



MESENCHYMAL STROMAL CELLS

Repeated subarachnoid administrations of autologous mesenchymal stromal cells supported in autologous plasma improve quality of life in patients suffering incomplete spinal cord injury

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Abstract

Background aims. Cell therapy with mesenchymal stromal cells (MSCs) offers new hope for patients suffering from spinal cord injury (SCI). **Methods.** Ten patients with established incomplete SCI received four subarachnoid administrations of 30×10^6 autologous bone marrow MSCs, supported in autologous plasma, at months 1, 4, 7 and 10 of the study, and were followed until the month 12. Urodynamic, neurophysiological and neuroimaging studies were performed at months 6 and 12, and compared with basal studies. **Results.** Variable improvement was found in the patients of the series. All of them showed some degree of improvement in sensitivity and motor function. Sexual function improved in two of the eight male patients. Neuropathic pain was present in four patients before treatment; it disappeared in two of them and decreased in another. Clear improvement in bladder and bowel control were found in all patients suffering previous dysfunction. Before treatment, seven patients suffered spasms, and two improved. Before cell therapy, nine patients suffered variable degree of spasticity, and 3 of them showed clear decrease at the end of follow-up. At this time, nine patients showed infra-lesional electromyographic recordings suggesting active muscle reinnervation, and eight patients showed improvement in bladder compliance. After three administrations of MSCs, mean values of brain-derived neurotrophic factor, glial-derived neurotrophic factor, ciliary neurotrophic factor, and neurotrophin 3 and 4 showed slight increases compared with basal levels, but without statistically significant difference. **Conclusions.** Administration of repeated doses of MSCs by subarachnoid route is a well-tolerated procedure that is able to achieve progressive and significant improvement in the quality of life of patients suffering incomplete SCI.

Key Words: cell therapy, mesenchymal stromal cells, SCI

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Introduction

As a result of the experience provided in literature, in recent years various techniques of cell therapy have been implemented, mainly using mesenchymal stromal cells (MSCs) in patients with traumatic spinal cord injury (SCI), and early clinical trials have confirmed the absence of significant side effects [1–3].

However, at present the advantages of using exclusively adult MSCs or a mixture of MSCs and other bone marrow mononuclear cells for these transplants are not clear [1–15] and the advantages of either of the two options have been discussed extensively in recent publications from our own research group [8,16].

Cell therapy is clearly a current therapeutic promise in this field of research [1–3,14–23] but is still subject to many uncertainties, with significant confusion due to the disparity of protocols, subject selection, cell type, doses and routes of administration used.

MSCs have the advantage of easy expansion and low antigenicity, which may allow, at least theoretically, the use of allogeneic MSCs in human clinical practice, but there are still evident uncertainties about the mechanisms through which this type of cell therapy achieves neurological recovery, both in experimental animals and in the few patients treated so far. In experimental studies carried out, it is noteworthy that the functional recovery of paraplegic animals after MSC transplantation starts before tissue regeneration occurs, allowing the passage of ascending and descending axons [6–8,16], a finding that has also been discussed in clinical trials [23].

Therefore, it is obvious that after MSC transplantation, various repair processes must exist, including the release of neurotrophic factors by the transplanted stem cells [24–28], or the activation of endogenous mechanisms of the spinal cord, able to partially restore neurological functions previously abolished, as has been suggested in experimental models of brain damage [29,30].

On the other hand, various experimental studies have shown that MSCs can reach areas of SCI after being deposited in the subarachnoid space, providing a safe method for minimally invasive cell transplantation [8,10–12,31,32], and this finding has been confirmed in patients [33].

In humans, the first subarachnoid administration of MSCs for the treatment of SCI was described in 2008, as the first pilot case of a clinical trial in which cell therapy was administered early after SCI [17]. Since then, the intrathecal route has been generally used in human clinical trials [34,35] with variable results.

Our preclinical experience using a paraplegic mini pig model [36] showed that direct intralesional administration of MSCs is the most effective route to allow a large number of cells in areas of the SCI, but because the subarachnoid route is a safe method for minimally

invasive cell transplantation, it should clearly be considered in patients with incomplete SCI to avoid the possibility of any surgical complication that could cause a loss of residual neurological function. However, the analysis of the reported clinical trials using subarachnoid injections of MSCs reveals a great variability in the dose and timing of administration, with a number of cells being scarce. Our previous studies suggest that transplanting a great number of cells is advisable because cell therapy seems to show a dose-dependent effect and that repeated cell therapy administration could be beneficial [23].

