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Stem Cell Transplantation in Amyotrophic Lateral Sclerosis Patients: Methodological Approach, Safety, and Feasibility

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Amyotrophic lateral sclerosis is characterized by the selective death of motor neurons. Stem cells have been proposed as a potential therapeutic strategy. The safety of stem cell transplantation into the frontal motor cortex to improve upper motor neuron function is described. Sixty-seven patients with definite amyotrophic lateral sclerosis were included. After giving their informed consent, the patients underwent magnetic resonance imaging, functional rating, pulmonary function test, and laboratory tests. Their bone marrow was stimulated with daily filgrastim (300 μg) given subcutaneously for 3 days. Peripheral blood mononuclear cells were obtained by leukapheresis. Isolated $CD133^+$ stem cells were suspended in 300 μl of the patient's cerebrospinal fluid and implanted into the motor cortex. Adverse events were recorded at each step of the procedure and were classified according to the Common Terminology Criteria for Adverse Events v3.0. The survival at 1 year was 90% after transplantation. with a mean long-term survival rate of 40.17 months from diagnosis. The most common adverse events were in grades I–II and involved transient skin pain (19.5% of patients) attributed to the insertion of the Mahurkar catheter into the subclavian vein, minor scalp pain (15.9%), and headache (12.2%) from the surgical procedure. Several patients (1.5–4.5%) reported diverse grade I adverse events. There were two deaths, one considered to be associated with the procedure (1.5%) and the other associated with the disease. Autologous stem cell transplantation into the frontal motor cortex is safe and tolerated well by patients. Further controlled studies are required to define the efficacy of this procedure.

Key words: ALS; Safety; Stem cell: Transplant

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a late-onset neurodegenerative disorder characterized by rapid deterioration and the selective death of motor neurons (MNs) in the cerebral cortex, brain stem, and spinal cord (29,33,38). A wide variety of clinical manifestations are present early in the course of ALS (23). The clinical features are attributable to the superimposition of motor deficits occurring in the upper motor neurons (UMNs) and lower motor neurons (LMNs) (34). The motor phenotypes are highly heterogeneous and are defined by (1) the body region of onset, (2) the relative mix of UMN and LMN involvement, and (3) the rate of progression (32). There is no specific diagnostic test, although the clinical diagnosis is probably correct in more than 95% of cases (32). The mean survival of ALS patients from diagnosis ranges from 15.7 to 47 months after presentation, according to different series (26,42).

There is no effective therapy for ALS patients. Riluzole is the only medication approved by the US Food and Drug Administration for the treatment of this disorder. However, this drug only slightly delays disease progression (23). Novel therapeutic strategies might be directed at replacing or repairing the damaged neurons. Stem cell therapy is considered an alternative method for treating neurodegenerative disorders, including ALS. Stem cell transplantation is a potential therapeutic strategy based not only on cell replacement but also on the modification of the extracellular motor neuronal environment through trophic and neuroprotective effects (18). A variety of cell

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sources have been considered for cell therapy (11,16), and adult and embryonic stem cells can differentiate into several specialized lineages.

Embryonic stem cells are totipotent or pluripotent stem cells that, under the appropriate conditions, can differentiate into almost any cell type, including neurons, hepatocytes, cardiomyocytes, and others. Adult stem cells are specialized cells found within many tissues of the body. These cells can be more easily isolated from the peripheral blood or bone marrow (BM), and they remain the main source of stem cells capable of differentiation into several types, such as osteoblasts, chondrocytes, endothelial cells, glia, neurons, and skeletal and cardiac myocytes (1,3,7,9,18,21,24,39).

It has been demonstrated that stem cells isolated from human BM can be induced to differentiate into neurons. These differentiated cells express nestin, neuron-specific enolase, neuron-specific nuclear protein, glial fibrillary acidic protein, neurofilaments, TAU, nuclear receptor-related 1 protein (NURR1), and neuron-specific tubulin 1 (3,6,12,24,39). Several studies have reported that these cells have synaptic transmission capacities (6,12).

