Intrathecal Autologous Bone Marrow-Derived Hematopoietic Stem Cell Therapy in Neurological Diseases

M. Zakerinia[1,2]*, A. Kamgarpour[3], H. Nemati[4], H. R. Zare[5], M. Ghasemfar[6], A. R. Rezvani[1,2], M. Karimi[1,2], H. Nourani Khojasteh[1,2], M. Dehghani[1,2], R. Vojdani[1,2], S. Haghighat[1,2], N. Namdari[1,2], J. Rekabpoor[1,2], M. Tavazo[1,2], S. Amirghofran[7], Z. Amirghofran[5], G. A. Yosefipour[8], M. Ramzi[1,2]

[1]*Bone Marrow Transplant Unit, Department of Internal Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
[2]*Hematology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
[3]*Department of Neurosurgery, Shiraz University of Medical Sciences, Shiraz, Iran*
[4]*Department of Pediatrics, Shiraz University of Medical Sciences, Shiraz, Iran*
[5]*Department of Immunology, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran*
[6]*Department of Anesthesiology, Shiraz University of Medical Sciences, Shiraz, Iran*
[7]*The International Branch, Shiraz University of Medical Sciences, Shiraz, Iran*
[8]*Department of Neurology, Shiraz University of Medical Sciences, Shiraz, Iran*

ABSTRACT

Background: Cellular transplantation is a promising treatment strategy for neurological diseases.

Objective: To report the results of intrathecal hematopoietic stem cell therapy in different neurological diseases in the past 6 years in a single center.

Methods: From October 2011 to September 2018, 220 patients with various neurological diseases were transplanted intrathecally by their bone marrow stem cells. To have a longer follow up, we only reported the first 80 patients, transplanted up to July 2015—10 patients had spinal cord injuries and paralysis, 12 had advanced Parkinson's disease, 28 had cerebral palsy, 7 had hypoxic brain damage, 2 had autism, 4 had multiple sclerosis, 5 had progressive cerebellar atrophy, and 12 had other neurological diseases. The patients were admitted to the Bone Marrow Transplant Unit. On the first day, 50–200 (median 100) mL bone marrow was aspirated from the patients' posterior iliac crests, mixed with 120 mL culture media (RPMI), and 12 mL heparin. The samples were then transferred to immunology lab in cold box. Mononuclear cells (MNCs) were separated by a Ficoll-Hypaque gradient, washed, and suspended in ringers. Cell viability was assessed with trypan blue viability test. Transplantation was performed 3–4 hours after bone marrow collection. 5–10 mL of the cerebrospinal fluids were aspirated and about 20 mL MNCs (containing stem cells) in ringers were injected intrathecally (IT). The patients were laid down on their back for 4–5 hours. The median number of MNCs was 4×10^7 (range $1–450\times10^7$). The median viability of the cells was 90% (range 60%–98%). The patients received intravenous ceftriaxone every 12 hours and were discharged from the hospital few days after autologous stem cell therapy.

Results: We noted clinical improvements in 9 of 12 patients with Parkinson's disease, 20 of 28 patients with cerebral palsy, 6 of 7 patients with hypoxic brain damage, 2 of 4 patients with multiple sclerosis, and

*Correspondence: Maryam Zakerinia, MD, Professor of Internal Medicine, Hematologist-Oncologist, Hematology Research Center, Department of Internal Medicine, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
Tel: +98-71-3647-4301,
E-mail: mzakerinia@yahoo.com

4 of 5 patients with cerebellar atrophy. The improvements were noted after 2–4 weeks of cell therapy. There were no improvements in patients with spinal cord injury and complete paralysis and those with autism. There were variable improvements in other patients treated.

Conclusion: Most patients with advanced Parkinson's disease, cerebral palsy, hypoxic brain damage, progressive cerebellar atrophy, and kernicterus neuropathy reported clinical effects of this safe intervention resulting in better functioning and an increased quality of life.

KEYWORDS: Neurological diseases; Bone marrow stem cells; Intrathecal

INTRODUCTION

Hematopoietic stem cells are adult precursor cells found mainly in the bone marrow, which provide the blood cells required for daily blood turnover and for confronting infections. Hematopoietic stem cell therapy has been shown to have considerable therapeutic potential for neurological diseases. In most animal studies, stem cells have been directly injected into the central nervous system injured tissues. Such approach is very difficult to perform in clinical practice [1].

Alternative and less invasive routes to deliver hematopoietic stem cells in animal models of spinal cord injury (SCI) have been described and the advantages of the percutaneous lumbar puncture (LP) technique have been demonstrated [2, 3]. This method had not been described in humans. Fernando Callera and colleagues from Sao Paulo, Brazil (2005) were the first who tested if autologous bone marrow precursor cells can be delivered into the spinal cord through LP in patients with SCI. The procedure was feasible, safe, and well tolerated in that group of patients [1].