Here we present the results of a phase II clinical trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02165904) NCT02165904, EudraCT 2011-005684-24) that studied the efficacy and safety of four doses of 30×10^6 MSCs in 10 patients suffering chronically established neurological dysfunction secondary to an incomplete SCI.

Methods

Study design and treatment

The present clinical trial included 10 patients (male/female: 8/2) suffering chronic and incomplete SCI (American Spinal Injury Association [ASIA] classification B, C or D). The mean age was 42.20 years (SD: 9.30 years), and time from SCI to treatment ranged from 2.43 to 34.59 years (mean: 14.21 years, SD: 9.88 years). [Table I](#) shows the main clinical and demographic data of the patients.

The clinical trial protocol was approved by the ethic committee of Puerta de Hierro-Majadahonda Hospital and by the Spanish Agency of Medicament and Health Products and conducted in accordance with the principles of the Declaration of Helsinki [37] and good clinical practice guidelines [38]. A flow chart of the patients can be seen in the supplementary Figure S1. Adverse events were collected throughout the follow-up

Table I. Clinical data of patients in our series.

Patient	Sex	Age	Level	ASIA	Years since SCI
1	M	37	L1	B	6.00
2	M	34	L1	C	8.17
3	M	41	L1-L2	C	13.06
4	M	56	D7-D8	C	34.59
5	M	38	D2	B	2.43
8	F	37	C5-C6	C	20.90
9	M	34	C5-C6	C	14.31
10	M	36	C5-C5	D	17.76
11	M	59	C3-C4	B	3.60
12	F	50	C5-C6	B	21.32

Age ranged between 34 and 59 years (mean: 42.20, SD: 9.30 years), and time from SCI to treatment ranged from 2.43 to 34.59 years (mean: 14.21, SD: 9.88 years).

and classified according to the Medical Dictionary for Regulatory Activities (MedDRA v. 18.1).

Treatment consisted of subarachnoid administration, by lumbar puncture, of 30×10^6 autologous MSCs obtained from bone marrow and supported in autologous plasma. It was repeated at months 4, 7 and 10, reaching a total administration of 120×10^6 MSCs for each patient. The patients were followed monthly, from the first administration of MSCs (month 1) through month 12.

Clinical scores were obtained from each patient by means of the following scales: The ASIA scale [39]; the SCI functional rating scale of the International Association of Neurorestoratology (IANR-SCIFRS scale) [40]; the Functional Independence Measure (FIM) scale [41] and the Barthel scale [42] for the study of functional independence in the activities of daily life (ADLs); the Visual Analog Scale (VAS) [43] for the evaluation of neuropathic pain; the Penn [44] and the modified Ashworth [45] scales for the evaluation of spasms and spasticity, respectively; the Geffner scale [46] for the study of bladder function; and the Neurogenic Bowel Dysfunction (NBD) scale [47] for the evaluation of symptoms related to neurogenic bowel dysfunction. Neurophysiological, urodynamic and magnetic resonance studies were also performed before and after treatment. Furthermore, the enzyme-linked immunosorbent assay technique was used to measure the neurotrophins brain-derived neurotrophic factor, glial-derived neurotrophic factor, nerve growth factor, ciliary neurotrophic factor, neurotrophin 3 and 4, in cerebrospinal fluid samples obtained before each administration of MSCs, at months 1, 4, 7 and 10 of the study. Technical details on the neurophysiological and urodynamic studies, and data about our cell therapy medicament, including genetic studies, culture, formulation, packaging and phenotypic characterization of the MSCs (supplementary Figure S2) are provided in the supplementary material.

Statistical analysis

To study the differences between the scores of the clinical scales, parameters of urodynamic studies, and changes in neurotrophic factors, the nonparametric Wilcoxon rank test was used, comparing the result of each time period with results at baseline. In the results deemed statistically significant, the size of the effect was calculated using Cohen's *d*, and the cutoffs proposed by Cohen [48] were used for the general interpretation of the cutoffs of this statistic. For the analysis of the section of neurophysiology, the chi-square test was used to study whether there were differences in the frequency distribution of each variable at each time point, and the McNemar test to study whether there were changes in each of the parameters evaluated between

6 and 12 months. Correlations were obtained using Spearman's rank correlation coefficient. Statistical analysis was performed using SPSS software (v. 21.0, IBM). The graphs were made with the GraphPad Prism program for Windows (v. 5.04, GraphPad Software). All inferential procedures used $\alpha = 0.05$ as the level of risk. The treatment of missing values in the neurotrophic factors section was done by *listwise*.

