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REVIEW ARTICLE

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Stem cell therapy in the treatment of Amyotrophic Lateral Sclerosis: systematic review

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ABSTRACT

Introduction: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by the progressive death of motor neurons, resulting in severe functional impairment. This study presents a systematic review of clinical trials published in the last decade regarding the use of stem cells in the treatment of ALS. **Objective:** To analyze the efficacy of cell transplantation, measured by the reduction of functional decline, using the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R), and the secondary objective was to identify the type of stem cell and the route of administration that yielded the most promising outcomes to date. **Methods:** Articles were selected from the PubMed database using the descriptors "Amyotrophic Lateral Sclerosis," "stem cell therapies," and "motor neuron disease." Clinical studies conducted in humans and published between January 2013 and March 2024 were included. **Results:** The search identified 26 articles, of which 18 were selected and analyzed according to inclusion and exclusion criteria. Sequentially transplanted mesenchymal stem cells demonstrated the best results in reducing functional decline, particularly in patients at early stages of the disease. The repeated intrathecal route of administration was found to be the most promising for slowing disease progression, with fewer severe adverse effects. **Conclusion:** This study proposes that future research focus on the repeated transplantation of autologous mesenchymal cells via the intrathecal route.

Keywords: Stem cell; therapies, investigational; mesenchymal stem cells; motor neuron disease; Amyotrophic Lateral Sclerosis; drug administration routes.

INTRODUCTION

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disorder that destroys motor neurons in the spinal cord, brainstem, motor cortex, and pyramidal and bulbar tracts[1,2]. Its initial symptoms vary according to disease onset, reflecting marked clinical heterogeneity[3]. Some patients present with spinal involvement, manifesting progressive limb weakness and altered reflexes (hypo- or hyperreflexia depending on lower or upper motor neuron compromise), while others develop bulbar symptoms such as dysarthria, dysphagia, and sialorrhea[4]. The disease course is marked by rapid, irreversible muscle atrophy, with respiratory failure as the leading cause of death[3,4]. Mean survival ranges from two to five years[1].

The global prevalence of ALS is approximately 5 cases per 100,000 people[4,5]. In Brazil, there is a lack of nationwide epidemiological data on the disease. However, a study conducted in the country identified an overall incidence of 0.89 per 100,000 inhabitants and 2.3 per 100,000 inhabitants in the age group between 45 and 80 years, demonstrating increased incidence in older age groups[6]. The same study reported an age-adjusted mortality rate of 0.61 to 0.89 per 100,000 person-years for individuals over 20 years of age, and 1.77 to 2.3 per 100,000 person-years for those over 45 years[6].

The diagnosis of ALS is clinical and requires excluding other causes of progressive muscle weakness through tests such as electromyography, cerebrospinal fluid analysis, and magnetic resonance imaging[4,7]. Diagnostic confirmation is often difficult, given the heterogeneity of clinical manifestations[3,4]. To aid this process, several criteria have been proposed, including the El Escorial, Revised El Escorial, Awaji, and Gold Coast (92% sensitivity, higher than the others)[7].

The Revised El Escorial Criteria, among the earliest developed, classify ALS as “definite,” “probable,” “possible,” or “suspected”[8]. The Awaji Criteria integrate clinical, electrophysiological,

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and neuroimaging findings to increase diagnostic sensitivity[9], assigning equal weight to electromyographic evidence of lower motor neuron dysfunction and clinical signs, thus considering fasciculation potentials as active denervation[9]. The Gold Coast Criteria defines ALS by progressive motor impairment with evidence of upper and lower motor neuron dysfunction in at least one body region, while excluding alternative diagnoses[10].

The pathophysiology of ALS is multifactorial and not yet fully understood. One of the processes involved is neuroinflammation and subsequent neuronal apoptosis, triggered by altered microglia producing pro-inflammatory cytokines[11]. Glutamate-induced neurotoxicity is another proposed mechanism in ALS pathogenesis, as this neurotransmitter activates calcium-dependent intracellular pathways, leading to motor neuron apoptosis[3]. Mitochondrial dysfunction is also implicated, resulting in reduced adenosine triphosphate (ATP) production and increased reactive oxygen species (ROS) levels[12].

Approximately 90% of amyotrophic lateral sclerosis (ALS) cases are sporadic, meaning they represent the first diagnosis within a family[2]. The remaining 10% are classified as familial ALS (fALS), characterized by the occurrence of the disease in at least two family members, usually with an autosomal dominant inheritance pattern. About half of FALS cases are associated with mutations in known genes, most frequently *SOD1, C9orf72, FUS,* and *TARDBP*[2]. In Brazil, *VAPB* and *C9orf72* mutations are the most prevalent[5].

