- Fidler J, Treleaven CM, Frakes A. Disease progression in a mouse model of amyotrophic lateral sclerosis: the influence of chronic stress and corticosterone. FASEB J 2011;(25): 4369-77.

29- Gianforcaro A, Solomon J, Hamadeh M. Vitamin D3 at 5-X Al Attenuates the decline in paw grip endurance, but not disease outcomes, in te G93A Mouse Modelo of ALS, and is Toxic in Females. PloS ONE 2013; 8(2): e30243.

30- Gianforcaro A, Hamadeh M. Dietary Vitamin D3 Supplementation at 10X the adequate intaque improves functional capacity in the G93A transgenic mouse model of ALS, a pilot study. Neurosci ther 2012; 18: 547-57.

31- Schuster J, Fu R, Heckman CJ. Fluoxetine treatment has modest effects on disease progression in the mSOD1G93A mouse model of ALS. Amyotroph Lateral Scler 2010; 11 suppl:(1) 72-3.

32- Couglan KS, Mitchem MR, Hogg MC, Prehn JH. "Preconditioning" with latrepirdine, an adenosine 5'-monophosphate-activated protein kinase activator, delays amyotrophic lateral sclerosis progression in SOD1(G93A) mice. Neurobiol Aging. 2015;36(2):1140-50.

33- Goursaud S, Schäfer S, Dumont AO, Vergouts M, Gallo A, Desmet N, et al. The anti-inflammatory peptide stearyl-norleucine-VIP delays disease onset and extends survival in a rat model of inherited amyotrophic lateral sclerosis. Exp Neurol. 2015;263:91-101.

34- Rudnicki SA, Berry JD, Ingersoll E, Archibald D, Cudkowicz ME, Kerr DA, et al. Dexpramipexole effects on functional decline and survival in subjects with amyotrophic lateral sclerosis in a phase II study: subgroup analysis of demographic

and clinical characteristics. Amyotroph lateral scler frontotemporal degener 2013; 14:44-51.

35- Chiò A, Mora G, La Bella V, Caponnetto C, Mancardi G, Sabatelli M, et al; STEMALS Study Group. Repeated courses of granulocyte colony-stimulating factor in amyotrophic lateral sclerosis: clinical and biological results from a prospective multicenter study. Muscle nerve 2011; 43:189-95.

36- Atassi N, Ratai EM, Greenblatt DJ, Pulley D, Zhao Y, Bombardier J, et al. A phase I pharmacokinetic dosage escalation study of creatine monohydrate in subjects with amyotrophic lateral sclerosis. Amyotroph lateral scler 2010; 11(6): 508-13.

37- Fondell E, O'Reilly ÉJ, Fitzgerald KC, Falcone GJ, McCullough ML, Thun MJ, et al. Non-steroidal anti-inflammatory drugs and amyotrophic lateral sclerosis: results from five prospective cohort studies. Amyotroph lateral scler 2012; 13: 573-9.

38- Grassinger J, Khomenko A, Hart C, Baldaranov D, Johannesen SW, Mueller G, et al. Safety and feasibility of long term administration of recombinant human granulocyte-colony stimulating factor in patients with amyotrophic lateral sclerosis. Cytokine 2014; 67:21-8.

39- Cudkowicz M, Bozik ME, Ingersoll EW, Miller R, Mitsumoto H, Shefner J, et al. The effects of dexpramipexole (KNS-760704) in individuals with amyotrophic lateral sclerosis. Nature medicine 2011; 17(12) 1652-6.

40- Cudkowicz ME, van den Berg LH, Shefner JM, Mitsumoto H, Mora JS, Ludolph A, et al; EMPOWER investigators. Dexpramipexole versus placebo for patients with amyotrophic lateral sclerosis (EMPOWER): a randomised, double-blind, phase 3 trial. Lancet Neurol 2013; 12(11): 1059-67.

41- Aggarwal SP, Zinman L, Simpson E, McKinley J, Jackson KE, Pinto H, et al; Northeast and Canadian Amyotrophic Lateral Sclerosis consortia. Safety and efficacy of lithium in combination with riluzole for treatment of amyotrophic lateral sclerosis: a randomized, double-blind, placebo-controlled trial. Lancet Neurol 2010; 9: 481-8.

42- Chiò A, Borghero G, Calvo A, Capasso M, Caponnetto C, Corbo M, et al; LITALS Study Group. Lithium carbonate in amyotrophic lateral sclerosis: lack of efficacy in a dose-finding trial. Neurology 2010; 75(7): 619-25.

43- Verstraete E, Veldink JH, Huisman MH, Draak T, Uijtendaal EV, van der Kooi AJ, et al. Lithium lacks effect on survival in amyotrophic lateral sclerosis: a phase IIb randomised sequential trial. J Neurol Neurosurg Psychiatry. 2012; 83(5): 557-64.

44- UKMND-LiCALS Study Group, Morrison KE, Dhariwal S, Hornabrook R, Savage L, Burn DJ, Khoo TK, et al. Lithium in patients with amyotrophic lateral sclerosis (LiCALS): a phase 3 multicentre, randomised, double-blind, placebo-controlled trial. Lancet Neurol 2013; 12(4): 339-45.

45- Duning T, Schiffbauer H, Warnecke T, Mohammadi S, Floel A, Kolpatzik K, et al. G-CSF prevents the progression of structural disintegration of white matter tracts in amyotrophic lateral sclerosis: a pilot trial. PLoS One 2011; 6(3): e17770.

46- Nefussy B, Artamonov I, Deutsch V, Naparstek E, Nagler A, Drory VE.Recombinant human granulocyte-colony stimulating factor administration for treating amyotrophic lateral sclerosis: A pilot study. Amyotroph Lateral Scler 2010; 11(1-2): 187-93.

47- Pascuzzi RM, Shefner J, Chappell AS, Bjerke JS, Tamura R, Chaudhry V, et al. A phase II trial of talampanel in subjects with Amyotrophic Lateral Sclerosis. Amyotroph Later Sclerosis 2010; 11: 266-71.

48- Min JH, Hong YH, Sung JJ, Kim SM, Lee JB, Lee KW. Oral solubilized ursodeoxycholic acid therapy in amyotrophic lateral sclerosis: a randomized cross-over trial. J Korean Med Sci. 2012; 27(2): 200-6.

49- Kaufmann P, Thompson JL, Levy G, Buchsbaum R, Shefner J, Krivickas LS, et al. ; QALS Study Group. Phase II trial of CoQ10 for ALS finds insufficient evidence to justify phase III. Ann Neurol 2009; 66(2): 235-44.

50- Parton M, Mitsumoto H, Leigh PN. Amino acids for amyotrophic lateral sclerosis / motor neuron disease. Cochrane Database Syst Rev. 2008 Apr 16 ;(2): CD003457.

51- Bongioanni P, Reali C, Sogos V. Ciliary neurotrophic factor (CNTF) for amyotrophic lateral sclerosis/motor neuron disease. Cochrane Database Syst Rev. 2004 ;(3): CD004302.

52- Pastula DM, Moore DH, Bedlack RS. Creatine for amyotrophic lateral sclerosis/motor neuron disease. Cochrane Database Syst Rev. 2012; 12:CD005225.

53- Saccà F, Quarantelli M, Rinaldi C, Tucci T, Piro R, Perrotta G, et al. A randomized controlled trial of growth hormone in amyotrophic lateral sclerosis: clinical, neuroimaging and hormonal results. J Neurol 2012; 259(1): 132-8.

54- Beghi E, Pupillo E, Bonito V, Buzzi P, Caponnetto C, Chiò A, et al; Italian ALS Study Group.Randomized double-blind placebo-controlled trial of acetyl-l-carnitine for ALS. Amyotroph Lateral Scler Frontotemporal Degener 2013; 14(5-6): 397-405.

55- Berry JD, Shefner JM, Conwit R, Schoenfeld D, Keroack M, Felsenstein D, et al; Northeast ALS Consortium. Design and initial results of a multi-phase randomized trial of ceftriaxone in amyotrophic lateral sclerosis. PLoS One 2013; 8(4): e61177.

56- Dupuis L, Dengler R, Heneka MT, Meyer T, Zierz S, Kassubek J, et al; GERP ALS Study Group. A randomized, double blind, placebo-controlled trial of pioglitazone in combination with riluzole in amyotrophic lateral sclerosis. PLoS ONE 2012; 7(6): e37885.

57- Lenglet T, Lacomblez L, Abitbol JL, Ludolph A, Mora JS, Robberecht W, et al; Mitotarget study group. A phase II-III trial of olesoxime in subjects with amyotrophic lateral sclerosis. Eur J Neurol 2014; 21(3): 529-36.

58- Boll MC, Bayliss L, Vargas-Cañas S, Burgos J, Montes S, Peñaloza-Solano G, et al. Clinical and biological changes under treatment with lithium carbonate and valproic acid in sporadic amyotrophic lateral sclerosis. J Neurol Sci 2014;15;340(1-2):103-8.

59- Cudkowicz ME, Titus S, Kearney M, Yu H, Sherman A, Schoenfeld D, et al; Ceftriaxone Study Investigators. Safety and efficacy of ceftriaxone for amyotrophic lateral sclerosis: a multi-stage, randomised, double-blind, placebo-controlled trial. Lancet Neurol 2014;13(11):1083-91.

60- Le Pichon CE, Dominguez SL, Solanoy H, Ngu H, Lewin-Koh N, Chen M, et al. EGFR inhibitor erlotinib delays disease progression but does not extend survival in the SOD1 mouse model of ALS. PLoS ONE 2013; 8(4): e62342.

61- Saenger S, Holtmann B, Nilges MR, Schroeder S, Hoeflich A, Kletzl H, et al. Functional improvement in mouse models of familial amyotrophic lateral sclerosis by PEGylated insulin-like growth factor I treatment depends on disease severity. Amyotroph Lateral Scler 2012; 13(5): 418-29.

62- Gerber YN, Privat A, Perrin FE. Gacyclidine improves the survival and reduces motor deficits in a mouse model of amyotrophic lateral sclerosis. Front Cell Neurosci 2013; 7:280.

63- Soon CPW, Donelly OS, Turner BJ. Diacetylbis (N(4)-methylthiosemicarbazonato) copper(II) (CuII(atsm)) protects against peroxynitrite-induced nitrosative damage and prolongs survival in amyotrophic lateral sclerosis mouse model. J Biol Chem 2011; 286(51): 44035-44.

64- Yoo YE, Ko CP. Dihydrotestosterone ameliorates degeneration in muscle, axons and motoneurons and improves motor function in amyotrophic lateral sclerosis model mice. PLoS ONE 2012; 7(5): e37258.

65- Henriques A, Pitzer C, Dittgen T, Klugmann M, Dupuis L, Schneider A. CNS-targeted viral delivery of G-CSF in an animal model for ALS: improved efficacy and preservation of the neuromuscular unit. Mol Ther 2011; 19(2): 284-92.

66- Ghoddoussi F, Galloway MP, Jambekar A, Bame M, Needleman R, Brusilow WS. Methionine sulfoximine, an inhibitor of glutamine synthetase, lowers brain glutamine and glutamate in a mouse model of ALS. J Neurol Sci 2010; 290(1-2): 41-7.

67- Bame M, Pentiak PA, Needleman R, Brusilow WS. Effect of sex on lifespan, disease progression, and the response to methionine sulfoximine in the SOD1 G93A mouse model for ALS. Gend Med. 2012; 9(6): 524-35.

68- Mancuso R, Oliván S, Rando A, Casas C, Osta R, Navarro X. Sigma-1R agonist improves motor function and motoneuron survival in ALS mice. Neurotherapeutics 2012; 9(4): 814-26.

69- Linares E, Seixas LV, dos Prazeres JN. Tempol moderately extends survival in a hSOD1 (G93A) ALS rat model by inhibiting neuronal cell loss, oxidative damage and levels of non-native hSOD1(G93A) forms. PLoS ONE 2013; 8(2): e55868.

70- Tanaka K, Kanno T, Yanagisawa Y, Yasutake K, Inoue S, Hirayama N, et al. A novel acylaminoimidazole derivative, WN1316, alleviates disease progression via suppression of glial inflammation in ALS mouse model. PLoS ONE 2014; 9(1): e87728.

71- Feng XH, Yuan W, Peng Y, Liu MS, Cui LY. Therapeutic effects of dl-3-n-butylphthalide in a transgenic mouse model of amyotrophic lateral sclerosis. Chin Med J (Engl). 2012; 125(10): 1760-6.

72- Wang L, Popko B, Tixier E, Roos RP. Guanabenz, which enhances the unfolded protein response, ameliorates mutant SOD1-induced amyotrophic lateral sclerosis. Neurobiol Dis 2014;71: 317-24.

73- Jiang HQ, Ren M, Jiang HZ, Wang J, Zhang J, Yin X. et al. Guanabenz delays the onset of disease symptoms, extends lifespan, improves motor performance and attenuates motor neuron loss in the SOD1 G93A mouse model of amyotrophic lateral sclerosis. Neuroscience. 2014;277:132-8.

74- Markert CD, Kim E, Gifondorwa DJ, Childers MK, Milligan CE. A single-dose resveratrol treatment in a mouse model of amyotrophic lateral sclerosis. J Med Food 2010; 13(5): 1081-5.

75- Song L, Chen L, Zhang X, Li J, Le W. Resveratrol ameliorates motor neuron degeneration and improves survival in SOD1(G93A) mouse model of amyotrophc lateral sclerosis. Biomed Res Int 2014;2014:483501.

76- Mancuso R, del Valle J, Modol L, Martinez A, Granado-Serrano AB, Ramirez-Núñez O, et al. Resveratrol improves motoneuron function and extends survival in DOD1(G93A) ALS mice. Neurotherapeutics 2014; 11(2): 419-32.

77- Staats KA, Van Rillaer M, Scheveneels W, Verbesselt R, Van Damme P, Robberecht W, et al. Dantrolene is neuroprotective in vitro, but does not affect survival in SOD1 (G93A) mice. Neuroscience. 2012; 220:26-31.

78- Cappello V, Vezzoli E, Righi M, Fossati M, Mariotti R, Crespi A, et al. Analysis of neuromuscular junctions and effects of anabolic steroid administration in the SOD1G93A mouse model of ALS. Mol Cell Neurosci 2012; 51(1-2): 12-21.

79- Dodge JC, Treleaven CM, Fidler JA, Hester M, Haidet A, Handy C, et al. AAV4-mediated expression of IGF-1 and VEGF within cellular components of the ventricular system improves survival outcome in familial ALS mice. Mol Ther 2010; 18(12): 2075-84.

80- Keller AF, Gravel M, Kriz J. Treatment with minocycline after disease onset alters astrocyte reactivity and increases microgliosis in SOD1 mutant mice. Exp Neurol 2011; 228(1): 69-79.

81- Yoo YE, Ko CP. Treatment with trichostatin A initiated after disease onset delays disease progression and increases survival in a mouse model of amyotrophic lateral sclerosis. Exp Neurol 2011; 231(1): 147-59.

82- Guo Y, Zhang K, Wang Q, Li Z, Yin Y, Xu Q, et al. Neuroprotective effects of diallyl trisulfide in SOD1-G93A transgenic mouse model of amyotrophic lateral sclerosis. Brain Res 2011; 1374:110-5.

83- Towne C, Setola V, Schneider BL, Aebischer P. Neuroprotection by gene therapy targeting mutant SOD1 in individual pools of motor neurons does not translate into therapeutic benefit in fALS mice. Mol Ther 2011; 19(2): 274-83.

84- Tanaka K, Kanno T, Yanagisawa Y, Yasutake K, Hadano S, Yoshii F, et al. Bromocriptine methylate suppresses glial inflammation and moderates disease progression in a mouse model of amyotrophic lateral sclerosis. Exp Neurol 2011; 232(1): 41-52.

85- Lucchetti J, Marino M, Papa S, Tortarolo M, Guiso G, Pozzi S, et al. A mouse model of familial ALS has increased CNS levels of endogenous ubiquinol9/10 and does not benefit from exogenous administration of ubiquinol10. PLoS ONE 2013; 8(7): e69540.

86- Lougheed R, Turnbull J. Lack of effect of methylene blue in the SOD1 G93A mouse model of amyotrophic lateral sclerosis. PLoS ONE 2011; 6(10): e23141.

87- Kaneb HM, Sharp PS, Rahmani-Kondori N, Wells DJ. Metformin treatment has no beneficial effect in a dose-response survival study in the SOD1 (G93A) mouse model of ALS and is harmful in female mice. PLoS ONE 2011; 6(9): e24189.

88- Hayes-Punzo A, Mulcrone P, Meyer M, McHugh J, Svendsen CN, Suzuki M. Gonadectomy and dehydroepiandrosterone (DHEA) do not modulate disease progression in the G93A mutant SOD1 rat model of amyotrophic lateral sclerosis. Amyotroph Lateral Scler 2012; 13(3): 311-4.

89- Goursaud S, Schäfer S, Dumont AO, Vergouts M, Gallo A, Desmet N, et al. The anti-inflammatory peptide stearyl-norleucine-VIP delays disease onset and extends survival in a rat model of inherited amyotrophic lateral sclerosis. Exp Neurol 2015;263:91-101.

90- Roberts BR, Lim NK, McAllum EJ, Donnelly PS, Hare DJ, Doble PA, et al. Oral treatment with Cu(II)(atsm) increases mutant SOD1 in vivo but protects motor neurons and improves the phenotype of a transgenic mouse model of amyotrophic lateral sclerosis. J Neurosci 2014;34(23):8021-31.

91- Ahn SW, Jeon GS, Kim MJ, Shon JH, Kim JE, Shin JY, et al. Neuroprotective effects of JGK-263 in transgenic SOD1-G93A mice of amyotrophic lateral sclerosis. J Neurol Sci. 2014;340(1-2):112-6.

92- Li J, Sung M, Rutkove SB. Electrophysiologic biomarkers for assessing disease progression and the effect of riluzole in SOD1 G93A ALS mice. PLoS ONE 2013; 8(6): e65976.

93- Pizzasegola C, Caron I, Daleno C. Treatment with lithium carbonate does not improve disease progression in two different strains of SOD1 mutant mice. Amyotroph Lateral Scler 2009; 10(4): 221-8.

94- Miller RG, Mitchell JD, Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). Cochrane Database Syst Rev. 1997; 3:CD001447.

95- Perrin S. Preclinical research: make mouse studies work. Nature 2014;507(7493):423-5.

96- Boillée S, Yamanaka K, Lobsiger CS, Copeland NG, Jenkins NA, Kassiotis G, Kollias G, Cleveland DW. Onset and progression in inherited ALS determined by motor neurons and microglia. Science. 2006; 312(5778): 1389-92.

97- Grad LI, Fernando SM, Cashman NR. From molecule to molecule and cell to cell: prion-like mechanisms in amyotrophic lateral sclerosis. Neurobiol Dis 2015;77:257-65.

98- Pasquali L, Longone P, Isidoro C, Ruggieri S, Paparelli A, Fornai F. Autophagy, lithium, and amyotrophic lateral sclerosis. Muscle Nerve. 2009; 40(2):173-94.

99- Traynor BJ, Bruijn L, Conwit R, Beal F, O'Neill G, Fagan SC, et al. Neuroprotective agents for clinical trials in ALS: a systematic assessment. Neurology 2006; 67:20-7.

100- Paganoni S1, Macklin EA, Lee A. Diagnostic timelines and delays in diagnosing amyotrophic lateral sclerosis (ALS). Amyotroph Lateral Scler Frontotemporal Degener. 2014 Sep; 15(5-6):453-6.

101- Gordon PH, Meininger V. How can we improve clinical trials in amyotrophic lateral sclerosis? Nat Rev Neurol 2011; 7:650-4.

102- Beghi E, Chiò A, Couratier P, Esteban J, Hardiman O, Logroscino G, et al; Eurals Consortium. The epidemiology and treatment of ALS: focus on the heterogeneity of the disease and critical appraisal of therapeutic trials. Amyotroph Lateral Scler. 2011; 12(1):1-10.

103- Fornai F, Longone P, Cafaro L, Kastsiuchenka O, Ferrucci M, Manca ML, et al. Lithium delays progression of amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A. 2008; 105(6):2052-7.

104- Schmitt F, Hussain G, Dupuis L, Loeffler JP, Henriques A. A plural hole for lipids in motor neuron diseases: energy, signaling and structure. Frontiers in Cell Neurosc. 2014 feb;8:1-10.

105- Dorst J, Cypionka J, Ludolph AC. High-caloric food supplements in the treatment of amyotrophic lateral sclerosis: a prospective interventional study. Amyotrophic Lateral Scler Frontotemporal Degener. 2013 dec;14(7-8): 533-6.

106- Ludolph AC, Bendotti C, Blaugrund E, Chio A, Greensmith L, Loeffler JP, et al Guidelines for preclinical animal research in ALS/MND: A consensus meeting.Amyotroph Lateral Scler 2010;11(1-2):38-45.

ARTIGO 3 – ARTIGO ORIGINAL EFFICACY OF STEM CELL THERAPY IN AMYOTROPHIC LATERAL SCLEROSIS: A SYSTEMATIC REVIEW AND META-ANALYSIS

Versão em inglês

Efficacy of stem cell therapy in amyotrophic lateral sclerosis: a systematic review and meta-analysis, publicado no periódico científico Journal of Clinical Medicine Research, 2016, in press.

EFFICACY OF STEM CELL THERAPY IN AMYOTROPHIC LATERAL SCLEROSIS: A SYSTEMATIC REVIEW AND META-ANALYSIS

Abstract

Published studies seeking to improve survival in amyotrophic lateral sclerosis have poor results in humans, although there are several studies in animal models with positive results. Here, we conducted a systematic review and meta-analysis of studies that were published between March 2009 and March 2015 on stem cell therapy and survival in animal models and patients with amyotrophic lateral sclerosis. A total of 714 articles were identified, and from these, we selected preclinical *in vivo* studies and retrospective clinical studies. A meta-analysis confirmed the efficacy of stem cell therapy in improving survival in preclinical trials, where a mean difference of 9.79 days (95% CI 4.45 to 15.14) in lifespan favored stem cell therapy. In contrast, the number of clinical studies is still insufficient to assess their effectiveness, and these studies only demonstrate the absence of serious adverse events. However, even this conclusion should be interpreted with caution because clinical studies are retrospective and heterogeneous and have an unsatisfactory quality.

KEY WORDS: ALS, amyotrophic lateral sclerosis, motor neuron, cell, treatment, survival.

Introduction

Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease. It is frequently sporadic and characterized by the progressive degeneration of both upper and lower motor neurons in the brain, brainstem and spinal cord(1). The incidence of ALS varies between 1.2 and 4.0 per 100,000 individuals per year, and ALS predominantly occurs in males(2,3). Death occurs between 2 and 4 years after onset due to respiratory insufficiency(1).

The mechanisms of ALS development are poorly understood, but the injury mechanisms of the disease may include both glial cells and neurons (4,5). The main known mechanisms of ALS pathogenesis are oxidative stress with damage to RNA, mitochondrial dysfunction, impairment of axonal transport, glutamate excitotoxicity, protein aggregation, endoplasmic reticulum stress, abnormal RNA processing, neuroinflammation and excitability of peripheral axons (6).

The clinical heterogeneity of ALS makes it difficult to identify the exact cause of the disease for the development of effective therapies. However, the drug riluzole may extend patient survival by a few months(7). In addition, multidisciplinary care, enteral nutrition and noninvasive ventilation can extend patient survival(8).

The results of studies on the use of stem cell therapy to preserve motor neuron function have been considered without any evidence of their effectiveness in humans, although the use of this therapeutic approach has been fairly efficacious in experimental animal models(9).

Given the need to identify new alternatives to treat ALS, the aim of the present study was to conduct a systematic literature review to assess the efficacy of stem cell therapy in clinical and preclinical studies.

Material and methods

Strategies used to identify studies for meta-analysis

In May 2015, we investigated primary preclinical *in vivo* studies and clinical studies and subsequent meta-analyses published between March 2009 and March 2015 in the following electronic databases: Medline, Embase, Cochrane Library and Lilacs. The following Medical Subject Headings (MeSH) and Health Science Descriptors (HScDe) were used: ‘Amyotrophic Lateral Sclerosis' OR 'Motor Neuron Disease' AND 'Treatment' AND ‘cell therapy’.

Two authors independently evaluated the titles and abstracts of all studies identified in the search of the aforementioned electronic databases based on the descriptors.

The inclusion criteria were the following:

i) clinical, prospective or retrospective studies of patients diagnosed with a motor neuron disease by means of anamnesis and electromyography according to the El Escorial (10) and Awaji criteria (11); ii) preclinical and *in vivo* studies with assessment of survival compared to a control group, and studies of treatment after the onset of weakness; and iii) studies based on the use of stem cell therapy to increase survival time compared to placebo or other treatments used by the control group.

The exclusion criteria were studies in which participants presented with respiratory failure or spinal muscular atrophy, studies in which the treatment was administered only prior to the disease onset, and narrative reviews, letters, editorials, case reports, duplicate publications or those without objective data to be evaluated.

Articles published in all languages were included.

The studies that met the inclusion criteria were obtained in full. References were also considered, and communication with the authors was established in cases of doubt. Disagreements were resolved by consensus, and when this was impossible, there was a subsequent analysis by two additional reviewers.

Data extraction

Data were obtained from each study using a review form with the following content: author, place where the work was conducted, year of publication, intervention, study design, number of participants, age, analysis by intention to treat, declaration of

conflict of interest, evaluation by a research ethics committee, and animal species used if the study was preclinical. The following outcomes were assessed:

i) comparison between different treatments;

ii) analysis of mean survival and absolute days of survival;

iii) mean duration of the disease until the start of the intervention;

iv) incidence of reactions and adverse effects of proposed treatments.

Assessing the quality of the studies

Quality was assessed by two independent authors, and in cases of disagreement, the situation was resolved by consensus among all authors. The GRADE (Grading of Recommendations, Assessment, Development and Evaluation) model(12) was used to access the quality of the studies. The following data were observed in the studies:

Methods: research question, treatment sequence, allocation confidentiality, post-intervention follow-up, blinded outcome assessment, primary clinical outcome measures, location of study, protection against contamination, calculation of statistical power, sample representativeness, conflict of interest, and ethical aspects.

Participants: inclusion criteria, exclusion criteria, age, gender, disease severity, and disease variants.

Interventions: procedures, follow-up time, and method for monitoring disease progression.

Outcomes assessed in the review: disease duration before intervention, survival time.

The results of the primary outcome were obtained based on the intention-to-treat principle. For a continuous outcome, the following variables were calculated: mean, standard deviation and number of participants in each group. Data from work that was published more than once were obtained from the more thorough study.

The authors related each primary study according to the overall quality of evidence as follows: A-high; B-moderate; C-low; and D-very low, assigning scores of 1-5 according to the number of biases.

For analytical purposes, the studies were grouped as i) interventions in animal models and ii) clinical studies.

Statistical analysis

Statistical analysis was performed in preclinical trials using RevMan software, version 5.3. All p-values < 0.05 were considered to be statistically significant.

For continuous variables, such as animal survival in days, the weighted mean difference (random effects model) was calculated based on the DerSimonian and Laird method, with a corresponding confidence interval of 95%.

To evaluate the heterogeneity among the studies, a heterogeneity test was performed by calculating both the Q-test of heterogeneity and the I^2 test of inconsistency. Heterogeneity was considered significant when $p < 0.10$. In addition, a sensitivity analysis was conducted using a funnel plot to quantify the presence of publication bias.

Results

Initially, 714 articles were obtained. Based on their abstracts, we selected 1 retrospective controlled clinical trial, 14 preclinical studies and 12 clinical uncontrolled descriptive studies.

After each original document was reviewed and data were obtained, 4 preclinical trials were excluded. Of these, 1 was an *in vitro* study(13), 2 did not include a quantitative evaluation of survival(14,15), and 1 only reported survival ratios and proportions(16).

Finally, 9 preclinical *in vivo* studies and 12 retrospective descriptive studies using stem cell therapy were included in the review. Although most of these studies were not controlled and randomized, they were included due to the limited number of studies that met this requirement. A flowchart illustrates the selection process that was adopted in the systematic review (Figure 1).

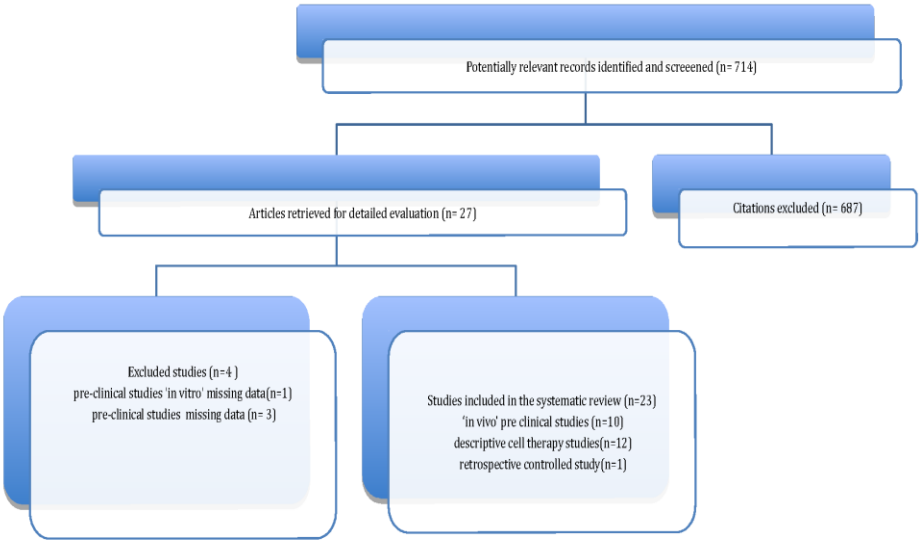


Figure 1 – Systematic review flowchart

Preclinical studies

Preclinical studies have been insightful for identifying the types of stem cells that offer therapeutic benefit in ALS. *In vivo* studies were conducted in transgenic mice expressing human mutated superoxide dismutase 1 and G93A (SOD1G93A) and present experimental ALS treatments using neuroprotective therapies. These studies can be considered homogeneous with respect to their methods and their evaluation of outcomes in animals, which allows for a comparison using meta-analytical methods that involve a network meta-analysis(12). All included studies analyzed survival using the Kaplan-Meier method with a log-rank test, but only those studies that clearly included the mean survival of animals in days and standard deviation were included in our meta-analysis. The authors of all of the studies that did not include such data were contacted via email.