Clinical studies using stem cells in humans have been described for the treatment of Huntington's disease, Parkinson's disease, spinal cord injury, stroke patients, and Batten's disease (13,18,19,22,30,36). Because no animal model reproduces all the salient features of ALS, particularly the involvement of the corticospinal and corticobulbar tracts, and experimental data have suggested, among other things, the possibility of mesenchymal stem cell differentiation into neurons in in vitro and in vivo studies (3,24,39), some researchers have attempted different stem cell-based approaches to the treatment of ALS patients. Current clinical trials are based principally on two main transplantation strategies: the systemic (2,5) and local approaches (8,14,25,27,37). Mesenchymal stem cell transplantation into the spinal cords of ALS patients has been described, and the authors reported this method as safe (27). Another study assessed the safety and therapeutic efficacy of autologous human BM stromal cell transplantation in patients with complete spinal cord injury (30). In the present study, the safety and feasibility of mesenchymal stem cell transplantation into the frontal motor cortex, to improve UMN function, were investigated in definite ALS patients in the largest unique uncontrolled, open-label nonrandomized clinical trial yet undertaken.

PATIENTS AND METHODS

Study Subjects

All patients were recruited and evaluated for their eligibility at the neurology service of the Hospital Universitario UANL, Monterrey, México, and the Neuroscience Center of the Hospital San José Tec de Monterrey, Monterrey, México, between June 2005 and May 2010. The institutional review board of the Hospital San Jose Tec de Monterrey and Tecnologico de Monterrey School of Medicine approved the protocol (registration number 01122005), and all the participating patients signed an informed consent form. A trained neurologist conducted examinations to confirm the diagnosis of ALS according to the well-established El Escorial clinical and neurophysiological criteria (4,28). Patients with a current or past history of neurological disease other than ALS and those enrolled in other clinical trials were excluded. The inclusion criteria were (a) confirmed ALS according to the El Escorial clinical and neurophysiological criteria; (b) no structural damage to the rain or spinal cord on cervical and cranial magnetic resonance imaging (MRI); (c) pulmonary function test showing a forced vital capacity (FVC) of at least of 30%; and (d) appropriate nutritional status, with a body mass index of at least 19 kg/m^2. The exclusion criteria were (a) severe bulbar affection (FVC less than 30%); (b) inadequate nutritional status or a body mass index lower than 18 kg/m^2; (c) tracheostomy; (d) presence of systemic disorders, such as malignant neoplasm, cardiovascular disease (decompensated hypertension, ischemic cardiopathy, and arrhythmia), previous stroke, or coagulation abnormalities; and (e) evidence of cervical spondylotic myelopathy or structural abnormalities on MRI.

The neurological examination consisted of testing for muscle tone, stretch reflexes, pathological reflexes, and the Medical Research Council Scale for grading muscle power and strength (17). The ALS Functional Rating Scale Revised (ALSFRS-R) (18), which is the most widely used and extensively validated global scale for assessing motor function in ALS, was administered to all ALS patients. This scale is weighted toward limb and bulbar function and gives a total severity score out of 48 possible points. Patients with greater disability have a lower score. A Mini Mental State Examination (MMSE) was performed for all ALS patients, and a cognitive neuropsychological test was performed for 10 patients before surgery. The entire clinical evaluation lasted 30–45 min and was performed at baseline and at 1–3 days and 1 month after surgery.

Stem Cell Preparation

After their informed consent was obtained, the patients in the treatment group received a subcutaneous daily dose of 300 μg of filgrastim (Neupogen, Basel, Switzerland) for a period of 3 days. This drug, a human granulocyte colony-stimulating factor (G-CSF) produced with recombinant DNA technology, acts on hematopoietic cells by binding to specific cell surface receptors and thus stimulating cellular proliferation, differentiation, and some end cell functional activation. Absolute monocyte and

lymphocyte counts have been reported to increase in both patients and normal subjects receiving G-CSF (10,20).

The day following the final dose of G-CSF, the patients were admitted to hospital, and their white blood cell count was measured. After admission, a Mahurkar catheter was placed into the right subclavian vein. Through this catheter, peripheral blood mononuclear cells were isolated by leukapheresis (Baxter CS 3000+, Deerfield, IL, USA, or Haemonetics MCS, Braintree, MA, USA). This procedure lasted for 2 h. A 2-ml sample of cerebrospinal fluid (CSF) was also obtained by lumbar puncture after the apheresis procedure. The cells obtained were washed three times with phosphate-buffered saline. The $CD133^+$ immunoreactive cells in the cell suspension were conjugated with anti-human $CD133^+$ superparamagnetic microbeads and isolated in a magnetic field over a MiniMACS separation column (Miltenyi Biotech, Gladbach, Germany). The enrichment of the $CD133^+$ cells in the patient samples was confirmed by fluorescence-activated cell sorting. The cells were counted with a Beckman Z2 Coulter Counter (Fullerton, CA, USA), and $2.5–7.5 \times 10^5$ cells from the first 10 patients and $3.0–5.0 \times 10^6$ cells from the subsequent patients were suspended in 0.3 ml of autologous CSF and dispensed into sterile tubes. The total preparation of the $CD133^+$ stem cells took 4 h, and the patients were then sent to the operating room.