The treatment of ALS is predominantly multidisciplinary and palliative, with only three drugs approved by the US Food and Drug Administration as of 2025: Riluzole (a glutamate antagonist), Edaravone (oxidative stress reducer), and Tofersen (SOD1 protein level reducer)[13]. In Brazil, ANVISA (regulatory agency) has only approved Riluzole and Edaravone, with the former as the sole available drug through the public healthcare system (SUS)[14].

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Given the incurable and highly debilitating nature of the disease, the search for new treatments has become an increasing priority within the medical and scientific communities. Various strategies have been proposed, ranging from gene therapy, glutamate antagonist drugs, antioxidant medications, to stem cell transplants[2,13].

Stem cells possess the capacity for self-renewal and differentiation into diverse cell types and can be derived from multiple sources[15]. These include embryonic stem cells (ESCs) from the inner cell mass of blastocysts, embryonic germ cells (EGCs) from primordial germ cells of post-implantation embryos, and induced pluripotent stem cells (iPSCs), generated from adult somatic cells such as fibroblasts through reprogramming genes[15]. Tissue-specific stem cells may also be isolated, including bone marrow–derived mesenchymal stem cells (BM-MSCs) and neural stem cells (NSCs), the latter being multipotent and able to differentiate into neurons[15].

In this context, stem cell therapy in ALS has emerged as a promising therapeutic strategy, supported by multiple clinical studies over recent years demonstrating safety and variable effects on disease progression[16].

The primary objective was to analyze the efficacy of cell transplantation, measured by the reduction of functional decline, using the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R), and the secondary objective was to identify the type of stem cell and the route of administration that yielded the most promising outcomes to date.

METHODS

This systematic review included experimental clinical studies, whether randomized or not (quasi-experimental), published between January 1, 2013, and March 1, 2024, that employed stem cells for the treatment of ALS in humans, focusing on safety and disease progression as assessed

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by the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R), a widely used and validated scale for the functional assessment of ALS patients[17].

The articles were searched through the PubMed, Lilacs, and SciELO databases, using the following English descriptors: "(Amyotrophic Lateral Sclerosis) and (stem cell therapies) and (motor neurone disease)." The search was conducted independently by two reviewers, using the same descriptors and inclusion and exclusion criteria, yielding identical results.

The inclusion criteria encompassed controlled clinical studies in humans that performed stem cell transplantation in ALS patients and assessed its efficacy using the ALSFRS-R scale. Literature reviews, gene therapy studies, treatments with isolated growth factors or experimental pharmacological treatments, as well as studies involving only animals or addressing other neurodegenerative diseases, were excluded from the analysis.

The risk of bias in the articles was assessed using the Joanna Briggs Institute (JBI) tool, checklists used to judge the internal validity and conclusion validity of quasi-experimental and randomized studies[18,19]. In this review, it was established that no negative responses on the checklist would classify the study as having a low risk of bias; one to three negative responses would classify it as moderate risk; and more than three negative responses would classify it as having a high risk of bias.

In addition to the JBI tool, the Robvis (Risk of Bias Visualization) aid, in the form of a "traffic light" (Figures 1 and 2), allowed for a clear visualization of the risk of bias in the articles. For quasi-experimental studies, the ROBINS-I version (Figure 1) was used, while for randomized studies, the RoB 2.0 Cluster version (Figure 2) was applied[20,21]. Studies with low or moderate risk of bias were included in the study, while those with high risk would be excluded.

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The data were extracted and tabulated using Microsoft Excel software. The articles were evaluated regarding the type of stem cell used (mesenchymal, neural progenitor, fetal neural, or other), the transplantation route (intrathecal, intramedullary, intravenous, or intramuscular), adverse effects, transplant tolerability, and efficacy as assessed by disease progression.

RESULTS

Data extraction and risk of bias analysis

In April 2024, 26 articles were identified on the PubMed platform, of which 18 were selected, and eight were excluded based on the exclusion criteria applied to titles and abstracts, as demonstrated in the PRISMA[22] diagram (Figure 3). No publications were found on the Lilacs and SciELO platforms using the descriptors. There was no disagreement between the reviewers' results. Of the included articles, three are randomized clinical studies, two of which are placebo-controlled, and 15 are quasi-experimental, totaling a sample of 623 patients, including controls and matched data.

All selected studies were classified as having moderate or low risk of bias and were included in the review. In the second phase, the articles were extensively analyzed, with data extraction and tabulation. The majority had safety and transplant tolerability as the primary endpoint, and functionality impact as the secondary endpoint, assessed using the ALSFRS-R scale.