Figure 2 shows an acyclic graph (forest) of cell therapy preclinical trials in animal models, demonstrating a mean meta-analytical difference of survival of 9.79 days (95% CI 4.45 to 15.14), which favored cell therapy over placebo.

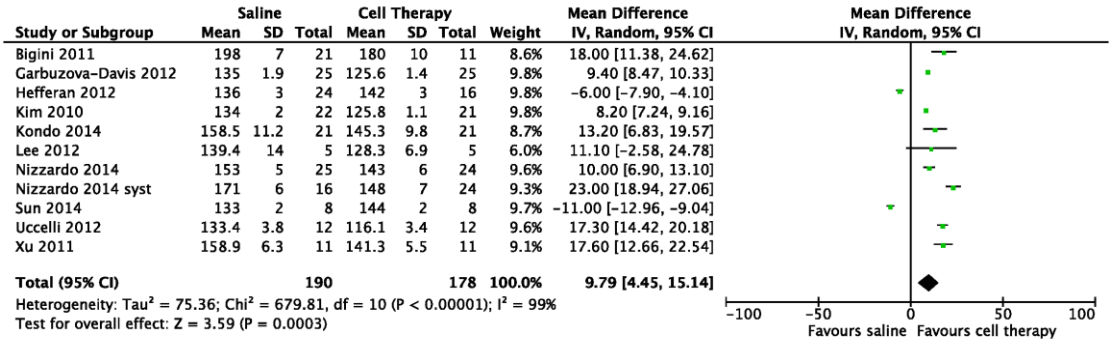


Figure 2: Preclinical studies in amyotrophic lateral sclerosis model with stem cell therapy – 2009 to 2015.

In 2010, a study conducted in South Korea(17) showed that mesenchymal cell transplantation (ALS-hMSCs) at three different doses in association with cyclosporine was effective in slowing disease progression at high doses using an intrathecal injection of 1 x 10^6 cells/ml into the cisterna magna ($p = 0.033$) and an intravenous injection of neurotrophic factors. In 2011, in Italy(18), intraventricular administration of mononuclear cells from human umbilical cord showed that treated animals had a significantly longer survival than controls ($p < 0.01$, Gehan-Breslow-Wilcoxon test). A study in 2012(19) showed that an intravenous injection of mesenchymal cells also led to increased survival of the animals ($p < 0.005$). In 2014, a study in Japan showed the efficacy of a transplantation of glial-rich neural progenitors in attenuating motor neuron degeneration and disease progression in rodent models(20).

In 2012, Garbuzova-Davis et al. (21) performed weekly intravenous injections of cells from human umbilical cord in the pre-symptomatic stage and in the 13th week of the established disease stage. Two dosages were analyzed, 1 x 10^6 cells and 2.5 x 10^6 cells. The results showed that the greatest increases in survival occurred with the administration at the pre-symptomatic stage using the lowest concentration of cells ($p = 0.0015$) and at a dose of 2.5 x 10^6 for symptomatic animals ($p = 0.0022$).

Three studies examined the transplantation of stem cells from human fetuses into the anterior horn of the lumbar cord in immunosuppressed mice, before the onset of ALS symptoms (14,22,23). In a study by Hefferan et al.(24), improvement was transient and not very significant ($p = 0.122$), while a study by Xu et al.(14) showed that a cervical and lumbar engraftment of cervical and thoracic spinal cord cells of an 8-week

human fetus significantly prolonged survival by 17 days. The study was not analyzed in conjunction with the others because there were missing data.

In 2012, Lee et al.(25) replaced microglia with a mutant SOD1 gene with microglia that expressed the wild-type gene using an injection of clodronate liposome and bone marrow transplants into the fourth cerebral ventricle and subsequently observed a 51.03% increase in survival.

In 2014, Sun et al. reported the behavioral improvement and extended lifespan of ALS model mice transplanted with fetal human neural cells into the spinal cord. It was suggested that intrathecal transplantation of motor neurons into the lumbar spinal cord of animals migrated into the ventral horn area and improved ambulatory function and survival(26).

Also in 2014, Nizzardo et al.(27) showed that survival in an animal model significantly benefited from both intrathecal and intravenous injections of specific neural stem cells that were derived from induced pluripotent stem cells. These positive effects are attributed to the activity of multiple mechanisms, including the production of neurotrophic factors and the reduction of microgliosis and macrogliosis.

In the studies described above, histopathological analyses of the nervous tissue of the spinal cord and brain were conducted after euthanasia of the animals, and a longer survival of motor neurons and glial cells was observed. Glial cells became branched after treatment but had a lower density and lower reactivity and showed less gliosis (18,19,21).

Clinical studies

Table 1 summarizes the cell therapy clinical trials. These studies were conducted with the main objective of assessing the adverse effects inherent to the procedure and the post-procedure clinical evolution of the disease. Some of these effects were assessed using the ALSFRS-R. The mean disease duration was 2.32 ± 1.1 years and ranged from 0.7 to 4.4 years. The follow-up period ranged from 6 to 47.2 months. The number of patients per study ranged from 6 to 24 with an average of 11. All but 1 study were uncontrolled, with a quality of C or D.

Table1-Studies in amyotrophic lateral sclerosis patients with stem cell therapy. 2009-2015

AUTHOR	YEAR	N	DISEASE (years)	FOLLOW UP(month)	OUTCOME	SURVIVAL	ALSFRS-R	ADVERSE EVENTS	GRADE
GLASS	2012	12	4.4	1.5	ALSFRS-R; ADVERSE EVENTS	NOT RELATED	STABLE	12	2D
KARUSSIS	2010	19	2.9	25	ADVERSE EVENTS	NOT RELATED	FALL LIGHT	11/FEVER	2D
BLANQUER	2012	11	1.8	6	ADVERSE EVENTS/ MOTONEURONS	NOT RELATED	STABLE	11- PAIN AND PARESTHESIA	2D
PRABHAKAR	2012	10	1.5	24	SURVIVAL	NOT RELATED	DECLINE AFTER 3 MONTHS	NO	2D
GAMEZ	2010	12	2.24	47.2	SURVIVAL/TRACHEO/GASTRIC TUBE	48 MONTHS	DECLINING EQUAL TO CONTROL	1- FEVER, IMPAIRED CONSCIOUSNESS	2D
MARTINEZ	2009	10	2	19	SURVIVAL/TRACHEO/GASTRIC TUBE	66 MONTHS	IMPROVEMENT IN 1 TO 2 MONHTS AND AT 6 MONTHS	NO	2C
RILEY	2013	6	3.7	6	ADVERSE EVENTS	NOT RELATED	FALL	2- HEMORRHAGE	2D
MAZZINI	2009	10	0.7	24	ADVERSE EVENTS/MUNE	NOT RELATED	FALL	7-PAIN 6-LOSS SENSITIVE	2D
DEDA	2009	13	2.6	12	ADVERSE EVENTS	NOT RELATED	NOT ASCERTAINED	NO	2D
TARELLA	2010	24	1	12	ADVERSE EVENTS	NOT RELATED	NOT ASCERTAINED	1 PROLACTINOMA E 1 TVPO	2C
RILEY	2012	12	3.1	18	ADVERSE EVENTS	NOT RELATED	NOT ASCERTAINED	HEMORRHAGE - 2 REOPERATED	2C
MAZZINI	2012	19	1.9	108	ADVERSE EVENTS/MORTALITY	52,5 MONTHS	6 PATIENTS FOR 74 MONTHS STABILIZED	NO	2D
SHARMA	2015	37	NOT RELATED	60	SURVIVAL	IMPROVED 87.76 (10.45) X 57.38 (5.31)	FALL	MINOR SIDE EFFECTS	2D

In 2009, Martinez et al.(28) performed a stereotactic autologous transplantation of CD133 (+) mononuclear cells into the frontal cortex, noting that the procedure is safe and well tolerated in patients with ALS. The survival time of the treated patients was statistically higher ($p = 0.01$) than that of the control group. In that same year, Mazzini et al.(29) and Deda et al.(30) injected autologous bone marrow cells into the anterior horn of the thoracic and cervical spinal cord, reporting pain and sensory loss as adverse effects; there was no significant functional decline compared to the control group, which confirmed the safety of the procedure.

An observational study conducted in Spain in 2010(31) in which the use of cell therapy in 12 patients was evaluated found no beneficial effect. In that same year, two authors (32,33) reported the effects of simultaneous intravenous and intrathecal space injections of autologous bone marrow cells(32). One of them used granulocyte colony-stimulating factor (G-CSF) before the procedure(33) in a study of 24 patients. Although the study addressed issues of feasibility and safety, there were signs of clinical improvement that were associated with cell mobilization following the use of G-CSF. The authors conclude that prolonged monitoring and a greater number of patients are necessary.

Recently, three separate studies (34–36) showed that the use of cells derived from fetal spinal cord or umbilical cord for microinjection into the lumbar spine after laminectomy were associated with immunosuppression. There was hemorrhage in 4

patients without an associated functional deterioration; the adverse effects were attributed to immunosuppression. Mazzini et al. used autologous mononuclear bone marrow cell transplantation with a 9-year follow-up and observed stabilization of FVC and ALSFRS-R scores(29). However, the percentage of young individuals in that study who had a better prognosis (above 60%) has been reported as a confounding variable.

In a retrospective analytical study, Sharma et al.(37) reported a better survival with the use of mononuclear stem cells; however, there were confounding factors in the experimental group, where the improvement of survival was also related to an age below 50 years and use of lithium. In this study, there were no important adverse events.

The remaining studies (31,38,39) also reported good tolerance to microinjections of fetal or autologous mesenchymal cells. In conclusion, only two studies (29,37) showed improvement of survival, but with an unsatisfactory quality.

Discussion

Stem cell therapy is a promising potential treatment option for ALS, given the remarkable plasticity of stem cells and their ability to differentiate into multiple neuronal lineages. Stem cells can be used as important models for molecular pathway studies, drug screening, and cell therapy studies. Notably, there are two actual clinical trials, NeuralStem(40) and BrainStorm (NCT01051882).

This systematic review uses a meta-analysis to show the efficacy of stem cell therapy in improving survival in preclinical trials, whereas the number of clinical studies is still insufficient to assess their effectiveness and demonstrates only the absence of serious adverse events. However, caution is required because most clinical studies are heterogeneous with an unsatisfactory quality. Only one study was controlled but was retrospective and with confounding factors, such as the concurrent use of lithium and the interference of age in the analysis of survival.

To replace the cells that have undergone degeneration, various sources of stem cells can be used, such as bone marrow cells, neural stem cells, mesenchymal cells, astrocyte precursor cells and pluripotent cells(41). Currently, there are basically two stem cell types for disease modeling, embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC)(42).

The assessed preclinical trials tend to use relatively young mutant SOD1G93A mice in homogeneous groups in a controlled environment in which the animals showed a similar clinical condition. Although animal models are very useful for mimicking human diseases, they have limitations because they present with a distinct disease progression and show diverse responses in trials with drugs(3,43). Moreover, the sample size and sex of the animals often vary between studies.

According to some authors(6,43), there are concerns about translating preclinical studies into effective human treatments. In preclinical studies, SOD1 animal models represented familial ALS more than sporadic ALS. In addition, ALS can be defined as a syndrome in which the pathophysiological mechanisms are poorly understood(4), and it is possible that familial and sporadic ALS differ in some fundamental mechanisms that determine the effectiveness of treatments.

Recent studies with ESCs have shown that the use of cell therapy to substitute motor neurons is not sufficient to impede the neurodegenerative process. In addition to the neuronal mechanisms, the toxic environment provided by glial cells contributes to motor neuron death. On the other hand, these findings have been limited to only one gene, SOD1, but the disease involves multiple pathways involving other genes such as C90ORF72, TDP-43, FUS and cytoplasmic aggregates, suggesting an underlying convergence of cellular processes(44,45).

Embryonic stem cells (ESCs) are found in the blastocyst and can differentiate into oligodendrocytes, astrocytes or neurons. Hematopoietic stem cells from the bone marrow (HSCs or MSCs) produce mesenchymal cells, as well as blood cells, which are also found in adipose tissue, umbilical cord, placenta and fetal tissues. According to Vercelli(46), mesenchymal cells can migrate to the spinal cord of mice, where they have neuroprotective actions, such as preventing the activation of microglia and the process of tissue gliosis and improving the count of motor neurons, which explains the positive results observed in all of the animal studies and the trend observed in human studies.

Another option for transplantation could be the use stem cells that are derived from the olfactory epithelium (OECs). OECs continue to multiply during the postnatal period, are multipotent, and serve as conductive connections between the central and peripheral nervous systems (47). In 2008, a clinical trial of 35 patients was conducted

(48) and found that olfactory cell transplantation may slow disease progression. OEC transplantation for ALS has been performed in China with positive effects in spinal cord injury studies, such as axonal regeneration, remyelination and functional improvements. Although a large Chinese study reports that OECs may offer a benefit to ALS, other reports criticize the observed outcomes and do not support the clinical translation of this therapeutic approach at this time.

The histological analyses performed in all of these cell therapy studies show an improved animal survival, which supports the potential of this approach for neuroprotection with greater tissue preservation. However, among both cell therapy preclinical trials and cell therapy clinical trials, there were no studies with negative results, which may indicate a possible publication bias.

The development of induced pluripotent stem cells (iPSCs) has led to remarkable changes in stem cell science. This technology has made it possible to obtain pluripotent stem cells directly from a patient's adult cells. These cells are usually induced to form embryonic bodies and subsequently form neural precursor cells (NPCs)(42), which holds new promise for the treatment of neurodegenerative diseases.

However, studies have shown that there are many similarities between iPSCs and ESCs, such as telomere renewal during cell reprogramming into iPSCs and telomere shortening upon differentiation into somatic cells(49). This similarity suggests that iPSCs could potentially be used as patient-specific ESCs, consequently preventing rejection and eliminating any ethical issues.

These recent studies in humans with ALS are summarized in Table 1 and include many differences, such as the number of patients, cell type, and delivery method and outcome measurement strategies; however, each study has the potential to contribute to increasing our current understanding of the safety and feasibility of stem cell therapies for ALS. These studies were considered to be of low quality because of biases.

Moreover, in most studies, the cell therapy procedure was uncontrolled and performed in patients with a very advanced stage of disease. The disease onset was variable and frequently prolonged at 2.32 ± 1.1 years. Most authors agree that the treatment must be performed early in the course of the disease(42). The goal of most of the studies was to assess adverse events and tolerability to treatment.

Guidelines have been introduced(50) that should reduce the number of false positives in preclinical studies and therefore prevent unnecessary clinical trials, which have occurred for various drugs. These recommendations include the following: 1) rigorously assessing an animals' physical and biochemical characteristics with respect to human disease; 2) characterizing disease symptoms and the occurrence of death and being alert to unexpected variations; and 3) creating a mathematical model to address questions about the experimental design, such as the number mice that must be included in a study. To reduce concerns about animal research, Perrin(43) suggested excluding irrelevant animals, balancing for gender, avoiding the use siblings in the same treatment group, and tracking genes that induce non-inherited disease.

In conclusion, ALS is a rare heterogeneous disease that is still poorly understood in terms of its pathophysiology. Moreover, from a clinical point of view, ALS is difficult to manage. Preclinical studies of stem cell therapy show great efficacy, whereas more prospective and controlled studies are needed to establish the effectiveness of clinical studies in improving survival. Nonetheless, the most effective cell type to be used in transplantation must be determined, and it should be the one that shows better potential for neurogenesis and not only neuroprotective mechanisms.

References

1. Gordon PH. Amyotrophic Lateral Sclerosis: An update for 2013 Clinical Features, Pathophysiology, Management and Therapeutic Trials. Aging Dis 4(5):295–310.

2. Chiò a., Logroscino G, Traynor BJ, Collins J, Simeone JC, Goldstein L, et al. Global epidemiology of amyotrophic lateral sclerosis: A systematic review of the published literature. Neuroepidemiology 2013;41(2) 118–30.

3. Marin B, Gil J, Preux PM, Funalot B, Couratier P. Incidence of amyotrophic lateral sclerosis in the Limousin region of France, 1997-2007. Amyotroph Lateral Scler 2009;10: 216-20.

4. Swinnen B, Robberecht W. The phenotypic variability of amyotrophic lateral sclerosis. Nat Rev Neurol. Nature Publishing Group; 2014;10(11):661–70.

5. D'Amico E, Factor-Litvak P, Santella RM, Mitsumoto H. Clinical perspective on oxidative stress in sporadic amyotrophic lateral sclerosis. Free Radic Biol Med 2013;65:509–27.

6. Mancuso R, Navarro X. Progress in Neurobiology Amyotrophic lateral sclerosis : Current perspectives from basic research to the clinic. Prog Neurobiol 2015;133:1–26.

7. Miller RG, Mitchell JD, Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). Cochrane database Syst Rev 2012;3:CD001447.

8. Miller RG, Rosenberg J a, Gelinas DF, Mitsumoto H, Newman D, Sufit R, et al. Practice parameter: The care of the patient with amyotrophic lateral sclerosis (An evidence-based review). Muscle Nerve. 1999;22(8):1104–18.

9. Lunn S, Sakowski SA, Feldman EL. Stem cell therapies for amyotrophic lateral sclerosis: Recent advances and prospects for the future J Stem Cells 2015;32(5):1099–109.

10. Ludolph A, Drory V, Hardiman O, Nakano I, Ravits J, Robberecht W, et al. A revision of the El Escorial criteria - 2015. Amyotroph Lateral Scler Front Degener 2015;(April):1–2.

11. Costa J, Swash M, de Carvalho M. Awaji criteria for the diagnosis of amyotrophic lateral sclerosis:a systematic review. Arch Neurol 2014;69(11):1410–6.

12. Puhan MA, Schunemann HJ, Murad MH, Li T, Brignardello-Petersen R, Singh JA, et al. A GRADE Working Group approach for rating the quality of treatment effect estimates from network meta-analysis. Bmj 2014;349(sep24 5):g5630–g5630.

13. 18- Gu R, Hou X, Pang R, Li L, Chen F, Geng J, et al. Human adipose-derived stem cells enhance the glutamate uptake function of GLT1 in SOD1G93A-bearing astrocytes. Biochem Biophys Res Com 2010;393:481–6.

14. Xu L, Shen P, Hazel T, Johe K KV. Dual transplantation of human neural stem

cells into cervical and lumbar cord ameliorates motor neuron disease in SOD1 transgenic rats. Neurosci Lett. 2011;494(3):222–6.

15. Boido M, Piras A, Valsecchi V, Spigolon G, Mareschi K, Ferrero I, et al. Human mesenchymal stromal cell transplantation modulates neuroinflammatory milieu in a mouse model of amyotrophic lateral sclerosis. Cytotherapy 2014;16:1059–72.

16. Dadon-Nachum M, Ben-Yaacov K, Ben-Zur T, Barhum Y, Yaffe D, Perlson E, et al. Transplanted Modified Muscle Progenitor Cells Expressing a Mixture of Neurotrophic Factors Delay Disease Onset and Enhance Survival in the SOD1 Mouse Model of ALS. J Mol Neurosci 2015;55(3):788-97.

17. Kim H, Kim HY, Choi MR, Hwang S, Nam KH, Kim HC, et al. Dose-dependent efficacy of ALS-human mesenchymal stem cells transplantation into cisterna magna in SOD1-G93A ALS mice. Neurosci Lett 2010;468(3):190–4.

18. Bigini P, Veglianese P, Andriolo G, Cova L, Grignaschi G, Caron I, et al. Intracerebroventricular administration of human umbilical cord blood cells delays disease progression in two murine models of motor neuron degeneration. Rejuvenation Res 2014;14(6):623–39.

19. Uccelli A, Milanese M, Principato MC, Morando S, Bonifacino T, Vergani L, et al. Intravenous Mesenchymal Stem Cells Improve Survival and Motor Function in Experimental Amyotrophic Lateral Sclerosis. Mol Med 2014;18(5):794–804.

20. Kondo T, Funayama M, Tsukita K, Hotta A, Yasuda A, Nori S, et al. Focal transplantation of human iPSC-derived glial-rich neural progenitors improves lifespan of ALS mice. Stem Cell Reports 2014;3(2):242-9

21. Garbuzova-Davis S, Rodrigues MCO, Mirtyl S, Turner S, Mitha S, Sodhi J, et al. Multiple intravenous administrations of human umbilical cord blood cells benefit in a mouse model of ALS. PLoS One 2014;7(2):e31254.

22. Hefferan MP, Galik J, Kakinohana O, Sekerkova G, Santucci C, Marsala S, et al. Human neural stem cell replacement therapy for amyotrophic lateral sclerosis by spinal transplantation. PLoS One 2014;7(8):e42614.

23. Lee HJ, Kim KS, Ahn J, Bae HM, Lim I, Kim SU. Human Motor Neurons Generated from Neural Stem Cells Delay Clinical Onset and Prolong Life in ALS Mouse Model 2014;9(5):1–9.

24. Hefferan MP, Galik J, Kakinohana O, Sekerkova G, Santucci C, Marsala S, et al. Human Neural Stem Cell Replacement Therapy for Amyotrophic Lateral Sclerosis by Spinal Transplantation. PLoS One. 2012;7(8):e42614.

25. Lee JC, Seong J, Kim SH, Lee SJ, Cho YJ, An J, et al. Replacement of microglial cells using Clodronate liposome and bone marrow transplantation in the central nervous system of SOD1(G93A) transgenic mice as an in vivo model of amyotrophic lateral sclerosis. Biochem Biophys Res Commun 2014;418(2):359–65.

26. Sun H, Hou Z, Yang H, Meng M, Li P, Zou Q, et al. Multiple systemic transplantations of human amniotic mesenchymal stem cells exert therapeutic effects in an ALS mouse model. Cell tissue Res 2015;357(3):571–82.

27. Nizzardo M, Simone C, Rizzo F, Ruggieri M, Salani S, Riboldi G, et al. Minimally invasive transplantation of iPSC-derived ALDHhiSSCloVLA4+ neural stem cells effectively improves the phenotype of an amyotrophic lateral sclerosis model. Hum Mol Genet. 2014;23(2):342–54.

28. Martinez HR, Gonzalez-Garza MT, Moreno-Cuevas JE, Caro E, Gutierrez-Jimenez E, Segura JJ. Stem-cell transplantation into the frontal motor cortex in amyotrophic lateral sclerosis patients. Cytotherapy 2009;11(1):26–34.

29. Mazzini L, Mareschi K, Ferrero I, Miglioretti M, Stecco A, Servo S, et al. Mesenchymal stromal cell transplantation in amyotrophic lateral sclerosis: a long-term safety study. Cytotherapy 2012 Jan;14(1):56-60.

30. Deda H, Inci MC, Kürekçi E, Sav A, Kayihan K, Ozgün E, et al. Treatment of amyotrophic lateral sclerosis patients by autologous bone marrow-derived hematopoietic stem cell transplantation: a 1-year follow-up. Cytotherapy 2009 ;11(1):18–25.

31. Gamez J, Carmona F, Raguer N, Ferrer-Sancho J, Martín-Henao G a, Martí-Beltrán S, et al. Cellular transplants in amyotrophic lateral sclerosis patients: an

observational study. Cytotherapy 2010;12(5):669–77.

32. Karussis D, Karageorgiou C, Vaknin- Dembinsky A, Gowda-Kurkalli B, Gomori JM, Kassis I, et al. Arch Neurol 2010;67(10):1187–94.

33. Tarella C, Rutella S, Gualandi F, Melazzini M, Scimè R, Petrini M, et al. Consistent bone marrow-derived cell mobilization following repeated short courses of granulocyte-colony-stimulating factor in patients with amyotrophic lateral sclerosis: results from a multicenter prospective trial. Cytotherapy 2010;12(1):50–9.

34. Deaconess BI, Arbor A. Lumbar Intraspinal Injection of Neural Stem Cells in Patients with Amyotrophic Lateral Sclerosis : Results of a Phase I Trial in 12 Patients. Stem Cells 2012;30(6):1144-51.

35. Riley J, Glass J, Feldman EL, Polak M, Bordeau J, Federici T, et al. Intraspinal stem cell transplantation in amyotrophic lateral sclerosis: a phase I trial, cervical microinjection, and final surgical safety outcomes. Neurosurgery. 2014;74(1):77-87.

36. Riley J, Federici T, Polak M, Kelly C, Glass J, Raore B, Taub J, Kesner V, Feldman EL BN. Intraspinal stem cell transplantation in amyotrophic lateral sclerosis: a phase I safety trial, technical note, and lumbar safety outcomes. Neurosurgery. 2012;71(2):405–16.

37. Sharma AK, Sane HM, Paranjape AA, Gokulchandran N, Nagrajan A. The effect of autologous bone marrow mononuclear cell transplantation on the survival duration in Amyotrophic Lateral Sclerosis - a retrospective controlled study. Am J Stem Cells 2015;4(1):50-65.

38. Blanquer M, Moraleda JM, Iniesta F, Gomez-Espuch J, Meca-Lallana J, Villaverde R, et al. Neurotrophic bone marrow cellular nests prevent spinal motoneuron degeneration in amyotrophic lateral sclerosis patients: A pilot safety study. Stem Cells 2014;30(6):1277–85.

39. Prabhakar S, Marwaha N, Lal V, Sharma RR, Rajan R, Khandelwal N. Autologous bone marrow-derived stem cells in amyotrophic lateral sclerosis : A pilot study. 2012;60(5):465–70.

40. Feldman EL, Boulis NM, Hur J, Johe K, Rutkove SB, Federici T, et al. Intraspinal neural stem cell transplantation in amyotrophic lateral sclerosis: phase 1 trial outcomes. Ann Neurol 2014;75(3):363–73.

41. McDonald JW, Liu XZ, Qu Y, Liu S, Mickey SK, Turetsky D, et al. Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Nat Med 1999;5(12):1410–2. /nm/journal/v5/n12/full/nm1299_1410.html

42. Coatti GC, Beccari MS, Olivio TR, Mitne-Neto M, Okamoto OK, Zatz M. Stem cells for amyotrophic lateral sclerosis modeling and therapy: Myth or fact? Cytometry Part A 2015;87(3):197–211.

43. Perrin S. Make mouse studies work. Nature 2014;507:423–5.

44. Ince PG, Highley JR, Kirby J, Wharton SB, Takahashi H, Strong MJ, et al. Molecular pathology and genetic advances in amyotrophic lateral sclerosis: An emerging molecular pathway and the significance of glial pathology. Acta Neuropathol 2011;122(6):657–71.

45. Blokhuis AM, Groen EJN, Koppers M, van den Berg LH, Pasterkamp RJ. Protein aggregation in amyotrophic lateral sclerosis. Acta Neuropathol 2013;125(6):777–94.

46. Vercelli A, Mereuta OM, Garbossa D, Muraca G, Mareschi K, Rustichelli D, et al. Human mesenchymal stem cell transplantation extends survival improves motor performance and decreases neuroinflammation in mouse model of amyotrophic lateral sclerosis. Neurobiol Dis 2008;31(3):395–405.

47. Mackay-Sim A, St John JA. Olfactory ensheathing cells from the nose: clinical application in human spinal cord injuries. Exp Neurol 2011;229(1):174–80.

48. Huang H, Chen L, Xi H, Wang H, Zhang J, Zhang F, et al. Fetal olfactory ensheathing cells transplantation in amyotrophic lateral sclerosis patients: A controlled pilot study. Clin Transplant 2008;22(6):710–8.

49. Gourronc FA, Klingelhutz AJ. Therapeutic Opportunities: Telomere Maintenance in Inducible Pluripotent Stem Cells. Mutat Res 2013;730:98–105.

50. Ludolph AC, Bendotti C, Blaugrund E, Chio A, Greensmith L, Loeffler J-P, et al. Guidelines for preclinical animal research in ALS/MND: A consensus meeting. Amyotroph Lateral Scler 2014;11(1-2):38–45.

ARTIGO 4 – ARTIGO ORIGINAL - IMPACT OF MULTIDISCIPLINARY CARE IN AMYOTROPHIC LATERAL SCLEROSIS HOSPITALIZATIONS IN THE PUBLIC HEALTH SYSTEM OF BRAZIL.

Versão publicada em inglês

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IMPACT OF MULTIDISCIPLINARY CARE IN AMYOTROPHIC LATERAL SCLEROSIS HOSPITALIZATIONS IN THE PUBLIC HEALTH SYSTEM OF BRAZIL

Abstract

There is evidence that multidisciplinary care improves quality of life and there is a growing appreciation of public policies in Brazil that favor home care with a multidisciplinary team in chronic diseases. This study aimed to determine the epidemiological profile of amyotrophic lateral sclerosis in the Federal District and the impact of tertiary reference center creation on patient care. A descriptive, cross-sectional study analyzing secondary data regarding mortality and hospitalizations for amyotrophic lateral sclerosis patients over 10 years and clinical and epidemiological profiles of patients evaluated at the Center of Reference for Neuromuscular Disease over three years was used. An incidence rate of 1.3/100,000 person-years over 20 years and an age at onset of 49.3 ± 15.1 years (Hospital Information System) and 57.2 ± 12.3 years (at the Center admission) was observed. The risk of death was greater in patients older than 75 years (RR = 4.05, p = 0.0018) and in bulbar-onset patients (RR = 2.53, p = 0.0027). Multidisciplinary care reduced 75% of hospitalization frequency and length of stay (p = 0.03) and 80 to 90% of the reimbursement value of hospitalization (p = 0.05). The adoption of multidisciplinary care has improved the efficiency of patient care for amyotrophic lateral sclerosis in the Brazilian public health system.