Results

Two patients initially selected to form part of the clinical trial (patients 06 and 07) were eliminated due to alterations in the genetic study and replaced by two other patients to make up the 10 patients of the present study. In our present clinical trial, the cell expansion process did not involve any alteration to the genome of the cells in any of the cases, according to the results obtained after analysis by the Array CGH platform.

Adverse events

During the study, 20 adverse events (AEs) were seen; and 8 (40%) were probably related to the administration of cell therapy. They generally consisted of headaches and pain in the area of the lumbar puncture. Regarding the degree of these AEs, 17 (84.21%) were considered mild and 3 (15.79%) moderate. There was one severe AE, which was not related to the administration of cell therapy (acute bronchitis). Details of collected AEs are provided in the supplementary material (supplementary Table SI).

Sensitivity and motor improvement

Sensitivity improvement according to the ASIA scale was already evident in the first assessment after the first administration of cells (at month 2 of the study) with a mean score of sensitivity in the patients that improved at this time from a basal value of 135.2 ± 42.79 points to 144.5 ± 47.90 points ($P = 0.03$). In 60% of cases, significant motor improvement was also found at an early stage after the first administration of cell therapy, which was confirmed by a mean motor score in the series, at month 2, of 55.10 ± 21.62 points, compared to the baseline 53 ± 20.45 points ($P = 0.027$). Throughout the follow-up period, progressive improvement was observed in both sensitivity and motor scores, reaching, at month 12, an improvement in the ASIA total score that ranged between 13 and 85 points from the baseline score, with a mean of 47.30 ± 28.81 points, and with a P value of 0.005 (effect size [ES]: 0.886) when the ASIA total score of the series, obtained at the end of the study, was compared with the basal ASIA total score. Figure 1 shows the progressive improvement obtained in the different scores of the ASIA scale.