Surgery

To avoid respiratory complications, the procedure was performed while the patients were awake, under mild sedation and local anesthesia. The stem cells were transplanted bilaterally into the frontal motor cortex with stereotaxy (Leksell G, Stereotactic System Elekta, Stockholm, Sweden) in the first four patients and with neuronavigation (Fig. 1) guidance (VectorVision 2, Brain Lab AG, Munich, Germany) in the subsequent patients. Based on a computed tomography (CT) scan for stereotaxy or an MRI scan for neuronavigation, the motor cortex strip was identified, and the target was defined 3–4 cm from the midline. The positions at which the bur holes were to be made were identified with the navigation system in the latter group of patients because it is less traumatic and more comfortable and has an accuracy similar to that achieved with stereotaxy. After the incision of the dura, the suspension of $CD133^+$ stem cells in CSF was injected into the cortex to a depth of 7 mm using a Hamilton syringe held by a mechanical arm to maintain its stability during the procedure. Ninety seconds was the injection time. After this period, the syringe was held on site for an additional 60 s. Finally, hemostatic gel was applied (Gelfoam, Pfizer, Belgium). The vascular structures and subarachnoid spaces were avoided. The patients were discharged on the following day.

Image Acquisition

MRI for neuronavigation and tractography (Fig. 2) of the corticospinal tract studies was performed in a lying position, without respiratory support, using 1.5-T magnetic resonance equipment (Philips Medical Systems, Eindhoven, Holland) with a whole-body gradient coil, and an extended MRI workspace with neuroimaging software. Conventional studies were made of axial, sagittal, and coronal views. The axial pulse sequences included three-dimensional fast field echo high-resolution T1- and T2-weighted images, fluid-attenuated inversion recovery (FLAIR) long TR images, and proton density-weighted images. T2-weighted turbo spin echo (TSE) images in sagittal and coronal sequences were also obtained, together with sagittal sequences on FLAIR. A neuroradiologist reviewed the images.

Adverse Events

Although all the ALS patients included in this study were followed up for a year, the adverse events (AEs) were only monitored from inclusion (baseline) to 1 month after surgery. During hospitalization, all AEs

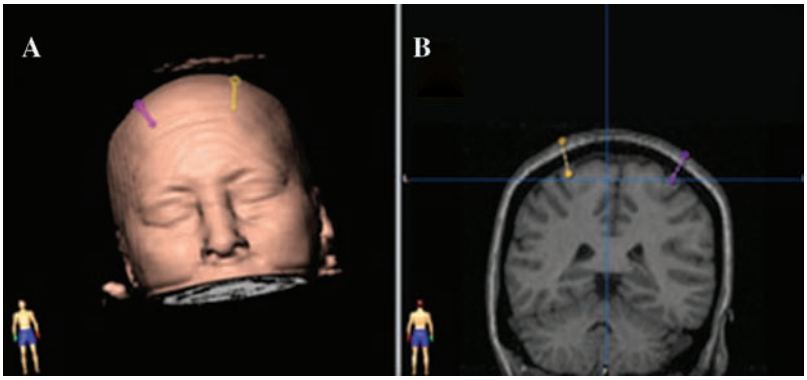

Figure 1. MRI neuronavigation brain 3D model. (A) Amyotrophic lateral sclerosis (ALS) subject and (B) MRI coronal posterior view showing frontal motor strips location bilaterally.