KEY WORDS: amyotrophic lateral sclerosis; multidisciplinary care; public health

Introduction:

Amyotrophic Lateral Sclerosis (ALS) has an average worldwide incidence of 2.08 / 100,000 person-years (Chiò and cols, 2013) and is a disease of unknown etiology that leads to motor disability, speech disorders, dysphagia and respiratory failure. The median survival time ranges from 24 to 48 months (Turner and cols 2013, Forbes and cols 2004).

In the last twenty years, numerous clinical trials with drugs and cell therapies have been conducted, but only Riluzole has shown efficacy in slowing the progression of symptoms (Hardiman and cols, 2011).

Since 1989, there is a large body evidence that shows multidisciplinary care improves quality of life, but there is still doubt as to if it increases survival time (Andersen and cols 2012, Miller and cols 1999). In European countries, this is well established, however, there is lack of evidence on the impact of the multidisciplinary treatment of ALS in Latin America, especially in relation to the public health system of Brazil.

There is currently a growing appreciation of public policies in Brazil that favor home care with a multidisciplinary team in non-specific chronic diseases (Leopoldina and cols, 2015).

The objective of this study was to determine the incidence of ALS, the epidemiological and clinical profiles of patients and their survival at three years of follow-up, analyzing the efficiency of a tertiary Reference Center for Neuromuscular Diseases (CRDN) in the Public Health System (SUS) in the Federal District, Brazil.

Method:

In September 2011, the CRDN was created; a multidisciplinary unit composed of Medical Neurologists, Pulmonologists, Physical Therapists, Occupational therapists, Psychologists, Nutritionists and Speech Therapists. The goals included: guiding patients and their families; reducing complications of immobility, dysphagia and respiratory failure; implementing the home ventilation program; and giving support to professionals

of the home care program (PID). In April 2013, the Secretariat of Health of Federal District (SES-DF) approved a dehospitalization protocol and started hiring home care, restricting the length of hospitalization of chronic patients in the ICU.

A cross-sectional, descriptive study was performed, using three data sources:

Mortality data were obtained using the TabWin application from the Mortality Information System (MIS) of the Federal District, Brazil, between January 2005 and December 2014, to estimate the incidence of disease. We used the 2010 Census (IBGE, 2014) for calculating incidence.

Admissions data were obtained using the TabWin application from the Reduced Hospitalar Information System (HIS) of the Federal District, between January 2005 and December 2014, related to hospital admissions in the SUS.

Records from the CRDN at the SES-DF were analized through the TrakCare software application, with data obtained between September 2011 and December 2014. Probable and defined ALS with diagnosis performed by neurologists using El Escorial criteria (Ludolph and cols, 2015) after exclusion of other diseases, were included. All patients used Riluzole and underwent electromyography.

The following parameters were analyzed: disease frequency, gender distribution, site of onset, age, diagnostic delay time, duration of disease, electromyography confirming the diagnosis, frequency and length of stay, reimbursements of Hospitalar Admission Authorizations (AIH), use of the Intensive Care Unit (ICU) in the SUS, and use of domiciliary mechanical ventilation.

The disease duration was defined as the time period between the first symptom and death or tracheostomy (in days).

Efficiency measures were frequency, length of stay, reimbursement of AIH and ICU costs.

Data were recorded in Excel 2010 and statistical analyses were performed using SAS 9.3 and SPSS19.0 applications.

Chi-square test was used for categorical variables and the two-tailed Student's t test for quantitative variables, with a 0.05 significance level. Multivariate analyses of

survival including all clinical variables was conducted. Initially, Cox regression univariate analysis with p <0.25 was selected for inclusion in the multivariate Cox regression analysis. The final multivariate regression model was built by successive exclusion variable-to-variable from the initial multivariate model, using the likelihood ratio test to determine the importance of each variable excluded (Collett, 2003). The level of significance was 0.05. The survival functions for the patients were estimated using the Kaplan-Meier method and compared using the log-rank test.

This study protocol was approved by the Ethics Committee of the Research and Education Foundation in Health Sciences - FEPECS / SES, number 820 117/2014 Protocol. The Ethics Committee waived the written consent form because the study consisted of analysis of patient records (many of them were deceased) and public domain databases.

Results:

To estimate the regional incidence of ALS in ten years, we used mortality data (MIS). Between 2005 and 2014, the MIS of the Federal District recorded 162 deaths above 20 years of age: 93 men and 69 women with mean ages of 61.5 ± 12.6 years and 65.1 ± 12.2 years, respectively, which was not significantly different (p = 0.059).

So, the adjusted incidence of the disease above 20 years of age was estimated in 1.3 / 100,000 person-years and 2.6 / 100,000 person-years, adjusted above 45 years of age, considering the population of 2,570,160 inhabitants, according to the 2010 Census of the Brazilian Institute of Geography and Statistics - IBGE[16]. It was observed that 97 (59.9%) deaths occurred between 60 and 79 years of age (data not shown).

In the same 10-year period, the HIS recorded 172 hospitalar admissions with the same diagnosis: 103 men and 69 women, with a mean age of 49.3 ± 15.1 years. In females, the average age was 45.6 ± 13.9 years, significantly lower than the average male age of 51.7 ± 15.4 years (p = 0.01).

Figure 1 compares the ALS incidence mortality-based with the hospitalar admissions and shows that the frequency of ALS hospitalization has fallen by 75% between 2012 and 2014, while the incidence based on mortality was sustained. Between 2005 and 2011, the average annual rate of hospitalizations was 21.6 ±5,6 hospitalizations/year (range:15-27) . Between 2012 and 2014, this average

dropped to 7±1,7 admissions per year, with a range of 5-8 (p = 0.03). The average length for an in-hospital stay was 17. 1±3. 2 days in the first period and 15.± 2.5 days in the second, which was not significant (p = 0.48).

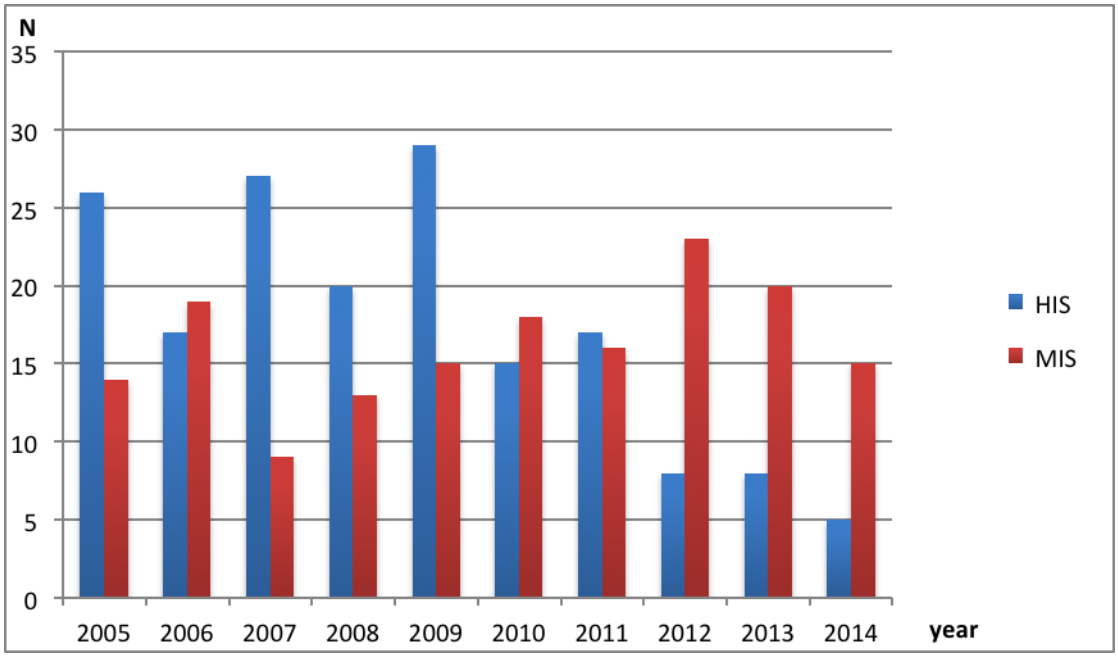

Figure 1 – Correlation between mortality (MIS) e frequencies of admissions (HIS) in Distrito Federal due to Amyotrophic Lateral Sclerosis. Period: 2005 to 2014.

Table 1 shows the annual change in frequency, length of stay and reimbursement amounts to the AIH, and amounts transferred by the SUS for ICU spending. It was observed that the higher frequency of hospitalizations occurred in the years 2009, 2007 and 2005 and there was a sharp decline between the years 2012 and 2014. Although the average length of stay did not show significant variation, the total days of hospitalization followed the 75% drop observed in frequency (p = 0.03).

The total SUS spending on AIH reimbursement also suffered a sharp decline, reaching 80-90% of all median and initial totals (Table 1).

In 2005, the average cost per patient was R$1,031, and the total sum was R$ 26,826. In 2014, a drop in AIH reimbursement was observed, with an average of R$447(p= 0,26), and a total of R$2,236.If we compare the period until 2011, the mean total AIH reimbursement was 22,780 ±14,599 and the mean cost of the second period

(2012-2014) was 3,416±1,550 (p=0,05). With regard to gender, there was no significant difference between spending with AIH (p = 0.79) or with ICU use (p = 0.81).

The period between September 2011 and 2014 was evaluated in the CRDN and included 135 patients with probable or definite diagnosis of the disease. There were 78 (57.8%) men and 57(42.2%) women, with male-female ratio of 1.4:1. The mean age was 57.2 ± 12.3 years, ranging from 25 to 86 years. The average age was 56.3 ± 12.3 years in males and 58.6 ± 12.3 years in females, with no difference between the sexes (p = 0.179).

Table 1 – Frequency, length of stay and reimbursement of hospitalization in Motor Neuron Disease in SUS of Distrito Federal, Brazil. Period: 2005 to 2014.

Year	N	length of stay(avg) days	SD	Length of stay (sum)	ICU (ammount R$)	ICU (avg R$)	AIH (avg R$)	AIH (total ammount R$)
2005	26	15.3	18.4	399	10,089	388	1,031	26,826
2006	17	17.8	23.6	303	0	0	602	10,250
2007	27	11.8	11.1	320	0	0	588	15,897
2008	20	15.4	12.8	309	0	0	628	12,571
2009	29	17.4	18.2	504	0	0	562	16,313
2010	15	20	23.2	300	14,361	957	1,645	24,681
2011	17	21.7	28	369	44,042	2,590	3,113	52,921
2012	8	16	10.9	128	0	0	646	5,173
2013	8	12,7	18,7	102	0	0	355	2,841
2014	5	17,8	10,6	89	0	0	447	2,236
Total	172	-	-	2,823	68,492	-	-	169,712

The distribution of patients on admission to the CRDN according to the El Escorial criteria is presented in Table 2. It was observed that 79 patients (58.5%) had a definite form of the disease and in 61 patients (45.6%) the site of onset was in the upper limb. Additionally, according to the table 2, 92 (68.1%) patients had diagnostic confirmation at admission (definite and probable forms), whereas in 43(31.9%) of them , the diagnosis was confirmed during follow-up. The median time to diagnosis from the first symptoms was 22.7 months in men and 23.5 months in women.

Table 2 – Patients profile at CRDN (Period: sep 2011 - dec 2014)*El Escorial Classification of ALS patients on admission . NIV: Non-invasive ventilation; IV: invasive ventilation; NV: no ventilation.

	Onset N (%)			
	Bulbar	**Leg**	**Arm**	**Total**
Possible*	8(24,2)	12(36,4)	12(39,4)	33(24,5)
Definite *	12(15,2)	28(35,4)	39(49,4)	79(58,5)
Probable *	1(7,1)	5(42,9)	7(50)	13(9,6)
Suspect *	5(50)	2(20)	3(30)	10(7,4)
NIV	3(14,3)	8(38,1)	10(47,6)	21(15,5)
IV	3(25)	1(8)	8(67)	12(8,9)
NV	20(19,6)	40(39,2)	42(41,2)	102(75,6)
Total	26(19,1)	48(35,3)	61(45,6)	135(100)

Table 2 also shows the frequency of home mechanical ventilation use. It was observed that a majority 102 (75.6%) of the patients did not use mechanical ventilation, while 21(15.5%) patients used non-invasive ventilation and only 12 (8.9%) used invasive ventilation by tracheostomy. There was no statistically significant difference between the use of ventilation and the form of onset.

Electroneuromyography helped confirm the diagnosis in 119 patients (88.1%), but in cases initially classified as suspected disease, the exam failed in 5 patients (50%). In patients with bulbar-onset, the exam was negative in 4 patients (15.4%) and in those with upper limb-onset, it failed in 10 patients (16.9%).

All patients received guidelines from the multidisciplinary team in at least two consultations, with a range of three to four months. Thirty-six patients (26.5%) received between 3 and 6 consultations.

Figure 2 shows the survival curve of patients according to age. In the log-rank test, it was observed a survival curve of an age group that differs from the others (p = 0.0043). With the Bonferroni adjustment, it was noted that the survival time for patients aged below 50 years was significantly higher than the survival time for patients over 75 years. For patients younger than 50 years old, approximately 83% were still alive one year after the start of follow-up, while only 22% of patients over 75 years of age were still
alive.

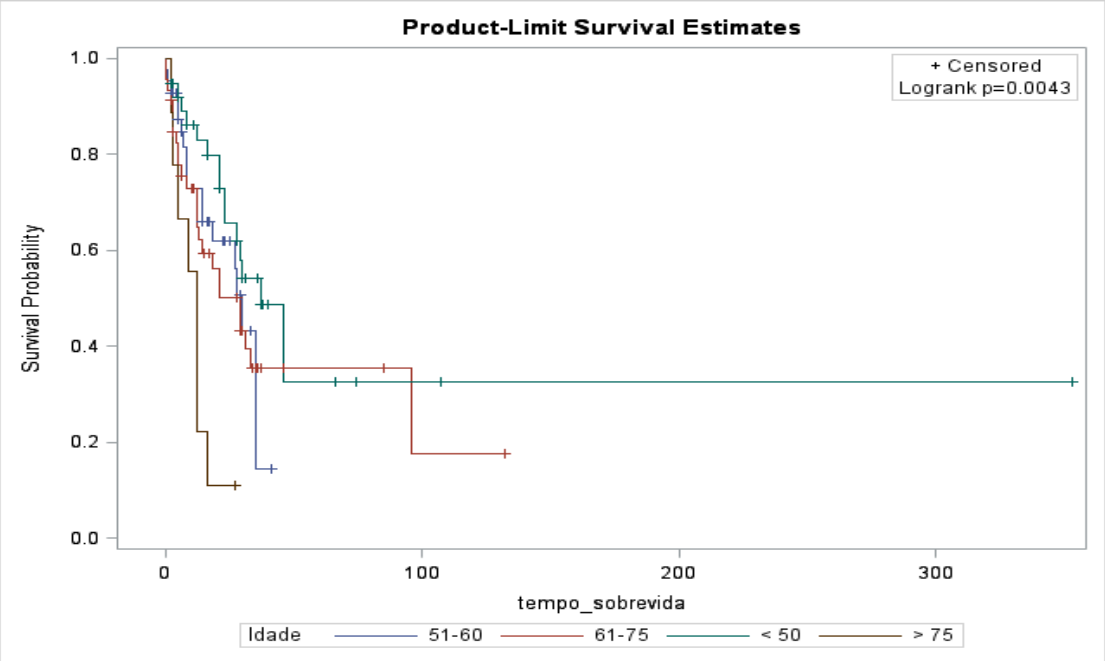

Figure 2 – Survival Kaplan Meier Analysis of 135 ALS patients evaluated in CRDN according to age of onset. Period: sep 2011 to dec 2014.

Table 3 shows the clinical features associated with lower survival in the group over 75 years of age. When analyzing the unadjusted hazard ratio, the following variables were not statistically significant and were excluded: sex, pyramidal syndrome, dementia, smoking, alcohol consumption, use of pesticides, heavy metals, clinical and diagnosis of ALS (data not shown). The multivariate Cox regression model showed that

only the age of onset and shape were significant risk factors for survival time. The risk of death among patients older than 75 years was approximately four times higher (RR = 4.05, p = 0.0018) than among patients younger than 50 years.

However, the risk of death in patients in the age groups 51-60 and 61-75 years did not differ significantly compared to patients younger than 50 years. The risk of death for patients with the early bulbar form of ALS was approximately two and a half times (RR = 2.53, p = 0.0027) that of patients with the early spinal form of ALS.

Table 3 –Crude and adjusted Risk Ratio for survival in Amyotrophic Lateral Sclerosis, by clinical selected variables in patients under multidisciplinar care and Riluzole. Period: set 2011 - dez 2014 [a] Adjusted for age and site of onset.

	Risk Ratio - RR (CI 95 %)			
	Crude (CI)	**p-value**	**Adjusted[a] (CI)**	**p-value**
Age (years)		0,0212		0,0135
≤ 50	1	-	1	-
51 - 60	1,54 (0,78 – 3,02)	0,2129	1,21 (0,60 – 2,44)	0,4854
61 – 75	1,60 (0,86 – 2,96)	0,1383	1,54 (0,81 – 2,91)	0,188
> 75	4,48 (1,88 – 10,72)	0,0007	4,05 (1,69 – 9,75)	0,0018
Onset		0,0052		0,0027
Spinal	1	-	1	-
leg	**1**	**-**	**1**	**-**
arm	1,08 (0,62 – 1,87)	0,7935	1,25 (0,69 – 2,27)	0,4615
Bulbar	2,43 (1,36 – 4,33)	0,0052	2,53 (1,38– 5,62)	0,0027
Diagnostic delay time (months)		0,0334	-	-
≤ 12	2,11 (1,12 – 3,96)	0,0205	-	-
13 - 24	1,96 (1,05 – 3,66)	0,034	-	-
> 24	1	-	-	-

Discussion:

Estimating disease incidence is important for determining the size of public health services. Prospective epidemiological studies based on case records associated with the use of secondary databases, such as mortality, using capture-recapture techniques are the ideal approach (Bresch and cols, 2015, Chiò and cols, 2013). However, the record of incident cases requires the existence of reference centers with the formation of a registration basis. In the absence of such, mortality data was used, which produced an incidence rate of the disease at 1.3 / 100,000 person-years over 20 years of age and 2.6 / 100,000 person-years over 45 years of age, which were similar to other studies (Chiò and cols, 2013). The use of adjusted rates by age is necessary because the incidence of ALS is more common over 45 years of age and because approximately 38% of the inhabitants of the Federal District are under 20 years of age (IBGE, 2014).

Mean age at hospitalization (49.3 ± 15.1 years), at death by disease (61.5 ± 12.6) and at admission to the Reference Center (57.2 ± 12.3 years) were significantly lower than the average age of onset of the disease observed in European countries, which is between 61 and 66.2 years (Chiò and cols 2009). These findings are corroborated by other studies in Latin American populations (Valenzuela and cols, 2015, Vázquez and cols, 2008, Zaldivar and cols, 2009).

The frequency of hospitalizations from disease has fallen by 75% between 2012 and 2014, after the creation of CRDN, despite the increased incidence-based mortality rate from the disease. There are studies showing improved quality of life (Andersen and cols, 2012,Miller and cols, 1999, Traynor and cols, 2003) from over the past decade, but it is still uncertain whether there is increased survival (Aridegbe and cols, 2012, Rodríguez de Rivera and cols, 2011) of ALS patients with the multidisciplinary care. This includes physical and occupational therapy to preserve patient autonomy, respiratory therapy for elimination of airway secretions and strengthening cough, nutritional support and speech therapy associated with assistive technologies (Andersen and cols, 2012, Miller and cols, 1999, Venkova-Hristova and cols, 2012).

Regarding the length of stay, the global average length of stay was 16.4 ± 18.4 days, with no significant change during the study period. Hospitalizations should be avoided whenever possible because both higher frequency and longer durations

predispose the patient to increased risk of infections and greater immobility, contributing to increased risk of respiratory complications (Miller and cols, 1999).

It was also observed that reduced financial expenses with hospitalizations as a decline in AIH reimbursements, which were reduced in their average sum, reaching approximately 10-20% of the initial average and total value, according to Table 2. Evaluate the AIH reimbursement may be useful and has been be used as an indirect measure of cost in Brazilian public health system, although it does not take into account the total direct and indirect costs of a health system (Bertó and cols, 2012), only the monetary reimbursement made by SUS.

The risk of death among patients older than 75 years was approximately four times higher (RR = 4.05, p = 0.0018) than among patients younger than 50 years. The risk of death for patients with the early bulbar form was approximately two and a half times (RR = 2.53, p = 0.0027) that of patients with early spinal form (Table 4). These findings are consistent with other studies (Aridegbe and cols, 2012, Turner and cols, 2010).

The ALS practice parameter of the American Academy of Neurology (AAN) recommended that, at an early stage of the disease, patients be sent to a multidisciplinary specialized center to assess their individual needs and to focus on their quality of life (Miller and cols, 1999). The time from diagnosis to referral for the CRDN was long (22.7 months in men and 23.5 months in women), surpassing the time described in European studies, where the average time to diagnosis was approximately 10 months (Chiò and cols, 2009).

There is great discussion about the adoption of the criteria El Escorial and Awaji in electroneuromyography for the diagnosis of ALS (Carvalho, 2009, Ludolph and cols, 2015). In cases initially classified as suspect and bulbar-onset, the exam did not help the diagnosis in 50% and 15.4% of cases, respectively, despite incorporating the Awaji criteria. This is confirmed by other studies showing, primarily in the bulbar form, that the procedure has a sensitivity of 16-19.5% (Bresch and cols, 2014, Okita and cols, 2011). In patients without diagnostic confirmation, follow-up in a specialized center has the objective of assisting with further diagnostic confirmation, based on clinical evolution, without delaying the introduction of multidisciplinary care.

A study conducted in England in 2012 with Cox regression in 437 patients with ALS showed that follow-up in specialized reference centers in relation to general outpatient care is an independent positive predictive factor for improved survival (Aridebge and cols, 2012). Another study conducted in Madrid claims that the specialized centers increase survival even in the bulbar-onset form because the treatment is provided early. Other recent European studies corroborate these findings (Chiò and cols, 2006, Miller and cols, 2009, Pouget and cols, 2013, Rodríguez de Rivera and cols, 2011, Traynor and cols, 2003).

In Barcelona the program for the treatment of ALS adds multidisciplinary teams to home care teams, considering the evolution of the disease, because of increased difficulties in walking to specialized centers (Guell and cols, 2013). In recent years, there has been significant growth in the scope of home care teams in the Federal District, which enabled this joint action (Leopoldina and cols, 2015).

Despite several clinical trials of drugs and cell therapy, ALS remains an incurable disease (Hardiman and cols, 2011). Respiratory failure is the natural course of the disease and advocates for the use of Non-Invasive Ventilation (NIV) (Andersen and cols, 2012, Miller and cols, 1999). In July 2008, the Ordinance of the Brazilian Ministry of Health (Brasil, 2008), recognizing the need for deinstitutionalization of patients, established the Program of Noninvasive Ventilator Assistance to Neuromuscular Disease Carriers, but did not create sources of funding for it.

Invasive ventilation by tracheostomy is always a second choice and must be requested by the patient, except for young patients with the early bulbar form of disease and inability to use NIV (Heritier and cols, 2013). A study conducted in Italy (Volanti and cols, 2011) with 44 patients with ALS showed that there are difficulties in the acceptance and adaptation of NIV equipment, which make use difficult, but it can be overcome by intensive training in multidisciplinary centers.

It was observed that there was a low frequency of use of home mechanical ventilation in this study, with 15.5% of patients using NIV and 8.9% using invasive ventilation by tracheostomy. In 2009, an extensive study conducted with ALS CARE database involving 5,600 patients in North America between 1997 and 2004 found that only 21% of patients used NIV (Miller and cols, 1999). More recent studies from

multidisciplinary and well-established centers showed greater adherence to the use of NIV, reaching 64.28% in early bulbar forms and 35.71% to 79% in the early spinal forms (Heritier and cols, 2013, Rodríguez de Rivera and cols, 2011, Volanti and cols, 2011).

Conclusion:

The incidence rates of ALS in the Federal District population was 1.3 / 100,000 person-years, adjusted for the population aged over 20 and 2.6 / 100,000 person-years for the population over 45.

Treatment by a multidisciplinary team showed increased efficiency with reduced hospitalization rates, length of stay and AIH reimbursement.

Thus, under the public health systems, the adoption of a comprehensive national public care policy for patients with ALS is suggested along with the creation of multidisciplinary reference centers combined with a deinstitutionalization policy of the Home Care Program (PID) and Non-Invasive Ventilation Program Homecare.

References

1. Andersen PM, Abrahams S, Borasio GD, de Carvalho M, Chio A, Van Damme P, et al. EFNS guidelines on the Clinical Management of Amyotrophic Lateral Sclerosis (MALS) - revised report of an EFNS task force. Eur J Neurol 2012;19(3):360–75.

2. Aridegbe T, Kandler R, Walters SJ, Walsh T, Shaw PJ, McDermott CJ. The natural history of motor neuron disease: Assessing the impact of specialist care. Amyotroph Lateral Scler 2012;(March 2012):1–7.

3. Bertó DJ BR. Gestão de custos e resultado na saúde. 5a ed. Sao Paulo-SP: Saraiva; 2012. 1-267 p.

4. Brasil Ministério da Saúde. Portaria MS 1370/2008 [Internet]. Available from: http://bvsms.saude.gov.br/bvs/saudelegis/sas/2008/prt0370_04_07_2008.html Last Accessed(07-04-2015)

5. Bresch S, Delmont E, Soriani MH, Desnuelle C. Apport de l'électromyogramme dans le diagnostic précoce des SLA à début bulbaire: comparaison des critères

d'El Escorial, d'El Escorial modifiés et d'Awaji. Rev Neurol (Paris) 2014;170(2):134–9.

6. Carvalho M De, Swash M. Awaji diagnostic algorithm increases sensitivity of El Escorial criteria for ALS diagnosis. Amyotroph Lateral Scler 2009;10(1):53–7.

7. Chiò a, Bottacchi E, Buffa C, Mutani R, Mora G. Positive effects of tertiary centres for amyotrophic lateral sclerosis on outcome and use of hospital facilities. J Neurol Neurosurg Psychiatry 2006;77(8):948–50.

8. Chiò A., Logroscino G, Traynor BJ, Collins J, Simeone JC, Goldstein LA., et al. Global epidemiology of amyotrophic lateral sclerosis: A systematic review of the published literature. Neuroepidemiology 2013;41(2):118–30.

9. Chiò A., Mora G, CalvoA., Mazzini L, Bottacchi E, Mutani R. Epidemiology of ALS in Italy: A 10-year prospective population-based study. Neurology 2009;72(8):725–31.

10. Costa J, Swash M, de Carvalho M. Awaji criteria for the diagnosis of amyotrophic lateral sclerosis:a systematic review. Arch Neurol 2012 Nov;69(11):1410-6.

11. Collett D. Modelling Survival Data in Medical Research. 2003. 2nd ed. Chapman and Hall/ CRC. Boca Raton, Florida.p. 1–390.

12. Forbes RB, Colville S, Cran GW, Swingler RJ. Unexpected decline in survival from amyotrophic lateral sclerosis/motor neurone disease. J Neurol Neurosurg Psychiatry 2004;75(12):1753–5.

13. Güell MR, Antón A, Rojas-García R, Puy C, Pradas J. Atención integral a pacientes con esclerosis lateral amiotrófica: Un modelo asistencial. Arch Bronconeumol 2013;49(12):529–33.

14. Hardiman O, van den Berg LH, Kiernan MC. Clinical diagnosis and management of amyotrophic lateral sclerosis. Nat Rev Neurol 2011;7(11):639–49.

15. Heritier Barras AC, Adler D, Iancu Ferfoglia R, Ricou B, Gasche Y, Leuchter I, et al. Is tracheostomy still an option in amyotrophic lateral sclerosis? Reflections of a multidisciplinary work group. Swiss Med Wkly 2013;143:1–9.

16. Instituto Brasileiro de Geografia e Estatística (IBGE) (2014). Censo Demográfico. Available at: http://www.ibge.gov.br/estadosat/perfil.php?sigla=DF Last Accessed(07-10-2015)

17. Leopoldina M, Villas DC, Shimizu HE, Sanchez MN. Clinical and epidemiological profile of patients from the home care program of Federal District Brazil. J Public Health and Epidemiology 2015;7(6): 189-97.

18. Ludolph A, Drory V, Hardiman O, Nakano I, Ravits J, Robberecht W, et al. A revision of the El Escorial criteria - 2015. Amyotroph Lateral Scler Front Degener 2015; 1–2. Available from: http://informahealthcare.com/doi/abs/10.3109/21678421.2015.1049183. Last Accessed(07-21-2015)

19. Marin B, Couratier P, Preux P-M, Logroscino G. Can mortality data be used to estimate amyotrophic lateral sclerosis incidence? Neuroepidemiology 2011;36(1):29–38.

20. Miller RG, Anderson F, Brooks BR, Mitsumoto H, Bradley WG, Ringel SP. Outcomes research in amyotrophic lateral sclerosis: Lessons learned from the amyotrophic lateral sclerosis clinical assessment, research, and education database. Ann Neurol 2009;65(SUPPL. 1):24–8.

21. Miller RG, Rosenberg JA, Gelinas DF, Mitsumoto H, Newman D, Sufit R, et al. Practice parameter: The care of the patient with amyotrophic lateral sclerosis (An evidence-based review). Muscle Nerve 1999;22(8):1104–18

22. Okita T, Nodera H, Shibuta Y, Nodera A, Asanuma K, Shimatani Y, et al. Can Awaji ALS criteria provide earlier diagnosis than the revised El Escorial criteria? J Neurol Sci 2011;302(1-2):29–32.