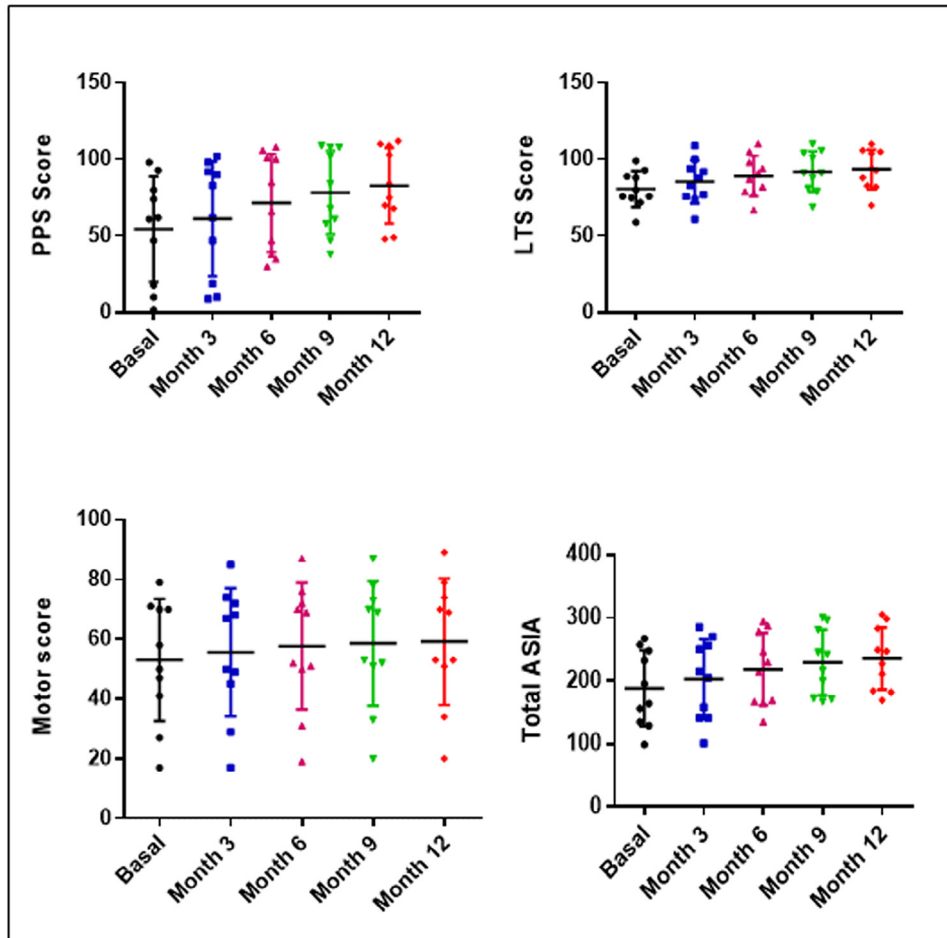


Figure 1. Graphs showing the progressive improvement in the different ASIA scores of the series, at different time points. PPS, Pin Prick Score; LTS, Light Touch Score; MS, Motor Score.

Motor score (MS) improved in the entire series between 0 and 12 points (mean: 6.20 ± 4.15 points) but did not correlate with the ASIA grade or chronicity of SCI. Nevertheless, when the level of SCI was analyzed, we found that higher levels of SCI correlated with greater improvement in ASIA total scores at the end of the follow-up ($P = 0.036$; $r = 0.6775$) due to the points added by the greater infralesional sensitivity improvement. On the other hand, MS improvement showed no significant correlation with respect to SCI level ($P = 0.240$; $r = 0.4078$).

In the entire series, the MS of the lower extremities improved during the study, in comparison with basal values, reaching an early statistical significance in the ASIA assessment. At month 3, after the first administration of MSCs, statistical analysis showed a P value of 0.028 (ES: 0.696), and at the end of the study, the p -value was 0.012 (ES: 0.798). This improvement supported the observation, in most of our patients, of a clear and progressive improvement in walking (supplementary Video S1).

In the ASIA assessment, the five tetraplegic patients in our series (patients 08, 09, 10, 11 and 12) showed variable degrees of improvement in muscle power of the upper extremities, and all except one showed motor improvement in muscle power of their lower extremities as well. The improvement in motor power of the upper extremities ranged between 1 to 5 points (mean \pm SD: 2.4 ± 1.67 points) and the motor power of the lower extremities ranged between 0 to 7 points (mean \pm SD: 5 ± 2.9 points). Table II shows the evolution of ASIA scores at different time points and the statistical analysis performed. Additional information is provided in the supplementary material (supplementary Tables SII–SIV and supplementary Figures S3–S9).

Overall spinal cord function

The IANR-SCIFRS scale evaluates spinal cord function through nine sections, with a final section that only applies to men and assesses sexual function.

Table II. ASIA scores at different time points.

Score subject	Time	Mean	SD	P value	ES
Motor Score	Before treatment	53.00	20.45	—	—
	At 3 months FU	55.60	21.45	0.028*	0.700
	At 6 months FU	57.70	21.15	0.008**	0.840
	At 9 months FU	58.60	20.83	0.008**	0.850
	At 12 months FU	59.20	21.15	0.008**	0.840
Pin Prick Score	Before treatment	54.50	34.36	—	—
	At 3 months FU	61.20	37.42	0.028*	0.696
	At 6 months FU	71.60	31.96	0.008**	0.844
	At 9 months FU	78.30	27.33	0.008**	0.844
	At 12 months FU	82.80	24.69	0.005**	0.886
Light Touch Score	Before treatment	80.70	11.70	—	—
	At 3 months FU	85.40	14.08	0.010*	0.811
	At 6 months FU	89.10	13.19	0.005**	0.890
	At 9 months FU	92.00	13.26	0.002**	0.886
	At 12 months FU	93.50	12.89	0.005**	0.887
ASIA total Score	Before treatment	188.20	60.00	—	—
	At 3 months FU	202.20	63.67	0.005**	0.887
	At 6 months FU	218.40	57.50	0.005**	0.886
	At 9 months FU	228.90	51.84	0.005**	0.886
	At 12 months FU	235.50	49.35	0.005**	0.886

Bold values indicate statistical significance. Statistical analysis showed early and progressive improvement in sensitivity and muscle power. FU, follow-up.