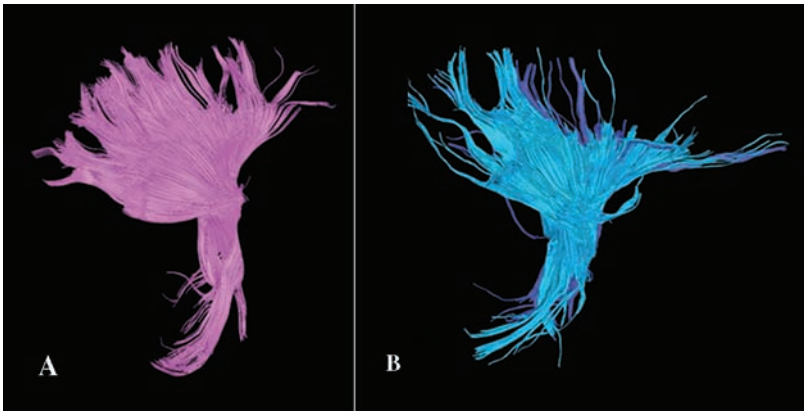

Figure 2. MRI tractography in sagittal view showing corona radiata and the corticospinal tract in (A) a healthy control subject and (B) ALS subject with a remarkable qualitative decrement in width and thickness in the corticospinal tract at baseline.

were recorded sequentially after every intervention, as described in the protocol: (1) filgrastim injection, (2) MRI study, (3) Mahurkar catheter insertion, (4) leukapheresis, (5) lumbar puncture, and (6) surgical procedure for stem cell transplantation. The Common Terminology Criteria for Adverse Events (CTCAE) was used to standardize the severity of the AEs (grades I–V) and to classify them appropriately (31).

Data Analysis

The variables are described as means ± standard deviations and medians (25th and 75th quartiles), for normal and non-normal data, respectively. Univariate comparisons of the demographic (age and sex) and clinical variables (baseline ALSFRS-R, FVC score, and site of onset) were analyzed with the chi-square test. Significance was tested at the 5% level. All statistical analyses were performed with the SPSS 16.0 software package (SPSS, Chicago, IL, USA).

RESULTS

Sixty-seven patients (45 men and 22 women) with definite ALS underwent autologous stem cell transplantation into the frontal motor cortex. The mean age of the total subject sample at the time of implantation was 49.2 ± 10.3 years. Bulbar-onset ALS was established in 28.4% ($n=19$) of patients, spinal-onset ALS in 70% ($n=47$), and only one patient (1.5%) had bulbospinal involvement at onset. The motor phenotypes were heterogeneous at the time of the baseline physical examination. In 26.9% ($n=18$) of patients, the LMNs–UMNs were equally affected, whereas 55.2% ($n=37$) of patients presented with predominant UMN involvement and only 18% of patients ($n=12$) had a predominantly LMN phenotype.

Among the 67 ALS patients included, the interval between clinical onset and diagnosis (median onset to diagnosis interval) was 9.5 months (25th, 75th quartiles: 7, 15). The pulmonary function test at baseline showed a median FVC of 55.5% (25th, 75th quartiles: 39.5, 74). The median baseline ALSFRS-R score was 29 points (25th, 75th quartiles: 24, 34.8). There were no abnormalities on the laboratory tests, which included coagulation profiles and electrocardiograms.

We observed no adverse events during MRI tractography (therefore, it was not included in Table 1) even in subjects requiring continuous positive airway pressure or with lower FVC. The mean absolute leukocyte number at the baseline workup for the entire group was $7.6 \pm 1.9 \times 10^3/\mu l$, and after the filgrastim injections, the mean absolute leukocyte number increased to $34.5 \pm 11.4 \times 10^3/\mu l$. Back pain was the only grade II adverse event observed after the filgrastim injection (Table 1). Sixteen patients reported right subclavian skin pain at the site of the Mahurkar catheter insertion ($n=16$, 19.5%). Other AEs associated to this catheter included right shoulder joint pain (6.1%), headache and insomnia (3.7%), and anxiety and generalized malaise (2.4%).