23. Pouget J. Les Centres dédiés à la sclérose latérale amyotrophique ont-ils changé les pratiques et la prise en charge? Rev Neurol (Paris) 2013;169(SUPPL.1):S39–44.

24. Rodríguez de Rivera FJ, Oreja Guevara C, Sanz Gallego I, San José Valiente B, Santiago Recuerda A, Gómez Mendieta M a., et al. Evolución de pacientes con esclerosis lateral amiotrófica atendidos en una unidad multidisciplinar. Neurologia 2011;26(8):455–60.

25. Traynor BJ, Alexander M, Corr B, Frost E, Hardiman O. Effect of a multidisciplinary amyotrophic lateral sclerosis (ALS) clinic on ALS survival: a population based study, 1996-2000. J Neurol Neurosurg Psychiatry 2003;74(9):1258–61.

26. Turner MR, Bowser R, Bruijn L, Dupuis L, Ludolph A, McGrath M, et al. Mechanisms, models and biomarkers in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2013;14 Suppl 1:19–32.

27. Turner MR, Scaber J, Goodfellow J a., Lord ME, Marsden R, Talbot K. The diagnostic pathway and prognosis in bulbar-onset amyotrophic lateral sclerosis. J Neurol Sci 2010;294(1-2):81–5. .

28. Valenzuela D, Zitko P, Lillo P. Amyotrophic lateral sclerosis mortality rates in Chile: A population based study (1994–2010). Amyotroph Lateral Scler Front Degener 2015;(February):1–6.

29. Vázquez MC, Ketzoián C, Legnani C, Rega I, Sánchez N, Perna A., et al. Incidence and prevalence of amyotrophic lateral sclerosis in Uruguay: A population-based study. Neuroepidemiology 2008;30(2):105–11.

30. Venkova-Hristova K, Christov A, Kamaluddin Z, Kobalka P, Hensley K. Progress in therapy development for amyotrophic lateral sclerosis. Neurol Res Int 2012;2012:187234

31. Volanti P, Cibella F, Sarvà M, De Cicco D, Spanevello A, Mora G, et al. Predictors of non-invasive ventilation tolerance in amyotrophic lateral sclerosis. J Neurol Sci 2011;303(1-2):114–8.

32. Zaldivar T, Gutierrez J, Lara G, Carbonara M, Logroscino G, Hardiman O. Reduced frequency of ALS in an ethnically mixed population: A population-based mortality study. Neurology 2009;72(19):1640–5.

ARTIGO 5 – ARTIGO ORIGINAL - AMYOTROPHIC LATERAL SCLEROSIS TREATMENT WITH LITHIUM ASSOCIATED WITH RILUZOLE VERSUS RILUZOLE ALONE: AN OPEN RANDOMIZED CONTROLLED TRIAL

Versão em inglês

Amyotrophic lateral sclerosis treatment with lithium associated with riluzole versus riluzole alone: an open randomized controlled trial, submetido ao periódico científico Journal of Negative Results in BioMedicine,

AMYOTROPHIC LATERAL SCLEROSIS TREATMENT WITH LITHIUM ASSOCIATED TO RILUZOLE VERSUS RILUZOLE ALONE: AN OPEN RANDOMIZED CONTROLLED TRIAL

Abstract

Background: there are still discrepancies about the role of lithium carbonate as a neuroprotector in reducing the progression of motor symptoms and improving survival in amyotrophic lateral sclerosis. Most previous trials have difficulties in finding a reliable biomarker to compare groups.

Methods: a 18 month randomized controlled clinical trial, phase 2-3 open parallel, with two arms. The experimental group (n=30) received riluzole 100 mg/day plus lithium carbonate (blood level: 0.4 to 0.8 mEq/l) and the control group (n=30) received riluzole 100 mg/day. The study was conducted each three to four month. The primary outcome was time for tracheostomy or death. The secondary outcomes were ALSFRS-R scale, muscle strenght, Forced Vital Capacity, hemoglobin saturation and neurophysiological biomarkers.

Results: in an intention to treat analysis, the survival functions did not differ significantly between groups (Kaplan-Meier, log-rank 0.38). In a per protocol analysis, where 8 patients really used lithium plus riluzole, the survival function was better in control group, with a mean survival of 14.72 months [95% CI 13.19-16.25]), whereas in the experimental group the mean survival was 10.59 months [95% CI 7.12-14.07] (log-rank 0.04). For the secondary outcomes, there were no differences between the groups, related to Forced Vital Capacity, muscle Strength, ALSFRS-R scale and neurophysiological parameters. Twenty-two patients who underwent use of lithium carbonate and riluzole discontinuated the medication, eight (26.7%) of them because they considered the drug ineffective and 14 (46.7%) by the occurrence of adverse events.

Conclusions: Lithium carbonate was innefective in reducing the progression and increasing survival in ALS, based on cumulative survival in a substantial cohort of

patients and the secondary endpoints. Besides, there were low compliance and a high incidence of adverse events.

Trial registration number: Rebec RBR-2n5mtq protocol

Keywords: amyotrophic lateral sclerosis, motor neuron disease, lithium, survival, CMAP, motor unit, treatment, drug.

Background

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease characterized by progressive loss of motor neurons in the brain, brain stem and spinal cord, resulting in generalized weakness, muscle atrophy, paralysis, and eventual mortality within 3-5 years of disease onset(1). Riluzole improves survival in patients, but its effect is only moderate(2).

Treatment with either lithium alone or in conjunction with other antioxidant drugs showed to improve motor function and slow disease progression in a mouse model of ALS(3). In addition, one preclinical study combined lithium and valproate and produced a greater and more consistent effect than monotreatment with either drug in delaying the onset of disease symptoms, decreasing neurological deficit scores, and prolonging life span(4).

Otherwise, in the past five years, three randomized clinical trials (5–7) showed high incidence of adverse events and no benefit with association of lithium and riluzole. Although these negative trials, some authors have raised methodological questions in clinical studies that access lithium efficacy in reducing ALS progression (8).

Beside this point, there is a consensus that in ALS, clinical trials have long been limited by problems in choosing reliable endpoints or biomarkers(9). In this context, neurophysiological biomarkers raises as novel quantitative electrophysiological techniques which provides indirect measures of the number of functional lower motor neurons in a muscle. It has been suggested that these techniques may serve as a marker of progressive loss of motor units, useful in ALS outcome (10,11).

The aim of this study is to clarify the discrepancies about the role of lithium carbonate as a neuroprotector in reducing the progression of motor symptoms of the disease and improving survival. It is suggested the combination of neurophysiological biomarkers to clinical parameters to assess the outcome of the patients.

Methods

It was performed a randomized controlled clinical trial, phase 2-3 open parallel, with two arms, at the Reference Center for Neuromuscular Diseases of the State Secretariat of Health of the Federal District, from March 2014 to September 2015.

It has obtained approval from the local Research Ethics Committee 525 241/2014 FEPECS Protocol. The Clinical Trial was registered under Rebec RBR-2n5mtq protocol.

Inclusion criteria were age above 18 years; diagnosis of ALS possible, probable or definite agreement with El Escorial Criteria (12); exclusion of other diseases through skull and spinal cord magnetic resonance, laboratory tests and electromyography; Forced Vital Capacity (FVC) above 50%. Exclusion criteria were FVC below 50% and the diagnosis of other diseases with similar clinical picture.

In a previous study (13), dead or tracheostromized patients after 24 months were expected to be approximately 60%. A total of 80 patients (40 in each arm) had to be randomized to have 80% power to detect a 30% absolute reduction (from 60% to 30%) in the primary endpoint in the group treated with lithium, with a 5% two-sided type 1 error. The allocation was randomized by iRandomizer application and the participants signed a consent form. The recruitment was carried out from March 2014 to March 2015.

Participants were randomly assigned in two groups: the first group (experimental group) with 30 patients receiving riluzole100 mg / day associated with lithium carbonate orally in scaled dose according to blood levels between 0.4 to 0.8 mEq/l and the second (control group) with 30 patients receiving riluzole 100 mg / day isolated. The study was conducted each three or four month, monitoring physical examination,

laboratory blood tests, breathing tests - spirometry, oxyhemoglobin saturation and surface electromyography.

The primary outcome was time for tracheostomy or death within 24 months or observing the development of respiratory failure in 24 months, with oxyhemoglobin saturation below 90% and decrease in PaO_2 below 60 mmHg.

The secondary outcomes were: Median and *ulnaris* right nerves CMAP (Compound Muscular Action Potential) area and amplitude, ICMUC- Ideal Case of Muscle Unit Counting (10) in right median and ulnar nerves in every three months; ALSFRS-R scores- Amyotrophic Lateral Sclerosis Fuctional Rating Scale –Revised (15) with a total score of 48 points, every three months for 24 months; muscle strenght - MRC - Medical Research Council (16) every three months for 24 months, with a total score of 70 points; Forced Vital Capacity (FVC) in supine position and oximetry in the supine position for 15 minutes every three months for 24 months.

The protocol for CMAP and ICMUC measurements, model and computation follow the model postulated by Nandedkar et al. (14). It was used self-adhesive disposal surface ground and two disc recording electrodes with 15 mm diameter. Measurements were performed using commercial Keypoint- Classic-electromyograph, it was performed in the right *Adbutor Pollicis Brevis* (APB) and *Abdutor Digiti Minimus* (ADM).

Statistical analysis

Data were allocated in Office Excel 2010 charts and analyzed using SPSS (Statistical Package for Social Sciences), version 19.0. The confidence intervals were calculated assuming a Poisson distribution. Categorical variables were analyzed using chi-square test with the two-tailed Z test and Fisher's Exact Test. Quantitative variables were analyzed by the Student T test and Kruskal-Wallis Non-parametric test. The significance level was 0.05.

The survival functions for the patients were estimated by Kaplan-Meier method and compared using the log-rank test, with p significance level of 0.05.

Results

Between March, 2014 and June 2015, 65 patients were screened, 60 of whom were recruited from Neuromuscular Reference Center (CRDN) and were randomly assigned to lithium carbonate plus riluzole - experimental group (n= 30) or to riluzole - control group (n=30).The groups were balanced for clinical characteristics at baseline (table 1).

Table 1. Baseline demographic and clinical characteristics of patients with amyotrophic lateral sclerosis treated with litthium plus riluzole or riluzole.

Characteristics*	Lithium+riluzole (n=30)	Riluzole (n=30)	p-value
Mean age ± SD (years)	55.1 ± 11.6	57.8 ± 10.3	0.35
Male (%)	18(60)	18(60)	1.0
Caucasian (%)	26(86.7)	25(83.3)	0.08
Bulbar onset (%)	6(20)	9(30)	0.55
Mean symptom duration ±SD (months)	26.6 ± 21.3	39.1 ± 46.1	0.18
Mean ALSFRS-R±SD	27.8 ± 7.1	26.2 ± 7.9	0.39
Mean FVC±SD	73.8 ± 20.8	65.8 ± 17.4	0.11
Mean BMI±SD (Kg/m^2)	25.46 ± 4.8	24.72 ± 3.2	0.49
Mean amp median CMAP±SD	2.71 ± 3.34	2.71 ± 2.8	0.99
Mean amp ulnaris CMAP±SD	3.01 ± 2.46	2.91 ± 2.44	0.88
Mean time to 2_{nd} site ±SD(months)	10 ± 11.5	10.9 ± 21.4	0.83
Mean sat O_2 ±SD	96.5 ± 1.4	95.2 ± 3.5	0.06
Mean MRC ±SD	52.9 ± 5.1	52.5 ± 5.7	0.76
Mean neck strenght (MRC)	4.40	4.47	0.74

*Acronysm: ALSFRS-R: Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; BMI: Body Mass Index; FVC: Forced Vital Capacity; CMAP: Compound Muscle Action Potential; sat: saturation; MRC: Medical Research Council; SD: Standard Deviation; amp: amplitude.

Compliance was acceptable with 44 patients (74%) taking at least 40% of the prescribed drugs. Compliance was better in riluzole group (93%) than in the lithium plus riluzole group (53.3%). During the study, no patient withdrew consent. Figure 1 shows the trial profile.

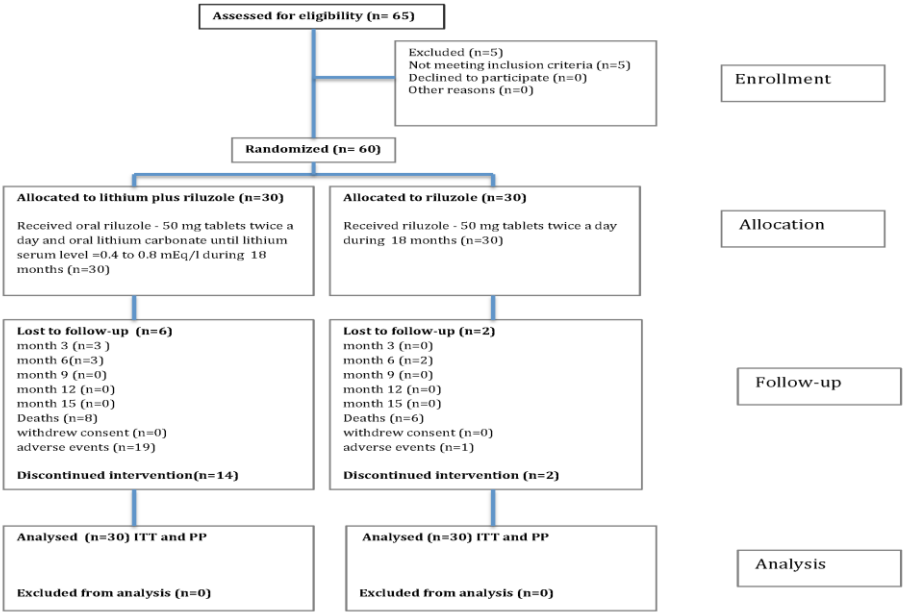

Figure 1. CONSORT diagram. Allocations, interventions, follow-up and analysis of 60 subjects with amyotrophic lateral sclerosis recruited into study.

All of 30 patients randomly assigned to lithium plus riluzole had at least one blood lithium concentration measurement between 0.4 to 0.8 mEq/l. The mean blood lithium concentration was 0.7 mEq/l in the experimental group and 0.6 mEq/l in the control group.

According to El Escorial criteria, in experimental group, 23 patients (80%) had definite or probable ALS and in control group, 26 patients (86.7%). The factors that influence survival did not differ between the groups (table 1). By the end of the study, at 18 months or at endpoint time (death or tracheostomy), 10 (33.3%) patients in experimental group and 5 (16.7%) patients in control group had received gastrostomy.

Nine (30%) patients in the experimental group and 11(36.7%) patients in control group had received non-invasive ventilation.

Fourteen (46.7%) patients in the experimental group and 19 (63.3%) patients in the control group were alive at 18 months.

The primary outcome, death or tracheostomy, was analysed by intention to treat (ITT) and per protocol (PP). Table 2 shows in an ITT analysis (figure 2), that the survival functions did not differ significantly between groups (Kaplan-Meier, log-rank

0.38). Table 2. Survival analysis in patients with amyotrophic lateral sclerosis treated with lithium carbonate plus riluzole versus riluzole.

	Lithium + riluzole			Riluzole			log-rank
	N	endpoint(%)	mean survival - months [CI95%]	N	endpoint(%)	mean survival - months [CI95%]	
ITT*	30	16(53.3)	13.12 [10.81-15.43]	30	11(36.7)	15.06 [13.43-16.70]	0.38
PP†	8	6(75)	10.59 [7.12-14.07]	50	21(42%)	14.72 [13.19-16.25]	0.04

*ITT: Intention to Treat; †PP: Per Protocol. Endpoint: death or tracheostomy

However, when it is observed in a PP analysis (figure 3), where eight patients really used lithium plus riluzole, the survival function was better in control group, with a mean survival of 14.72 months [95% CI 13.19-16.25]), whereas in the experimental group the mean survival was 10.59 months [95% CI 7.12-14.07] (log-rank 0.04).

Figures 2 and 3 show patients survival according to ITT and Per Protocol analysis.

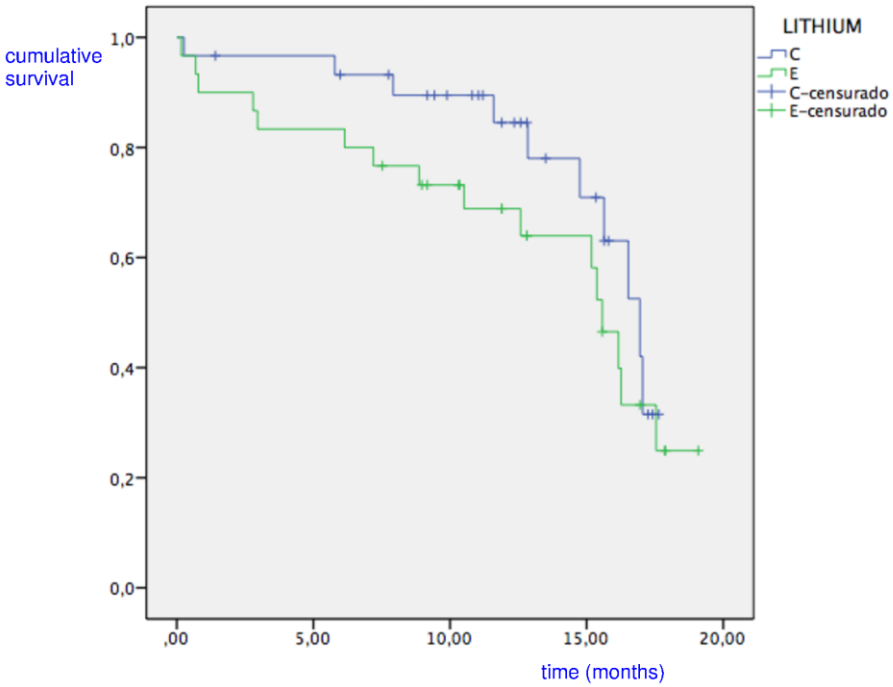

Figure 2. Kaplan-Meier survival analysis in patients with amyotrophic lateral sclerosis treated with lithium carbonate plus riluzole (E) versus riluzole (C). Intention To Treat analysis. Log Rank (Mantel-Cox): 0.38

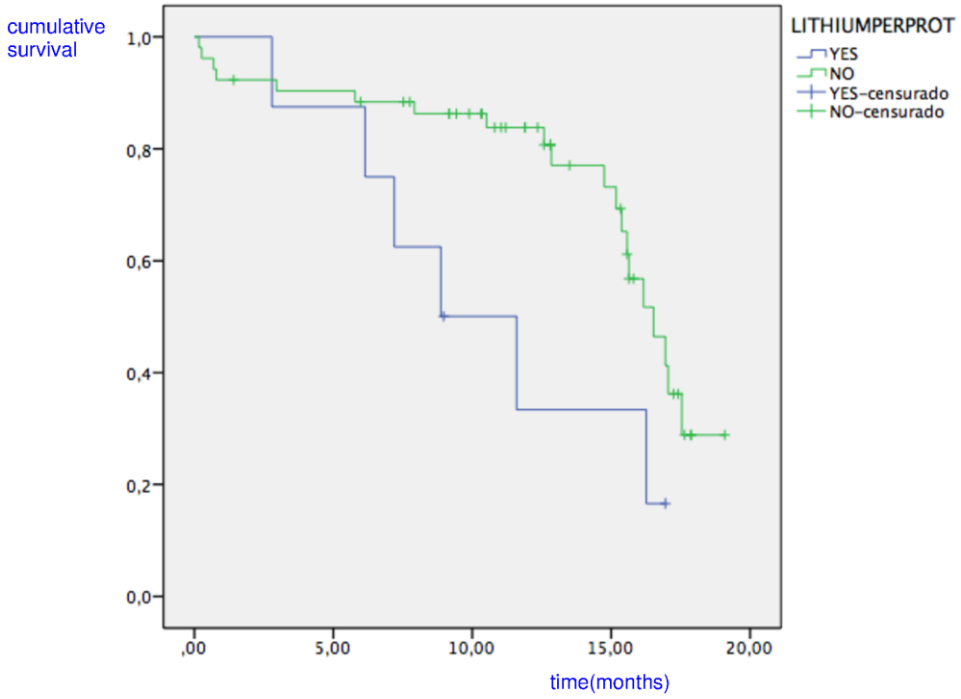

Figure 3. Kaplan-Meier survival analysis in patients with amyotrophic lateral sclerosis treated with lithium carbonate plus riluzole (E) versus riluzole (C). Per Protocol analysis. Log Rank (Mantel-Cox): 0.04

Table 3 shows the mean scores for the secondary outcomes. In an ITT analysis, there were no diferences between the groups, related to CFV, MRC scores, ALSFRS-R and median CMAP area and amplitude. In the unadjusted analysis for the ALSFRS-R , a functional scale which varies from 0 to 48, the annual rate of change was 8.15 ±5.24 in experimental group and 7.36± 4.14 in control group (p=0.66).

However, in a PP analysis, it was observed that the experimental group had minor slope in ALSFRS-R at 12 months (3.50 ±1.73), than control group (8.48± 4.59) (p=0.04).

Table 3. Secondary outcome measures, by time since randomization in patients with amyotrophic lateral sclerosis treated with lithium carbonate plus riluzole versus riluzole.

MEAN VARIATION(SD)*	INTENTION TO TREAT			PER PROTOCOL		
	L+R	R	p	L+R	R	p
MRC - 3 MONTHS	1.36(5.54)	2.90(5.76)	0.38	3.14(5.90)	4.31(5.65)	0.62
MRC - 6 MONTHS	3.15(3.68)	3.42(4.85)	0.85	2.80(4.76)	3.35(4.22)	0.79
MRC - 12 MONTHS	4.90(4.39)	4.77(8.10)	0.94	6.50(3.21)	5.88(6.85)	0.87
MRC - 18 MONTHS	10.75(16.76)	10.56(15.66)	0.67	9.56(5.50)	10.55(7.99)	0.88
ALSFRS-R 3 MONTHS	3.39(2.77)	3.74(3.03)	0.68	2.57(2.15)	3.74(2.98)	0.33
ALSFRS-R 6 MONTHS	4.84(3.99)	4.35(3.77)	0.71	2.80(3.11)	4.90(3.91)	0.26
ALSFRS-R 12 MONTHS	8.15(5.24)	7.36(4.14)	0.66	3.50(1.73)	8.48(4.59)	0.04
ALSFRS-R 18 MONTHS	9.09(4.46)	7.14(3.13)	0.33	6.00(5.66)	8.63(3.91)	0.40
FVC(%) -3 MONTHS	9.00(9.58)	4.42(6.13)	0.81	4.29(4.23)	7.41(8.99)	0.37
FVC (%)- 6 MONTHS	12.09(11.76)	12.73(11.16)	0.83	7.01(8.23)	13.26(11.63)	0.15
FVC(%) - 12 MONTHS	16.63 (16.36))	14.67(18.36)	0.22	23.12(19.90)	20.09(17.54)	0.75
FVC(%) - 18 MONTHS	26.29(19.16)	24.53(14.97)	0.83	14.30(20.22)	26.80(16.45)	0.33
CMAP AREA - 3 MONTHS	2.22(2.15)	4.32(8.44)	0.27	2.26(1.69)	3.41(6.56)	0.63
CMAP AREA - 6 MONTHS	4.22(4.07)	4.23(8.66)	0.16	4.81(2.19)	4.14(6.99)	0.83
CMAP AREA - 12 MONTHS	3.82(4.05)	8.70(13.70)	0.18	6.73(5.13)	6.00(10.56)	0.89
CMAP AREA - 18 MONTHS	5.06(9.64)	6.36(14)	0.86	6.95(13.49)	5.51(11.41)	0.75

*Acronysm: L+R: lithium plus riluzole; R: riluzole; MRC: Medical Research Council; ALSFRS-R: Amyotrophic Lateral Sclerosis Rating Scale-Revised; FVC: Forced Vital Capacity; CMAP: Median nerve Compound Motor Action Potential; SD: Standard Deviation.

Table 4 describes these five patients who had better functional outcome in 12 months by PP analysis. It is observed that bulbar-onset and a prolonged time from onset of symptoms to diagnosis (above 48 months) were present in three of them.

Table 4. Patients using lithium and riluzol with better outcome in Per Protocol analysis*

Age(years)	Gender	Time 1	Time 2	Onset	death	tracheostomy	ALSFRS-R (1)	ALSFRS-R(12)	BMI
39	M	94	12	spinal (leg)	no	no	33	2	21.5
43	M	48	0	bulbar	no	yes	32	0	24.4
45	M	36	12	spinal (arm)	yes	no	20	1	24.8
56	M	6	5	bulbar	no	yes	32	1	23.0
65	F	72	60	bulbar	yes	no	31	2	22.7

*Acronysm: M: male; F: female; ALSFRS-R: Amyotrophic Lateral Sclerosis Rating Scale-Revised (1)= first score; (12)= variation at 12 months; BMI: Body Mass Index; Time 1: onset of symptoms; Time 2: progression to another site.

Analyzing only patients with bulbar-onset, it is observed that those who used lithium carbonate and had higher scores in ALSFRS-R scale had 46.67±26.03 months of disease progression and control patients had 13.56± 4.95 months of progression (p=0.031). The variables age, race, FVC, Sat O2, BMI, MRC, neck strenght, CMAP area and amplitude and ICMUC did not showed any significant diferences in bulbar-onset patients.

Twenty-two patients who underwent use of lithium carbonate and riluzole discontinuated the medication, eight (26.7%) of them because they considered the drug ineffective and 14 (46.7%) by the occurrence of adverse events. Table 4 lists the main adverse events observed with lithium carbonate. Most events were considered minor, but one patient developed a serious event, respiratory distress with O2 saturation below 90 %, that ameliorates after discontinuation of lithium carbonate. So, the study stopped earlier for futility and by the occurrence of serious adverse events.

Table 5. Adverse events observed in patients with amyotrophic lateral sclerosis in use of lithium carbonate plus riluzole.

N	ADVERSE EVENT
8	HEADACHE
5	NAUSEA AND VOMITING
3	DIARRHEA
3	POOR APPETITE AND WEIGHT LOSS
2	MUSCLE WEAKNESS
2	TREMOR
1	RESPIRATORY FAILURE*
1	INSOMNIA
25	TOTAL

* Reversible when the drugs were discontinued.

Discussion

In experimental model os ALS, lithium has demonstrated antiapoptotic and antiglutamatergic activity(4); its ability to promote autophagy has been shown in several in-vitro and in-vivo models of neurological diseases(17). Moreover, lithium used for more than 50 years in the treatment of bipolar disorder and, more recently, cluster headache, is a secure drug because it has well known pharmacokinetics, pharmacodynamics, side-effects, and drug interactions in human beings(4,8).

However, this study showed that lithium, in combination with riluzole, did not improve survival in patients with ALS and, in addition, showed adverse events so important that reduced compliance and led to interruption of study.

Secondary outcome measures supported the equivalence of lithium and riluzole based on disease progression, as measured by the ALSFRS-R, neurophysiological measures, clinical parameters and vital capacity. Additionally, safety analyses did reveal a major safety issue and discontinuation of trial medication due to adverse effects occurred significantly more often in patients taking lithium compared with placebo. There is no evidence that treatment with lithium results in increase in survival at 18 months.

It was observed an improvement in functional analysis using ALFRS-R scale, in some patients with bulbar-onset. However, these patients had a confounding factor, a significant longer time of disease, which may suggest that they would have a more slow progression of the disease.

Overall, there is still doubt about neuroprotective lithium effect and Motor Neuron Disease. Lithium has demonstrated neuroprotective effect in cell and ALS animal models. Mice expressing mutant Cu/Zn superoxide dismutase 1 (SOD1) exhibit ALS-like phenotypes, including the formation of intracellular aggregates of SOD1 in the brain and spinal cord, behavioral abnormalities, and premature death(3,17,18,19). In organotypic slice cultures of spinal cord, chronic treatment with lithium dose-dependently prevents excitotoxic cell death of MNs by inhibiting the GSK-3β signaling pathway(19).

Table 6 shows the previous studies of lithium treatment in amyotrophic lateral sclerosis patients.

In 2007, Fornai et al. (3) found that daily doses of lithium, leading to plasma levels ranging from 0.4 to 0.8 mEq/liter, delay ALS progression in 16 human patients. There were no deaths in the experimental group. In the same study, the authors observed in genetic ALS animal model, the G93A mouse, a marked neuroprotection by lithium, which delayed disease onset and duration and augmented the life span (3).

Recently, a retrospective controlled cohort study with patients with ALS who received autologous bone marrow mononuclear cell transplantation (20) showed neuroprotective effect of lithium with improvement of survival time.

Table 6. Previous studies of Lithium treatment of amyotrophic lateral sclerosis

Year	Author(ref)	N(Exp)	N(Control)	GRADE	Study design	Analysis	Statistics	Results
2008	Fornai et al. (3)	16	28	1C	RCT single-blind	Survival, ALSFRS-R, FVC, MRC, Norris Scale	ANOVA and Kaplan-Meier	Favors Lithium-Survival and all secondary endpoints
2010	Aggarwal et al. (5)	40	44	1A	RCT double-blind	Time to event: death or six points in ALSFRS-R	sequential design - number of events predicted	Futility boundary was crossed, no benefit.
2010	Chiò et al. (22)	87	84	1B	RCT single-blind dose finding	Survival and ALSFRS-R at 18 months	Kaplan-Meier ant Student t test	Stopped early - 117 patients(68.4%) discontinued - no evidence in any direction
2011	Miller et al. (21)	107	249	2	historical placebo control	ALSFRS-R, FCV, QOL,weight loss, time to failure	Linear mixed-effects model and Fisher exact test	lack of therapeutic benefit in 13 months.
2012	Verstraete et al. (6)	66	67	1A	RCT double-blind	Survival, ALSFRS-R, FVC	sequential design, Kaplan-Meier, linear fixed effect model	no differences between the two groups
2013	Morrison et al. (7)	107	107	1A	RCT double-blind	Death at 18 months, ALSFRS-R, EuroQoL	Fleiss method, Cox Regression model	no difference between the two groups.

