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Effects of Hematopoietic Autologous Stem Cell Transplantation to the Chronically Injured Human Spinal Cord Evaluated by Motor and Somatosensory Evoked Potentials Methods

A. A. Frolov and A. S. Bryukhovetskiy

NeuroVita Clinic of Interventional and Restorative Neurology and Therapy, Moscow, Russia

International standards for stem cell treatment of neurological disorders have not yet been established. In particular, specific quantitative methods have not yet been adopted to assess the effectiveness of stem cell treatment. The aim of this study is to evaluate the functional changes detectable by conventional neurophysiologic methods in an injured spinal cord during stem cell therapy. Twenty adult patients with chronic spinal cord injury at C4–C8 level were examined by somatosensory evoked potentials (SEPs) and motor evoked potentials (MEPs) methods, the first time prior to the treatment and then regularly during its course (1–4 years). The treatment consisted of repeated intrathecal transplantations of autologous hematopoietic stem cells. After at least 1 year of treatment, four effects were detected: 1) restoration of the initially absent short-latency SEP (three patients); 2. N20P23 interpeak amplitude increase in SEP elicited by median nerve stimulation (four patients); 3) P38 latency reduction in SEP elicited by tibial nerve stimulation (two patients); 4) appearance of MEP (three patients). The nonidentical effects of stem cell transplantation in different patients presumably reflect the variety of the regeneration processes in different pathways of the spinal cord, depending on the extent and nature of lesion of the spinal cord pathways in different patients. The local effects of stem cell treatment at the cervical level were evaluated by median SEP and wrist muscle MEP demonstrate the ability of stem cells to spread within the spinal cord at least from lumbar to the cervical level, home there, and participate in the neurorestoration processes.

Key words: Spinal cord injury (SCI); Hematopoietic stem cell; Somatosensory evoked potential (SEP); Motor evoked potential (MEP); Neurorestoration

INTRODUCTION

The prospects of stem cell transplantation for the treatment of various neurological disorders have been intensively studied in the last two decades (12). A number of experiments have demonstrated the promising potential of stem cells in spinal cord injury (SCI) treatment (10,11,13). Yet the benefit of cell therapy in SCI is debatable. The mechanisms of stem cell action within the damaged spinal cord remain unknown. At present, only a few clinical trials have been conducted. Some results were confirmed by quantitative methods, although such evaluation was unsystematic.

A limited number of quantitative methods are available to assess the function of spinal cord pathways. Somatosensory evoked potentials (SEPs) and motor evoked potentials (MEPs) enable direct estimation of the nervous impulse conduction via afferent and efferent spinal cord pathways, respectively (3,5,6). Compared with other available methods, SEPs and MEPs are truly objective (i.e., they do not require the patient's report or voluntary effort to contract the muscles). Compared to clinical examination, neurophysiological methods are more precise and give important additional information concerning the processes underlying the recovery. For example, the nervous impulse conduction velocity can be estimated only by these methods.

Previous studies have shown the positive effects of stem cell transplantation in SCI patients by the measurement of SEPs and MEPs. The appearance of the initially absent response and its amplitude increase were reported (14). However, most of the described cases were attributed to the acute or subacute (within 3 months) stage of SCI. In such cases the stem cells' effect cannot be differentiated from spontaneous recovery. Some cases of SEP restoration after stem cell transfusions in chronic

Address correspondence to A. S. Bryukhovetskiy, NeuroVita Clinic of Interventional and Restorative Neurology and Therapy, 23 Kashirskoye shosse, Moscow, Russia. Tel: +7 495 3249339; Fax: +7 495 9801373; E-mail: neurovita-as@mail.ru or neurovitaclinic@gmail.com

SCI patients have also been previously reported (13). However, the results are not conclusive because the observed changes may reflect small fluctuations in conduction. Valid assessment of such subtle changes requires stability during repeated testing.

We review the data obtained by regular neurophysiologic examination of 20 chronic SCI patients, who had taken the course of stem cell treatment in our clinic. Intrathecal injections of stem cells were performed with the interval of several months. The transplanted hematopoietic autologous stem cells ($CD34^+$) are multipotent stem cells able to transform into neuroprotective glia, myelin-producing oligodendrocytes, and astrocytes. They are considered to be capable of concentration in an injured area where they participate in neuroregenerative process.