The properties of self-renewal and multi-lineage differentiation make stem cells attractive candidates for use in cellular reparative therapy, particularly in neurological diseases where there is a paucity of treatment options. However, clinical trials using fetal material in Parkinson's disease have been disappointing and highlighted problems associated with the use of embryonic stem cells, including ethical issues and practical concerns regarding teratoma formation. Understandably, this has led investigators to explore alternative sources of stem cells for transplantation. The expression of neuroectodermal markers by cells of bone marrow origin brought attention to these adult stem cells. Although early enthusiasm has been tempered by dispute regarding the validity of reports of in vitro (trans) differentiation, the demonstration of functional benefit in animal models of neurological disease is encouraging [4].

The XCell-Center group in Germany treat patients with different neurological diseases including Alzheimer, multiple sclerosis (MS), stroke, SCI, cerebral palsy (CP), and Parkinson's disease (PD) by injecting the autologous bone marrow stem cells into the cerebrospinal fluid through LP since 2007 [5].

In this study we report on the results of intrathecal autologous hematopoietic stem cell therapy in different neurological diseases in the past six years in our center.

PATIENTS AND METHODS

From October 2011 to September 2018, 220 patients with various neurological diseases were transplanted intrathecally by their own bone marrow stem cells. To have a longer follow up, we only reported the first 80 patients who were transplanted up to July 2015 in Bone Marrow Transplant Unit, Nemazee Hospital, affiliated to Shiraz University of Medical Sciences, Shiraz, Iran.

Patients with neurologic disorders, including those with congenital disorders or severe and progressive degenerative diseases with no effective therapy; or those in whom the drugs became ineffective as the disease worsen, were referred from different cities of Iran to our

center.

Few days before admission, each patient underwent a comprehensive physical and cognitive assessment at the clinic by our neurologist. The assessment included gait, standing, and balance analysis, and isometric maximum strength measuring. Patients underwent magnetic resonance imaging (MRI), if they had not had it before.

Isolation of Autologous Bone Marrow Cells for Administration

A written informed consent was obtained from each patient or the parents of the pediatric patients. The patients were then admitted to the Bone Marrow Transplant Unit. A general physical exam was done by a hematologist-oncologist and blood tests were done. On the first day, 50–200 (median 100) mL bone marrow (BM) was aspirated from the patients' posterior iliac crests under regional or light general anesthesia, mixed with 120 mL culture media (RPMI) and 12 mL sodium heparin (5000 U/mL). The BM samples were then transferred immediately to the immunology lab in a cold box. In the lab, under sterile conditions, the BM samples were diluted at a ratio of 1:1 with sterile buffer solution (ringers or RPMI). The diluted samples were smoothly placed over the Ficoll in same volume. Then, they were centrifuged at 800×g for 20 min. After centrifuge, five layers were seen—red blood cells (RBCs), neutrophils, Ficoll, ring (mononuclear cells), plasma, and platelets. The ring layer was collected carefully, and was transferred to a centrifuge tube. Ten mL ringers solution was added, and the mixture was centrifuged (at 400×g for 5 min). The supernatant was discarded and the pellet cells were resuspended in ringers solution (for 20 min). The ring layer was washed three times using the above-mentioned method. The cells were counted by Neubauer chamber. The cell's viability was determined by trypan blue staining (1:1 cell suspension and trypan blue). The ring layer contains mononuclear cells including stem cells (10–20 mL). The relative content of hematopoietic stem cells was evaluated by the number of $CD34^+$ $CD38^-$ cells. The number of mesenchymal stromal stem cells (MSCS) was evaluated by the number of $CD45^-$ $CD146^+$ cells using dual color flowcytofluorometry (FACS Calibur, Becton Dickinson). After collecting and preparing the cells, they were injected intrathecally. The patients, including those who received light general anesthesia were instructed to lie down for 24 hours. They were discharged from the hospital few days later. They were evaluated for the clinical effects of the intervention on day 10 after the treatment, and then every three months in the first year, and every six months later on, by our neurologist. The response to therapy was evaluated by the clinical improvements before and after the intervention.

RESULTS

The median yield for mononuclear cells was 4×10^7; it was 1.4% for $CD34^+$ $CD38^-$ cells and 0.001% for $CD45^-$ $CD146^+$ cells. The characteristics of the patients with neurological diseases who were transplanted intrathecally by their marrow stem cells in our center are shown in Table 1.

Motor Parkinsonism (bradykinesia, hypokinesia, rigidity, tremor, and loss of postural reflexes) is the clinical hallmark of Parkinson's disease. Most (9 of 12) patients with advanced Parkinson's disease reported a decrease of motor Parkinsonism resulting in improved speech (n=9), dexterity (n=8), swallowing (n=9), handwriting (n=8), and stability (n=8). Six patients noticed increased motivation too.