Other controlled studies that analysed the lithium effect in survival and functional progression showed any benefit of lithium(5–7,21).

In a large negative ALS clinical trial- UKMND-LiCALS Study Group et al. (7) plasma lithium levels were 0.4–0.8 meq/L, barely at the lower therapeutic range for psychiatric disorders and significantly lower than the serum concentration for lithium of its major hypothesized targets (≥1 mM). Indeed, in the antidepressant like rodent model,

the forced-swim test, only plasma lithium levels above 1.3 meq/L significantly reduced immobility-time (23). Importantly, it has recently been shown (24) that lithium-induced neuroprotection is antagonized by riluzole, suggesting that the drug's neurotoxic effects may mask the potential neuroprotective activity of lithium (24,18).

In the same study(7), there was also no advantage of the active drug over placebo for the primary endpoint of survival rate at 18 months. In contrast to the actual study, there were no differences in adverse events between experimental and control group (hazard ratio for all serious adverse events 1·14, 95% CI 0·79–1·65) and compliance with treatment was good (65%), but was higher in the placebo group (71%) than in the lithium group (60%).

An important aim of this study was to assess the safety and the adverse events of lithium for the treatment of patients with ALS. The well known side effects of lithium, for example, nausea and cephalalgia do occurred more frequently in the lithium treated group. In fact, more patients in the lithium group experienced side effects which led to discontinuation of trial medication, which was related in another study (6).

As for the narrow therapeutic range of lithium and therefore the increased risk of toxicity, it was refrained from including a higher dosage group out of concern for the safety of our patients. The dropout rate for lithium trials tends to be higher compared with trials with other compounds in ALS, which might have obscured the observed effects.

It is noteworthy that little is known about potential interactions between lithium and riluzole. However, the limited evidence available suggests a potentiating rather than a detrimental effect of riluzole on the neuroprotective properties of lithium(24).

There is a large discussion about the primary endpoint of the studies and their biomarkers. Early trials focused on mortality, but it is even difficult to define death and the real time of tracheostomy need, once some patients undergone tracheostomy or gastrostomy and others do not accept the procedures. This study choose as the primary endpoint time to death or tracheostomy, because it is a more objective measure and easily comparable to other studies.

As secondary endpoints, the current study focuses on clinical markers such as functional scale ALSFRS-R, MRC index, respiratory function and neurophysiological biomarkers such as amplitude and area of CMAP and ICMUC, that also considers the recruitment potential of Motor Unit. There was no difference between the two groups.

Quality of life measures and ALSFRS-R functional scale are even more imprecise, because they also reflect emotional state and can be biased by frontotemporal dementia. The most commonly utilized measure, the ALS Functional Rating Scale, is a non-linear scale made up of questions related to bulbar, ventilatory, upper limb and lower limb function. However, the sensitivity of the ALSFRS-R to change remains somewhat uncertain and it has not adequate to be used as the only one measure of change in clinical trials.

So, a more sensitive measure is needed to improve clinical trials. A major feature of the progressive course of ALS relates to loss of functional motor units, and reinnervation in remaining but anormal motor units, resulting in the characteristic concentric needle electromyography feature of chronic partial denervation. The combination of denervation and reinnervation by axonal sprouting brings the CMAP relatively insensitive to change. Force measurements and quantitative motor unit analysis are useful for diagnosis of neurogenic change but similarly not sensitive to progression in the disease.

A method for directly counting functional motor unit numbers in a muscle was introduced by McComas and colleagues, the Motor Unit Number Estimation – MUNE (25). The original method is difficult, in a methodological point of view. Several modified techniques has been introduced by various authors, in order to increase the sensitivity of the method and to manage its major problem, that of variance in the measurement and the technical issue.

Neuwirth et al. have established the sensitivity and reliability of MUNIX (11), allowing it to be used as a biomarker inferring lower motor neuron involvement, although how it may fluctuate in relation to changes in the upper motor neuron system has yet to be determined. However, this technique needs major technical training to be replicated, a relatively preservation of patient muscle strength and a mathematical model coupled to electromyogram equipment.

Recently, it was (26) suggested the use of a cumulative index that is composed by the summation of the CMAP amplitudes, as a way to estimate the motor neuron loss in amyotrophic lateral sclerosis or a CMAP scan (27), which allows the study of the remaining motor units, considering both denervation e reinnervation processes.

In conclusion, this randomized controlled trial demonstrated the inefficacy of lithium in reducing the progression and increase survival in ALS, based on cumulative survival in a substantial cohort of patients and the secondary endpoints. Besides, there were low compliance and a high incidence of adverse events, which led to the early termination of the study.

References

1. Hardiman O, van den Berg LH, Kiernan MC. Clinical diagnosis and management of amyotrophic lateral sclerosis. Nat Rev Neurol. Nature Publishing Group; 2011;7(11):639–49.
2. Miller RG, Mitchell JD MD. Riluzole for amyotrophic lateral sclerosis (ALS)/ motor neuron disease (MND) (Review). Cochrane Database Syst Rev 2012 Mar 14;3CD001447. 2007;(3).
3. Fornai F, Longone P, Cafaro L, Kastsiuchenka O, Ferrucci M, Manca ML, et al. Lithium delays progression of amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A. 2008;105(6):2052–7.
4. Boll M-C, Bayliss L, Vargas-Cañas S, Burgos J, Montes S, Peñaloza-Solano G, et al. Clinical and biological changes under treatment with lithium carbonate and valproic acid in sporadic amyotrophic lateral sclerosis. J Neurol Sci 2014;340(1-2):103–8.
5. Aggarwal SP, Zinman L, Simpson E, McKinley J, Jackson KE, Pinto H, et al. Safety and efficacy of lithium in combination with riluzole for treatment of amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. Lancet Neurol 2014;9(5):481–8.
6. Verstraete E, Veldink JH, Huisman MHB, Draak T, Uijtendaal E V, van der Kooi AJ, et al. Lithium lacks effect on survival in amyotrophic lateral sclerosis: a phase IIb randomised sequential trial. J Neurol Neurosurg Psychiatry 2014 ;83(5):557–64.
7. Morrison KE, Dhariwal S, Hornabrook R, Savage L, Burn DJ, Khoo TK, et al. Lithium in patients with amyotrophic lateral sclerosis (LiCALS): a phase 3 multicentre, randomised, double-blind, placebo-controlled trial. Lancet Neurol 2014 ;12(4):339–45.

8. Agam G, Israelson A. Why lithium studies for ALS treatment should not be halted prematurely. Front Neurosci 2014 Sep 2;8:267.

9. Simon NG, Turner MR, Vucic S, Al-Chalabi A, Shefner J, Lomen-Hoerth C,et al. Quantifying disease progression in amyotrophic lateral sclerosis. Ann Neurol 2014 Nov;76(5):643-57.

10. Nandedkar SD, Nandedkar DS, Barkhaus PE, Stalberg EV. Motor unit number index (MUNIX). IEEE Trans Biomed Eng. 2004 Dec;51(12):2209-11.

11. Neuwirth C, Barkhaus PE, Burkhardt C, Castro J, Czell D, de Carvalho M, et al. Tracking motor neuron loss in a set of six muscles in amyotrophic lateral sclerosis using the Motor Unit Number Index (MUNIX): a 15-month longitudinal multicentre trial. J Neurol Neurosurg Psychiatry 2015 Nov;86(11):1172-9.

12. Ludolph A, Drory V, Hardiman O, Nakano I, Ravits J, Robberecht W, et al. A revision of the El Escorial criteria - 2015. Amyotroph Lateral Scler Front Degener 2015;(April):1–2.

13. Moura MC, Novaes MR, Eduardo EJ, Zago YS, Freitas Rdel N, Casulari LA. Prognostic Factors in Amyotrophic Lateral Sclerosis: A Population-Based Study. PLoS One 2015 Oct 30;10(10):e0141500.

14. Nandedkar SD, Nandedkar DS, Barkhaus PE, Stalberg E V. Motor unit number index (MUNIX). IEEE Trans Biomed Eng. 2004;51(12):2209–11.

15. Cedarbaum JM, Stambler N, Malta E, Fuller C, Hilt D, Thurmond B NA. 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(1-2):13–21.

16. Medical Research Council. Aids to the examination of the peripheral nervous system, Memorandum no. 45, Her Majesty's Stationery Office, London, 1981.

17. Pasquali L, Longone P, Isidoro C, Ruggieri S, Paparelli A, Fornai F. Autophagy, lithium, and amyotrophic lateral sclerosis. Muscle Nerve 2014 ;40(2):173–94.

18. Ren M, Wang J, Yin X, Qi Y. Notch pathway is activated in cell culture and mouse models of mutant sod1-related familial amyotrophic lateral sclerosis , with suppression of its activation as an additional mechanism of neuroprotection for lithium and valproate. Neuroscience 2015; 301:276-88.

19. Calderó J, Brunet N, Tarabal O, Piedrafita L, Hereu M, Ayala V, et al. Lithium prevents excitotoxic cell death of motoneurons in organotypic slice cultures of spinal cord. Neuroscience 2014;165(4):1353–69.

20. Sharma AK, Sane HM, Paranjape AA, Gokulchandran N, Nagrajan A. The effect of autologous bone marrow mononuclear cell transplantation on the survival duration in Amyotrophic Lateral Sclerosis - a retrospective controlled study. Am J Stem Cells 2015 Mar 15;4(1):50-65.

21. Miller RG, Moore DH, Forshew D a., Katz JS, Barohn RJ, Valan M, et al. Phase II screening trial of lithium carbonate in amyotrophic lateral sclerosis: Examining a more efficient trial design. Neurology 77(10):973–9.

22. Chiò A, Borghero G, Calvo A, Capasso M, Caponnetto C, Corbo M, et al. Lithium carbonate in amyotrophic lateral sclerosis: lack of efficacy in a dose-

finding trial. Neurology. 2010 Aug 17;75(7):619-25.

23. Bersudsky Y, Shaldubina A BR. Lithium's effect in forced-swim test is blood level dependent but not dependent on weight loss. Behav Pharmacol 2007;18(1):77–80.

24. Yáñez M, Matías-Guiu J, Arranz-Tagarro JA, Galán L, Viña D, Gómez-Pinedo U, et al. The neuroprotection exerted by memantine, minocycline and lithium, against neurotoxicity of CSF from patients with amyotrophic lateral sclerosis, is antagonized by riluzole. Neurodegener Dis 2014;13(2-3):171–9.

25. McComas AJ. Invited review: motor unit estimation: methods, results, and present status. Muscle Nerve. 1991 Jul;14(7):585-97

26. Nandedkar SD, Barkhaus PE, Stålberg EV. Cumulative motor index: an index to study progression of amyotrophic lateral sclerosis. J Clin Neurophysiol. 2015 Feb;32(1):79-85.

27. Maathuis EM, Drenthen J, van Doorn PA, Visser GH, Blok JH. The CMAP scan as a tool to monitor disease progression in ALS and PMA. Amyotroph Lateral Scler Frontotemporal Degener. 2013 Apr;14(3):217-23.

ARTIGO 6 – ARTIGO ORIGINAL - PROGNOSTIC FACTORS IN AMYOTROPHIC LATERAL SCLEROSIS: A POPULATION - BASED STUDY.

Versão publicada em inglês

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PROGNOSTIC FACTORS IN AMYOTROPHIC LATERAL SCLEROSIS: A POPULATION-BASED STUDY

Abstract

Objective: To determine the prognostic factors associated with survival in amyotrophic lateral sclerosis at diagnosis.

Methods: This retrospective population-based study evaluated 218 patients treated with riluzole between 2005 and 2014 and described their clinical and demographic profiles after the analysis of clinical data and records from the mortality information system in the Federal District, Brazil. Cox multivariate regression analysis was conducted for the parameters found.

Results: The study sample consisted of 132 men and 86 women with a mean age at disease onset of 57.2±12.3 years; 77.6% of them were Caucasian. The mean periods between disease onset and diagnosis were 22.7 months among men and 23.5 months among women, and the mean survival periods were 45.7±47.0 months among men and 39.3±29.8 months among women. In addition, 80.3% patients presented non-bulbar-onset amyotrophic lateral sclerosis, and 19.7% presented bulbar-onset. Cox regression analysis indicated worse prognosis for body mass index (BMI) <25 kg/m^2 (relative risk [RR]: 3.56, 95% confidence interval [CI]: 1.44–8.86), age >75 years (RR: 12.47, 95% CI: 3.51–44.26), and bulbar-onset (RR: 4.56, 95% CI: 2.06–10.12). Electromyography did not confirm the diagnosis in 55.6% of the suspected cases and in 27.9% of the bulbar-onset cases.

Conclusions: The factors associated with lower survival in amyotrophic lateral sclerosis were age >75 years, BMI <25 kg/m^2, and bulbar-onset.

KEY WORDS: Motor Neuron Disease; Amyotrophic Lateral Sclerosis; prognosis; survival

Introduction

Amyotrophic lateral sclerosis (ALS) is a degenerative disease characterized by the progressive loss of upper and lower motor neurons in the brain and spinal cord and progresses with muscle weakness and atrophy with or without pyramidal syndrome. Its incidence is estimated at 2.08 per 100,000 person-years, and the average age at symptom onset is 61.8±3.8 years (range of 54–67 years)[1].

The disease is associated with poor prognosis, and its etiology has been attributed to the temporal interaction between genetic and environmental factors [2,3]. Death is due to respiratory failure, with an average survival period after diagnosis of 24–36 months [3].

With respect to the clinical trials of treatments aimed at increasing survival, only riluzole, an anti-glutamatergic drug, exhibited moderate efficacy[4]. The possible causes of the failure of the clinical trials include clinical heterogeneity of the disease, because ALS may represent a spectrum of diseases with diverse causes and etiologies, and there is also difficulty in identifying evolution and prognostic factors[5] .

Moreover, considering that no effective therapy is available for ALS, the establishment of a prognosis during diagnosis is important for the development of a treatment plan for each patient.

The present study aimed to determine the factors associated with survival in ALS at the time of diagnosis.

Methods

This descriptive, population-based study was conducted using retrospective data from the medical records of patients treated at the Reference Center for Neuromuscular Diseases (CRDN) located in the Federal District, Brazil, and from riluzole-dispensing records between 2005 and 2014.

All patients selected for the study were treated with riluzole. According to Ministry Of Health of Brazil protocol[6], only patients with definite or probable diagnosis in accordance with the El Escorial diagnostic criteria[7,8] are allowed to use riluzole. They were enrolled in the study after excluding the diagnosis of other diseases.

The data extracted included age, sex, time of onset of symptoms, onset site, form of progression to another affected limb, family history of ALS, presence of pyramidal syndrome, time between onset of symptoms and diagnosis, disease course duration, self-reported weight and height, diagnosis confirmed by electromyography, smoking, alcohol consumption, contact with pesticides or heavy metals, intense physical activity (athlete), history of malignancy, associated dementia, and a set of socioeconomic indicators included in the Municipal Human Development Index in 2010 (IDHM-2010)[9].

Disease onset was regarded as the time from symptom onset to the endpoint or disease course duration in months, or the time from symptom onset to death or indication for tracheostomy.

Dementia was defined as the story of cognitive and behavioral deficits according to Lund and Manchester Groups criteria[10] in patient records and frontotemporal atrophy in neuroimaging.

The database from the Mortality Information System of the Unified Health System (SIM/DATASUS) was reviewed for the same period, and the data were cross-referenced with those collected in the CRDN to confirm the dates of death for the patients.

The BMI at the onset of symptoms was calculated as weight (kg)/height^2 (m^2).[11]

Statistical analysis

Descriptive statistics were performed using two-tailed Student's t-tests and $\chi 2$ tests at a significance level of 5%. Multivariate analysis was performed, including all variables. Initially, Cox univariate regression analysis was used for socio-demographic and clinical variables in relation to the survival period. Variables with $p<0.25$ in the univariate analysis[12] were included in the Cox multivariate regression analysis. The final multivariate regression model was built by the successive exclusion of each variable from the initial multivariate model. The likelihood ratio test was used to evaluate the importance of each excluded variable[12]. The level of significance was set at 5%. The survival functions for the patients were estimated using the Kaplan-Meier method and were compared using the log-rank test. All analyses were performed using

Statistical Analysis System software version 9.3 and Statistical Package for the Social Sciences software version 19.0.

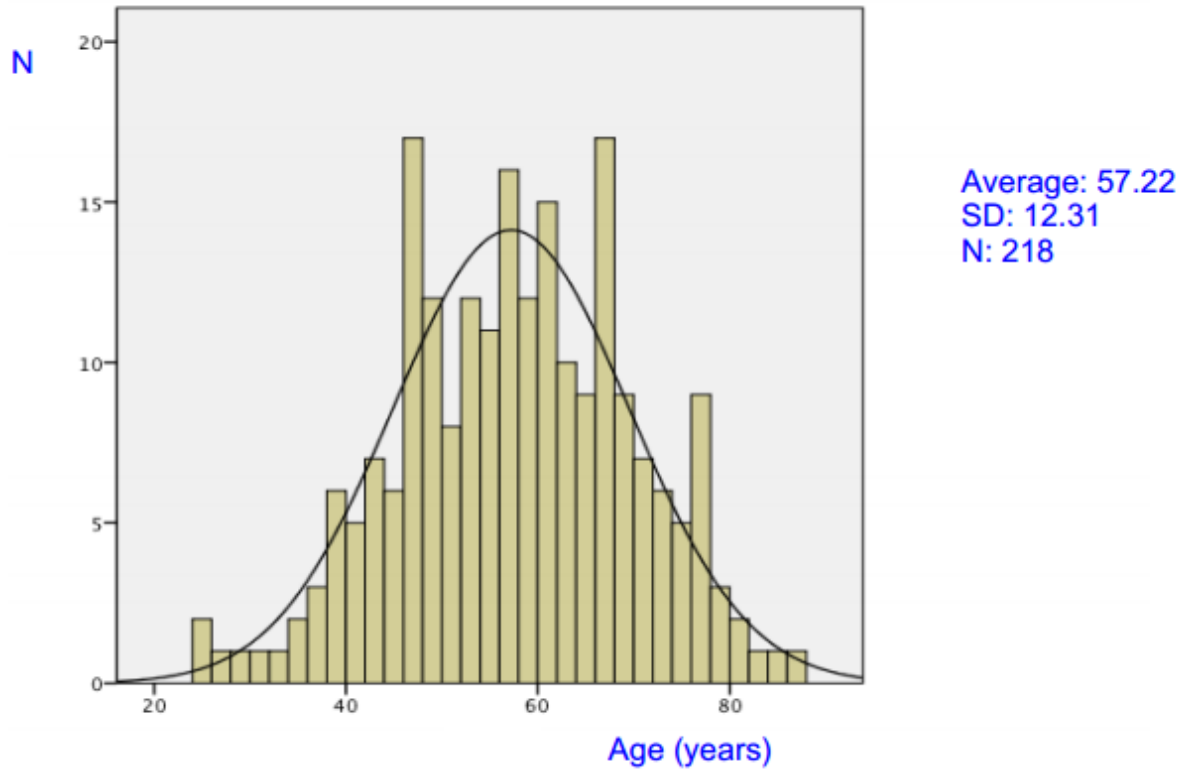
The study was approved by the Research Ethics Committee of Foundation of Teaching and Research in Health Sciences -FEPECS in the Federal District, Brazil, under protocol No. 820.117/2014. The Ethics Committee waived the written consent form because the study consisted of analysis of patient records (many of patients were deceased) and secondary databases (mortality data).

Results

From 2005 to 2014, 145 medical records and 73 riluzole-dispensing records were found, totaling 218 cases. The adjusted incidence rate for the population aged >20 years in the Federal District according to the IBGE census of 2010[13] was 1.26 cases per 100,000 person-years, considering a population of 1,740,922 inhabitants. The adjusted incidence rate for the population aged >45 years was 2.38 cases per 100,000 person-years, considering a population of 925,334 inhabitants.

The study sample consisted of 132 men (60.6%) and 86 women (39.4%), but this sex difference was not significant (p=0.06). The frequency distribution according to sex is shown in Figure 1. The mean age considering both sexes was 57.2±12.3 years, with a range of 25–86 years. The average ages were 56.3±12.3 years among men and 58.6±12.3 years among women, without a significant difference between the sexes (p=0.179).

Figure 1 - Histogram showing age distribution of patients with amyotrophic lateral sclerosis in Federal District, taking riluzole. 2005-2014.



A retrospective analysis of the records showed that 109 patients (50%) were classified as Definite; 41 (18.8%) were Probable; 50 (22.9%) were Possible and 18 (8.3%) were Suspect.

With regard to ethnicity, 169 (77.6%) patients were Caucasians, 45 (20.6%) were mestizos, and 4 (1.8%) patients were Black. In local general population, according to Brazilian Institute of Geography and Statistics – IBGE 2010 Census[13], above 20 years of age, there are 742,139 Caucasians (42.63%), 144,901 Blacks (8.32%) and 818,212 Mestizos (47%).

BMIs at disease onset were calculated for 138 patients from their medical records and drug-dispensing records and ranged between 14.9 and 34.9 kg/m^2, with an average of 23.5±4.1 kg/m^2, without significant difference between the sexes (p=0.15).

The IDHM-2010 ranged between 0.577 and 0.955, with an average of 0.83, without significant difference between the sexes (p=0.46).

Family history of the disease was reported by 11 (5%) patients.

The mean periods of diagnosis from symptom onset were 22.7 months in men and 23.5 months in women.

In the same period, SIM/DATASUS data indicated the occurrence of 162 deaths due to ALS in the region. Of these deaths, 99 cases were also present in the CRDN database. The deaths occurred with similar frequencies between 2005 and 2014, except in 2007, when nine deaths were reported. The highest frequency of deaths occurred in the age groups 60–69 years and 70–79 years, accounting for 59.8% of deaths. No deaths were reported in the age group <30 years. Deaths occurred in 90(55.6%) men and 72 (44.4%) women. The mean survival periods were 45.7±47.0 months among men and 39.3±29.8 months among women, without significant difference between the sexes (p=0.26).

Table 1 presents the distribution of patients according to the form of presentation. The most frequent form of presentation involved signs and symptoms in the upper limbs, followed by symptoms in the lower limbs and in bulbar muscles. The average age at disease onset did not differ significantly among the spinal or bulbar-onset forms (p=0.06).

Table 1. Amyotrophic Lateral Sclerosis patients profile considering the site of onset, age and gender.

Site of Onset	Age average (SD)*	CI 95%	Men (%)	Women (%)	Total (%)
Arm	55.1 (12.9)	52.5 – 57.7	62 (47)	36 (41.9)	98 (45)
Leg	58.2 (12.0)	55.5 – 61.0	48 (36.4)	29 (33.7)	77 (35.3)
Bulbar	60.0 (10.6)	56.7 – 63.3	22 (16.7)	21 (24.4)	43 (19.7)
Total	57.2 (12.3)	55.6– 58.86	132 (100)	86 (100)	218

The frequency of disease presentation was similar between the sexes. The frequency of impairment of another body region is shown in Table 2. Impairment of the contralateral limb was more frequent, followed by impairment of the ipsilateral limb and bulbar region. These frequencies were not different between the sexes.

Table 2. Clinical progression in patients with Amyotrophic Lateral Sclerosis

Clinical Progression	Male(%)	Female(%)	Total
Contralateral	87(65.9)	48(55.8)	135(61.9)
Ipsilateral	40(30.3)	35(40.7)	75(34.4)
Bulbar	5(3.8)	3(3.5)	8(3.7)
Total	132	86	218

Electroneuromyography (ENMG) was performed on all patients, and the diagnosis was confirmed in 181 cases (83%). However, in 18(8.3%) cases initially classified as Suspected, the procedure did not confirm the diagnosis in 55.6% cases. Moreover, ENMG did not confirm the diagnosis of the bulbar-onset form in 12 (27.9%) patients, of which 15.3% cases involved the upper limbs and 13% cases involved the lower limbs.

The following signs were observed in the course of the disease: pyramidal in 153(70.2%) patients; dementia in 16(7.3%) patients; Urinary disorders in 5(2,3%) of which 2 (0.9%) and 3(1.4%) presented with incontinence and urinary retention, respectively, not explained by urologic disease.

The analysis of the unadjusted risk ratio shown in table 3 indicates that the following variables were not statistically significant and were excluded: sex, IDHM-2010, clinical progression, presence of pyramidal signs, family history, urinary disorders, dementia, smoking, alcoholism, exposure to pesticides and heavy metals, intense physical activity, and diagnostic delay.

Table 3. Crude and adjusted Risk Ratio for survival, by demographic and selected clinical variables and adjusted[a] by age , BMI and site of onset .

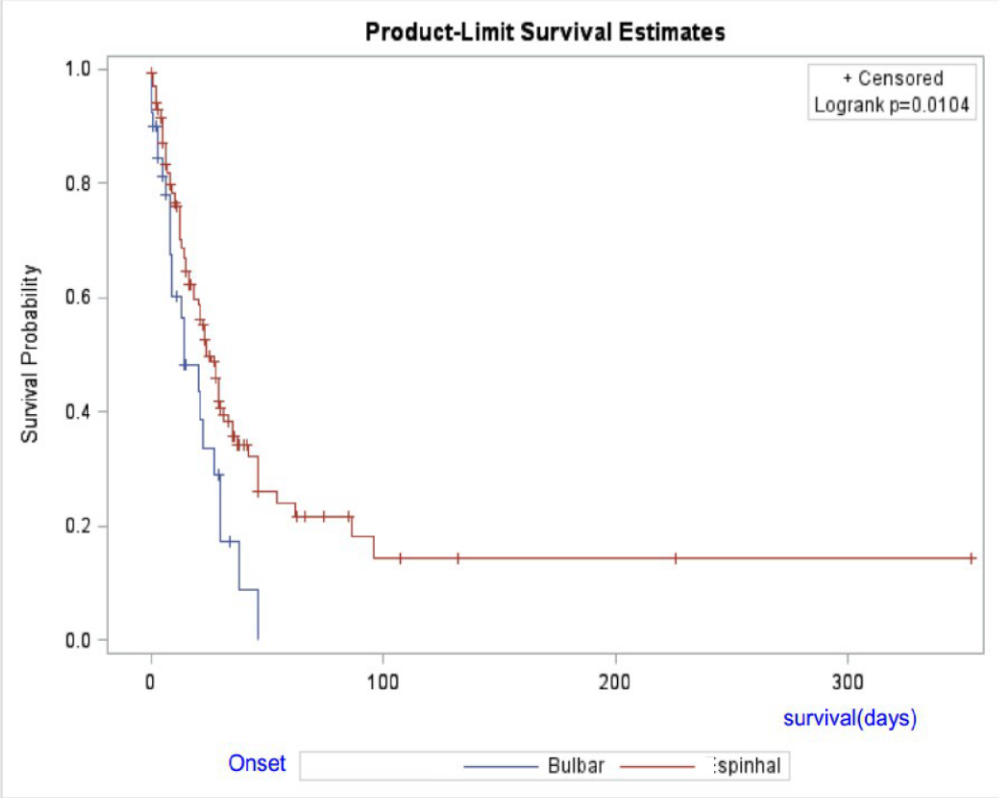
	Risk Ratio - RR (CI 95 %)			
	Crude	**p-value**	**Adjusted[a]**	**p-value**
Age (years)		0.01		0.001
< 50	1	-	1	-
51 - 60	1.53 (0.66 – 3.56)	0.31	1.37 (0.59 – 3.19)	0.46
61 – 75	1.83 (0.80 – 4.17)	0.15	1.90 (0.83 – 4.39)	0.13
> 75	6.70 (2.02 – 22.24)	0.001	12.47 (3.51 – 44.26)	< 0.0001
BMI (kg/m^2)		0.03		0.006
< 25	2.59 (1.08 – 6.23)	0.03	3.56 (1.44 – 8.86)	0006
≥ 25	1	-	1	-
Site of onset		0.003		0.0002
Spinal	1	-	1	-
Bulbar	2.97 (1.44 – 6.14)	0.003	4.56 (2.06 – 10.12)	0.0002

The Cox multivariate regression model indicated that only age, BMI, and the initial form of presentation were risk factors significantly associated with the survival period (Table 3). The risk of death among patients aged >75 years was approximately 12-fold higher (relative risk [RR]: 12.47, p<0.0001) than that of patients aged <50 years. In contrast, the risk of death among patients in the age groups 51–60 and 61–75 years did not differ significantly compared with the age group <50 years. The risk of death among patients with a BMI <25 kg/m^2 was approximately 3.5-fold higher than that of patients with a BMI ≥25 kg/m^2 (RR: 3.56, p=0.006). The risk of

death among patients with bulbar-onset ALS was approximately 4.5-fold higher (RR: 4.56, p=0.0007) than that of patients with spinal-onset ALS. No differences were observed in relation to the onset sites in the upper and lower limbs.

The results of the log-rank test, shown in Figure 2, indicate that the survival period in patients with spinal-onset ALS was significantly longer than that in patients with bulbar-onset ALS (p=0.01). Accordingly, approximately 70% of the patients with spinal-onset ALS were still alive one year after initiation of follow-up, whereas only 56% of those with bulbar-onset ALS were still alive in the same period.

Figure 2– Kaplan-Meier method estimation of survival of amyotrophic lateral sclerosis patients taking riluzole in Federal District, Brazil, from 2005 to 2014.



Discussion

The observation of clinical events is important for the evaluation of prognosis in ALS, which has been previously demonstrated [14-16]. Most studies have reported that age, bulbar-onset ALS, and cervical weakness are factors associated with poor prognosis and lower survival, whereas longer

diagnostic delay and use of riluzole were positively correlated with increased survival [14-17].

The study was conducted in a population ethnically distinct from populations living in Europe and North America [13]. We noted that the average age of 57.2±12.3 years at disease onset was similar in both sexes and was below the average age of populations from the northern hemisphere (61.8±3.8 years)[1]. In addition, 50% of the study patients were aged between 48 and 66 years. This finding was corroborated by other studies in Latin America [18-20] and may indicate the presence of yet unidentified genetic mutations or environmental factors involved in the disease etiology.