In our patients, the mean score in overall IANR-SCIFRS before treatment was 29.10 points (SD: 9.96), and at end of the study it was 36.90 points (SD: 8.21), showing a clear and statistically significant improvement ($P = 0.005$, ES: 0.889). The mean improvement during the follow-up ranged between 4 and 19 points, with a mean of 8.80 ± 4.96 points) (Table III). Additional information is provided in the Supplementary Appendix (Figures S10 and S11).

According to the IANR-SCIFRS scale, before treatment, five patients of the series showed a “slight degree of functional disability,” three patients showed a “medium degree of functional disability” and two patients showed a “severe degree of functional disability,” while at the end of the follow-up, six patients showed a “slight degree of functional disability,” and the four remaining patients showed a “medium degree of functional disability” (Figure 2).

Table III. Scores in overall IANR-SCIFRS scale, at different time points, with statistical analysis.

Time	Mean	SD	P value	ES
Before treatment	29.10	9.96	—	—
At 3 months FU	31.50	8.89	0.017*	0.755
At 6 months FU	33.90	9.73	0.005*	0.890
At 9 months FU	35.90	9.01	0.005*	0.890
At 12 months FU	36.90	8.21	0.005*	0.889

Bold values indicate statistical significance. FU, follow-up.

Sexual function

Sexual function was evaluated in the eight male patients of the series, according to the IANR-SCIFRS scale. In two of them (25%) sexual function improved, mainly as a consequence of improved sensitivity in the genital area. See supplementary Table SV and Supplementary Figure S12.

Activities of daily living

The FIM and Barthel scales studied ADL in our study. Both scales showed significant improvement at 12 months of follow-up. At this time point, the difference from the baseline overall score showed a P value of 0.027 (ES: 0.700) for the FIM scale, and a P value of 0.039 (ES: 0.651) for the Barthel scale. See supplementary Tables SVI and SVII and supplementary Figures S13 and S14.

Neuropathic pain

Neuropathic pain was studied using the VAS scale. Only 4 patients in our series (40%) suffered neuropathic pain (patients 01, 03, 04 and 05). Patients 01 and 03 showed clear improvement after the first administration of cell therapy, with the disappearance of neuropathic pain at months 7 and 2, respectively. Patient 04 showed no improvement, and patient 05 improved slightly as of month 2 (supplementary Table SVIII and supplementary Figure S15).

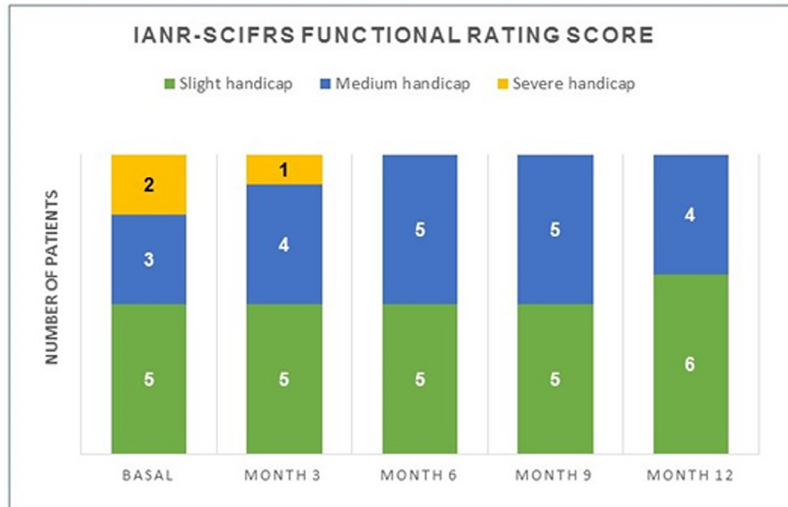


Figure 2. Evolution of the functional rating score of the patients, according to the IANR-SCIFRS scale. On this scale, a global score that ranged between 34 and 47 represents a slight handicap, between 17 and 33 represents a medium handicap and between 0 and 16, a severe handicap.

Spasms and spasticity

The evolution of spasms and spasticity was studied by the Penn and modified Ashworth scales, respectively. Although our patients generally described improvement in spasms and spasticity throughout the study, the low number of patients showing these symptoms precludes obtaining conclusions from a statistical point of view. Only seven patients in our series suffered spasms before treatment, and in two of them (28.57%), the spasms reduced over the course of follow-up, according to the scores in the Penn scale. See supplementary Table SIX and supplementary Figure S16.