These AEs were transient and grades I–II events (Table 1), as was bleeding from the incision (1.2%). During leukapheresis, only one AE was observed (vomiting). After the lumbar puncture, the AEs included nasal reaction, anxiety, back pain, and transient headache in one patient each (grades I–II). Scalp pain at the site of incision ($n=13$, 15.9%) and headache ($n=10$, 12.2%) were the most frequent AEs experienced in the period after stem cell transplantation (Table 1). No other serious AEs were recorded, including pneumothorax, seizures, central nervous system infection, focal neurological symptoms, or changes in cognitive function, as demonstrated with MMSE at 1 month after the procedure in the whole group. Besides the subjects describe as complications no other individual has presented a long-term neurological

Table 1. Adverse Events for the Entire Group

Adverse Event (CTCAE v3.0)	No. AEs (%)	Grade
A. Filgrastim		
Back pain (6531)	1 (1.2%)	II
B. Mahurkar catheter		
Skin pain (6512)	16 (19.5%)	II
Joint pain (6536)	5 (6.1%)	I–II
Headache (6541)	3 (3.7%)	II
Insomnia (2503)	3 (3.7%)	II
Generalized malaise (2500)	2 (2.4%)	II
Anxiety (6020)	2 (2.4%)	II
Bleeding from the incision (4499)	1 (1.2%)	II
Palpitations (1760)	1 (1.2%)	I
Back pain (6531)	1 (1.2%)	II
Vomiting (3572)	1 (1.2%)	II
Abdominal pain NOS (6514)	1 (1.2%)	II
C. Leukapheresis		
Vomiting (3647)	1 (1.2%)	II
D. Lumbar puncture		
Nasal reaction (6770)	1 (1.2%)	I
Anxiety (6020)	1 (1.2%)	II
Back pain (6531)	1 (1.2%)	II
Headache (6541)	1 (1.2%)	II
E. Stem cell transplantation		
Scalp pain (6511)	13 (15.9%)	II
Headache (6541)	10 (12.2%)	II
Abdominal pain NOS (6514)	3 (3.7%)	I–II
Hypotension (2005)	2 (2.4%)	II
Bleeding from the incision (4499)	2 (2.4%)	I
Nausea (3572)	2 (2.4%)	II
Subdural hematoma (4250)	1 (1.2%)	V
Respiratory failure (6999)	1 (1.2%)	V
Hematoma from site (4250)	1 (1.2%)	I
Insomnia (2503)	1 (1.2%)	II
Unspecified URI (5008)	1 (1.2%)	II
Vomiting (3647)	1 (1.2%)	II
Hypertension (2004)	1 (1.2%)	II
Urinary retention (7064)	1 (1.2%)	II
Total	82 (100%)	I–V

CTCAE, Common Terminology Criteria for Adverse Events; AE, adverse event; NOS, not specified; URI, upper respiratory infection.

complication inherited to the procedure (up to 2 years). MRI studies did not indicate tumor nor other brain structural changes.

The subject survival was of 90% at 1 year and 52% at 2 years after transplantation. Until now, 38 patients of the whole group remain alive and subject to long-term follow-up, and from the 29 deceased subjects, the mean survival from diagnosis was 33.5 months. Of those, 29 subjects have completed at least 2 years after transplantation with a mean survival rate of 40.17 months from diagnosis. From the 29 deaths and 38 surviving patients, 32% and 26% had bulbar onset ALS, respectively. Respiratory failure ($n=15$) and pneumonia ($n=8$) were the most common causes of death among our study subjects. Other causes of death included sepsis ($n=1$), bronco aspiration ($n=1$), influenza AH1N1 ($n=1$), and unknown ($n=1$), excluding two cases that are described below.

In the entire group, a total of 82 AEs were reported by 45 patients (67%). The median number of AEs was 1, and almost all of them were classified as grade I or grade II according to the CTCAE v3.0. The presence of AEs did not differ significantly when analyzed by age ($p=0.13$), sex ($p=0.81$), baseline ALSFRS-R score ($p=0.83$), or FVC ($p=0.83$). An analysis of clinical heterogeneity, leukocyte, and mononuclear cell responses after filgrastim, FVC, and ALSFRS-R in relation to the age at onset is described (Table 2). The median number of AEs was the same in the ALS patients younger than 45 years and in those older than 55 years. The mortality rate was 3% ($n=2$) for the entire group. The causes of death were myocardial infarction with subdural hematoma after infarct treatment in one patient and respiratory failure in the other. Further details are described below as patient 1 and patient 2, respectively.