Types of stem cells and transplants

For the analysis of results, the studies were grouped according to the type of stem cell and the route of administration (Table 1). Of the 18 clinical trials, nine used mesenchymal stem cells

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(MSCs) from bone marrow[23-31]. In three of these studies, the cells were induced to secrete neurotrophic factors (MSC-NTF)[27,28,31]. Only one study used mesenchymal stem cells from Wharton's jelly (WJ-MSC)[32].

In addition, other types of stem cells were employed: fetal neural spinal cord (NFME)[33], fetal neural (hNSC)[34,35], spinal cord (HSSC)[36,37], glial neural progenitors (GDNF)[38], mononuclear bone marrow (ABMNC)[39], and stress-resistant multi-lineage differentiated (MUSE) cells[40].

As for the administration routes used in the transplants, seven studies opted for the intramedullary (IMd) or intraspinal (IE) route[33-39], seven studies exclusively used the intrathecal (IT) transplant route[23,24,26,29-32], two chose the IT and/or intramuscular (IM) route[27,28], one performed the intravenous (IV) transplant[40], and another combined the IMd and IV routes[25].

Safety and Adverse Effects

Most of the articles included in this systematic review had the primary objective of assessing the safety and tolerability of stem cell transplantation. All studies reported this therapeutic modality as safe and tolerable, although they observed some adverse effects (AEs). The most frequent AEs were: pain at the puncture site[31], headache and/or nausea after lumbar puncture[24-27,29,30,32], neuropathic pain[38], low-grade fever[23-25,30], paresthesia[38], myalgias[23], low back pain[23,24,27], fatigue and malaise[30], muscle spasms[35], pain in the dermatome corresponding to the transplant site[34], and infection at the surgical site[33]. All these symptoms were appropriately treated and did not cause further problems.

On the other hand, in four studies, serious AEs related to the transplant were observed, including cervical kyphosis[33,36], iatrogenic diabetes[35], acute neurological deterioration due to edema in the spinal cord, and refractory pain[37]. These complications occurred in studies that used

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the IMd transplant route, suggesting that this route carries a higher risk compared to the IT route. No study reviewed reported deaths directly attributable to stem cell treatment.

Effectiveness

The efficacy of the transplant was assessed in all studies through the decline in ALSFRS-R score on the ALSFRS-R scale. This scale ranges from 48 points (complete functionality) to 0 points (total dysfunction) and is widely validated to classify the functional status of patients with ALS[17].

Cudkowicz *et al.*[31] conducted a phase III study with MSC-NTF transplant via IT but did not obtain statistically significant results (p=0.45). In the MSC-NTF group, participants with ALSFRS-R ≥26 points (early disease) progressed an average of 1.20 points less compared to the placebo group after 28 weeks, although without significance (p>0.05). Another study[28] with MSC-NTF showed no difference in the global functionality curve evolution between the placebo and intervention groups, except in the subgroup of patients with rapid progression (n=21) at weeks 4 and 12 (p=0.004 and 0.046, respectively).

Petrou *et al.*[27], in a study with MSC-NTF transplanted via IM and/or IT, observed a slowing of functional decline only in the subgroups that received intrathecal injections. In phase I group (n=12), the average monthly reduction in ALSFRS-R score was 1.56 in the three months before the transplant, dropping to 0.28 in the six months following. In the phase IIa group (n=14), the drop was from 1.4 to 0.6. *Post hoc* analysis indicated a trend toward reduced average decline in the scale, almost reaching statistical significance (p=0.052).

Rushkevich *et al.*[25] demonstrated that transplanting MSCs induced to neuronal differentiation route IV followed by IT resulted in a trend toward reduced functional decline in the

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intervention group, with a decrease of 0.38 (0.29–0.80) compared to 1.47 (1.0; 1.58) in the control group (p=0.003). In another study[32], patients treated with WJ-MSC via IT were compared to peers from the PROACT database. A significant difference in overall survival was observed between the two groups: 1,183 days (WJ-MSC) versus 640 days (PROACT) (p=0.002).

Kim *et al.*[23] evaluated biomarkers to identify patients who could benefit from intrathecal transplantation of autologous BM-MSCs, classifying them as responders and non-responders. In the first three months, responders showed a slower decline in ALSFRS-R score (0.79±1.71) compared to non-responders (3.06±1.81) ($p<0.01$). Significantly higher levels of vascular endothelial growth factor (VEGF), angiogenin (ANG), and transforming growth factor-beta (TGF-β) were observed in the cultures of BM-MSC cells from responder patients ($p<0.05$).

A South Korean[24] study used BM-MSC via IT in two injections with a 26-day interval. Six months after treatment, the average rate of functional decline was 0.02±0.18, significantly lower compared to the pre-treatment period (1.38±0.45) ($p<0.001$)[24]. However, the intrathecal transplant of three stem cell injections performed by Siwek *et al.*[29], with the first two being MSC and the last one WJ-MSC or allogenic MSC, showed no difference in the monthly rate of functional reduction before, during, and after treatment (p=0.57).