Nine patients of the series showed variable degrees of spasticity, according to the modified Ashworth scale, and three of them (33.3%) showed improvement over the course of follow-up (patients 02, 03 and 04). One of them (patient 04) was carrying a baclofen pump,

the administration of which was gradually reduced during follow-up, with no increase in spasticity. See supplementary Table SX and supplementary Figure S17.

Sphincter function

Sphincter function was studied using the Geffner scale (bladder dysfunction) and the NBD scale, for the study of bowel control. All patients except one (90%), suffered bladder dysfunction before treatment, and eight of them (88.8%) improved over the follow-up period. The statistical study showed a significant difference between the baseline score of the Geffner scale and the score at the end of follow-up ($P = 0.024$, ES: 0.712) (see Figure 3, supplementary Figure S18 and supplementary Table SXI).

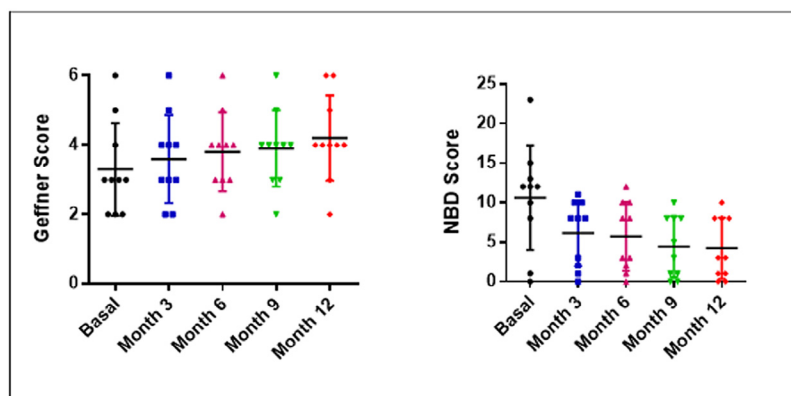


Figure 3. Evolution of the progressive improvements observed in the Geffner (bladder dysfunction) and NBD (bowel dysfunction) scales, at different time points. At the end of the study (month 12) statistical differences with basal scores were found. For the Geffner scale, $P = 0.024$ and ES was 0.712. For the NBD scale, $P = 0.018$, and ES was 0.750.

Table IV. Scores in NBD scale, at different time points, with statistical analysis.

Time	Mean	SD	<i>P</i> value	ES
Before treatment	10.60	6.64	—	—
At 3 months FU	6.10	4.15	0.042*	0.643
At 6 months FU	5.70	4.35	0.018*	0.748
At 9 months FU	4.40	3.86	0.018*	0.751
At 12 months FU	4.20	3.88	0.018*	0.750

Bold values indicate statistical significance.
FU, follow-up.

The analysis of the NBD scale showed an early and progressive improvement in NBD symptoms of our patients, with a *P* value, at the end of the study, of 0.018 (ES: 0.750) (Table IV). In the series, all patients except one (90%) showed clear symptoms of bowel dysfunction, and seven of them (77.7%) showed clear improvement over the follow-up period (see Figure 3, Table IV and supplementary Figure S19).

According to the rating score of the NBD scale, before cell therapy, two patients had severe neurogenic bowel dysfunction, five had moderate dysfunction, one had mild dysfunction and two had minimal dysfunction. At the end of the follow-up, six patients had absent or minimal dysfunction, three patients had mild dysfunction and one patient had moderate dysfunction (Figure 4).

Neurophysiological studies

All patients showed neurophysiological improvement during the follow-up period. In eight patients, somatosensory evoked potentials showed progressive improvement in parameters of latency and/or amplitude

in comparison with the basal study. Improvement in motor evoked potentials was seen in four patients at month 6 and in five patients at month 12 of follow-up. With respect to basal recordings, improvement in sensitive nerve conduction, in terms of conduction velocity and amplitude, was only recorded in two patients at month 6. They showed progressive improvement in the study performed at month 12, and at this time point, another patient showed improvement with respect to the basal study. Similarly, five patients showed improvement in motor nerve conduction at month 6 compared with baseline, and seven patients at month 12. In comparison with basal studies, improvement in electromyography parameters showing voluntary muscle contraction was recorded in four patients of the series at month 6, and in six patients at the end of the follow-up (see supplementary Video S2). Moreover, infralesional polyphasic motor potentials, considered typical of active muscle reinnervation, were recorded in seven patients at month 6, and in all patients except one at the end of the follow-up (*P* = 0.011). Additional information is provided in supplementary Tables S12 and S13).