In a recent study of Cristance et al. (4), hematopoietic autologous stem cells were found to be rather effective in the treatment of chronic SCI. These authors report recovery of the somatosensory evoked response elicited by tibial nerve stimulation in more than half of the patients with previously "electrophysiologically complete" (no response) SCI. In our trial "electrophysiologically incomplete" patients were included as well, but the region of interest was limited to cervical cord injury.

To increase the reliability of the data we used a series of MEP and SEP examinations, repeated on average every 4–5 months during at least 1 year of follow-up since the beginning of stem cell therapy. The aim of this study is to evaluate and to measure functional changes in an injured spinal cord of patients treated by stem cell transplantation.

MATERIALS AND METHODS

Patients

Twenty patients (15 men, 5 women, ranging from 18 to 55 years old, mean age ± SD, 32.41 ± 11.67) with chronic complete and incomplete traumatic SCI were enrolled in this study. The time postinjury varied from 1 to 9 years (3.39 ± 3.11). The level of injury varied from C4 to C8. The control group included 10 patients matched by sex and age and type of SCI. Before being admitted to our clinic all patients had passed the course of standard therapy and rehabilitation with or without any effect, but exhibited no progress during several months before the beginning of stem cell therapy. None of them underwent surgical treatment just before or during the course of stem cell therapy. During the period of observation they did not change principally their usual physical rehabilitation courses. The study was approved by the local ethics committee. All patients gave informed written consent.

Autologous stem cell mobilization and harvesting were performed by standard techniques. Recombinant human granulocyte-colony stimulating factor (G-CSF; filgastrim, Neupogen®) administered for 4 days twice a day at a dosage of 2.5–6.8 μg/kg (mean 4.3 μg/kg) was used as a colony-stimulating factor. The peripheral hematopoietic stem cells (PHSCs) were collected on the fifth day by a standard procedure of leukapheresis using a blood cell separator (COBE Spectra, Gambro BCT, USA). The amount of all mononuclear cells and $CD34^+$ cells collected in one patient was $15–20 \times 10^9$ and $56–171 \times 10^6$, respectively. The material was divided into 14–20 aliquots, each containing $4–8.5 \times 10^6$ $CD34^+$ cells and $1.3–1.5 \times 10^9$ leukocytes in the volume of 2 ml. The cryopreservation was performed in sterile conditions using 10% dimethyl sulfoxide (DMSO) and polyglycine. For every patient all material was preserved in this way except for one aliquot that was taken for standardization and certification purposes.

One aliquot of PHSCs was used per dose. Before transplantation, the material was prepared by defrosting and elimination of DMSO and polyglycine using centrifugation with 0.9% saline solution and suspended in ~2 ml of cerebrospinal fluid gathered by spinal tap. The prepared PHSCs were delivered intrathecally to the subarachnoid space during standard lumbar puncture twice over an 8-day period, with an interval of several months (usually 3–5 months) before the next two doses. The duration of the course was 1–4 years (usually 1.5–2.5 years).

SEP and MEP Examination

All SEP and MEP recordings were made and interpreted by the same person. Medtronic keypoint (USA) was used to perform the neurophysiologic examination. SEP recordings were performed prior to stem cell therapy and later with an interval of several months (on average 4–5 months) during the treatment course. The evoked potentials were elicited by rectangular pulse (0.2 ms duration) stimulation delivered unilaterally to the median (wrist level) or tibial (medial malleolus) nerves. The rate of stimulation was 3.3 and 0.47 Hz, respectively. The intensity was 25 and 50 mA, respectively. SEPs were elicited by right/left median nerve stimulation (median SEP) or tibial stimulation (tibial SEP). Median SEP was recorded from surface scalp electrodes placed 10 mm posterior to C3/C4 according to international 10–20% system (7). Tibial SEP was recorded from surface scalp electrodes placed 10 mm posterior to Cz. Fz was the reference electrode. The test was performed for every patient. The epoch of analysis was 200 ms for median SEP and 500 ms for tibial SEP. Two averaged series (at least 300 stimulations each) were obtained during every test. All the SEP recordings were carefully inspected and those containing visually detected artifacts [e.g., electromyographic (EMG) artifact] were excluded from further analysis. The assessment of the stability of SEP dynamics was based on visual expert analysis of series of the recordings.