Twenty out of 28 patients with cerebral palsy (perinatal hypoxia) reported clinical improvements after treatment, better swallowing (n=6), neck holding (4 of 4), complete drooling stoppage (4 of 4), decreased spasticity (n=10), improved sitting (n=5), standing (n=10), walking (n=6), posture stability (n=8), improvement in mental function resulting in better communication (n=7), and improvement in speech (n=9). More than 90% of all the improvements started within eight weeks after of the treatment.

Six out of seven patients with hypoxic brain

Table 1: Characteristics of the patients with neurological diseases who were transplanted intrathecally by their marrow stem cells, during 2011–2015

Disease	n	Age (yrs) range (median)	Sex M:F	The duration of the disease (yrs) range (median)	Mononuclear cells range (median),	BM volume (mL) range (medium)	Post-treatment follow-up (yrs) range (median)	Clinical improvement, ratio (%)
Advanced Parkinson's disease	12	42–73 (64)	6:6	2–14 (5)	1.2–450 (3.0)×10^7	90–200 (100)	3–6 (4)	9/12 (75)
Complete paralysis	6	23–53 (35)	2:4	1/2–5 (2)	2–29 (4.0)×10^7 ,	100–200 (100)	3–6(3.5)	0/6
Incomplete paralysis	4	35–40 (36)	3:1	2–4 (3)	2.4–16 (7.0)×10^7	100–200 (100)	4–5.5 (4)	0/4 (25)
Cerebral palsy	28	2.5–30 (11)	16:12	2.5–30 (11)	2.0–19.0 (2.8)×10^7	50–160 (100)	3.5–5.5 (3)	20/28 (71)
Hypoxic brain damage	7	6–28 (15)	4:3	2–25 (5)	1.4–8 (2.0)×10^7	100–120 (100)	3.5–4 (3)	5/7 (71)
Autism	2	8,9	1:1	5, 8	0.7, 22.4×10^7	50, 100	5.5,5	0/2 (0)
Multiple sclerosis	4	29–40 (36.5)	2:2	6–18 (13)	3–10 (6.4)×10^7	100–120 (100)	3.5–5 (3.5)	4/4 (100) Relapse 2/4(50)
Progressive cerebellar atrophy	5	9–50 (34)	4:1	3–25 (9)	1.2–65 (2.6)×10^7	100–140 (100)	3–6 (3.5)	4/5 (80)
Hereditary spinocortico-cerebellar atrophy	1	30	F	28	3.2×10^7, 120		5	+ve
Hereditary neuropathy	1	54	M	8	8×10^7, 100		3.5	+ve
Friedreich's ataxia	1	23	M	7	1.6×10^7, 75		4	-ve
Amyotrophic lateral sclerosis	1	56	F	12	2×10^7, 100		3.5	-ve
Primary lateral sclerosis	1	61	M	4	4×10^7, 100		3.5	-ve
Kernicterus neuropathy	2	7,30	1:1	7, 30	8.8, 6.4×10^7	100, 140	3, 4.5	2/2 (100)
Head injury (colpocephaly)	1	34	M	9	14×10^7, 160		4	+ve
Head trauma	1	21	M	4	4×10^7, 100		3.5	-ve
Cerebrovascular accident	1	55	M	10	6.4×10^7, 90		4	-ve
Neurological sequel of meningitis	1	39	F	8	3.8×10^7, 70		4	+ve
Transverse myelitis	1	38	M	8	1.72×10^7, 160		3.5	-ve
Total	80				Mean MNCs=4.0×10^7 Mean $CD34^+$ $CD38^-$ cells=1.4% Mean $CD45^-$ $D146^+$ cells=0.0007%			48/80 (60)

n: Number, BM: Bone marrow, +ve: positive: some improvements, -ve: negative (no improvement), MNCs: mononuclear cells

damage had clinical improvements after treatment showing as decreased spasticity (n=3), improved sitting (n=2), standing (n=2), posture stability (n=2), better walking (n=1), neck holding (3 of 3), stopped drooling (n=3), and improved mental function (n=3).

Four out of five patients with cerebellar atrophy (autoimmune or hereditary) responded to the treatment as improvement in gait (n=4), unclear or "scanning" speech (n=4), visual blurring due to nystagmus (n=2), hand incoordination (n=4), and tremor with movement (Romberg sing) (n=2).

Of the four patents with SCIs and incomplete paralysis, only one, a 35-year-old woman, responded to the treatment and could walk after four years of inability. She could not move her right ankle before the treatment; after one month, she could do that. She could stand and walk by the time this report was written. No response was noted in the six patients with SCIs and complete paralysis.

All of the patients with MS responded to the treatment after 2–4 weeks. Three of them, however, had relapse of the disease after one month. One patient, a 40-year-old man with MS for 14 years, developed better talking and intelligence, decreased spasticity of the extremities, and stool and urine control one month after stem cell therapy. Another patient, a 29-year-old woman with MS for 12 years with decreased vision, positive cerebellar signs, and urinary retention, developed good vision, increased power, and normal urination two weeks after the treatment. Three weeks later with restarting Avonex (interferon b) therapy, she had relapse of the disease. She underwent second autologous stem cell therapy one year later. She has been stable for the past 3.5 years with improved vision and power, decreased tremor, and no more urinary retention. She was on Avonex therapy in the last visit. Therefore, two out of the four patients with MS had very good clinical improvements.