Patient 1

A 62-year-old woman had been diagnosed with definite ALS 13 months earlier. She suffered hypertension, which was treated with diuretics and an angiotensin receptor blocker. She had previously been treated with riluzole, celecoxib, and minocycline, with progressive deterioration of her muscular, motor, and respiratory functions. Her baseline ALSFRS-R was 18 points. After signature of her informed consent, laboratory tests, a CT scan for stereotaxy, and filgrastim injections were performed. She underwent autologous stem cell transplantation with 1.5×10^6 cells without complications and stayed alert and conscious during the postimplantation period. The patient was transferred to her room 2 h later, and she ate a normal meal at dinner. At that time, her clinical examination was normal. Twenty-four hours after surgery, the patient suddenly developed shortness of breath, anxiety, and somnolence. She was transferred to the intensive care unit. An electrocardiogram revealed an acute anterior myocardial infarct with elevated cardiac enzymes. Because thrombolytic therapy and percutaneous transluminal angioplasty were contraindicated, a dose of enoxiparin was injected subcutaneously, and noninvasive helmet ventilation was instituted. The patient recovered, and 24 h later, she was discharged from the intensive care unit. Twenty-four hours after her discharge, she again experienced sudden loss of consciousness caused by an acute left subdural hematoma, which is evident on a head CT scan. Craniotomy and drainage of the hematoma were performed.

During this procedure, the site of stem cell transplantation in the left frontal motor strip was visualized and excluded as a source of bleeding. Two days later, the patient experienced hypotension, bradycardia, cardiac arrest, and death.

Table 2. Demographic and Clinical Features and Workup Evaluation

			Involvement	Onset	Baseline				After Filgrastim	
Age	Sex M:F	BMI (kg/m^2)	UMN vs. LMN	Bulbar vs. Spinal	Mean ALSFRS	Median FVC	Mean ANL	Mean MNC (%)	Mean ANL	Mean MNC (%)
<45	4.5:1	24.3 ± 4.5	18% vs. 3%	9% vs. 22%	28 (26, 34)	48.5 (38.5, 70.8)	7.5 ± 1.2	31.9 ± 8.4	32.8 ± 10.1	13.3 ± 6
45–55	2.2:1	24.1 ± 4.9	24% vs. 10%	10% vs. 33%	30 (23, 35)	58 (37, 74)	7.4 ± 1.8	30.4 ± 8.7	33.2 ± 9.8	13.9 ± 6
>55	1.6:1	22 ± 2.8	13% vs. 4.5%	9% vs. 15%	29.5 (27.8, 34.8)	57 (46, 74)	8.2 ± 2.7	33.1 ± 6.7	38.9 ± 14.8	11.9 ± 4.3
Total	2:1	23.6 ± 4.4	55% vs. 18%	28% vs. 70%	29 (24, 34.8)	55.5 (39.5, 74)	7.6 ± 1.9	31.6 ± 8	34.5 ± 11.4	13.2 ± 5.6

M, male; F, female; BMI, body mass index; UMN, upper motor neuron; LMN, lower motor neuron; ALSFRS, ALS Functional Rating Scale; ANL, absolute number of leukocytes ($\times 10^3/\mu l$); MNC, mononuclear cells; FVC, functional vital capacity; AE, adverse event.

Patient 2

A 41-year-old man with no past medical history was diagnosed with definite ALS 12 months before our evaluation. The patient had been treated with coenzyme Q10 and lithium since his diagnosis. His baseline ALSFRS-R was 27 points, with moderate respiratory compromise and intermittent use of bilevel positive airway pressure. After signature of his informed consent, the patient underwent laboratory tests and MRI for neuronavigation. Autologous stem cell transplantation into the motor cortex was performed with 3×10^6 cells without complications. The patient progressed satisfactorily, and 2 h after surgery, he returned to his room and had dinner, which was tolerated well. At that time, a general physical examination and his vital signs were normal. He was discharged from hospital 48 h after the transplantation.

Thirty-six hours after his discharge, the patient experienced moderate shortness of breath and was admitted to hospital for evaluation and management. He remained for 2 days with respiratory distress and excessive tracheal and bronchial secretions. His respiratory status declined after admission, but the patient and his wife rejected endotracheal intubation, including bilevel positive airway pressure. The respiratory status of the patient declined progressively, leading to respiratory failure and death.

DISCUSSION

There is no effective treatment for ALS patients. Their life expectancy ranges from 15 to 47 months after presentation (26,42), even after the administration of riluzole. Stem cell transplantation is a potential therapeutic strategy for ALS patients, as for other neurodegenerative disorders (13,14,19,21,22,25,27,30,36,37), and may act by cell replacement or by modifying the extracellular motor neuronal environment (21). Adult stem cells isolated in vivo from human BM or peripheral blood can give rise to neural cells, and therefore, these stem cells represent an alternative to embryonic stem cells for therapeutic transplantation. CD133$^+$ stem cells isolated from the peripheral blood can differentiate into neurons (3,39).