MEPs were elicited by 100% transcranial magnetic stimulation using Schwarzer mags 2 magnetic stimulator (Germany) of primary motor cortex and recorded in contralateral abductor digiti minimi (ADM) muscle. MEPs were not performed if the response of ADM was absent during ulnar nerve stimulation. Also, some patients refused to have transcranial magnetic stimulation. Finally, 15 patients were examined by MEP. This test was made prior to the treatment and every 4–5 months during it. Cases were grouped according to their prior to treatment MEP examination results: group I, MEP absence; group II, nonstructured MEP of unstable form in which principle components could not be identified; group III, MEP of stable form in a sequence of five stimulations.

RESULTS

Patients With Initially Absent Short-Latency (N20P23) Median SEP

In right-hand median SEP of two patients' N20P23 was initially absent; restoration of short-latency SEP was noted in none of the patients after stem cell therapy. In left-hand median SEP of three patients' N20P23 was initially absent; restoration of short-latency SEP was noted in one patient after 1 year of stem cell therapy.

Patients With Initially Absent Short-Latency (P38N45) Tibial SEP

In right foot tibial SEP of 15 patients' P38N45 was initially absent; restoration of short-latency SEP was noted in two patients after stem cell therapy. In left foot tibial SEP of 16 patients' P38N45 was initially absent; restoration of short-latency SEP was noted in two patients after 1–1.5 years of stem cell therapy.

One patient demonstrated bilateral tibial SEP restoration (Fig. 1) and one had tibial SEP plus medial SEP restoration on the same (left) side.

Conventional SEP pattern analysis includes only short-latency SEP assessment, while so-called middle-latency and long-latency potentials are usually neglected [except for the nociception studies (13)]. Nevertheless, it must be noted that in all studied SEP patterns these potentials were always found in the recordings preceding the restoration of short-latency SEP. In some patients only restoration of middle- and long-latency SEP was found.

Patients With Initially Present Short-Latency (N20P23) Median SEP

The latency value of N20 was within the normal range in all the examined patients. During the course

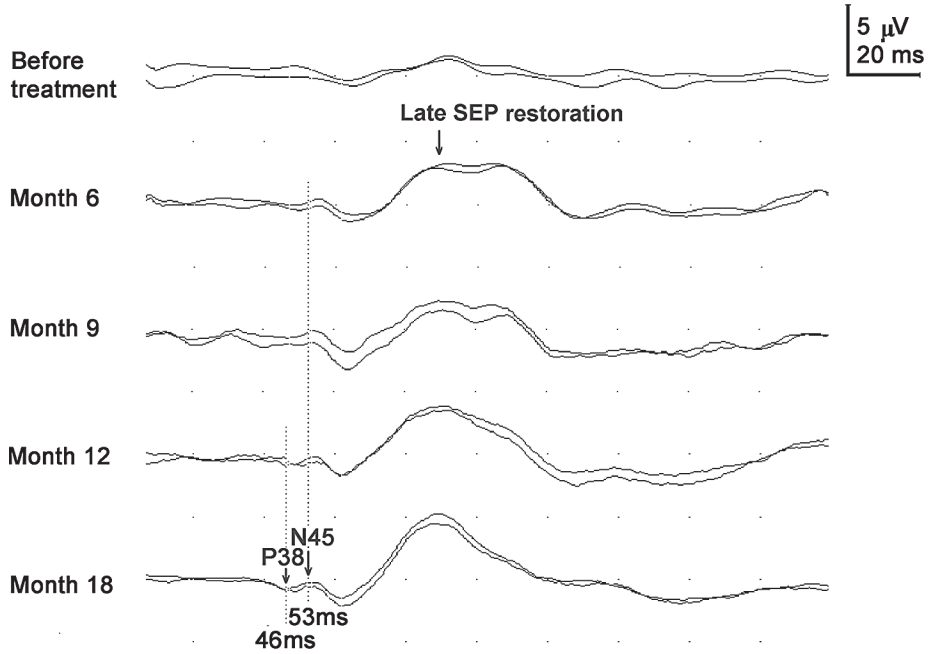

Figure 1. The dynamics of somatosensory evoked potentials (SEPs) elicited by stimulation of the left tibial nerve of a C5 level spinal cord injury (SCI) patient. The stem cell treatment was started 4 years after injury. Note the restoration of short-latency components—firstly N45 and then P38. The simultaneous latency reduction of the appeared components is seen. Note also the increase of late components amplitude.