Two patients with autism and those with cerebrovascular accident (CVA, thrombotic type), Friedreich's ataxia, primary lateral sclerosis (PLS), amyotrophic lateral sclerosis (ALS), transverse myelitis, and head trauma did not have any improvements with stem cell therapy. However, one patient with hereditary spinocorticocerebellar atrophy had improvement in talking and power. One patient with kernicterus had improvement in talking and power. In another patient with kernicterus, urine and stool sensation and control were recovered seven months after stem cell therapy. In one patient with colpocephaly due to head trauma because of boxing, vision was improved and spasticity decreased. In another patient with neurological sequela of meningitis, better talking, decreased tremor, improved swallowing, power, taste, sense of smell, and walking with help (after 3–4 months of therapy) were improved. And, in the last patient with hereditary neuropathy, clinical improvements were achieved in sensation of legs and power.

Evaluation of all patients with advanced/progressive neurological diseases who underwent auto-bone marrow transplant in our center revealed that 48 (60%) of 80 patients had clinical improvements, especially those with PD, CP, hypoxic brain damage, and cerebellar atrophy. More than 90% of all the improvements started within 8–12 weeks of the treatment.

The adverse events reported were limited to mild headaches and vomiting in a few patients for just 2–3 days.

DISCUSSION

Most of the advances made in stem cell research have been directed at treating degenerative diseases. While many treatments aim at limiting the damage of these diseases, in some cases scientists believe that the damage can be reversed by replacing lost cells with new ones derived from cells that can mature into nerve cells, the so-called "neural stem cells." Using rodents and primates as model species to treat Parkinson's dieses was attempted in the 1970s [6].

Adults' stem cells (or more accurately, tissue stem cells) are regenerative cells of the human

body that possess the characteristic of plasticity—the ability to specialize and develop into other tissues of the body. Beginning in an unspecialized and undeveloped state, they can be coaxed to become heart tissue, neural matter, skin cells, and a host of other tissues. They are found in our own organs and tissues such as fat, bone marrow, umbilical cord blood, placenta, neuronal sources, and olfactory tissues that reside in the upper nasal cavity. This simple fact has remarkable implications for medicine as diseased or damaged tissues can become healthy and robust through the infusion of such cells [7]. This finding has consequently attracted the attention of many researchers as well as those suffering from various diseases [8].

Cell-based therapy in neurological diseases is an attractive option, but presents a difficult challenge, given the diversity of the central nervous system (CNS) cell types, the complex and precise interactions amongst them and the availability of appropriate cellular sources. Sources for cell transplantation in the nervous system include fetal neural tissues, embryonic stem (ES) cells, induced pluripotent stem (IPS) cells, neural stem cells (NSCs), non-neural somatic stem cells, or even direct conversion of non-neural cells into neurons. Each of these cell types has the potential to replace cells lost to injury or disease or to modulate brain or spinal cord function while each has its own advantages and disadvantages [9].

The strategy to use new cells to replace the lost ones is not new. Surgeons first attempted to transplant dopamine-releasing cells from a patient's own adrenal glands in the 1980s. The US surgeons were only able to achieve modes and temporary improvements [10].

Researchers of Tiantan Puahu Hospital in Beijing, China use four types of stem cells to treat different neurological conditions: (1) Human retinal pigment epithelial (hRPE) stem cells, which are used to treat Parkinson's disease, stroke patients, and other targeted neurological conditions, derived from donated tissues; (2) Neural stem cells, which are used to treat a large variety of diseases, derived from donated fetal tissues; (3) Mesenchymal stem cells from bone marrow and cord blood, derived from donated tissues; and, (4) Autologous stem cells, derived from the patients' own bone marrow.

hRPE stem cells are the only part of the central nervous system that is visible. The cells are collected from donated material and injected via stereotactic brain injection into a specific target in the brain where dopamine is produced. hRPE cells have the function of producing dopamine, and are used for patients suffering from Parkinson's disease. hRPE stem cells are administered via a direct stereotactic brain injection [11].

There are only a few countries, besides China, where stem cell treatments are used for different kinds of diseases. They include the USA [10], Germany [5, 19, 21, 23, 26], India, Russia, Hungary, Mexico [11], Brazil [1], Iraq [12], Turkey [13], *etc.*

Regarding the application of autologous bone marrow stem cells (SC), consisting of hemopoietic SC ($CD34^+$ $CD\ 38^-$ cells) and stromal mesenchymal cells (fibroblast CFU [CFU-F]), in the population of isolated mononuclear cells, for treatment of neurological diseases, and particularly for spinal cord injury, different routes of administration such as intravenous injection and/or performing meningomyeloradiculolis and transplantation of BM stem cells into the cystic and/or atrophic degeneration cavity, have been tried [14]. Administration of BM stem cells is done through multiple routes such as direct injection into the spinal cord or into the spinal canal, and/or intravenously [15].