On the other hand, Syková *et al.*[26] observed, in a study with BM-MSC via IT in a single dose, a significant reduction in ALSFRS decline three months after administration ($p<0.02$) when comparing pre- and post-treatment data, although this reduction was less pronounced at six months ($p<0.05$). The effect was most evident in the subgroup (n=12) of patients with worsening in the functional scale in the six months before the transplant, and was not observed in patients with stable values.

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A second study conducted by Petrou *et al.*[30], with autologous MSC via IT, showed a deceleration of functional loss after each of the four cycles, compared to the pre-treatment period: –1.054±0.86/month during the adaptation period versus –0.051±1.03 after the first transplant; +0.019±0.39 after the second MSC injection, –0.253±0.26 after the third injection, and an overall reduction of –0.445±0.81. The overall beneficial effect was statistically significant (p=0.0038).

The transplant via IE of NFME did not show a reduction in disease progression[33]. Similarly, two different studies[34,35] conducted by the same group of Italian researchers with fetal hNSC did not indicate a significant long-term reduction in the decline of functionality. However, the second study[35] showed a transient decrease in ALSFRS-R decline immediately after the transplant, between the first and fourth months (p=0.0136), but this effect was not sustained throughout the follow-up.

Two American studies[36,37] employed HSSC stem cells in intramedullary transplants in the cervical and/or lumbar spine. In the first study[36], half of the patients showed disease progression slower than expected, but without statistical significance. In the second study[37], there was no significant difference in progression between the treatment and control groups. Another study[38] used GDNF stem cells in unilateral intramedullary transplants, showing a slower loss of strength in the treated limb, although without statistical significance (p=0.16).

Ruiz-López *et al.*[39], in a study with autologous ABMNC via intramedullary transplantation, did not observe a reduction in the decline of the score, comparing the six months before treatment (-0.6) with the six months after (-0.7). In another study[40], MUSE stem cells were administered via the intravenous route in five patients, with three showing a reduction in the rate of decline, one with an increase, and one with stabilization, with no significant difference (p=0.094).

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DISCUSSION

In the selected studies, various combinations of stem cell types, collection methodologies, administration routes, number of cells per session, number of sessions, and intervals between them were observed. Eight studies conducted phase I of the research, three only phase II, six published phase I and II tests jointly, and only one study, conducted by Harvard University, advanced to phase III[31]. These data indicate that the use of stem cells for ALS treatment is still in a preliminary phase, although the safety of the procedure has already been established.

In the 18 articles analyzed, similar selection criteria were applied: most studies required participants to be between 30 and 80 years of age, have a possible, probable, or confirmed ALS diagnosis, and the absence of tracheostomy or gastrostomy. These criteria aimed to ensure the most accurate diagnosis possible and avoid advanced stages of the disease, which could compromise both the interventions and the analysis of the results.

Similarly, patients with other neurological, psychiatric, or systemic diseases, as well as those who had participated in previous stem cell studies or had a history of bone marrow transplantation, were excluded. Furthermore, users of Riluzole or Edaravone were required to maintain a stable dose for at least one month before the study interventions. Only patients capable of understanding the risks and consenting to participate in the study, as well as women without pregnancy risk, were included. These criteria resulted in a total sample of 623 patients across 18 studies.

None of the studies, including those that performed multiple injections (ranging from 20 to 40 doses at different surgical times) or employed doses with increasing cell numbers, reported intolerance or safety concerns to the transplantation procedures. Most adverse effects were self-

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limiting and related to the procedure. Severe adverse effects were rare (n=14) and associated with post-transplant inflammatory responses[33,35,37].

Regarding the functional response to stem cell treatment, only four studies[24-26,30] presented statistically significant results ($p<0.05$). Berry *et al*.[28], using MSC-NTF cells induced neuronal differentiation, observed a significant response only in the subgroup of patients with rapid progression, suggesting that this patient profile may benefit more from this treatment.

Similarly, a study conducted by Petrou *et al.*[27], with MSC-NTF via IM and IT, showed a trend toward significance ($p=0.052$). This result may be due to the small number of patients in phase I (n=12) and phase II (n=4) involved in the trial. Another subsequent study, carried out by the same group with autologous MSC via IT, observed a slowdown[30] in the loss of function compared to the pre-treatment period ($p=0.003$).

All the studies that showed a significant clinical functional response ($p<0.05$) used MSC[24-26,30], suggesting that these cells are more effective in reducing functional loss in ALS patients. Among these four studies, one used MSC-NTF[30], another used MSC-induced neuronal differentiation[25], and the other two used autologous bone marrow MSC[24,26]. However, no clear differences were observed in the benefits provided by each cell subtype.