Urodynamic studies

Supplementary Table S14 shows the improvement in urodynamic parameters obtained for each patient of the series when compared with baseline. The possibility of voluntary micturition, which was not present at the basal study, was recorded in five patients (50%) at the end of the follow-up. Compared with baseline, at this time point, 60% of patients improved in first sensation at filling, 50% improved in maximum cystometric capacity and 60% improved in the

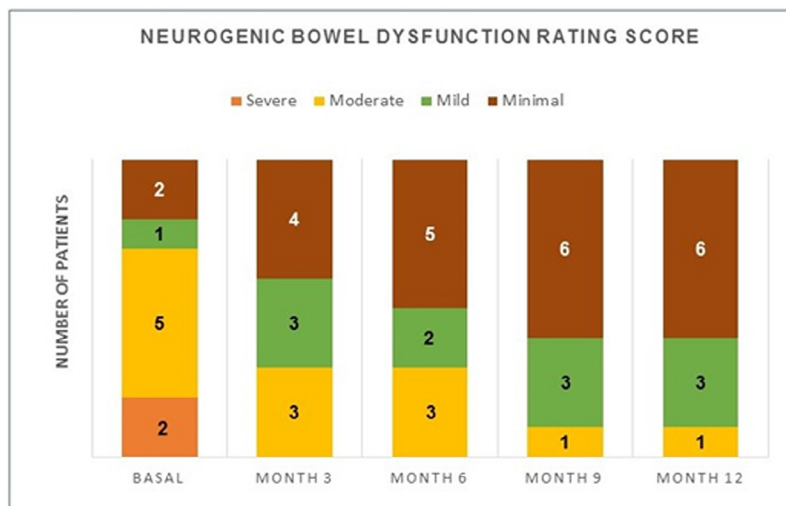


Figure 4. Evolution of the functional rating score of our patients, according to the NBD scale. On this scale, a global score between 0 and 6 represents a minimal NBD dysfunction, between 7 and 9 mild dysfunction, between 10 and 13 moderate dysfunction and 14 or more severe NBD dysfunction.

parameter of detrusor pressure. Furthermore, at the end of the study, 80% of our patients showed significant improvement in bladder compliance ($P = 0.037$, ES: 0.661). Additional information is provided in the supplementary material (supplementary Tables S15–S17 and supplementary Figures S20–S22).

Neuroimaging studies

Neuroimaging studies (conventional magnetic resonance imaging and myelography) were performed before cell therapy and at the end of the follow-up (at month 12) and failed to show changes in the morphology of SCI zones compared with basal images.

Neurotrophins in CSF

CSF samples obtained before each administration of cell therapy showed great variability in the expression of neurotrophins. In samples of CSF obtained at month 10 (after 3 administrations of MSCs), mean values of brain-derived neurotrophic factor, glial-derived neurotrophic factor, nerve growth factor, ciliary neurotrophic factor and neurotrophin 3 and 4 showed slight increases in comparison with basal levels. Statistical analysis failed to obtain statistical significance, except for the finding of a P value of 0.011 (ES: 0.850) for ciliary neurotrophic factor levels at month 7 of follow-up, but this statistical significance was not maintained in the CSF samples obtained at month 10 (see supplementary Table S18 and supplementary Figure S23).

Discussion

In this clinical trial, and as a result of our experience gained using animal models [6–9,16,22,23,29,30,36,49] and in humans [23], we administered a cell therapy medicament consisting of autologous MSCs supported by autologous plasma to patients suffering incomplete SCI, and assuming that these patients might show improvement after injury, we only included patients with longstanding SCI and with established neurological dysfunction. With regard to the dose of MSCs used, at present, clinical experience with cell therapy in SCI is limited, and there are no clear criteria in the literature to recommend dosage or administration intervals. Doses of 30×10^6 MSCs were already used by us in intrathecal administration in a previous clinical trial with perfect tolerance [23]. The hypothesis that injected MSCs can die after administration is also valid. Because of these considerations, we repeated administrations to a total dose of 120×10^6 MSCs.