In a study done in Philadelphia, USA, on rats that underwent partial cervical hemisection injury and in whom BM stromal cells (BMSCs) were transplanted intravenously, intraventricularly, or intrathecally 24 hours later, the researchers observed that BMSCs selectively moved to the site of injury of the spinal cord after transplantation regardless of the route of administration [2]. Very few or no cells were present within the injured spinal segments in rats that received BMSCs intra-

venously. In contrast, considerably more cells were detected in the injured tissues after both intrathecal and intraventricular delivery. The number of cells within the injured cord tissues increased over the time. Few cells were noted three days after transplantation. However, many more cells were seen 10–14 days after the transplantation.

The basal ganglia use dopamine as one of their primary neurotransmitters. Loss of dopamine-secreting cells in the substantia nigra, one of the structures in the basal ganglia, is responsible for dopamine reduction and, ultimately, for the Parkinson's symptoms. However, the cause of this neuropathology remains unclear [16]. The "on phenomenon" is referred to the period during which the patient responds to the medication and the "off" represents the period the patient does not respond.

The NIH has funded two large and well-controlled clinical trials in the past 15 years in which researchers transplanted tissue from aborted fetuses into the striatum of patients with Parkinson's disease [17, 18]. These studies, performed in Colorado and New York, included controls where patients received sham surgery with no tissue implanted; neither the patients nor the scientists who evaluated their progress knew which patients received the implants. The patients' progress was followed for up to eight years. Unfortunately, both studies showed that the transplants offered little benefit to the patients as a group. While some patients showed improvement, others began to suffer from dyskinesia, jerky involuntary movements that are often side effects of long-term L-dopa treatment. This effect occurred in 15% of the patients in the Colorado study [17], and in more than half of the patients in the New York study [18]. Additionally, the New York study showed evidence that some patients' immune systems were attacking the grafts. However, promising findings emerged from these studies as well. Younger and milder Parkinson's patients responded relatively well to the grafts. PET scan of patients showed that some of the transplanted dopamine neurons survived and matured. Additionally, autopsies on three patients who died of unrelated causes, years after the surgeries, indicated the presence of dopamine neurons from the graft. These cells appeared to have matured in the same way as normal dopamine neurons, which suggested that they were acting normally in the brain.

Nearly 50% (36 of 75) of the patients with Parkinsonism treated with autologous adult stem cells in XCell-Center, in Germany reported clinical effects of this safe intervention resulting in better functioning and an increased quality of life. On the other hand 30% of the patients with Parkinsonism reported a stable situation concerning their health condition, which also suggests a positive effect. The reported improvements suggest clinical efficacy after adult autologous stem cell treatment in patients with Parkinsonism [19].

Nine of 12 our patients reported a decrease in the symptoms of motor Parkinsonism. Most of our patients were unable to talk well to be understood by others, which had led to a lot of stress on their lives. Within the first 2–3 weeks after treatment, they noticed dramatic changes as they regained the ability to speak. The rigidity and hypokinesia (slowness of movement) gradually improved as did the "on/off" phenomena. Our first patient with PD decided to have second and third operations with 1.5 years apart, each time, to have more improvements.

In CP, damage to the motor control centers in the brain causes increased motor tone, leading to muscle stiffness. In a report from Lebanon, 17 sequential patients with CP treated with intrathecal administration of bone marrow mononuclear cells (BMMC). All patients had an uneventful post-injection course with 12 of the evaluable patients treated having a good response using the Gross Motor Function Classification System (GMFCS). The average improvement was 1.3 levels on the GMFCS with cognitive improvements as well [20].

Almost all of our patients with CP had very spastic extremities. After treatment, 10 of 28 patients developed some relaxation of the extremities. All those who could not hold their

neck or had drooling, could hold their neck and drooling were stopped completely. Overall 20 of our CP patients reported clinical improvements after treatment. In XCell-Center 66.4% of the 104 treated patients reported improvements [21].

Eighty-six percent of our patients with hypoxic brain damage responded to the treatment, with stopping of drooling and development of neck holding in all those who had such problems.

Embryonic neural transplants have become clinically relevant over the past 25 years for their possible application in the treatment of cerebellum-related neurodegenerative diseases. While highlighting the important role that fetal neural progenitors have in meeting these challenges, we define rationales for all types of cell therapy involving adult stem cells as well as human embryonic stem cells (hESC) and human induced pluripotent stem (iPS) cells. The recent advances in the field of hESC and iPS cells, including their capacity for differentiation toward regional specific neural lineages, could open a new era of transplantation in cell-based therapy for cerebellar ataxias [22].

Eighty percent of our patients with cerebellar atrophy had significant clinical improvements after receiving the treatment. One patient was transplanted three times, each 1.5 years apart, to have more improvements.