Both, genetic and environmental factor are still unclear in ALS. It was related [21] a repeat expansion in C9orf72 hexanucleotide in 6% of sporadic ALS and 44% of familiar ALS patients of European ancestry, with younger age at onset, shorter disease duration and higher frequency of Frontotemporal Dementia, in different ethnic populations. With respect to environmental factors, to our knowledge, in Latin America has no study.

With regard to ethnicity, the disease predominated in Caucasians (77.6%); however, the differences in the survival rate were not significant between the ethnicities. Similar findings have been previously reported [22-24]. In contrast, an Asian study monitored 73 patients over a 10-year period and reported a lower survival rate among Ethnic Indians compared with other ethnic groups [24].

It was observed that the risk of death among patients aged >75 years was approximately 12-fold higher (RR: 12.47, $p<0.0001$) than that of patients aged <50 years. In contrast, lower survival was not observed in the age group 51–75 years compared with the age group <50 years. A recent study in the United States evaluated 456 incident cases and reported that the survival rate decreased linearly with the increase in age [25].

This same study [25] reported survival rates of 67% at 12 months and 46% at 24 months. In the same period, it was observed survival rates of 70% for spinal-onset ALS and 56% for bulbar-onset ALS. Previous studies have reported better prognosis when the onset site was in the lower limbs [26,27] or bi brachial variant [28]. However, the present study found that the risk of death in bulbar-

onset ALS was 4.5-fold higher (RR: 4.56, p=0.0007) than that of spinal-onset ALS, independently of whether the onset site was in the arms or legs.

A Scottish study involving 1,226 patients [3] also reported worse prognosis associated with bulbar-onset ALS - although of lower magnitude (hazard ratio [HR]: 1.25, 95% confidence interval [CI]: 1.09–1.46) - presence of pyramidal signs (HR: 1.23, 95% CI: 1.01–1.49), and advanced age. The present study did not observe worse prognosis with the presence of signs of impairment of the first motor neuron.

There is controversy with regard to the association between dementia and ALS prognosis. In 2011, a study evaluated the association between motor neuron disease and frontotemporal dementia and indicated that the presence of signs of dementia in the frontal lobe were associated with bulbar-onset ALS and lower survival [29], however, this result was not observed in other studies [29]. In this series, it was observed related dementia in 7.3% cases; however, there was no increased risk of death in these patients.

A study conducted in Taiwan reported a correlation between low socioeconomic status and lower survival rates [31]. In the Federal District, the socioeconomic factors included in the Brazilian IDHM corresponded to the same three variables contemplated in the global HDI: longevity, education, and income. No significant difference in survival was observed between the regions with higher and lower IDHN, but this difference was higher than 0.8, a value considered high for Brazil.

No correlation was observed between survival period and longer diagnostic delay; however, in our study, the diagnostic delay was longer (averages of 22.74 months among men and 23.50 months among women) compared with other studies, wherein the average delay was <15 months [6,32,33] .This result may have occurred because the patients were sent to the CRDN only after confirmation of the diagnosis by ENMG.

Nerve conduction and electromyography studies (ENMG) are important tools for the identification of the degree and extent of impairment of the lower motor neurons and neuronal loss [34,35] and to discard diagnoses of other diseases; however, in the present study, the examination failed to confirm the disease in 55.6% of cases initially classified as suspicious and in 27.9% of cases

of bulbar-onset ALS, which reinforces the assumption that the clinical events should always be considered. A recent study in France [34] showed that the maximum sensitivity of the ENMG for bulbar-onset ALS was 49.98% using the criteria of Awaji-shima.

No significant difference in survival was observed between the sites of disease progression in the ipsilateral limbs, contralateral limbs, or bulbar region. A Japanese study involving 150 patients reported that the average survival rate was lower when bulbar manifestations were present in the first year of disease onset ($p<0.001$), in cases of rapid progression to another onset site, and when symptoms progressed with longitudinal or ipsilateral patterns [27]. The results of Turner [24] reinforce the hypothesis that the period of progression to another site is more important for disease prognosis than the anatomical distribution.

The poorer prognosis in the disease forms that prematurely affect the bulbar muscles may be due to patient malnutrition. In the present study, it was found that the survival rate of patients with a BMI <25 kg/m^2 was approximately 4-fold lower (RR=3.56, 95% CI: 1.44–8.86) than that of patients with a BMI ≥25 kg/m^2. Recent studies have indicated that poor nutritional status at the beginning and in the course of disease was associated with worse prognosis. A French study involving 63 patients indicated that the mean disease duration was lower among those with weight loss >10% during the disease course [35]. Other studies have shown that a BMI of 30–35 kg/m^2 was associated with a higher survival rate and that the rapid decrease in this index was associated with poor prognosis. Although these studies suggest that weight gain may have a protective effect in ALS, they did not confirm that an excessively high BMI was beneficial [37-39].

With regard to environmental factors, no correlations were detected between alcohol consumption, smoking, contamination with pesticides and heavy metals, intense and prolonged exercise, malignancy, and disease prognosis. In a population-based study conducted in the Netherlands with 494 patients with ALS and 1,599 controls [40], a multivariate analysis indicated increased risk for ALS and lower survival rates among smokers (HR: 1.51, 95% CI: 1.07–2.15). These factors were evaluated because of the possible correlation between the occurrence of oxidative stress and disease pathogenesis in which

DNA injury mechanisms and pathological changes in lipid and protein metabolism may lead to cell death via necrosis or apoptosis. However, the roles of these factors on disease prognosis have not been characterized when a degenerative process has already been established [40,41].

It was observed that 5% of patients had a family history for the disease, a frequency that is in accordance with the incidence of the familial forms of the disease in most studies, with a range between 1.6% and 5.6%[32], but this variable did not influence disease prognosis. Finding a precise genetic diagnosis in ALS is difficult because of the large phenotypic variability of the disease. Several studies have described mutations in more than 22 genes; in addition, more than one mutation may be present in the same patient, and most of these mutations may have high or moderate penetrance. The most frequent mutations reported encode the enzyme copper/zinc superoxide dismutase (SOD1), transactive-response DNA-binding protein 43 (TARDBP), C9orf72In, ubiquitin-like protein 2 (UBQLN2), profilin 1 (PFN1), optineurin (OPTN), angiogenin (ANG), and the RNA-binding protein FUS (fused in sarcoma)[42,43].

In conclusion, the factors that most impacted prognosis and decreased the survival rate in ALS were age >75 years, BMI <25 kg/m^2, and bulbar-onset ALS. For the earliest possible diagnosis and interventions aimed at prolonging survival, the consideration of clinical events rather than electromyography is essential, primarily in cases of bulbar-onset ALS and in cases classified as suspect according to the El Escorial diagnostic criteria.

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References

1. Chiò A, Logroscino G, Traynor BJ, Collins J, Simeone JC, et al. Global epidemiology of amyotrophic lateral sclerosis: a systematic review of the published literature Neuroepidemiology 2013;41:118–130.

2. Al-Chalabi A, Hardiman O. The epidemiology of ALS: a conspiracy of genes, environment and time. Nat Rev Neurol 2013;9:617–628.

3. Forbes RB, Colville S, Cran GW, Swingler RJ, Scottish Motor Neurone Disease Register. Unexpected decline in survival from amyotrophic lateral sclerosis/motor neurone disease. J Neurol Neurosurg Psychiatry 2004;75:1753–1755.

4. Zinman L, Cudkowicz M. Emerging targets and treatments in amyotrophic lateral sclerosis. Lancet Neurol 2011;10:481–490.

5. Beghi E, Chiò A, Couratier P, Esteban J, Hardiman O, et al. The epidemiology and treatment of ALS: focus on the heterogeneity of the disease and critical appraisal of therapeutic trials. Amyotroph Lateral Scler 2011;12:1–10.

6. Brasil. Ministério da Saúde. Secretaria de Atenção à Saúde. Protocolos Clínicos e diretrizes terapêuticas v.1. Brasilia, 2010. 608 p.

7. Okita T, Nodera H, Shibuta Y, Nodera A, Asanuma K, et al. Can Awaji ALS criteria provide earlier diagnosis than the revised el Escorial criteria? J Neurol Sci 2011;302:29–32.

8. Finsterer J, Stöllberger C. Apply Awaji-shima Consensus Conference Criteria before diagnosing Amyotrophic lateral sclerosis. Open Neurol J. 2013;7:4–6.

9. Chediek J. Atlas do Desenvolvimento Humano nas Regiões Metropolitanas Brasileiras – Brasília: PNUD, Ipea, FJP, 2014. 120 p.

10. Lund and Manchester Groups. Clinical and neuropathological criteria for frontotemporal dementia. J Neurol Neurosurg Psychiatry 1994;57:416e418.

11. Flegal KM, Kit BK, Orpana H, Graubard BI. Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. JAMA 2013;309:71-82.

12. Collett D. Modelling Survival Data in Medical Research, 2nd ed. Boca Raton: CRC Press; 2003.

13. Instituto Brasileiro de Geografia e Estatistica (IBGE) 2014. Censo Demográfico. Available at: http://www.ibge.gov.br/estadosat/perfil.php?sigla=DF Last accessed (07-10-2015).

14. Rooney J, Byrne S, Heverin M, Corr B, Elamin M , et al. Survival analysis of irish amyotrophic lateral sclerosis patients diagnosed from 1995-2010. PLOS One 2013;8:e74733.

15. Nakamura R, Atsuta N, Watanabe H, Hirakawa A, Watanabe H, et al. Neck weakness is a potent prognostic factor in sporadic amyotrophic lateral sclerosis patients. J Neurol Neurosurg Psychiatry 2013;84:1365–1371.

16. Chiò A, Logroscino G, Hardiman O, Swingler R, Mitchell D, et al. Prognostic factors in ALS : A critical review. Amyotroph Lateral Scler 2009 ;10:310-323.

17. Turner MR, Brockington A, Scaber J, Hollinger H, Marsden R, et al. Pattern of spread and prognosis in lower limb-onset ALS. Amyotroph Lateral Scler 2010;11:369-373.

18. Dietrich-Neto F, Callegaro D, Dias-Tosta E, Silva HA, Ferraz ME, et al. Amyotrophic lateral sclerosis in Brazil: 1998 national survey. Arq Neuropsiquiatr 2000;58:607–615.

19. Valenzuela D, Zitko P, Lillo P. Amyotrophic lateral sclerosis mortality rates in Chile: a population based study (1994–2010). Amyotroph Lateral Scler Frontotemporal Degener 2015;1–6.

20. Zaldivar T, Gutierrez J, Lara G, Carbonara M, Logroscino G, et al. Reduced frequency of ALS in an ethnically mixed population: A population-based mortality study. Neurology 2009;72:1640–1645.

21. García-Redondo A, Dols-Icardo O, Rojas-García R, Esteban-Pérez J, Cordero-Vázquez P, et al. Analysis of the C9orf72 Gene in Patients with Amyotrophic Lateral Sclerosis in Spain and Different Populations Worldwide. Hum Mutat 2013;34(1):79–82.

22. Jordan H, Fagliano J, Rechtman L, Lefkowitz D, Kaye W. Effects of demographic factors on survival Time after a diagnosis of Amyotrophic lateral sclerosis. Neuroepidemiology 2015;44:114–120.

23. Georgoulopoulou E, Fini N, Vinceti M, Monelli M, Vacondio P, et al. The impact of clinical factors, riluzole and therapeutic interventions on ALS survival: A population based study in Modena, Italy. Amyotroph Lateral Scler Frontotemporal Degener 2013;14:338–345

24. Goh KJ, Tian S, Shahrizaila N, Ng CW, Tan CT. Survival and prognostic factors of motor neuron disease in a multi-ethnic Asian population. Amyotroph Lateral Scler Frontotemporal Degener 2011;12:124–129.

25. Caller TA, Andrews A, Field NC, Henegan PL, Stommel EW, et al. The epidemiology of Amyotrophic lateral sclerosis in New Hampshire, USA, 2004-2007. Neurodegener Dis 2015. [Epub ahead of print].

26. Turner MR, Brockington A, Scaber J, Hollinger H, Marsden R , et al. Pattern of spread and prognosis in lower limb-onset ALS. Amyotroph Lateral Scler 2010;11:369–373.

27. Fujimura-Kiyono C, Kimura F, Ishida S, Nakajima H, Hosokawa T, et al. Onset and spreading patterns of lower motor neuron involvements predict survival in sporadic amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2011;82:1244–1249.

28. Katz JS, Wolfe GI, Andersson PB, Saperstein DS, Elliott JL, et al. Brachial amyotrophic diplegia: a slowly progressive motor neuron disorder. Neurology 1999 22;53(5):1071-6.

29. Coon EA, Sorenson EJ, Whitwell JL, Knopman DS, Josephs KA. Predicting survival in frontotemporal dementia with motor neuron disease. Neurology 2011;76:1886–1892.

30. Hu WT, Shelnutt M, Wilson A, Yarab N, Kelly C, et al. Behavior matters - cognitive predictors of survival in Amyotrophic lateral sclerosis. PLOS ONE 2013;8:e57584.

31. Lee CT, Chiu YW, Wang KC, Hwang CS, Lin KH, et al. Riluzole and prognostic factors in Amyotrophic lateral sclerosis Long-term and short-term survival: A population-based study of 1149 cases in Taiwan. J Epidemiol 2013;23:35–40.

32. Ganesalingam J, Stahl D, Wijesekera L, Galtrey C, Shaw CE, et al. Latent cluster analysis of ALS phenotypes identifies prognostically differing groups. PLOS One 2009;4:e7107.

33. Chiò A, Mora G, Calvo A, Mazzini L, Bottacchi E, et al. Epidemiology of ALS in Italy: A 10-year prospective population-based study. Neurology 2009;72(8):725–31.

34. Bresch S, Delmont E, Soriani M, Desnuelle C. Apport de l'électromyogramme dans le diagnostic précoce des SLA à début bulbaire : comparaison des critères d'El Escorial, d'El Escorial modifiés et d'Awaji . Revue Neurologique 2014;170:134–139.

35. Costa J, Swash M, de Carvalho M. Awaji criteria for the diagnosis of amyotrophic lateral sclerosis:a systematic review. Arch Neurol 2012;69:1410–1416.

36. Marin B, Desport JC, Kajeu P, Jesus P, Nicolaud B, et al. Alteration of nutritional status at diagnosis is a prognostic factor for survival of amyotrophic lateral sclerosis patients. J Neurol Neurosurg Psychiatry 2011;82:628–634.

37. Ngo ST, Steyn FJ, McCombe PA. Body mass index and dietary intervention: implications for prognosis of amyotrophic lateral sclerosis. J Neurol Sci 2014;340:5–12.

38. Paganoni S, Deng J, Jaffa M, Cudkowicz ME, Wills AM.. Body mass index, not dyslipidemia, is an independent predictor of survival in Amyotrophic lateral sclerosis. Muscle Nerve 2011;44:20-24.

39. Limousin N, Blasco H, Corcia P, Gordon PH, De Toffol B, et al. Malnutrition at the time of diagnosis is associated with a shorter disease duration in ALS. J Neurol Sci 2010;297:36–39.

40. de Jong SW, Huisman MH, Sutedja NA, van der Kooi AJ, de Visser M, et al. Smoking, alcohol consumption, and the risk of amyotrophic lateral sclerosis: A population-based study. Am J Epidemiol 2012;176:233–239.

41. D'Amico E, Factor-Litvak P, Santella RM, Mitsumoto H. Clinical perspective on oxidative stress in sporadic amyotrophic lateral sclerosis. Free Radic Biol Med 2013;65:509–527.

42. Wingo TS, Cutler DJ, Yarab N, Kelly CM, Glass JD. The heritability of amyotrophic lateral sclerosis in a clinically ascertained United States research registry. PLOS One 2011;6:6–10.

43. Harms MB, Baloh RH. Clinical neurogenetics: Amyotrophic lateral sclerosis. Neurol Clin 2013;31:929–950.

ARTIGO 7 – ARTIGO ORIGINAL - PREDICTORS FOR PROGNOSIS IN AMYOTROPHIC LATERAL SCLEROSIS: A PROSPECTIVE OBSERVATIONAL SINGLE CENTER STUDY

Versão em inglês

Predictors for prognosis in amyotrophic lateral sclerosis: a prospective observational single center study, submetido ao periódico científico PloS ONE, Qualis CAPES A2.

PREDICTORS FOR PROGNOSIS IN AMYOTROPHIC LATERAL SCLEROSIS: A PROSPECTIVE OBSERVATIONAL SINGLE CENTER STUDY

Abstract

Objective: Amyotrophic Lateral Sclerosis is a neurodegenerative and fatal disorder characterized by muscular weakness. The lack of prognostic factors reduces the efficiency of clinical trials and hinders the therapeutic planning.

Methods: a cohort study evaluated 101 patients with amyotrophic lateral sclerosis treated with riluzole and described their clinical, respiratory and neurophysiological outcome each three-four months for predictive factors in relation to death or tracheostomy. Cox multivariate regression analysis related to survival or tracheostomy was conducted for the parameters found and the most significant factors created a prognosis model that was submitted to an accuracy analysis by ROC curve.

Results: 58 men and 43 women with a mean age at disease onset of 57.2 ± 11.7 years and lower age in men; 80.2% of them were Caucasian. 77 patients (76.2%) the disease had spinal onset, with the predominance of leg as the initial site (41.6%) and 24 patients (23.8%) had bulbar onset. The mean diagnosis delay time was 25 ± 5.6 months and the mean survival time was 43.5± 5.7 months (CI 95% 32.3-54.8). Cox regression analysis

indicated significantly worse prognosis for age > 65 years (RR=2,50 CI 95%1,23 – 5,08), involvement of second site in < six months (RR=2,02 CI 95%1,04 – 3,94), supine FVC < 63% (RR= 2,78 CI 95%1,03 – 7,48), neck weakness (RR=2,28 CI 95% 1,03 – 5,05) and pyramidal syndrome associated (RR= 2,36 CI 95%1,05 – 5,33).

Interpretation: Accurate prediction of outcome is possible in most patients. A combination of five factors available prospectively predicted death or tracheostomy with 74% of accuracy in 12 months. The created model needs external validation in a wider cohort study.

KEY WORDS: motor neuron disease, amyotrophic lateral sclerosis, prognosis, survival, ALS, biomarker.

Introduction

Amyotrophic Lateral Sclerosis (ALS) is an adult-onset neurodegenerative disease characterized by progressive loss of upper motor neurons in the brain and lower motor neurons in brain stem and spinal cord, resulting in generalized weakness, muscle atrophy and possible respiratory failure within 3-5 years of disease onset(1). Most forms are sporadic and its incidence may vary between 1.2- 4.0 per 100,000 individuals per year(2).

The pathophysiology of the disease is still poorly understood and, consequently, there are few treatment options to reduce disease progression. The main mechanisms involved affect both motor neurons as glial cells and have the final change the abnormal accumulation of glutamate by abnormal activity of the enzyme Cu / Zn superoxide dismutase 1(SOD1), but are also described oxidative stress, formation of protein aggregates, impairment of autophagic mechanisms, alterations in mitochondrial function and inflammatory process(3).

The clinical variability in ALS complicates measurement of disease progression and clinical trials are limited by problems in choosing reliable endpoints or biomarkers and predicting the individual prognosis. Accurate measurement of disease burden remains a critical priority to facilitate efficient clinical trial design and to enable further insights into disease pathogenesis. Most studies endpoints have relied on revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) (4) and quality of life scales and the occurrence of death, non-invasive ventilation usage time and tracheostomy, endpoints now considered linear, inaccurate and subject to bias setting(5).

By improving prognostic determination, individual clinical care can be planned, including discussions of placing of gastrostomy tube and use of noninvasive ventilation. Besides, it is also valuable to give insights into health care policy, in order to assess the comparative effectiveness of systems of care, as helping to formulate service and policy development.

Prognostic biomarkers also could have a meaningful effect on the conduct of clinical trials, allowing the determination of subgroup analysis. Biomarker subgroup

analyses in clinical trials has the potential to permit the stratification of clinical response results.

In recent years, it was observed the attempt to establish a biomarker for ALS, without success. This study aim to create a predictive model by combining some markers possible to be used in the routine evaluation of the patient.

Methods

It was performed a descriptive prospective cohort study on prognostic factors in 101 patients with ALS diagnosed according to El Escorial (6) and Awaji-Shima Criteria(7), at Neuromuscular Disease Reference Center of Federal District (CRDN).

Data were derived from a randomised controlled clinical trial on the efficacy of lithium carbonate in ALS that failed to demonstrate differences between active treatment and placebo, in primary and in secondary outcomes. All patients selected for the study were treated with riluzole and 30 were enrolled in the experimental group.

The inclusion criteria were: a) diagnosis of Motor Neuron Disease done by a neurologist, that discarded other similar diseases; b) age over 18 years; c) patients signed a written informed consent term; d) Forced Vital Capacity (FVC) greater than or equal to 50%, except in Bulbar Progressive Palsy; d) El Escorial diagnostic criteria ALS as Definite, Probable an Possible.

Patients with Bulbar Progressive Palsy and FVC below 50% that had facial weakness were submitted to nocturnal oximetry and were included when the procedure was normal.

The exclusion criteria were: a) other forms of disease that affects the anterior horn of the spinal cord; b) nerve conduction studies showing motor nerve blockade; c) respiratory failure defined by oxyhemoglobin saturation lower than or equal to 90% and / or less than or equal PaO2 of 60 mm Hg.

The patients were admitted in CRDN between March 2014 and September 2015 and the evaluations were performed every three to four months during 20 months. The following variables were evaluated: age, gender, race/color for heterodetermination,

Body Mass Index - BMI(8), time of onset of symptoms, time and direction of the first spread of the motor deficit, functional scale: ALSFRS-R(4), , with maximum score of 40, CMAP (Compound Muscular Action Potential) area in right median and ulnar nerves (9) , muscle strength - MRC - Medical Research Council (10), with a total score of 70 points, flexors cervical muscles weakness defined as a MRC score ≤ 3, Forced Vital Capacity (FVC) in supine position, oximetry in the supine position, predominance of signs of upper motor neuron, smoking, familiar history and frontotemporal dementia, defined as the story of cognitive and behavioral deficits according to Lund and Manchester Groups criteria (11)and frontotemporal atrophy in neuroimaging.

It was used the protocol for CMAP (Compound Motor Action Potential) amplitude and ICMUC (Ideal Case Motor Unit Count) and MUNIX (Motor Unit Number Estimation Index) measurements, model and computation model postulated by Nandedkar and cols (14). It was used self-adhesive disposal surface ground and two disc recording electrodes with 15 mm diameter. Measurements were performed using commercial Keypoint-Classic-electromyograph, MUNIX was performed in the right *Adbutor Pollicis Brevis* (APB) and *Abdutor Digiti Minimus*(ADM).

The authors used also a delta CMAP with the summation of *ulnaris* and *medianus* nerves CMAP negative peak amplitude, performing the subtraction between the first and second measurement(9).

Disease onset was regarded as the time from symptom onset to the endpoint or disease course duration in months, or the time from symptom onset to death or indication for tracheostomy.

The Body Mass Index (BMI) was calculated as weight (kg)/height^2 (m^2).

Data were allocated in Office Excel 2010 charts and analyzed using SPSS (Statistical Package for Social Sciences) version 19.0. and SAS 9.3. The confidence intervals were calculated assuming a Poisson distribution. Categorical variables were analyzed using chi-square test the two-tailed Z test and the quantitative variables, the Student t test, with a significance level was $p < 0.05$.

Related variables were subjected to multivariate analysis including all variables. Initially, univariate Cox regression analyzes were used for clinical variables partner

with respect to survival time. Variables with p <0.25 in the univariate analysis were selected for inclusion in the multivariate Cox regression analysis. The final multivariate regression model was built by the successive exclusion of the variable from the initial multivariate model, using the likelihood ratio test to determine the importance of each variable excluded. The level of significance was set at 0.05.

The internal validation was performed calculating the sensitivity and specificity of individual variables and the final model by the standard means and Receiver Operating Characteristic (ROC) Curve.

It has obtained approval from the Research Ethics Committee under number 525 241 FEPECS Protocol / 2014 requiring signed Informed Consent Term.

Results

Between March 2014 and December 2015, 101 patients were followed. No patient withdrew consent. There were 43 women (42.6%) and 58 men (57.4%), with the proportion men: women 1:1.3. The age range was 25-80 years, the mean age was 57.2 ± 11.7 years and the median age, 58 years. In women, age at onset of symptoms was significantly higher, 60.5 ± 10.8 years, compared to men, 54.8 ± 11.8 years (p = 0.015).

Figure 1 shows the patients distribution according to gender.

Figure 1- Box plot showing age distribution of patients with amyotrophic lateral sclerosis (n= 101) in Federal District, Brazil; Mean age: 57.4 ± 11.7 years

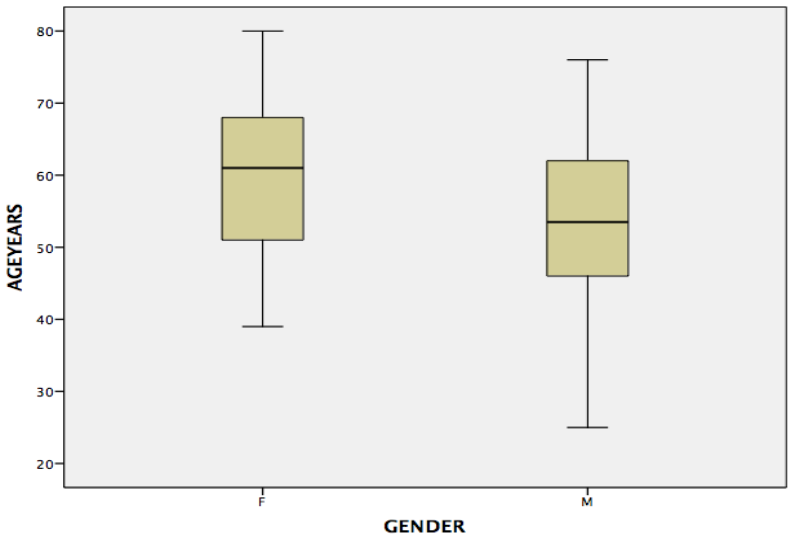

Table 1 shows the main characteristics of the patients, according to gender. Eighty-four patients (83.2%) had possible or probable ALS, according to El Escorial Criteria. In 77 patients (76.2%), 48 men and 29 women, the disease had spinal onset, with the predominance of leg as the initial site (41.6%). Twenty-four patients (23.8%), which 14 were women (58.3%, p=0.07) had bulbar onset.

Table 1 – Baseline Characteristics of the patients with amyotrophic lateral sclerosis (n=101) in Federal District, Brazil, 2014-2015.

Parameter	Men(n=58)	SD	Women(n=43)	SD	p
Follow-up time	9.72	5.86	6.98	5.5	0.019
BMI	24.89	3.62	25.37	4.45	0.551
Neck strenght (MRC)	4.55	0.65	4.67	4.97	0.853
Age (years)	54.83	11.79	54.83	10.78	0.015
Onset of symptoms (months)	27.59	68.01	21.51	35.05	0.594
Time to second site (months)	10.6	19.86	8.42	6.37	0.489
ALSFRS-R slope	5.47	7.41	4.47	7.75	0.519
initial ALSFRS-R	28.07	7.49	24.98	7.4	0.042
MRC slope	3.49	5.52	3.62	4.47	0.902
initial MRC	31.79	6.64	30.09	6.46	0.201
Supine FVC	69.02	22.66	58.63	24.54	0.031
SatO2	96.16	2.52	95.79	2.25	0.454
CMAP *medianus* amplitude	2.64	2.84	2.46	2.7	0.765
CMAP *ulnaris* amplitude	2.92	2.81	2.97	2.51	0.935
Survival	47.31	68.73	38.49	35.78	0.445
Caucasian (%)	39(48.1)	-	42(51.9)	-	0.002
Familial	6(50)	-	6(50)	-	0.76
El Escorial (Definite+Probable)	48(47.6)	-	36(36.3)	-	0.62
Crossed progression	17(43.5)	-	22(56.4)	-	0.41

Ipsilateral progression	22(44)	-	28(56)	-	-
Bulbar progression	3(27.2)	-	8(72.8)	-	-

Only two patients (2%) had the flail arm variant, five patients (5%) had symptoms of frontotemporal dementia. The signs of involvement of upper motor neuron were present in 70 patients (69.3%). Twelve (11.9%) had positive familiar history.

In relation to race, 81(80.2%) patients were Caucasian, 12(11.9%) were Mestizos, 7 (6.9%) were Black and 1(1%) was Asian.

The average time from onset of symptoms to diagnosis (diagnosis delay time) and treatment was 25 ± 5.6 months (CI 95% 23.9 - 46.1), with averages of 27.59 ± 8.9 months among men and 21.51 ± 5.3 months among women; p=0.6. The range was 4 to 500 months.

The average time of involvement to second member was 9.7 ± 15.5 months (CI 95% 6.6 -12.7), with a range of one to 144 months.

The ALSFRS-R mean score was 26.75 ± 7.6 and the MRC mean score was 52 ± 1.2. There were no differences between men and women.

The mean flexor cervical strength (MRC score) was 4.6 (CI 95% 3.9-5.2) and 50% of patients had scores ≤ 3, considered neck weakness.

Body mass index range was 15.9 to 39.0 Kg/m^2 and the average BMI at admission was 26.75 ± 7.6 Kg/m^2 and 50% of patients were between 22.9 and 27.5 Kg/m^2. In four to six months, 34 patients had weight loss and the average BMI was 24.80 ± 2.01 Kg/m^2, with no significance for survival time (p=0.96).

The mean supine FVC was 64.5 ± 23.9 % (CI 95% 59.8-69.3) and the mean oxyhemoglobin saturation was 96 ± 2.4%.