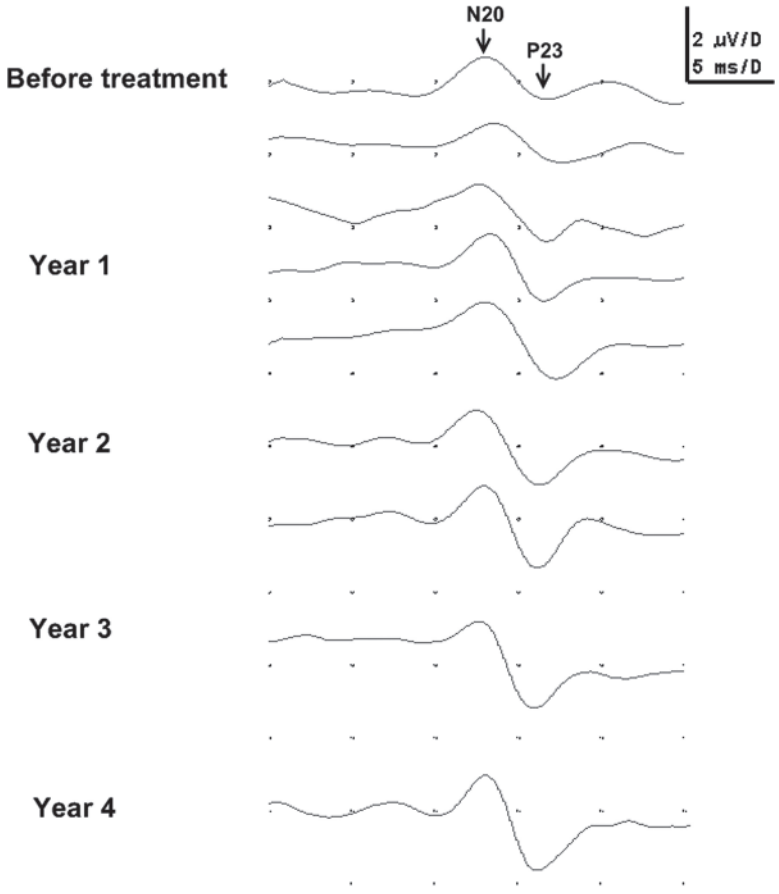

Figure 2. The dynamics of SEPs elicited by stimulation of left median nerve of C6 level SCI patient. The stem cell treatment was started at 2 years after injury. Note that amplitude of short-latency SEP (N20P23), being abnormally low before the treatment increased during the course of the treatment.

of therapy no pathologic increase of latency values was observed in any of the analyzed SEP patterns.

A clear interpeak N20P23 amplitude increase (Fig. 2) was detected in some patients: bilateral in one patient and unilateral in three patients (all in left median SEP). This effect was constant only in SEP patterns initially characterized as low amplitude. In patients, who initially had an N20P23 of abnormally low amplitude (less than 2 μV), mean amplitude increase was 0.22 ± 0.70 μV/year. No equivalent amplitude decrease or N20P23 disappearance was noted.

Patients With Initially Present Short-Latency (P38N45) Tibial SEP

Four of five patients had abnormally increased values of P38 latency (range 40.5–63.3 ms) before treatment. After 1–1.5 years of therapy the gradual reduction (from test to test) of P38 latency was evident (Fig. 3) in one patient bilaterally and in one patient unilaterally (in right tibial SEP). The mean P38 latency reduction was 1.2 ± 2.8 ms/year.

No stable changes in P38N45 interpeak amplitude were found. Any stable changes in SEP pattern of the control group were not detected.

MEP Restoration

After 1–1.5 years of treatment the following progress was observed in right ADM: MEP appeared in one of three patients of group I (Fig. 4); stable initial MEP components had appeared in five of nine patients of group II.