Recently, several authors have described their preliminary data on stem cell therapy in ALS patients (2,5,8,14,25,27,37). Different local routes for stem cell transplantation have been reported in these patients, including intraspinal (27) and intrathecal routes (15), and into the frontal motor cortex (25). Previous studies using a systemic approach (2,5) in ALS patients were based on the impairment of the blood–brain barrier observed in animal models of ALS (40), but this topic remains controversial (41). Our methodological approach is intended to improve UMN function with CD133$^+$ stem cell transplantation into the frontal motor cortices, and it avoids the contentious status of the blood–brain barrier in ALS patients.

We recently described autologous CD133+ stem cell transplantation into the frontal motor cortices of 10 patients with definite ALS based on the scientific rationale of improving the UMN function in these patients (25). In the present study, the safety and feasibility of CD133+ stem cell transplantation into the frontal motor cortices of 67 definite ALS patients are described in an uncontrolled, open-label nonrandomized clinical trial. We decided to use autologous CSF as a vehicle for suspending CD133+ mesenchymal stem cells according to previous reports described by Mazzini et al. (27). These authors used CSF as the optimal vehicle, reporting viability of stem cells in CSF. The viability of stem cells of each ALS subject included in our research protocol was confirmed. These mesenchymal stem cells were viable for up to 4 h after dilution. This was an important issue in our protocol since the time elapsed between the enrichment of the sample and the surgery was 2 h.

We observed a total of 82 AEs, most of which were mild and transient, and required no special measures. They usually disappeared 1–3 days after their first appearance. The majority of AEs occurred after the insertion of the Mahurkar catheter or after surgical stem cell transplantation. The AEs observed after insertion of the Mahurkar catheter may have been related to the filgrastim injection, and they included headache, insomnia, generalized malaise, back pain, vomiting, and abdominal pain. However, all these AEs were transient and grades I–II. The AEs reported by some patients after the lumbar puncture may also have been secondary to the filgrastim injection (Table 1). The AEs observed after surgery were scalp pain (15.9%) and headache. These AEs were transient and required only oral analgesics for a day or two. Two patients died in the month after transplantation, and their AEs observed during this period were considered to be related to the procedure or perioperative complications. One death was considered to be associated with the procedure (1.5%), resulting from an acute subdural hematoma, which appeared after treatment for myocardial infarction. The other death (1.5%) was considered to be ALS related, because this patient remained alert with no AE after surgery and died from respiratory insufficiency a week later. Although the observed mortality was similar to the one expected by craniotomies, they were still considered as transplant-related deaths (35).

We consider stem cell transplantation into the frontal motor cortex a safe and feasible procedure. No serious life-threatening AEs were observed in most cases. Our preliminary results suggest that this procedure depicts a positive tendency towards disease stabilization due to survival rates of 90% at 1 year and 52% at 2 years after transplantation. Although the focus of this article circumscribes to a description of methodology, safety, and feasibility of a clinical research protocol, preliminary observations in 29 patients reaching at least a 2-year follow-up revealed survival rates of 40.17 months from diagnosis. The survival rate in ALS patients, as described in the literature, ranged from 15.7 to 47 months (26,41) with a median survival of around 29.1 months after presentation (42).

In certain ALS patients, more complete cardiac and respiratory function evaluations are necessary to avoid serious AEs. The scientific rationale for the methodology described is that CD133+ stem cells obtained from the peripheral blood are capable of differentiating into neurons, and their transplantation into the frontal motor cortex is intended to improve the UMN function. This procedure is performed under local anesthesia and light short-term sedation to avoid the AEs that may be expected in ALS patients under general anesthesia. Future analysis of the outcomes of ALS patients with predominant UMN involvement will be presented. It will be necessary to evaluate the efficacy of this procedure further in ALS patients in controlled studies.

CONCLUSION

Stem cell transplantation into the frontal motor cortices is a safe and feasible procedure. Large, controlled clinical trials should be conducted to establish its efficacy. Increasing the number of patients who undergo the procedure might reveal new AEs that should be reported in future publications.

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