By the end of the study, at 20 months, 44 patients (43.6%) had reached endpoint. Thirty patients (29.7 %) died, two of them died for stroke and 28 from respiratory distress and/or pneumonia. At the same time, 18 patients (17.8 %) had received gastrostomy tube and 27 (26.7%) had received non-invasive ventilation. The mean

follow-uptime was 8.55 ± 5.84 (CI 95% 7.4-9.7) months and there were no loss of follow-up. The mean survival time was 43.5± 5.7 months (CI 95% 32.3-54.8)

When analyzing the unadjusted hazard ratio (table 2), the following variables with p <0,25 were included in the multivariate analysis: neck weakness, age, gender, site of onset, time of onset (months), time to second member, ALSFRS-R slope, ALSFRS-R at admission, MRC score and MRC slope, and supine FVC, presence of signs of upper motor neuron (UMN). The multivariate Cox regression model showed that only age, time to second member, supine FVC and signs of UMN are significant risk factors for the survival time. The bivariate analysis showed that neck weakness and ALSFRS-R at admission ≪ 24 also were relevant in a minor degree.

Table 2 - Crude and adjusted risk ratio for survival, by selected clinical variables. Cohort of patients with amyotrophic lateral sclerosis (n=101), 2014-2015, Federal District, Brazil.

	Risk Ratio (CI 95 %)			
	Crude	**p**	**Adjusted[a]**	**p**
BMI		0.8392		
< 25	1.07 (0.57 – 2.02)	0.8392		
≥ 25	1	-		
Race		0.2918		
Caucasian	1.66 (0.65 – 4.27)	0.2918		
Others	1	-		
Familial		0.3897		
No	1.87 (0.45 – 7.76)	0.3897		
Yes	1	-		
Neck Weakness		0.0414		
No	1	-		
Yes	2.28 (1.03 – 5,05)	0.0414		
AGE (Years)		0.0032		0.0111
≤ 65	1	-	1	-
> 65	2.75 (1.40 – 5.40)	0.0032	2.50 (1.23 – 5.08)	0.0111
Gender		0.1793		
Male	1	-		
Female	1.54 (0.82 – 2.91)	0.1793		
EL Escorial		0.4141		
Definite	1.67 (0.50 – 5.62)	0.4061		
Possible	1	-		

Probable	2.62 (0.74 – 9.23)	0.1331		
Suspect	1.97 (0.20 – 19.06)	0.5581		
Yes	1.02 (0.57 – 1.82)	0.8867		
ONSET		0.1488		
Bulbar	1.72 (0.82 – 3.59)	0.1488		
Spinal	1	-		
Onset Months		0.1598		
≤ 12	2.09 (0.91 – 4.79)	0.0823		
13 – 24	1.82 (0.86 – 3.83)	0.1175		
> 24	1	-		
Clinical Progression		0.7457		
Bulbar	1.22 (0.37 – 3.98)	0.7457		
Crossed and ipisilateral	1	-		
Second Member		0.1483		0.039
< 6 meses	1.60 (0.85 – 3.01)	0.1483	2.02 (1.04 – 3.94)	0.039
≥ 6 meses	1	-	1	-
ALSFRS slope		0.077		
0	1.83 (0.94 – 3.56)	0.077		
> 0	1	-		
ADMISSION ALSFRS		0.0035		
≤ 24	2.57 (1.36 – 4.85)	0.0035		
> 24	1	-		
MRC slope		0.1027		
0	1.75 (0.89 – 3.42)	0.1027		
> 0	1	-		
ADMISSION MRC		0.2509		
≤ 24	1.55 (0.73 – 3.26)	0.2509		
> 24	1	-		
Supine FVC		0.0079		0.0186
≤ 50	4.85 (1.78 – 13.22)	0.002	3.80 (1.31 – 11.00)	0.0137
50.1 – 62.9	3.39 (1.30 – 8.84)	0.0125	2.78 (1.03 – 7.48)	0.0433
63 – 82	1.69 (0.57 – 5.06)	0.3457	1.14 (0.36 – 3.63)	0.8195
> 82	1	-	1	-
SAT O2		0.2626		
≤ 96	1.43 (0.76 – 2.70)	0.2626		
> 96	1	-		
UMN		0.0453		0.0387
No	1	-	1	-
Yes	2.14 (1.02 – 4.52)		2.36 (1.05 – 5.33)	0.0387
DELTA CMAP		0.3104		
< 0	1	-		
≥ 0	1.71 (0.61 – 4.81)	0.3104		
Smoking		0.6553		
No	1.27 (0.45 – 3.57)	0.6553		

Yes	1	-
Athlete		0.4408
No	1.75 (0.42 – 7.28)	0.4408
Yes	1	-

The average amplitudes of the CMAP of median and *ulnaris* nerves were respectively 2.56 ± 2.8 mV and 2.94 ± 2.8 mV. The average values of maximum effort ICMUC of median and *ulnaris* nerves are shown in table 3 and were respectively, 3.06 ± 3.65 and 3.24 ± 3.57, with a slope at 4-6 months of 4.77 ± 30.1 and -0,046 ± 4.47. MUNIX calculation was not possible to be done in most patients because they had great weakness in affected muscles and inability to perform five epochs of sustained and replicable muscle contraction, maintaining the SIP area above CMAP area. So, the authors considered CMAP amplitude and maximum effort ICMUC in multivariate analysis.

Table 3 – Variation of Ideal Case of Motor Unit Counting (ICMUC) of median and ulnar nerves at maximum effort in patients with amyotrophic lateral sclerosis in Federal District, Brazil, 2014-2015.

	Group		**P**
Variable	**Control(n=54)**	**Experimental(n=47)**	*****
ICMUC Medianus			
Basal	3,40 ± 0,81	3,12 ± 0,83	
Six months	1,75 ± 1,32	1,99 ± 1,41	
12 months	-0,79 ± 0,48	0,31 ± 0,51	0,69
ICMUC Ulnaris			
Basal	3,08 ± 0,38	3,72 ± 0,74	
Six months	0,02 ± 0,99	0,92 ± 1,10	
12 months	-0,61 ± 0,79	-0,51 ± 0,83	0,60

* interaction between time and group. Experimental: endpoint at 8-12 months; Control: alive at 8-12 months.

The risk of death among patients with supine FVC less than or equal to 50 % is about four times (RR = 3.80, p = 0.0137) than in patients with supine FVC greater than 82%.

The authors excluded FVC < 50% and ALSFRS-R ≪ 24 because these factors are widely recognized as biomarkers and do not need to be validated. The ALSFRS-R slope did not show any significance in bivariate analysis (table1).

The risk of death among patients older than 65 years are two and a half times (RR = 2.50, p = 0.0111) than in patients aged less than 65 years.

The risk of death among patients with involvement of second member <6 months is about two times higher than among patients with second member greater than or equal to 6 months (RR = 2.02, p = 0.0390).

The risk of death among patients with supine FVC between 50 and 63% is about three most times (RR = 2.78, p = 0.0437) than among patients with supine FVC greater than 82%. Furthermore, patients with supine FVC between 63 and 82% do not presented difference in the risk of death (RR = 1.14, p = 0.8195) compared to patients with supine FVC greater than 82%.

The risk of death among patients with occurrence of UMN is about two times higher (RR = 2.36, p = 0.0387) than patients without occurrence of UMN.

The relative risk of death among patients with neck flexors weakness was 2.28 (CI 95% 1.03 – 5.05, p=0.04).

The parameters included in the predictive model were: age above 65 years, supine FVC below 63%, progression to second member within in less than six months, associated pyramidal syndrome and neck flexors weakness.

The analysis of sensibility and specificity of the five significant variables is shown in figure 2 and table 4, where the final model accuracy obtained by a Receiver Operating Characteristic (ROC) curve is 74 %, considered satisfactory.

As the study was performed in a single center, in a specific population, it is necessary major validation including other populations, in a wider prospective cohort study. In the other hand, the gold standard considered in the analysis of study accuracy, death or tracheostomy, is subject to bias because often the patients refuse the necessary procedures that improve survival.

Figure 2 – Receiver Operating Characteristic (ROC) Curve for model created with significant variables of Cox Regression. Cohort of patients with amyotrophic lateral sclerosis 2014-2015, (n=101) in Federal District, Brazil.

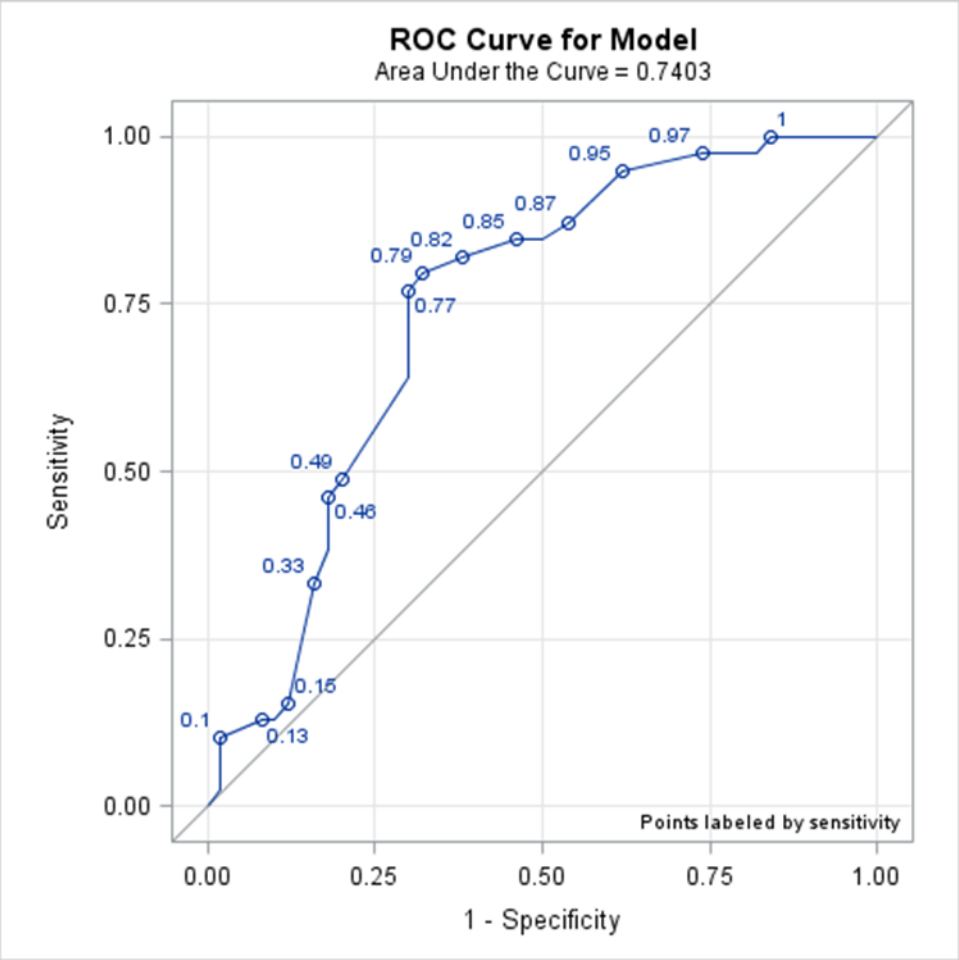

Table 4 – Internal validation of the prediction model according to each parameter considered in Cox Regression. Cohort of patients with amyotrophic lateral sclerosis 2014-2015, (n=101), in Federal District, Brazil.

Parameter	ROC Area	SD*	95% Confidence Interval	
Age > 65 years	0.60	0.05	0.50	0.69
Second site < 6 months	0.57	0.05	0.46	0.67
Supine FVC < 63 %	0.66	0.05	0.56	0.76
Signs of upper motor neuron	0.58	0.04	0.49	0.68
Neck weakness	0.54	0.04	0.46	0.62

*SD: standard deviation

Discussion

ALS may represent a spectrum of diseases with diverse causes and etiologies, and so there is difficulty in identifying a single biomarker to determine disease progression. The authors suggest a combination of clinical and laboratory prognostic factors in order to create a set of markers that may help in clinical decision-making. In order to identify prognostic factors in ALS, various studies have been performed.

This study was preceded by a retrospective population-based study that analyzed prognostic factors in 218 patients over 10 years, and identified by Cox regression, three independent factors related to lower survival: bulbar onset, onset above 75 years of age and BMI below 25 Kg/m^2. A similar prospective study performed in Germany (12) confirmed age above 75 years and fall of BMI and added the interval between symptom onset and diagnosis below 7 months, and ALSFRS-R below 31 points as independent factors predicting early death.

In the present study, the authors identified five independent prognostic factors with easy observation to routine clinical evaluation, with the power to predict the outcome of patients with amyotrophic lateral sclerosis in relation to the development of respiratory failure within eight to 12 months. The combination of these factors resulted in a model that was internally validated demonstrating 74% of accuracy.

We noticed that the average age of 57.2 ± 11.7 years at disease onset and significantly minor in males, was below the average age of populations from the northern hemisphere (61.8±3.8 years)(2). In addition, 50% of the study patients were aged between 49 and 66.5 years.

It was found that age above 65 years old was an independent factor for endpoint. In our retrospective study with 218 patients(13), it was observed that the risk of death among patients aged above 75 years was approximately 12-fold higher (RR: 12.47, $p<0.0001$) than that of patients aged below 50 years. A recent study in the United States evaluated 456 incident cases and reported that the survival rate decreased linearly with the increase in age (14). Other studies, as the present study, showed a lower age of onset of ALS in Latin America (15,16) and it may suggest the presence of yet unidentified genetic mutations or environmental factors involved in the disease etiology.

Moreover, genetic and environmental factor need further studies in ALS. Some authors related a repeat expansion in C9orf72 hexanucleotide in 6% of sporadic ALS and 44% of familiar ALS patients of European ancestry, with younger age at onset, shorter disease duration and higher frequency of Frontotemporal Dementia, in different ethnic populations(17), but there are no studies in Latin American populations.

In the present study, the authors observed that the presence of signs of upper motor neuron increases risk of death in two times. A recent study about clinical biomarkers estimating prediction to respiratory insufficiency correlated reduced survival with a lower motor neuron score(18), whereas others (19) reported worse prognosis with the presence of a pyramidal syndrome (HR: 1.23, 95% CI: 1.01–1.49) .

Other important prognostic factor was cervical weakness, with a relative risk of 128%. Other studies (20,21) show that cervical weakness is a factor associated with poor prognosis and lower survival, whereas longer diagnostic delay and use of riluzole were positively correlated with increased survival.

The risk of death among patients with involvement of a second site or member in less than six months was about two times higher than later, but not in relation to the site of progression. The results of Turner (22) reinforce the hypothesis that the period of progression to another site is more important for disease prognosis than the anatomical distribution.

A Japanese study involving 150 patients reported that the average survival rate was lower when bulbar manifestations were present in the first year of disease onset ($p<0.001$), in cases of rapid progression to another onset site, and when symptoms progressed with longitudinal or ipsilateral patterns(23). In this study, no significant difference in survival was observed between the sites of disease progression in the ipsilateral limbs, contralateral limbs, or bulbar region. Previous studies have reported better prognosis when the onset site was in the lower limbs(22,23) or bi brachial variant(24). A Scottish study involving 1,226 patients (19)also reported worse prognosis associated with bulbar-onset ALS, association with pyramidal signs and advanced age.

Forced vital capacity (FVC) has been used as an index of respiratory failure in most ALS trials(34,35). The authors correlate values below 50 % to 60% of the

standardized one to a poor prognosis, but it may not be an ideal test to diagnose respiratory dysfunction alone in the early phase of ALS. It may not fall until the development of a severe muscle weakness because specially patients with bulbar involvement might not be able to perform correctly the spirometry test (36,37). However, supine FVC (37) and Sniff nasal inspiratory pressure (SNIP) , which is known to predict hypoventilation and hypercapnia (36) are important predictors of respiratory failure in ALS patients. Most respiratory laboratory does not perform SNIP test routinely, but it is quite easy to perform supine FVC. This study shows that the risk of death among patients with supine FVC below 63% is about three most times (RR = 2.78, p = 0.0437).

No correlation was observed in this study between survival period and longer diagnostic delay time; however, the diagnostic delay time was longer (averages of 27.59 ± 8.9 months among men and 21.51 ± 5.3 months among women) compared with other studies, wherein the average delay was less than 15 months(26,27).

The disability score ALS Functional Rating Scale-Revised (ALSFRS-R) has been used to measure the course of the disease and to assess the efficacy of candidate treatments in clinical trials(4). However, the present study did not identify the ALSFRS-R as a potential marker of ALS prognosis. As postulated by some authors (43), various clinical factors affects ALSFRS-R decline, as age of onset, degree of diagnostic certainty according to Revised El Escorial criteria, BMI and FVC at diagnosis, suggesting that survival and ALSFRS-R can be influenced by different biomarkers.

Recently, two functional scales were compared in evaluating ALS progression from baseline to 6 months, the ALS-MITOS system, and ALSFRS-R, predicting death, tracheotomy or >23-hour non-invasive ventilation at 12 months and at 18 months. The analysis of ALS- MITOS and ALSFRS-R progression at 6-month follow-up showed that the best cut-off to predict survival at 12 and 18 months was loss of at least one function for the ALS-MITOS and a decline ranging from 6 to 9 points for the ALSFRS-R(44).

Lower motor neuron dysfunction in ALS is characterized by loss of motor units and subsequent reinnervation, which causes muscle weakness that develops relatively late, not synchronous with the neurophysiologic findings. The maximum CMAP

(Compound Muscle Action Potential) amplitude and area reflects muscle fiber innervation, but they can suffer the interference of reinnervation process and maintain its normality. Several studies have found MUNE-Motor Unit Number Estimation(47) and MUNIX-Motor Unit Number Estimation Index(48) mores sensitive markers , because they consider the functional subpopulation of motor units.

Although the neurophysiological parameters have been cited as potential biomarkers to predict prognosis in ALS and used in clinical trials(5,45), this study failed to correlate these variables with patients survival.

The amplitude of the CMAP is a standard measure in motor nerve conduction studies. When the CMAP is recorded at supramaximal intensity, its amplitude reflects the number and size of the motor units. In ALS, it can progressively diminish because of ongoing motor unit loss, useful to study ALS progression, but it may remain normal until 30-50 % loss of strength (9,45,49). The main reasons for lack of correlation in the present study were the technical issues to obtain values to calculate MUNIX in patients with great weakness and difficulties in performing a sustained contraction and on the other hand, limitations to disponibilize the electromyography and to create the necessary mathematical model in most clinical centers. The use of CMAP alone demonstrated to have limitations because reinnervation e poor correlation with survival(46).

Some authors have found CMAP decrement at repetitive stimulation (at least 10%) in ALS subjects, that arise from failure of conduction down axon branches or neuromuscular junctions of regenerating neurons undergoing collateral sprouting, but there is doubt if the decrement is associate with survival(46).

The actual study did not identified in a independently way the BMI as a prognostic factor, neither loss of weight. The fact is, nowadays in the CRDN, with the adoption of multidisciplinary care(40), the nutritional status of the patients was significantly better in this study. In the anterior study, it was found a strong correlation between the survival rate of patients and a BMI <25 kg/m2 (RR=3.56, 95% CI: 1.44–8.86) (13). A poor nutritional status at the beginning and in the course of disease is usually associated with worse prognosis(12,41). There is a French study involving 63 patients indicated that the mean disease duration was lower among those with weight loss >10% during the disease course(42). Other studies have shown that a BMI of 30–35

kg/m^2 was associated with a higher survival rate and that the rapid decrease in this index was associated with poor prognosis(43,44).

There is controversy with regard to the association between dementia and ALS prognosis. In 2011, a study evaluated the association between motor neuron disease and frontotemporal dementia and indicated that the presence of signs of dementia in the frontal lobe were associated with bulbar-onset ALS and lower survival(45), however, this result was not observed in other studies.

In a population-based study conducted in the Netherlands with 494 patients with ALS and 1,599 controls(46), a multivariate analysis indicated increased risk for ALS and lower survival rates among smokers (HR: 1.51, 95% CI: 1.07–2.15). These factors were evaluated because of the possible correlation between the occurrence of oxidative stress and disease pathogenesis in which DNA injury mechanisms and pathological changes in lipid and protein metabolism may lead to cell death via necrosis or apoptosis. However, the roles of these factors on disease prognosis have not been characterized when a degenerative process has already been established.

It was observed that 5% of patients had a family history for the disease, a frequency that is in accordance with the incidence of the familial forms of the disease in most studies, with a range between 1.6% and 5.6%, but this variable did not influence disease prognosis. Finding a precise genetic diagnosis in ALS is difficult because of the large phenotypic variability of the disease. Several studies have described mutations in more than 33 genes or chromosomes (47); in addition, more than one mutation may be present in the same patient, and most of these mutations may have high or moderate penetrance. The most frequent mutations reported encode C9orf72In, the enzyme copper/zinc superoxide dismutase (SOD1), transactive-response DNA-binding protein 43 (TARDBP), ubiquitin-like protein 2 (UBQLN2), profilin 1 (PFN1), optineurin (OPTN), angiogenin (ANG), and the RNA-binding protein FUS (fused in sarcoma) (48,49).

Most studies seeking ALS biomarkers in blood and cerebrospinal fluid have been focused primarily on diagnosis rather than clinically relevant prognostic endpoints and generally followed a targeted rather than unbiased discovery approach. One study performed a multivariate analysis in patients with the disease and identified several

biomarkers with predictive value that are biologically relevant to ALS, including inflammatory cytokines, growth factors, and markers of iron metabolism, but further prospective studies are needed(50).

In conclusion, the present study identified five biomarkers useful in a predictive model for worse prognosis in ALS, which are: age > 65 years (RR=2,50 CI 95%1,23 – 5,08), involvement of second site in < six months (RR=2,02 CI 95%1,04 – 3,94), supine FVC < 63% (RR= 2,78 CI 95%1,03 – 7,48), neck weakness (RR=2,28 CI 95% 1,03 – 5,05) and associated pyramidal syndrome (RR= 2,36 CI 95%1,05 – 5,33). The created model can easily be used in clinical practice with 74% of accuracy, but needs external validation in populations with diverse features.

References

1. Hardiman O, van den Berg LH, Kiernan MC. Clinical diagnosis and management of amyotrophic lateral sclerosis. Nat Rev Neurol. Nature Publishing Group; 2011;7(11):639–49.
2. Chiò a., Logroscino G, Traynor BJ, Collins J, Simeone JC, Goldstein L a., et al. Global epidemiology of amyotrophic lateral sclerosis: A systematic review of the published literature. Neuroepidemiology. 2013;41(2), 118–130.
3. Ravits J, Appel S, Baloh RH, Barohn R, Brooks BR, Elman L, et al. Deciphering amyotrophic lateral sclerosis: what phenotype, neuropathology and genetics are telling us about pathogenesis. Amyotroph Lateral Scler Frontotemporal Degener 2013;14 Suppl 1:5–18.
4. Cedarbaum JM, Stambler N, Malta E, Fuller C, Hilt D, Thurmond B NA. 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(1-2):13–21.
5. Swash M, Kiernan MC. Measuring change in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2015;0(0):1–2.
6. Ludolph A, Drory V, Hardiman O, Nakano I, Ravits J, Robberecht W, et al. A revision of the El Escorial criteria - 2015. Amyotroph Lateral Scler Front

Degener 2015:1–2.

7. Costa J, Swash M, de Carvalho M. Awaji criteria for the diagnosis of amyotrophic lateral sclerosis:a systematic review. Arch Neurol 2014;69(11):1410–6.

8. Flegal KM, Kit BK, Orpana H. Association of All-Cause Mortality with overweight and obesity using standard Body Mass Index Categories. A Systematic Review and Meta-analysis. 2015;309(1):71–82.

9. Nandedkar SD, Barkhaus PE, Stålberg E V. Cumulative Motor Index : An Index to Study Progression of Amyotrophic Lateral Sclerosis 2015;32(1).

10. Medical Research Council. Aids to the examination of the peripheral nervous system, Memorandum no. 45, Her Majesty's Stationery Office, London, 1981.

11. Miller BL, Ikonte C, Ponton M, Levy M, Boone K, Darby A, et al. A study of the Lund-Manchester research criteria for frontotemporal dementia: clinical and single-photon emission CT correlations. Neurology 1997;48(4):937–42.

12. Wolf J, Safer A, Wöhrle JC, Palm F, Nix WA, Maschke M, et al. Factors prortality in amyotrophic lateral sclerosis patients - data from a population-based registry.edicting one-year m. BMC Neurol 2014;14(1):197.

13. Moura MC, Novaes MRCG, Eduardo EJ, Zago YSSP, Freitas RDNB, Casulari LA. Prognostic Factors in Amyotrophic Lateral Sclerosis: A Population-Based Study. PLoS One 2015;10(10):e0141500.

14. Caller T a., Andrews A, Field NC, Henegan PL, Stommel EW. The Epidemiology of Amyotrophic Lateral Sclerosis in New Hampshire, USA, 2004-2007. Neurodegener Dis 2015;15(4):202-6.

15. Valenzuela D, Zitko P, Lillo P. Amyotrophic lateral sclerosis mortality rates in Chile: A population based study (1994–2010). Amyotroph Lateral Scler Front Degener 2015;1–6.

16. Zaldivar T, Gutierrez J, Lara G, Carbonara M, Logroscino G, Hardiman O. Reduced frequency of ALS in an ethnically mixed population: A population-based mortality study. Neurology 2009;72(19):1640–5.

17. García-Redondo A, Dols-Icardo O, Rojas-García R, Esteban-Pérez J, Cordero-Vázquez P, Muñoz-Blanco JL, et al. Analysis of the C9orf72 Gene in Patients

with Amyotrophic Lateral Sclerosis in Spain and Different Populations Worldwide. Hum Mutat 2013;34(1):79–82.

18. Devine MS, Ballard E, O'Rourke P, Kiernan MC, Mccombe PA, Henderson RD. Targeted assessment of lower motor neuron burden is associated with survival in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Front Degener 2015;8421:1–7.

19. Forbes RB, Colville S, Cran GW, Swingler RJ. Unexpected decline in survival from amyotrophic lateral sclerosis/motor neurone disease. J Neurol Neurosurg Psychiatry 2004;75(12):1753–5.

20. Sato Y, Nakatani E, Watanabe Y, Fukushima M, Nakashima K, Kannagi M, et al. Prediction of prognosis of ALS : Importance of active denervation findings of the cervical-upper limb area and trunk area. Intractable Rare Dis Res 2015;4(4):181–9.

21. Nakamura R, Atsuta N, Watanabe H, Hirakawa A, Watanabe H, Ito M, et al. Neck weakness is a potent prognostic factor in sporadic amyotrophic lateral sclerosis patients. J Neurol Neurosurg Psychiatry 2013;1365–71.

22. Turner MR, Brockington A, Scaber J, Hollinger H, Marsden R, Shaw PJ, et al. Europe PMC Funders Group Pattern of spread and prognosis in lower limb-onset ALS. Amyotroph Lateral Scler 2010 Aug;11(4):369-73.

23. Fujimura-Kiyono C, Kimura F, Ishida S, Nakajima H, Hosokawa T, Sugino M, et al. Onset and spreading patterns of lower motor neuron involvements predict survival in sporadic amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2011;82(11):1244–9.

24. Katz JS, Wolfe GI, Andersson PB, Saperstein DS, Elliott JL, Nations SP, et al. Brachial amyotrophic diplegia: A slowly progressive motor neuron disorder . Neurology 1999;53 (5):1071.

25. Coon E a, Sorenson EJ, Knopman DS. Predicting survival in frontotemporal dementia with motor neuron disease. Neurology 2011 ;76(22):1886-93.

26. Nzwalo H, De Abreu D, Swash M, Pinto S, De Carvalho M. Delayed diagnosis in ALS: The problem continues. J Neurol Sci 2014;343(1-2):173–5.

27. Ganesalingam J, Stahl D, Wijesekera L, Galtrey C, Shaw CE, Leigh PN, et al.

Latent cluster analysis of ALS phenotypes identifies prognostically differing groups. PLoS One. 2009;4(9).

28. Beghi E, Chiò A, Couratier P, Esteban J, Hardiman O, Logroscino G, et al. The epidemiology and treatment of ALS: focus on the heterogeneity of the disease and critical appraisal of therapeutic trials. Amyotroph Lateral Scler 2011;12(1):1–10.

29. Chiò a., Mora G, Calvo a., Mazzini L, Bottacchi E, Mutani R. Epidemiology of ALS in Italy: A 10-year prospective population-based study. Neurology 2009;72(8):725–31.

30. Ngo ST, Steyn FJ, McCombe P a. Body mass index and dietary intervention: Implications for prognosis of amyotrophic lateral sclerosis. J Neurol Sci 2014;340(1-2):5–12.

31. Marin B, Desport JC, Kajeu P, Jesus P, Nicolaud B, Nicol M, et al. Alteration of nutritional status at diagnosis is a prognostic factor for survival of amyotrophic lateral sclerosis patients. J Neurol Neurosurg Psychiatry 2014;82(6):628–34.

32. Paganoni S, Deng J, Jaffa M, Cudkowicz ME, Wills AM. Body mass index, not dyslipidemia, is an independent predictor of survival in amyotrophic lateral sclerosis. Muscle Nerve 2011;44(1):20-4

33. Limousin N, Blasco H, Corcia P, Gordon PH, De Toffol B, Andres C, et al. Malnutrition at the time of diagnosis is associated with a shorter disease duration in ALS. J Neurol Sci 2010;297(1-2):36–9.

34. Cudkowicz ME, van den Berg LH, Shefner JM, Mitsumoto H, Mora JS, Ludolph A, et al. Dexpramipexole versus placebo for patients with amyotrophic lateral sclerosis (EMPOWER): a randomised, double-blind, phase 3 trial. Lancet Neurol 2013 Nov [cited 2014;12(11):1059–67.