In the ASIA scale assessment, scores showed progressive improvement during the study, including improvement in the motor power of the upper extremities in tetraplegic patients, a finding supported by neurophysiological studies, suggesting that motor

benefit can be obtained in cervical SCI after intrathecal administration of MSCs in the lumbar region. Although tetraplegic patients improved their motor power in the upper extremities, the improvement was scarce, and, at least in our present study, in no case did we obtain complete muscle recovery. This observation requires further study with a greater number of patients suffering cervical SCI.

Our results showed that all our patients experienced gradual improvement in clinical parameters without reaching a plateau at the end of the follow-up period. Recovery of infra-lesional sensitivity occurred early after the first administration of cell therapy, a finding we recently described after the intralesional administration of MSCs in complete chronic paraplegia [23], suggesting a possible effect through the cytokines released by the transplanted cells that activate preserved but non-functional circuits, rather than a mechanism of nerve pathway regeneration.

On the other hand, in the present clinical trial, the patients showed progressive improvement in scores of the IANR-SCIFRS scale, with a clear parallel between this improvement and that obtained from the ASIA scale, a finding we previously described when our cell therapy medicament was applied to patients with complete SCI [23]. The important improvement obtained in sphincter dysfunction supports our previously reported findings in patients suffering chronic complete paraplegia [23] and its obvious impact on quality of life.

Scales evaluating ADLs (MIF and Barthel scales) are not useful for the assessment of patients with chronic SCI because they have generally adapted to the dysfunction and are able to perform most activities without assistance [23], but we found significant improvement in our series at the end of the follow-up, supporting the effectiveness of the treatment.

Improvement in neuropathic pain was difficult to ascertain in our present study because only four patients had significant neuropathic pain before treatment. However, we did observe a tendency for neuropathic pain to decrease as of the first administrations of cell therapy, with one patient (patient 01) showing an important decrease after the first MSC administration and a complete disappearance of neuropathic pain at month 6 of follow-up.

Furthermore, patients with spasms and spasticity improved, but conclusions could not be drawn because of the limited number of patients suffering these symptoms in the present study.

In neurophysiological studies, although the sample size prevents obtaining statistically significant results in most of the parameters studied, all patients showed improvement during the follow-up period, mainly in somatosensory evoked potentials and motor nerve conduction. Electromyography recordings showing progressive improvement in voluntary muscle

contraction with signs of infra-lesional active muscle reinnervation represent an objective finding supporting the efficacy of the treatment.

Urodynamic studies showed variability between patients, but 80% of them showed improvement in bladder compliance, reflecting the improvement in bladder function after cell therapy.

In our study, magnetic resonance imaging studies failed to show changes in the morphology of SCI after cell therapy, suggesting that subarachnoid administration of MSCs is not able to modify the morphology of established spinal cord lesions and that improvement may be mainly due to the release of neurotrophic factors without changing the neuroimage associated with SCI.

With regard to the values of neurotrophins, it is difficult to obtain conclusions in the present study because of limitations due to the number of patients studied, the low expression of these factors in CSF and its variability. Despite the great variability among patients that prevented our obtaining statistically significant results, our findings show slight increases in some neurotrophic factors when the average values were compared with those obtained before MSC administration. It is well known that neurotrophic factors can be secreted by MSCs, and they have been linked to their beneficial effects [24–28]. In the present study the increase of ciliary neurotrophic factor with respect to baseline seems to be greater than other neurotrophic factors that we have studied. It is a protein that promotes neurotransmitter synthesis and survival and/or differentiation of a variety of neuronal cell types [50], and its possible role in the functional recovery of patients subjected to cell therapy requires further study. On the other hand, the possibility that other neurotrophic factors released by MSCs may play a role in the functional recovery of our patients must be taken into account.

Conclusions

In conclusion, our cell therapy treatment is a safe procedure that significantly improves neurological dysfunction and increases the quality of life of patients suffering incomplete SCI. The experience obtained from the present clinical trial shows the benefit of this simple procedure in patients with incomplete SCI and suggests the desirability of studying whether this form of cell therapy may be useful in other diseases with similar clinical features, such as severe spondylotic myelopathy.

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Appendix: Supplementary material

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