In the randomized clinical trial[28] (n=48), placebo-controlled, with MSC-NTF administered via IM and IT in a single dose, there was no reduction in the global functional decline over the follow-up period, except in the subgroup of patients with rapid progression (n=21), who showed improvement in the first few weeks after transplantation (four and twelve weeks; $p=0.004$ and 0.046, respectively). These findings suggest that the approach may be promising in trials with repeated monthly doses in patients with rapid disease progression[28].

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The only clinical trial included in this systematic review that extrapolated the endpoints of safety and efficacy was conducted by Kim *et al*.[23], who also evaluated biomarkers to identify which patients would benefit from intrathecal transplantation of autologous BM-MSC, classifying them as responders and non-responders. In the first three months, the responders showed a deceleration in the decline of ALSFRS-R score (0.79±1.71) compared to the non-responders (3.06±1.81) ($p<0.01$). Significantly higher levels of vascular endothelial growth factor (VEGF), angiogenin (ANG), and transforming growth factor-beta (TGF-β) were observed in the cultures of BM-MSC cells from the responder patients ($p<0.05$). This suggests that the trophic profile may influence the functional outcome and that the analysis of these biomarkers in MSC cell cultures could be useful for determining treatment indications.

An Italian study[35] with fetal hNSC administered via intramedullary in a single dose showed a transient reduction in functional decline up to four months after transplantation ($p=0.0136$), but with no sustained effect after that period. This suggests that repeated doses could improve patient functionality. On the other hand, the IT transplantation of WJ-MSC, in three bi-monthly doses, significantly increased the survival of participants compared to the PROACT control group ($p=0.002$), although with no considerable impact on functionality, indicating a possible survival benefit of WJ-MSC in patients[32].

Additionally, it is noteworthy that the number of doses in studies with statistically significant results ranged from two to four, with intervals between 26 days and seven months (Table 1). This reinforces the idea that repeated administration of cell transplantation may prolong functional benefits[24,25,30,31]. Only one study[26] with a significant result, performed a single transplantation session, and in this case, the functional decline was less at two months than at six, supporting the hypothesis that repeated administrations may prolong functional effects.

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In contrast to the promising results observed with MSC cells in other studies, Siwek *et al.*[29], with the same cell type in three quarterly IT administrations, failed to demonstrate functional benefits (p=0.57). This result may stem from the small number of participants and significant loss to follow-up (initial n=15; final n=8), as well as the fact that in two patients, the third dose was WJ-MSC and in two others, allogeneic MSC.

Studies with other types of stem cells (NFME, fetal hNSC, HSSC, fetal GDNF, ABMNC, and MUSE) did not show statistically significant functional benefits in reducing disease progression, as indicated by the decline in ALSFRS-R scores[33,34,37-40]. In this context, it can be inferred that autologous mesenchymal stem cell transplantation is the approach with the best potential efficacy.

It is important to highlight that three of the four studies that had statistically significant results used the IT route[24,26,30]. This same route was employed in studies with promising initial results[23,27,28], with adverse effects, in all of them being non-severe. In contrast, severe adverse effects occurred in studies using intramedullary transplantation[33,35-37]. These data suggest that IT administration not only provides better functional outcomes but also presents a reduced risk of severe adverse effects.

Conclusion

In summary, this systematic review indicates that intrathecal transplantation of mesenchymal stem cells (MSC) stands out as a promising approach to reducing functional decline in patients with Amyotrophic Lateral Sclerosis (ALS). Most of the analyzed studies followed rigorous participant selection criteria and did not report severe adverse effects, with repeated dose administration showing prolonged functional benefits. In this regard, the evaluation of trophic

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factors such as VEGF, ANG, and TGF-β may help identify patients with greater potential for treatment response. However, the heterogeneity among studies, the need for more advanced-phase clinical trials, and the scarcity of studies in emerging countries highlight the urgency of further research to consolidate this therapeutic approach.

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Table 1: Clinical trials according to the type of stem cell used.