Multiple sclerosis is a disease characterized by multifocal areas of demyelination in the brain and spinal cord, with associated inflammatory cell infiltrates, reactive gliosis, and axonal degeneration. Fifty patients suffering from multiple sclerosis worsening despite pharmacological treatment were treated by means of several intrathecal injections of peripheral blood cells harvested by aphaeresis after G-CSF (granulocyte colony stimulating factor) treatment in Iraq, 24 patients (48%) had a reduction of EDSS score; eight patients had a relapse, but it was milder than usual and more easily controlled by cortisone. In conclusion, since mesenchymal cells increase in the peripheral blood after G-CSF stimulation, a peripheral blood harvest seems easier and cheaper than mesenchymal cells cultivation prior to the injection. It seems a reasonable treatment for progressive multiple sclerosis [12].

Although three of the four patients in our center with multiple sclerosis had relapse of the disease, one patient developed stool and urine control, and better talking and intelligence; one of them who had relapse of the disease, with second stem cell therapy one year later, developed good vision, decreased cerebellar signs and no more urinary retention.

Two of our patients with kernicterus, one with neurological sequelae of meningitis, two with heriditary neuropathies, and one with colpocephaly due to boxing trauma, had clinical improvements.

Autism is a brain development disorder characterized by impaired social interaction and communication, and by restricted and repetitive behavior. All of these signs begin before a child is three years old. Autism involves many parts of the brain. How it occurs is not well understood. In Xcell-center, Germany, follow-up statistics from seven treated patients with autism show that five of seven patients experienced improvements after stem cell therapy. The median age of the patients was 9.5 (range 5–16) years. There was no apparent correlation between positive outcome and the number of stem cells administered. Overall, patients reported improvements in cognition, language, social contact, eye contact, coordination, motor skills, and awareness [23].

In our center, two patients with autism, and each patient with CVA, Friedreich's ataxia, PLS, ALS, transverse myelitis, and head trauma did not have any response to stem cell therapy.

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is characterized by a progressive destruction of motor neurons in the spinal cord. Patients with ALS develop increasing muscle weakness over time, which ultimately leads to paralysis and death. The cause of ALS is largely unknown, and

there are no effective treatments. Researchers recently have used different sources of stem cells to test in rat models of ALS to test for possible nerve cell-restoring properties. In one study, researchers injected cell clusters made from embryonic germ (EG) cells into the spinal cord fluid of the partially-paralyzed rats [24]. Three months after the injection, many of the treated rats were able to move their hind limbs and walk with difficulty, while the rats that did not receive cell injections remained paralyzed. Moreover, the transplanted cells had migrated throughout the spinal fluid and developed into cells that displayed molecular characteristics of mature motor neurons. However, too few cells matured in this way to account for the recovery, and there was no evidence that the transplanted cells formed functional connections with muscles. The researchers suggest that the transplanted cells may be promoting recovery in some other ways, such as producing trophic factors.

In a study eight patients with definite or probable ALS were enrolled. After a 3-month lead-in period, autologous MSCs were isolated two times from the BM at an interval of 26 days and were then expanded in vitro for 28 days and suspended in autologous cerebrospinal fluid. Of the eight patients, seven received two intrathecal injections of autologous MSCs (1×10^6 cells/kg) 26 days apart. Clinical or laboratory measurements were recorded to evaluate the safety 12 months after the first MSC injection. The ALS Functional Rating Scale-Revised (ALSFRS-R), the Apple ALS score, and forced vital capacity were used to evaluate the patients' disease status. No serious adverse events were observed during the 12-month follow-up period. Most of the adverse events were self-limited or subsided after supportive treatment within four days. Decline in the ALSFRS-R score was not accelerated during the six-month follow-up period. Two repeated intrathecal injections of autologous MSCs were safe and feasible throughout the duration of the 12-month follow-up period [25].

In Xcell-center, Germany, 162 (88%) of 184 treated patients with spinal cord injury returned the post-treatment questionnaires. Clinical improvements were reported in 56 (34.6%) of the patients. Improved bladder and bowel function was reported in 37.7% and 35.7% of the improved patients, respectively. In these patients, neurogenic pain and muscle spasm were also improved in 52.7% and 50.7% of the patients, respectively. In two patients, the Baclofen® pump (for treatment of spasm) could be removed permanently after the treatment. Completed pre- and post-treatment ASIA Score forms could be collected in 25% of the patients (n=46). After treatment, the mean ASIA motor score increased significantly ($p<0.001$) by 6.5 points, and the mean SAIA sensory score increased by more than 11 points ($p<0.001$). In four patients, there was a change in ASIA classification—ASIA-A (complete motor/sensory loss bellow SCI) to -B (complete motor loss) in three patients and ASIA-C (major motor loss) to -D (minor motor loss) in one. None of the 46 patients did deteriorate during the study [26].

