

## Clinical Outcome of 50 Progressive Multiple Sclerosis Patients Treated with Cellular Therapy in Iraq

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**Background and Objectives:** 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. It typically presents in young adults with episodic neurologic dysfunction, our aim is to find new simple method to treat multiple sclerosis by hematopoietic stem cells derived from peripheral blood.

**Methods and Results:** 50 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. 24 patients (48%) had a reduction of EDSS score. 8 patients had a relapse, but it was milder than usual and more easily controlled by cortisone.

**Conclusions:** 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.

**Keywords:** Multiple sclerosis, Mesenchymal cells, Cellular therapy

### Introduction

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. It typically presents in young adults with episodic neurologic dysfunction. Although the exact origin of MS remains enigmatic, evidence suggests that it is an immune mediated attack on myelin, with secondary disruption of axons leading to progressive disability over time in the majority of afflicted patients. The main clinical scoring system is the Expanded Disability

Status Scale (EDSS) which is a rating system that is frequently used for classifying and standardizing the condition of people with multiple sclerosis.

Despite early introduction of disease-modifying treatments, the disease is not contained in some patients. For these people, aggressive immunosuppression or even immunoablative therapies are important therapeutic options. Very high doses of chemotherapy can be used to ablate effectively the entire immune system, which is then replaced de novo with frozen hematopoietic stem cells derived from the patient himself. An immunoablation followed by the infusion of autologous hemopoietic stem cells is able to cure multiple sclerosis, but this procedure has a measurable risk for the patient (1).

There is a growing interest in cellular therapy with mesenchymal cells injected both intrathecally and intravenously (2). This therapy should be devoided of any risk for the patient. The limiting factor is the need of a GMP laboratory for the production of these cells.

Some adult stem cells have the capability to differ-

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entiate into tissues other than the ones from which they originated; this is referred to as plasticity.

Their immunosuppressive-immunomodulating effect was exploited in the treatment of a series of autoimmune diseases, such as diabetes, rheumatoid arthritis, and system lupus erythematosus. A large cohort of patients with GVHD has been treated so far (3), and other studies are ongoing in this clinical situation.

Adult stem cells are able to differentiate *in vitro* and *in vivo* into neurons. Furthermore they are able to produce soluble factors that could stimulate the growth and the function of the recipient neurons. Both effects are the bases of so called “neuroregenerative” effect that was exploited for treating patients with Parkinson, Multiple System Atrophy, spinal cord lesions and some genetic diseases involving the CNS (4, 5). In multiple sclerosis a cellular therapy could work through two effects:

1) Immunomodulating effect, thus curing the immune imbalance at the basis of the disease.

2) Neuroregenerative affect, thus (hopefully) repairing the already existing brain lesions.

Even if most centres are aiming to treat patients with multiple sclerosis with bone marrow derived mesenchymal cells, recently it was demonstrated that mesenchymal cells appear in substantial numbers in the peripheral blood after G-CSF therapy (6). G-CSF in conjunction with the receptor of Urokinase (uPAR) mobilizes mesenchymal cells into the peripheral blood (7).

A treatment with mesenchymal cells needs to cultivate these cells in an expensive GMP laboratory in order to follow the directives of the international health authorities. If the same result could be accomplished by means of a cheaper processing of peripheral blood, there would be an advantage for both patients and institutions (8).

## Materials and Methods

50 Iraqi patients; 25 males, 25 females, with primary or secondary progressive multiple sclerosis were assessed clinically in the multiple sclerosis clinic, the Neurology Department of the Medical City complex in Baghdad, after getting patient consensus according to the ethical committee plan which is headed by high authorities in Iraq Health Ministry.

The age of the patients ranged from 25 to 63 years, and mean age was 44 years. The EDSS scoring system was more than 6 in all cases (from 6 to 8). All the patient had an increase of EDSS score of >1 point in the previous year, despite any immunosuppressive treatment. Then all the patients stopped any other treatment modality. The

mean period from diagnosis till starting cell therapy is 8 years.

The procedure of peripheral hematopoietic stem cell separation was done by blood cell separator (Cobe Spectra version 7 lrs turbo).

1. The process of cell separation was done after using G-CSF (Neupogen-Roche) which was given in doses of 5 mg per kilogram body weight daily for 3~4 days subcutaneously followed by collection of peripheral hematopoietic stem cells on the fourth-fifth day using WBC protocol of blood cell separation.

2. The second step was the buffy coat separation; then total white blood cell count was done and mononuclear cells counted by manual method followed by CD34 counting to identify the exact CD34 count in the whole product.

Mean total mononuclear cell count per product was  $5 \times 10^8$  (range 1~8).

Mean CD34 count per product was  $5 \times 10^6$  (range 2~7).

3. The last step was the spinal injection of the cell product intrathecally through the fifth lumbar vertebrae in sitting position under local anesthesia by lidocaine within 24 hours of collection.

The procedure was repeated 1~8 times (mean 2.14) within 6~8 weeks. The period of follow up is 12 months.

## Results

A subjective clinical improvement was referred by 42 patients soon after 2 weeks of starting therapy (84%), while an objective reduction in the EDSS score of more than 1 (from 1 to 1.5) was observed in 24 patients (48%). No major changes of MR imaging was seen. However there was no increase of Gadolinium enhancement in 40 patients during the period of follow up.

This cell therapy did not prevent relapses in 8 cases, but they were milder than usual, and quickly controlled by cortisone treatment.

Complications: the only complication in 90% of cases was transient backache and meningism. No serious side effects.

## Discussion

Cell therapy for multiple sclerosis is a new concept as a part of regenerative medicine field. Articles in the available sources are scarce, solid proof of benefit from stem cell therapy is difficult to obtain.

It is beyond doubt that there is lots of research work to be done on the nature of brain damage and natural repair mechanisms of nerve tissue, on the interaction be-

tween immune system and stem cells and on various other aspects in this complex neurobiological arena.

However, in our opinion the preclinical phase of cell therapy in multiple sclerosis is over.

There is growing, although sometimes inconclusive or casuistic, evidence of clinical relevant brain-repair and protective properties of transplanted stem cells. Given the urgency of finding a cure for this widespread, disabling disease, most scientists argued that it is justifiable to arrange a rapid onset of well managed trials.

Our results seem to assess that intrathecally injected peripheral blood stem cells are first of all safe for the patients, and could be as effective as the bone marrow's derived stem cells. Our procedure would be feasible in any institution which has the normal facilities of any Haematology or Transplantation dept.

Further studies should solve some questions.

1) Number of cells that is reasonable to inject, and number of injections. We used a huge number, that guaranteed that a relevant number of mesenchymal cells (since less than one in thousand should be in the cell mixture) could be injected. Since half billion cells is the threshold of discomfort for the patient (fever, vomit, headache, drowsiness), we wonder if a reduced number could be effective as well.

2) Involvement of CD34 haemopoietic stem cells in the immunoregulatory and neuroregenerative effect. Literature is rich in this regard, but there are still controversial opinions. Many centres aimed to treat patients with different diseases (mainly cardiac diseases) with CD34 cells. The results seemed difficult to evaluate in many methanalysis studies. We wonder if the positive results that someone reported were effect of the mesenchymal cells contaminating the cell suspension, instead of a direct effect of CD34 cells.

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### Potential conflict of interest

The authors have no conflicting financial interest.

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