Author/Year	Study type	Patients	Cell type	Number of cells per Administration	Number of Administrations	Total number of doses and dosing interval	Total cell number	Route of administration
Kim *et al.* 2014[23]	Quasi-experimental	45	Autologous MSC	1.0x10^6	1	2 (1 month interval)	2.0x10^6	Intrathecal L2-3 level
Riley *et al.* 2014[33]	Quasi-experimental	18	Fetal neural spinal cord (NFME)	1.0x10^5	5 (unilateral) or 10 (bilateral)	single dose	5.0x10^5 or 1.0x10^6	Intraspinal: Cervical C3-5 level or Cervical + thoracolumbar T10-11 level
Feldman *et al.* 2014[36]	Quasi-experimental	15	HSSC	1.0x10^5	5 (unilateral) or 15 (bilateral)	2 (1 month interval)	5.0x10^5 or 1.5x10^6	Intramedullary: Lumbar L2-4 and/or cervical C3-5
Mazzini *et al.* 2015[34]	Quasi-experimental	6	hNSCl fetal neural	7.5x10^5	3 (unilateral) or 6 (bilateral)	2 (3 months interval)	2.25x10^6 or 4.5x10^6	Intramedullary: T8-11 level (anterior horn)
Oh *et al.* 2015[24]	Quasi-experimental	8	BM-MSC autologous	Between 4.8^107 and 8.6^107	1	2 (26-day interval)	between 9.6x10^7 and 1.72x10^8	Intrathecal L2-4 level
Rushkevic *et al.* 2015[25]	Quasi-experimental	25	Autologous BM-MSC with induction of neural differentiation	4.2x10^7 to 1.0x10^8 + 5.0x10^6 to 9.7x10^6	2 (one IV and 7 days after, one Intramedullary)	single dose or 2 (5-7 months interval)	between 4,7x10^7 and 1.18x10^8	Intravenous and intrathecal L3- L4 level
Glass *et al.* 2016[37]	Quasi-experimental	15	HSSC	1.0x10^6 to 8.0x10^5	10 to 40	single dose or 2 (1 month interval)	between 2.9x10^6 and 1.6x10¨7	Intramedullary: C3-5 in all patients. A group of 3 patients also received cells in the L2-4 level.
Ruiz-Lopez *et al.* 2016[39]	Quasi-experimental	11	ABMNC autologous	Cells not counted	2	single dose	Data not available	Intramedullary: T3-T4 level, gray substance of the posterior horn
Sykova *et al.* 2016[26]	Quasi-experimental	26	BM-MSC autologous	Berteen 1.5x^7 to 4.5x10^7	1	single dose	Between 1,5x10^7 to 4.5x10^6/kg	Intrathecal via lumbar puncture
Petrou *et al.* 2016[27]	Quasi-experimental	26	NTF-MSC autologous	IM 1.0x10^6 or IT 1.0x10^6/kg. A second group received 1.0-2.0x10^6 IM and 1.5-2.0x10^6	24 IM spots (biceps and triceps) and/or 1 IT	single dose	1.0x10^6 to 2.0x10^6 + 2.0x10^6/kg	Intramuscular and/or intrathecal
Berry *et al.* 2019[28]	Randomized clinical trial	48	MSC-NTF	1.2x10^8 IT. 4.8x10^7 IM	1 IT and 24 IM	single dose	1.68x10^8	Intrathecal and intramuscular
Mazzini *et al.* 2019[35]	Quasi-experimental	18	hNSC fetal	7.5x10^5	1 oe 2	single dose	7,5x10^5	Intramedullary: microtransplant T8-11 mono- or bilateral. C3-5 mono- and bilateral
Siwek *et al.* 2020[29]	Quasi-experimental	15	BM-MSC autologous	1.0x10^7	1	3 (3 months interval)	3.0x10^7	Intrathecal lumbar
Barczewsk *et al.* 2020[32]	Quasi-experimental	134	WJ-MSC	3.0x10^7	1	3 (bimonthly)	9.0x10^7	Intrathecal L3-4 level
Petrou *et al.* 2021[30]	Quasi-experimental	20	BM-MSC autologous	1.0x10^6/kg	1	1-4 (quarterly or semi-annually)	1.0-4.0x10^6/kg	Intrathecal L4-5 level
Baloh *et al.* 2022[38]	Quasi-experimental	18	GDNF fetal cortex	2.0x10^5 or 5.0x10^5	10	single dose	2.0x10^6 or 5.0x10^6	Intramedullary: Unilateral lumbar in the transition zone of the spinal cord
Cudkowicz *et al.* 2022[31]	Randomized clinical trial	196	MSC-NTF	Data not available	1	3 (bimonthly)	Data not available	Intrathecal
Yamashita *et al.* 2023[40]	Quasi-experimental	5	MUSE allogenous	1.5x10^7	1	6 (monthly)	1.2x10^8	Intravenous

Total N: 623 patients

IT: intrathecal. IV: intravenous. IM: intramuscular. Intramedullary: intramedullary. MUSE: multilineage-differentiated stress-resistant stem cells. MSC: mesenchymal stem cells. BM-MSC: bone marrow-derived mesenchymal stem cells. ABMNC: autologous bone marrow mononuclear cells. hNSC: human neural stem cells. GDNF: neural progenitor glial cells transduced with glial cell line-derived neurotrophic factor. BM-MSC: bone marrow-derived mesenchymal stem cells. WJ-MSC: Wharton's jelly-derived mesenchymal stem cells. MSC-NTF: bone marrow-derived mesenchymal stem cells induced to secrete neurotrophic factors. HSSC: human spinal cord stem cells. NFME: fetal spinal cord neural cells.