To evaluate the therapeutic effects of autologous bone marrow cell transplantation (BMT) in conjunction with the administration of granulocyte macrophage-colony stimulating factor (GM-CSF) in six complete SCI patients in south Korea, BMT (1.1×10^6 cells/μL in a total of 1.8 mL) applied to the injury site and subcutaneous GM-CSF administration was performed in five patients. One patient was only treated with GM-CSF. The follow-up period ranged from 6 to 18 months. Sensory improvements were noted immediately after the operations. Sensory recovery in the sacral segment was noted mainly three weeks to seven months, postoperatively. Significant motor improvements were noted 3–7 months postoperatively. Four patients showed improvements in their American Spiral Injury Association Impairment Scale (AIS) grades (from A to C). One patient improved from AIS grade A to B; the last patient remained in AIS grade A. No immediate worsening of neurologic symptoms was found. However, side effects of GMCSF treatment such as a fever and myalgia were noted. Serious complications increasing mortality and morbidity were not found. The follow-up study with magnetic resonance imaging 4–6 months after the injury showed slight

enhancement within the zone of BMT [27].

Despite reports of sensory and motor improvements after transplantation of bone marrow cells into the injured spinal cord, only one out of our four patients with SCI and incomplete paralysis responded to the therapy. She could stand and walk, while was unable to do it before therapy. No response was noted in six patients with SCIs and complete paralysis.

In a study, mono-nuclear fraction of bone marrow (mnBM) did not increase mature immune cells after transplantation into SCI, or evoke an increased host immune response or tissue loss compared with bone marrow stromal cells (MSC)-transplanted animals. In contrast, the host macrophage/microglia response was increased early after MSC transplantation, perhaps due to exposure of cells to serum-containing media. The glial scar was less prominent after mnBM transplantation on the 4th day. After 21 days, differences had subsided and MSC and mnBM macrophage responses and effects on glial scarring were comparable. MSC and mnBM engraftment efficiencies were also similar. Cellular transplantation is a promising treatment strategy for SCI. However, most cells need to be cultured before transplantation introducing burdensome steps for clinical application. Cells immediately available for transplantation, like mnBM, would be preferable [28].

Embryonic stem cells, fetal mesenchymal neurons, and neural stem cells have been introduced as restorative strategies in PD animals and patients, but ethical and immunological problems as well as the serious side effects of tumor genesis and disabling dyskinesia have limited clinical application of these stem cells. Meanwhile, cell therapy using mesenchymal stem cells (MSCs) is attractive clinically because these cells are free from ethical and immunological problems [29].

The marrow-derived mesenchymal stem cells function mainly by providing a microenvironment through various cytokines that induce cell growth and stimulate vascularization or by fusing with local cells, rather than by transdifferentiation into specific differentiated cells of the organ undergoing repair [30].

Isolated bone marrow mononuclear cell fractions include hematopoietic stem cells, macrophages, lymphocytes, as well as marrow stromal cells. Mechanisms regulating lineage commitment and cellular differentiation in the neural and hematopoietic systems are similar. Hematopoietic stem cells excrete many types of cytokines including thrombopoietin and interleukin 11. These cytokines are also known as essential factors for the survival and differentiation of neuronal progenitor cells. Colony-stimulating factor I is one of the important hematopoietic cytokines that also acts as a growth factor in the central nervous system. A recent study shows that activated microglial cells or macrophages enhance axonal regeneration by removal of myelin debris in injury site. These are suggestive modes of action of bone marrow cells transplantation [27].

In conclusion, we showed the safety and efficacy of bone marrow mononuclear cells (BMMCs) injected intrathically to patients with various neurological diseases. The results however, should be confirmed in large studies. The injections may theoretically be repeated.

REFERENCES

1. Callera F, do Nascimento RX. Delivery of autologous bone marrow precursor cells into the spinal cord via lumbar puncture technique in patients with spinal cord injury: a preliminary safety study. *Exp Hematol* 2006;**34**:130-1.
2. Bakshi A, Hunter C, Swanger S, *et al*. Minimally invasive delivery of stem cells for spinal cord injury: advantages of the lumbar puncture technique. *J Neurosurg Spine* 2004;**1**:330-7.
3. Lepore AC, Bakshi A, Swanger SA, *et al*. Neural precursor cells can be delivered into the injured cervical spinal cord by intrathecal injection at the lumbar cord. *Brain Res* 2005;**1045**:206-16.
4. Rice CM, Scolding NJ. Autologous bone marrow stem cells- properties and advantages. *J Neurol Sci* 2008;**265**:59-62.
5. Cerebral Palsy Patient Still Improving after 2nd Round of Stem Cells in Germany. Availabe from *www.prweb.com/releases/2010/01/prweb3430684.htm.* (Accessed August 16, 2016)