35. Morrison KE, Dhariwal S, Hornabrook R, Savage L, Burn DJ, Khoo TK, et al. Lithium in patients with amyotrophic lateral sclerosis (LiCALS): a phase 3 multicentre, randomised, double-blind, placebo-controlled trial. Lancet Neurol 2014;12(4):339–45.

36. Capozzo R, Quaranta VN, Pellegrini F, Fontana A, Copetti M, Carratù P, et al. Sniff nasal inspiratory pressure as a prognostic factor of tracheostomy or death in

amyotrophic lateral sclerosis. J Neurol 2014;262(3):593–603.

37. Baumann F, Henderson RD, Morrison SC, Brown M, Hutchinson N, Douglas J a, et al. Use of respiratory function tests to predict survival in amyotrophic lateral sclerosis. Amyotroph Lateral Scler 2010;11(1-2):194–202.

38. De Jong SW, Huisman MHB, Sutedja N a., Van Der Kooi AJ, De Visser M, Schelhaas HJ, et al. Smoking, alcohol consumption, and the risk of amyotrophic lateral sclerosis: A population-based study. Am J Epidemiol 2012;176(3):233–9.

39. Coatti GC, Beccari MS, Olivio TR, Mitne-Neto M, Okamoto OK, Zatz M. Stem cells for amyotrophic lateral sclerosis modeling and therapy: Myth or fact? Cytometry Part A 2015;87(3):197–211.

40. Moura MC, Novaes MRCG, Eduardo EJ, Zago YS, Freitas R DelNB, Casulari LA . Impact of multidisciplinary care in amyotrophic lateral sclerosis hospitalizations in the public health system of Brazil. J Public H Epidemiol 2015;7(7):206–16.

41. Wingo TS, Cutler DJ, Yarab N, Kelly CM, Glass JD. The heritability of amyotrophic lateral sclerosis in a clinically ascertained United States research registry. PLoS One 2011;6(11):6–10.

42. Harms MB BR. Clinical Neurogenetics: Amyotrophic Lateral Sclerosis. Neurol Clin 2013;31(4):1–24.

43. Su XW, Simmons Z, Mitchell RM, Kong L, Stephens HE, Connor JR. Biomarker-based predictive models for prognosis in amyotrophic lateral sclerosis. JAMA Neurol 2013;70(12):1505–11.

44. Mandrioli J, Biguzzi S, Guidi C, Sette E, Terlizzi E, Ravasio A, et al. Heterogeneity in ALSFRS-R decline and survival: a population-based study in Italy. Neurol Sci 2015 ;36(12):2243-52.

45. Tramacere I, Dalla Bella E, Chiò A, Mora G, Filippini G, Lauria G. The MITOS system predicts long-term survival in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2015;1–6.

46. Maathuis EM, Drenthen J, van Doorn P a., Visser GH, Blok JH. The CMAP scan as a tool to monitor disease progression in ALS and PMA. Amyotroph Lateral Scler Front Degener. 2012;1–7.

47. Henderson R, Baumann F, Hutchinson N MP. CMAP decrement in ALS. Muscle and Nerve 2009;39(4):555–6.

48. Furtula J, Johnsen B, Christensen PB, Pugdahl K, Bisgaard C, Christensen MK, et al. MUNIX and incremental stimulation MUNE in ALS patients and control subjects. Clin Neurophysiol 2014;124(3):610–8.

49. Neuwirth C, Nandedkar S, Stålberg E, Weber M. Motor Unit Number Index (MUNIX): A novel neurophysiological technique to follow disease progression in amyotrophic lateral sclerosis. Muscle and Nerve 2010;42(3).

50. Neuwirth C, Barkhaus PE, Burkhardt C, Castro J, Czell D, de Carvalho M, et al. Tracking motor neuron loss in a set of six muscles in amyotrophic lateral sclerosis using the Motor Unit Number Index (MUNIX): a 15-month longitudinal multicentre trial. J Neurol Neurosurg Psychiatry 2015;1–8.

5. CONCLUSÃO

A Esclerose Lateral Amiotrófica (ELA) é uma doença neurodegenerativa, melhor definida como uma síndrome clinicamente heterogênea, caracterizada pelo acometimento progressivo do primeiro e do segundo neurônio motor, de etiologia desconhecida, que cursa com fraqueza e atrofia muscular. Mesmo havendo grande interesse em se determinar a fisiopatologia da doença, o fato de ainda não se ter encontrado um conjunto satisfatório de biomarcadores deixa uma lacuna quanto à caracterização dos seus diferentes estágios.

Em seus mecanismos fisiopatogênicos se destacam o estresse oxidativo, a formação de agregados protéicos, a disfunção mitocondrial, a excitotoxicidade do glutamato por alteração de função da enzima Cu/Zn superóxido dismutase, entre outros fatores.

A incidência da Esclerose Lateral Amiotrófica (ELA) no mundo é cerca de 1 a 4 por 100.000 pessoas ano. No Brasil, estimamos sua incidência bruta em 0.89/100.000 pessoas ano e ajustada à população acima de 45 anos a 2.3/100.000 pessoas ano, a partir de estudo de base populacional baseado em dados do Sistema de Informação de Mortalidade (SIM) . A doença predomina ligeiramente no sexo masculino, em uma razão 1:1.4 e entre os anos de 2004 a 2014 a média de idade foi de 62.7±13.2 anos, inferior a dos países europeus. A doença demonstrou predomínio na raça branca, em locais de colonização européia, com Odds Ratio (OR) de 2.92 em relação às demais raças no país.

Até o momento, existe desde 1996 um único medicamento que reduz a progressão dos sintomas e prolonga a sobrevida em cerca de 2 a 4 meses, o riluzol. Vários medicamentos têm demonstrado eficácia em aumentar a sobrevida em estudos pré-clínicos, mas o mesmo não ocorre em estudos clínicos. Os estudos pré-clínicos são realizados com um modelo animal modificado geneticamente chamado SOD1G93A, com mutação no gene da enzima Cu/Zn Superóxido Dismutase, que não é a única alteração observada na fisiopatologia da doença. Além disso, os estudos clínicos envolvem pacientes em vários estágios da doença, o que traz uma dificuldade extra na

estratificação dos grupos dos estudos. Portanto, os achados positivos dos estudos pré-clínicos devem ser avaliados com cautela.

Em relação à terapia celular, os estudos ainda são inconclusivos, porque há escassez de estudos clínicos controlados e a maior parte das evidências, mesmo que fortemente positivas, está restrita a estudos pré-clínicos e devem ser interpretadas com cautela.

Ensaio clínico randomizado com carbonato de lítio associado ao riluzole não demonstrou efetividade na redução da progressão dos sintomas ou na melhora da sobrevivência. Além disso, houve grande número de efeitos colaterais, o que levou à suspensão do estudo.

Um dos grandes problemas que existem em relação a ELA é a determinação de biomarcadores para a doença. A maior parte dos estudos utiliza como biomarcadores a morte por insuficiência respiratória, a realização de traqueostomia ou escalas funcionais com a Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R). Existe atualmente um grande esforço na busca de novos biomarcadores que auxiliem os ensaios clínicos e que ajudem a determinar o prognóstico da doença.

O estudo de fatores prognósticos inicialmente revelou forte correlação com idade avançada, estado nutricional e forma de inicio bulbar da doença. A maior parte dos estudos realizados no mundo corrobora a relação linear de pior prognóstico com o avançar da idade. A desnutrição vem sendo atribuída ao pior prognóstico em vários estudos. Com relação à forma de início bulbar, há controvérsias, porque a sobrevivência depende também do acesso ao adequado cuidado multidisciplinar e da tomada de medidas no tempo correto.

A avaliação prognóstica prospectiva buscou estabelecer um conjunto de variáveis clínicas de fácil uso, que pudessem auxiliar na tomada de decisão e no planejamento das condutas como uso de gastrostomia e dos sistemas de ventilação. O estudo comprova que fatores como idade acima de 65 anos, Capacidade Vital Forçada supina abaixo de 63%, tempo de progressão para o segundo segmento corporal menor que seis meses, associação de sinais do primeiro neurônio motor e fraqueza da musculatura flexora cervical são indicadores de prognóstico desfavorável e se

associados, têm alto valor preditivo de morte ou traqueostomia (74%) em oito a 12 meses.

Finalmente, o estudo comprova a eficiência do Centro de Referência de Doenças Neuromusculares da Secretaria de Estado de Saúde do Distrito Federal na redução das internações pela doença e no custo de cuidado dos pacientes para o sistema público de saúde brasileiro.

Esta tese reforça a necessidade de constituição de centros de referência multidisciplinares com a finalidade de promoção do cuidado paliativo aos pacientes com ELA, no âmbito do Sistema Único de Saúde, com a finalidade de melhorar a qualidade de vida, a sobrevivência e reduzir os custos dos sistemas de saúde.

6. REFERÊNCIAS

1. Gordon PH, Cheng B, Katz IB, Pinto M, Hays AP, et al. The natural history of primary lateral sclerosis. Neurology. 2006; 66:647-53.

2. Gordon PH. Amyotrophic Lateral Sclerosis: An update for 2013 Clinical Features, Pathophysiology, Management and Therapeutic Trials. Aging and Disease. 2013;4: 5.

3. Logroscino G, Traynor BJ, Hardiman O, Chio A, Mitchell D, et al. Incidence of amyotrophic lateral sclerosis in Europe. J Neurol Neurosurg Psychiatry 2010; 81:385-90.

4. Marin B, Gil J, Preux PM, Funalot B, Couratier P. Incidence of amyotrophic lateral sclerosis in the Limousin region of France, 1997-2007. Amyotroph Lateral Scler 2009; 10:216-20.

5. Ragonese P, Filippini G, Salemi G, Beghi E, Citterio A, et al. Accuracy of death certificates for amyotrophic lateral sclerosis varies significantly from north to south of Italy: implications for mortality studies. Neuroepidemiology 2004; 23:73-7.

6. Logroscino G, Traynor BJ, Hardiman O, Chio' A, Couratier P, et al. Descriptive epidemiology of amyotrophic lateral sclerosis: new evidence and unsolved issues. J Neurol Neurosurg Psychiatry 2008; 79:6-11.

7. Dietrich-Neto F, Callegaro D, Dias-Tosta E, Silva HA, Ferraz ME, et al. Amyotrophic lateral sclerosis in Brazil: 1998 national survey. Arq Neuropsiquiatr 2000; 58: 607–15.

8. Al-Chalabi A, Hardiman O. The epidemiology of ALS: a conspiracy of genes, environment and time. Nat Rev Neurol 2013; 9: 617–28.

9. Kanouchi T, Ohkubo T, Yokota T. Can regional spreading of amyotrophic lateral sclerosis motor symptoms be explained by prion-like propagation? J Neurol Neurosurg Psychiatry 2012; 83:739-45.

10. Ravits J, Appel S, Baloh RH, Barohn R, Brooks BR, et al. Deciphering amyotrophic lateral sclerosis: what phenotype, neuropathology and genetics are telling us about pathogenesis. Amyotroph Lateral Scler Frontotemporal Degener 2013; 14 Suppl 1:5-18.

11. Gordon PH, Meininger V. How can we improve clinical trials in amyotrophic lateral sclerosis? Nat Rev Neurol 2011; 7:650-4.

12. Miller RG, Jackson CE, Kasarskis EJ, England JD, Forshew D, et al. Practice parameter update: The care of the patient with amyotrophic lateral sclerosis: drug, nutritional, and respiratory therapies (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 2009; 73:1218-26.

13. Miller RG, Mitchell JD, Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). Cochrane Database Syst Rev. 2012; 3:CD001447.

14. Morrison KE, Dhariwal S, Hornabrook R, Savage L, Burn DJ et al. Lithium in patients with amyotrophic lateral sclerosis (LiCALS): a phase 3 multicentre, randomized, double-blind,placebo-controlled trial. Lancet Neurology 2013;12(4): 339-45.

15. Beghi E, Pupillo E, Bonito V, Buzzi P, Caponnetto C et al. Randomized double-blind placebo-controlled trial of acetyl-L-carnitine for ALS. Amyotroph Lateral Scler Frontotemporal Degener 2013; 14(5-6): 397-405.

16. Marin B, Desport JC, Kajeu P, Jesus P, Nicolaud B, et al. Alteration of nutritional status at diagnosis is a prognostic factor for survival of amyotrophic lateral sclerosis patients. J Neurol Neurosurg Psychiatry 2011; 82:628-34

17. Swingler, RJ. The prognosis of motor neurone disease. J Neurol Neurosurg Psychiatry 2010; 81:1062.

18. Turner MR, Brockington A, Scaber J et al. Pattern of spread and prognosis in lower limb-onset ALS. Amyotroph Lateral Scler 2010; 11(4): 369–73.

19. Ravits JM, La Spada AR. ALS motor phenotype heterogeneity, focality, and spread: deconstructing motor neuron degeneration. Neurology 2009; 73:805–11.

20. Inghilleri M, Iacovelli E. Clinical Neurophysiology in ALS. Arch Ital Biol. 2011; 149(1):57-63.

21. de Carvalho M, Swash M. Sensitivity of electrophysiological tests for upper and lower motor neuron dysfunction in ALS: a six-month longitudinal study. Muscle Nerve 2010; 41(2):208-11.

22. Costa J, Swash M, de Carvalho M. Awaji criteria for the diagnosis of amyotrophic lateral sclerosis:a systematic review. Arch Neurol 2012; 69(11): 1410-6.

23. Brasil. Esclerose Lateral Amiotrófica. Protocolos Clínicos e Diretrizes Terapêuticas. 2002. disponível em http://dtr2001.saude.gov.br/sas/dsra/protocolos/do_e16_01.pdf. Acesso em 28/10/2013.

24. Medical Research Council. Aids to examination of the peripheral nervous system. Memorandum no. 45. London: Her Majesty's Stationary Office; 1976.

25. Instituto Brasileiro de Geografia e Estatistica (IBGE) 2014. Censo Demográfico. Available at: http://www.ibge.gov.br/estadosat/perfil.php?sigla=DF. Último acesso (10-07-2015).

26. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ 2008; 336; 924-926.

27. Kung J, Chiappelli F, Cajulis OO, Avezova R, Kossan G et al. From systematic reviews to clinical recommendations for evidence-based health care: validation of revised assessment of multiple systematic reviews (R-AMSTAR) for grading of clinical relevance. The Open Dentristy Journal, 2010,4,84-91.

28. Collett D. Modelling Survival Data in Medical Research, 2nd ed. Boca Raton: CRC Press; 2003. 204p.

29. Cedarbaum JM, Stambler N, Malta E, Fuller C, Hilt D, et al. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BNDF ALS Study Group (Phase III). J Neurol Sci 1999; 169: 13-21.

30. Guedes Keyte, Pereira Cecília, Pavan Karina, Valério Berenice Cataldo Oliveira. Cross-cultural adaptation and validation of als Functional Rating Scale-Revised in Portuguese language. Arq NeuroPsiquiatr 2010; 68(1): 44-7.

31. Zhou P, Li X, Rymer WZ. Computing Motor Unit Index of the First Dorsal Interosseous Muscle with Two Different Contraction Tasks. Med Eng Phys 2012; 34(8): 1209-12.

32. Bromberg MB. MUNIX and MUNE in ALS. Clin Neurophysiol 2013; 124(3): 433-4.

33. Nandedkar SD, Barkhaus PE, Stalberg EV. Motor Unit Number Index(MUNIX): principle, method, and findings in healthy subjects and in patients with motor neuron disease. Muscle nerve 2010;42:798-807.

34. Chediek J. Atlas do Desenvolvimento Humano nas Regiões Metropolitanas Brasileiras – Brasília: PNUD, Ipea, FJP, 2014. 120 p.

35. Flegal KM, Kit BK, Orpana H, Graubard BI. Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. JAMA 2013;309:71-82.

36. Marin B, Couratier P, Preux PM, Logroscino G. Can mortality data be used to estimate amyotrophic lateral sclerosis incidence? Neuroepidemiology2011;36:29-38.

37. Steyerberg EW, Moons KGM, van der Windt DA, Hayden JA, Perel P, et al. Prognosis research strategy (PROGRESS) 3: Prognostic Model Research. PLoS Med 2013; 10(2):e1001381.

7. ANEXOS

Anexo A – Modelo para coleta de dados com escala ALSFRS-R

Anexo B - Documento 1 de aprovação do Comitê de Ética em Pesquisa da FEPECS

Anexo C - Documento 2 de aprovação do Comitê de Ética em Pesquisa da FEPECS

Anexo D – Termo De Consentimento Livre e Esclarecido

DATA
NOME **SEXO**
SES **DATA DE NASCIMENTO / / IDADE**
EXPERIMENTAL ( ) CONTROLE ( )
TELEFONE DE CONTATO

CRITÉRIOS DE INCLUSÃO:
DIAGNÓSTICO:
( )ELA DEFINITIVA-NMS e inferior NMI em 3 regiões
( )ELA PROVÁVEL- NMS e NMI em 2 regiões com algum sinal de NMS rostral.
( )ELA POSSÍVEL- NMS e NMI em uma região somente
( )ELA SUSPEITA- NMS em uma ou mais regiões ou Sinais de NMI em uma ou mais regiões.
MRI :
ENMG:
ANTI GM1:

1ª AVALIAÇÃO:
INICIO DOS SINTOMAS
PESO
1º MEMBRO ACOMETIDO
TEMPO PARA ACOMETIMENTO DO 2º MEMBRO
2º MEMBRO ACOMETIDO
EXAME NEUROLOGICO:

SEGMENTO	PROXIMAL	DISTAL	NMS	CERVICAL
MSD				
MSE				
MID				
MIE				

CVF supina: **prona:**
FLUXO DE PICO:
SAT OXIHB:
AMPLITUDE DO PAMC NERVO MEDIANO ABP
AMPLITUDE DO PAUM ABP
AMPLITUDE DO PAMC NERVO ULNAR ADM
AMPLITUDE DO PAUM ADM
LITEMIA

ALFRS :

MEDIDA	ALTERAÇÃO	PONTOS
FALA	Normal	4

	Alterações leves da fala	3
	Inteligivel com repetições	2
	Fala combinada com gestos	1
	Anartria	0
SALIVAÇÃO	normal	4
	Leve excesso noturno	3
	Excesso moderado engasgo minimo	2
	Excesso com disfagia	1
	Marcado,com disfagia constante	0
DEGLUTIÇÃO	normal	4
	Disfagia ocasional	3
	Alteração da consistencia	2
	Suplemetação por tubos	1
	Enteral ou parenteral	0
USO DA MÃO P ESCREVER	Normal	4
	Lenta com letras legíveis	3
	Algumas palavras legiveis	2
	Pega, mas não escreve	1
	Não segura a caneta	0
CORTAR E COMER, UTENSILIOS	SG, normal	4
	SG,Lento, mas sozinho	3
	SG,alguma ajuda p cortar	2
	SG, ajuda p cortar tudo	1
	SG, acamado	0
	G, normal	4
	G,Dificuldade leve	3
	G, independente mas com dificuldade	2
	G, executa com ajuda	1
	G, não executa	0
VESTUÁRIO E HIGIENE	Normal	4
	Independente com esforço	3
	Assistencia intermitente	2
	Assistencia necessária	1
	dependente	0
VIRAR NA CAMA	normal	4
	Lento e sem ajuda	3
	Com muita dificuldade	2
	Inicia mas não completa	1
	Com ajuda	0
MARCHA	normal	4
	Dificuldade precoce	3
	Com assistência	2
	Movimenta, mas não anda	1
	Não se movimenta	0
SUBIR ESCADAS	Normal	4
	Lento	3
	Fadiga ou instabilidade	2
	Assistência	1
	Não sobe	0
RESPIRAÇÃO	Normal	4
	Dispneia aos esforços	3
	Dispneia em repouso	2
	Assistência ventilatória intermitente, p ex noturna	1
	Dependente de VNI ou VI	0

ESCORE TOTAL:

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Difusão clínica, progressão neurofisiológica e prognóstico de pacientes com Doença do Neurônio Motor submetidos à associação de riluzol e carbonato de lítio - um ensaio clínico randomizado.

Pesquisador: Mirian Moura

Área Temática:

Versão: 1

CAAE: 24461313.8.0000.5553

Instituição Proponente: FUNDAÇÃO DE ENSINO PESQUISA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 525.241

Data da Relatoria: 27/01/2014

Apresentação do Projeto:

Ensaio clínico prospectivo randomizado fechado em pacientes com diagnóstico de Esclerose Lateral Amiotrófica no Centro de Referência para Doenças Neuromusculares do Hospital Regional da Asa Norte, observando-se a difusão clínica dos sintomas motores, os achados neurofisiológicos e a progressão da capacidade Vital Forçada com uso de riluzol associado ou não ao carbonato de lítio.

Objetivo da Pesquisa:

Objetivo Primário:

Avaliar a correlação entre a difusão clínica, a progressão neurofisiológica e o prognóstico da Doença do Neurônio Motor adquirida nos pacientes
tratados com associação de riluzol e carbonato de lítio.

Objetivo Secundário:

Correlacionar a distribuição e a difusão da fraqueza muscular determinado pelo exame neurológico e pelo cálculo do Índex do número de Unidades Motoras funcionantes (MUNIX) à Eletroneuromiografia e a Capacidade Vital Forçada (CVF), com a velocidade de progressão da doença;

Correlacionar a difusão da fraqueza muscular, as alterações neurofisiológicas e a função respiratória inicial com o desenvolvimento de insuficiência respiratória; ¿ Estabelecer o valor da associação de carbonato de lítio ao tratamento com riluzol na progressão da Esclerose Lateral Amiotrófica, através de escalas funcionais e achados neurofisiológicos.

Avaliação dos Riscos e Benefícios:

Qualquer pesquisa que envolva seres humanos deve observar os princípios da BENEFICÊNCIA, DA NÃO MALEFICÊNCIA, DA AUTONOMIA e da JUSTIÇA.

A análise dos riscos inerentes a qualquer pesquisa deve considerar a possibilidade de danos não somente na esfera física, como perda de janela terapêutica, sequelas, cicatrizes ou a própria morte, como também aquelas da estatura moral, psíquica, intelectual, cultural, espiritual em qualquer dos momentos da pesquisa.(CONSELHO NACIONAL DE SAÚDE, Resolução nº 466, 12 de dezembro de 2012, Diário Oficial da União)

Estas dimensões são apreciadas nos Conselhos de Ética em Pesquisa, que são colegiados com múnus público e que defendem os interesses dos sujeitos de pesquisas, sua integridade e dignidade contribuindo para o controle dos padrões éticos das pesquisas.

Assim, os riscos associados à pesquisa confrontados com os que os sujeitos normalmente estão expostos;as medidas tomadas para mitigar os riscos previsíveis; os prováveis benefícios da pesquisa; a razoabilidade entre os riscos e benefícios; verificar se a descrição e informação dos riscos, desconfortos ou benefíciosantecipados serão recebidos pelos sujeitos; os intervalos dos relatórios a serem apresentados, pelo pesquisador ao CEP, necessários ao acompanhamento da pesquisa são verificados pelas Comissões de Ética em Pesquisa.

As pesquisas isentas de risco são aquelas em que se não são realizadas intervenções, invasão da intimidade ou modificações nas variáveis fisiológicas ou psicológicas dos sujeitos.

O presente projeto, por conseguinte, não é isento de risco, contudo, por suas características o risco é mínimo guardando uma RELAÇÃO RISCO X BENEFÍCIO ADEQUADA.

Precaução especial deverá ser dado em qualquer evento adverso que deverá ser comunicado imediatamente a este Conselho de Ética em Pesquisa.

Comentários e Considerações sobre a Pesquisa:

Trata-se de pesquisa que busca verificar a difusão clínica dos sintomas motores, os achados neurofisiológicos e a progressão da capacidade Vital Forçada com uso de riluzol associado ou não ao carbonato de lítio em paciente com Esclerose Lateral Amiotrófica.

Considerações sobre os Termos de apresentação obrigatória:

Folha de Rosto; Projeto de Pesquisa em Português; Critérios de Inclusão e Exclusão; Termo de Consentimento Livre e Esclarecido; Orçamento do Projeto; Curricullum vitae dos pesquisadores; Termos de Concordância. FORAM APRESENTADOS

Recomendações:

Precaução especial deverá ser dado em qualquer evento adverso que deverá ser comunicado imediatamente a este Conselho de Ética em Pesquisa.

Conclusões ou Pendências e Lista de Inadequações:

Diante do exposto opino pela APROVAÇÃO do protocolo.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

BRASILIA, 10 de Fevereiro de 2014

Assinador por:
luiz fernando galvão salinas
(Coordenador)

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Perfil epidemiológico da Doença do Neurônio Motor no Distrito Federal
Pesquisador: Mirian Moura
Área Temática:
Versão: 1
CAAE: 36791514.4.0000.5553
Instituição Proponente: FUNDAÇÃO DE ENSINO PESQUISA
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 820.117
Data da Relatoria: 06/10/2014

Apresentação do Projeto:
sem alterações

Objetivo da Pesquisa:
sem alterações

Avaliação dos Riscos e Benefícios:
sem alterações

Comentários e Considerações sobre a Pesquisa:
sem alterações

Considerações sobre os Termos de apresentação obrigatória:
A planilha de orçamento deve ser anexada ao projeto, mesmo que os custos (gastos) sejam baixos e de responsabilidade da própria pesquisadora

Recomendações:
Acrescentar na estrutura da pesquisa os custos inerentes à sua execução

Conclusões ou Pendências e Lista de Inadequações:
Seguir a recomendação acima

Situação do Parecer:
Aprovado

Endereço: SMHN 2 Qd 501 BLOCO A - FEPECS
Bairro: ASA NORTE **CEP:** 70.710-904
UF: DF **Município:** BRASILIA
Telefone: (61)3325-4955 **Fax:** (33)3325-4955 **E-mail:** comitedeetica.secretaria@gmail.com

Continuação do Parecer: 820.117

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

BRASILIA, 06 de Outubro de 2014

Assinado por:
LUIZ FERNANDO GALVÃO SALINAS
(Coordenador)

1 via.

Termo de Consentimento Livre e Esclarecido - TCLE

O (a) Senhor(a)---------- está sendo convidado(a) a participar do projeto: **DIFUSÃO CLÍNICA, PROGRESSÃO NEUROFISIOLÓGICA E PROGNÓSTICO DE PACIENTES COM DOENÇA DO NEURÔNIO MOTOR SUBMETIDOS À ASSOCIAÇÃO DE RILUZOL E CARBONATO DE LÍTIO – UM ENSAIO CLÍNICO RANDOMIZADO.**

O objetivo desta pesquisa é: determinar a progressão clínica e à eletroneuromiografia da Esclerose Lateral Amiotrófica em pacientes utilizando o tratamento medicamentoso com Riluzol associado ou não a Carbonato de Lítio.

O(a) senhor(a) receberá todos os esclarecimentos necessários antes e no decorrer da pesquisa e lhe asseguramos que seu nome não aparecerá, sendo mantido o mais rigoroso sigilo pela omissão total de quaisquer informações que permitam identificá-lo(a)

A sua participação será através de duas entrevistas com exame físico e eletroneuromiografia de superfície no Setor de Doenças Neuromusculares do Hospital Regional da Asa Norte na data combinada com um tempo estimado para sua realização de 120 minutos. Não existe obrigatoriamente, um tempo pré-determinado para a avaliação e respeitado o tempo de cada um. Informamos que o(a) Senhor(a) pode se recusar a responder qualquer questão que lhe traga constrangimento, podendo desistir de participar da pesquisa em qualquer momento sem nenhum prejuízo para o(a) senhor(a).

O Sr Poderá ou não ser selecionado para utilizar o medicamento carbonato de lítio.

Caso o Sr apresente manifestações clinicas e alteração aos exames laboratoriais periódicos, o(s) medicamento(s) poderão ser suspenso(s).

Para realização dos exames laboratoriais, será necessária uma cooperação maior, já que envolve a coleta de sangue venoso que, apesar de causar pequeno desconforto, é rápida e segura, feita por profissionais habilitados. Dentre os exames realizados, estarão basicamente o hemograma, litemia e enzimas hepáticas e uma pequena quantidade de sangue, em geral, será suficiente. Será também realizada espirometria para mediar a capacidade de soprar.

2 via.

Em relação à eletroneuromiografia, será realizada por médico neurofisiologista devidamente habilitado, com formação em neurologia e especialização em eletromiografia. O exame é totalmente seguro e consiste em duas etapas: o estudo da condução nervosa e o eletromiograma com eletrodo de superfície, sendo necessário o uso de eletrodo de agulha apenas para os pacientes que não tiverem seu diagnóstico confirmado. Na primeira fase, o Sr poderá receber, em pontos específicos do corpo, um estímulo elétrico, semelhante a choque, que não causará nenhum dano, exceto dor tolerável. Na ultima etapa, poderão ser introduzidas agulhas em músculos selecionados e o Sr. devera contrair o músculo em estudo. Neste caso, poderá ocorrer, além da dor pela introdução de agulha, pequenos sangramentos.

Os resultados da pesquisa serão divulgados na Fundação de Ensino e Pesquisa em Ciências da Saúde do Governo do Distrito Federal, podendo ser publicados posteriormente. Os dados e materiais utilizados na pesquisa ficarão sobre a guarda do pesquisador.

Se o(a) Senhor(a) tiver qualquer dúvida em relação à pesquisa, por favor telefone para: **Dra Mírian Moura no Ambulatório do Hospital Regional da Asa Norte – Setor de Doenças Neuromusculares no telefone – 33254357,** no horário compreendido entre 8 e12 h e das 14 às 17 h.

Este projeto foi Aprovado pelo Comitê de Ética em Pesquisa da SES/DF. As dúvidas com relação à assinatura do TCLE ou os direitos do sujeito da pesquisa podem ser obtidos através do telefone: (61) 3325-4955.

Este documento foi elaborado em duas vias, uma ficará com o pesquisador responsável e a outra com o sujeito da pesquisa.

_____

Nome / assinatura

_____

Pesquisador Responsável

Nome e assinatura

Brasília, ___ de _____de _____