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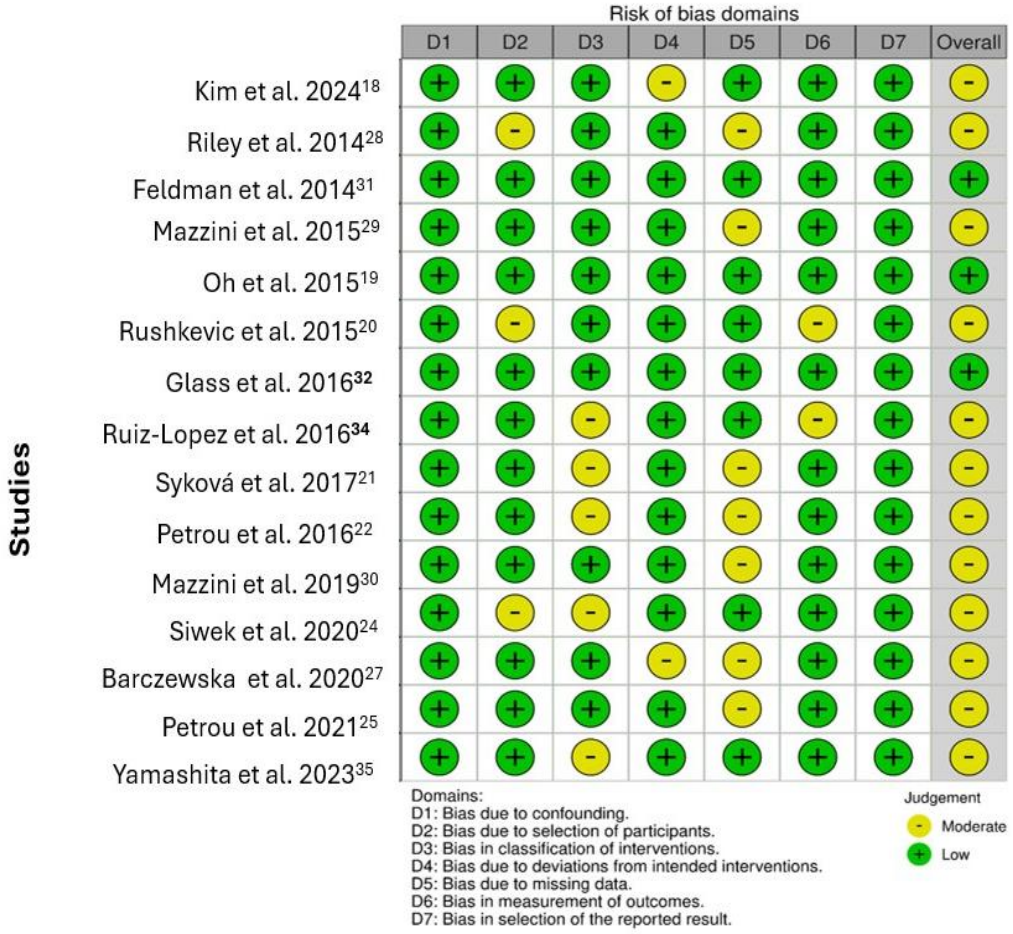
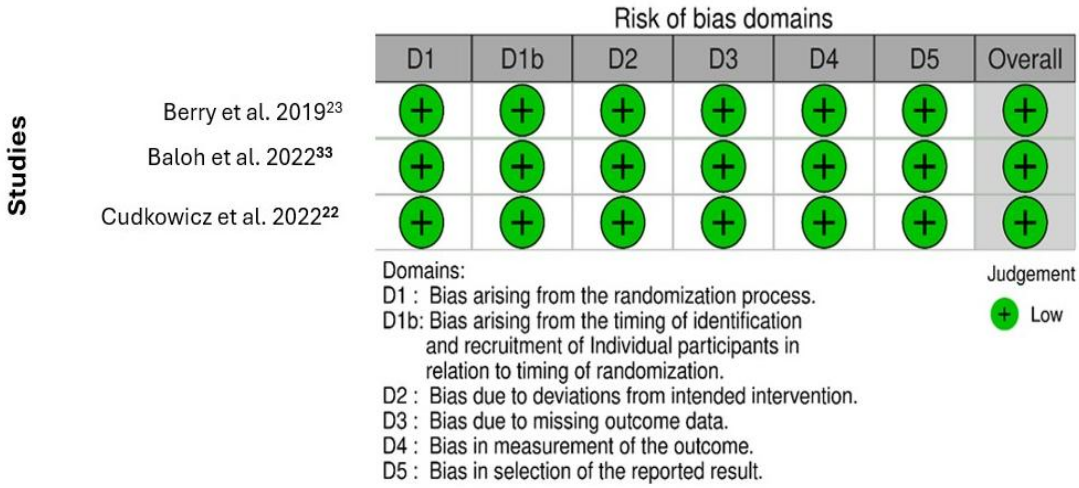
Figure 1: Risk of bias in quasi-experimental clinical trials (ROBINS-I) diagram