6. Panchision DM. Repairing the Nerous system with stem cells. Available from *https://stemcells.nih.gov/info/Regenerative_Medicine/2006Chapter3.htm.* (Accessed August 16, 2016)
7. Theise ND, Krause DS. Toward a new paradigm of cell plasticity. *Leukemia* 2002;**16**:542-8.
8. Hughes BR. Real-World Successes of Adult Stem Cell Treatment. Available from *www.cogforlife.org/adultStemCellSuccess.htm*. (Accessed August 17, 2016)
9. Azari H, Osborne GW, Yasuda T, *et al*. Purification of immature neuronal cells from neural stem cell progeny. *PLoS One* 2011;**6**:e20941.
10. Backlund EO, Graniberg PO, Hamberger P, *et al*. Transplantation of adrenal medullary tissue to striatum in Parkinsonism. First clinical trials. *J Neurosurg* 1985;**62**:169-73.
11. Tiantan Puhua, Stem Cell Treatment Center, Frequently Asked Questions. Available from *www.stemcellspuhua.com/qanda.shtml.* (Accessed August 18, 2016)
12. Hammadi AM, Marino A, Farhan S. Clinical outcome of 50 progressive multiple sclerosis patients treated with cellular therapy in iraq. *Int J Stem Cells* 2011;**4**:113-5.
13. Deda H, Inci MC, Kurekci AE, *et al*. Treatment of chronic spinal cord injured patients with autologous bone marrow-derived hematopoietic stem cell transplantation: 1-year follow-up. *Cytotherapy* 2008;**10**:565-74.
14. Chernykh ER, Stupak VV, Muradov GM, *et al*. Application of autologous bone marrow stem cells in the therapy of spinal cord injury patients. *Bull Exp Biol Med* 2007;**143**:543-7.
15. Geffner LF, Santacruz P, Izurieta M, Flor L, *et al*. Administration of autologous bone marrow stem cells into spinal cord injury patients via multiple routes is safe and improves their quality of life: comprehensive case studies. *Cell Transplant* 2008;**17**:1277-93.
16. Gupta K. Fetal Neural Transplants in Parkinson's. Available from *www.lef.org/magazine/mag2000/april00-report2.html*. (Accessed August 18, 2016)
17. Freed CR, Greene PE, Breeze RE, *et al*. transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med* 2001;**344**:710-19.
18. Olanow CW, Goetz CG, Kordower JH, *et al*. A double- blind controlled trial of bilateral fetal neural transplantation in Parkinson's disease. *Ann Neurol* 2003;**54**:403414.
19. Stem Cell Treatment of Parkinson's disease. Available from *www.xcell-center.com/treatments/diseases-treated/parkinson.aspx.* (Accessed August 20, 2016)
20. Abi Chahine N.H, Wehbe T.W. Hilal R A, *et al*. Treatment of cerebral palsy with stem cells: a report of 17 cases. *Int J of stem cells* 2016;**9**:90-95.
21. Stem Cell Treatment for Cerebral Palsy. Available from *www.xcell.center.com/treatments/diseases-treated/cerebral-palsy.aspx*. (Accessed August 18, 2016)
22. Erceg S, Moreno-Manzano V, Garita-Hernandez M, *et al*. Concise review: stem cells for the treatment of cerebellar-related disorders. *Stem Cells* 2011;**29**:564-9.
23. Stem Cell Treatment for Autism. Available from *www.xcell-center.com/treatments/diseases-treated/autism.aspx.* (Accessed August 20, 2016)
24. Kerr DA, Liado J, Shamblott MJ, *et al*. Human embryonic germ cell derivatives facilitate motor recovery of rats with diffuses motor neuron injury. *J Neurosci* 2003;**23**:5131-40.
25. Oh KW, Moon C, Kim HY, *et al*. Phase I trial of repeated intrathecal autologous bone marrow- derived mesenchymal stromal cells in amyotrophic lateral sclerosis. *Stem Cells Transl Med* 2015;**6**:590-7. Doi:10.5966/sctm.2014-0212.
26. Park HC, Shim YS, Ha Y, *et al*. Treatment of complete spinal cord injury patients by autologous bone marrow cell transplantation and administration of granulocyte-macrophage colony stimulating factor. *Tissue Eng* 2005;**11**:913-22.
27. Stem Cell Treatment of Spinal Cord Injurie. Available from *www.xcell-center.com/treatments/disease-treated/spinal-cord-injuries.aspx.* (Accessed August 18, 2016)
28. Samdani AF, Paul C, Betz RR, *et al*. Transplantation of human marrow stromal cells and mononuclear bone marrow cells into the injured spinal cord: a comparative study. *Spine (Phila Pa 1976)* 2009;**34**:2605-12.
29. Lee PH, Park HJ. Bone marrow –derived mesenchymal stem cell therapy as a candidate disease-modifiying strategy in parkinson's disease and multiple system atrophy. *J Clin Neurol* 2008;**1**:1-10. doi:10.3988/jcn. 2009.5.1.1.
30. Phinney DG, Prockop DJ. Concise review: Mesenchymal stem /multipotent stromal cells: the state of transdifferentiation and modes of tissue repair-current views. *Stem Cells* 2007;**25**:2896.