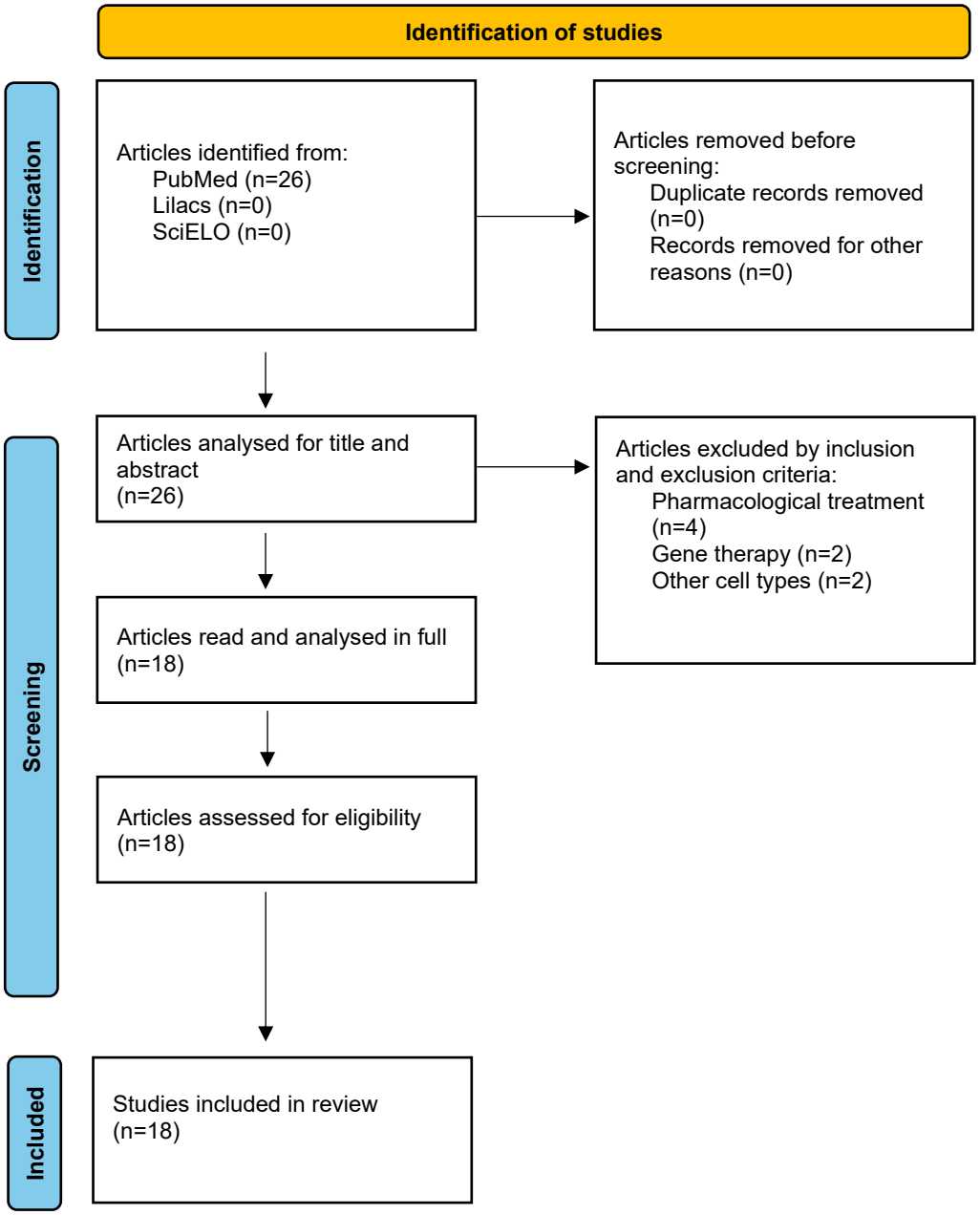
https://doi.org/10.7322/abcshs.2025061.3094

Figure 2: Risk of bias diagram in randomized clinical trials (RoB2 Cluster



https://doi.org/10.7322/abcshs.2025061.3094

Figure 3: PRISMA 2020 flowchart for identification, screening, eligibility, and inclusion of studies in the systematic review.



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