In left ADM, MEP appeared in two of five patients of group I; stable MEP components appeared in one of six patients of group II. The MEP did not disappear in any of the cases. MEP estimated in series of examinations with the interval of several months revealed the apparent temporal instability of MEP characteristics.

Any stable MEP changes in the control group were not detected.

DISCUSSION

That the effects of stem cell transplantation vary with patients presumably reflect the variety of the regeneration processes in different pathways of the spinal cord, depending on the extent and nature of the lesion of the spinal cord pathways in different patients.

One of the observed effects—the increase of short-latency median SEP amplitude—must indicate the enlargement of the amount of the conducting axons. Changes in peripheral axon can hardly cause the increase of SEP amplitude. On the contrary, the slow axonal loss in peripheral nerves often takes place in SCI patients (9). The peripheral axon and the body of the peripheral sensory neuron are located outside of the CNS where the stem cells are delivered. We performed electroneurography on the sural nerve of 48 patients before and after 1 year of stem cell therapy. The results (unpublished data) showed that the decrease of action potential of peripheral nerve reflecting the axonal loss was typical for SCI patients during cell regenerative treatment as well.

It is important that the median SEP amplitude increase was noted predominantly in patients with an initially low amplitude SEP. This observation demonstrates that the true restorative effect was due to the stem cells, but not the general SEP amplitude enhancement. We consider that short-latency SEP restoration is simply the special case of the amplitude increase effect—the increase from zero level. The same is true not only for median SEP and tibial SEP restoration, but also for MEP restoration.

Another effect is the reduction of the SEP latency, which was pronounced mainly in tibial SEP. The latency value of the early median SEP was initially within the normal limits because median SEP (if present) was produced by the impulses conducted through the intact fibers corresponding to the spinal segments located

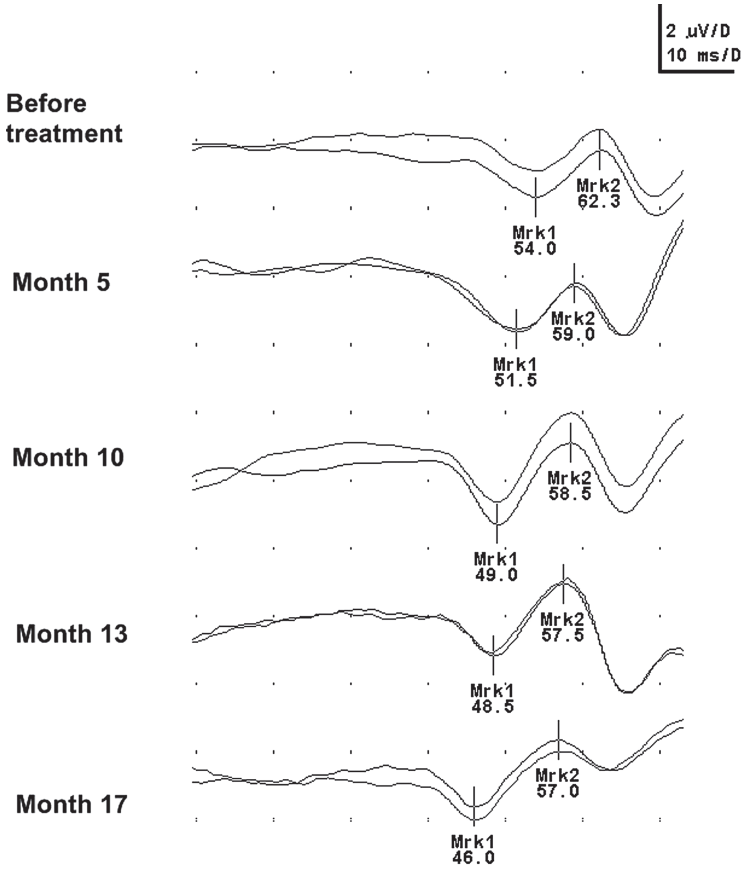

Figure 3. The dynamics of SEPs elicited by stimulation of left tibial nerve of C5 level SCI patient. The stem cell treatment was started 1 year after injury. Latency of SEP components is given in milliseconds. Short-latency components in each curve are well identified (so called anterior cord syndrome is characterized by preservation of dorsal columns). Note the reduction of P38 latency during stem cell therapy from 54 to 46 ms in 17 months.

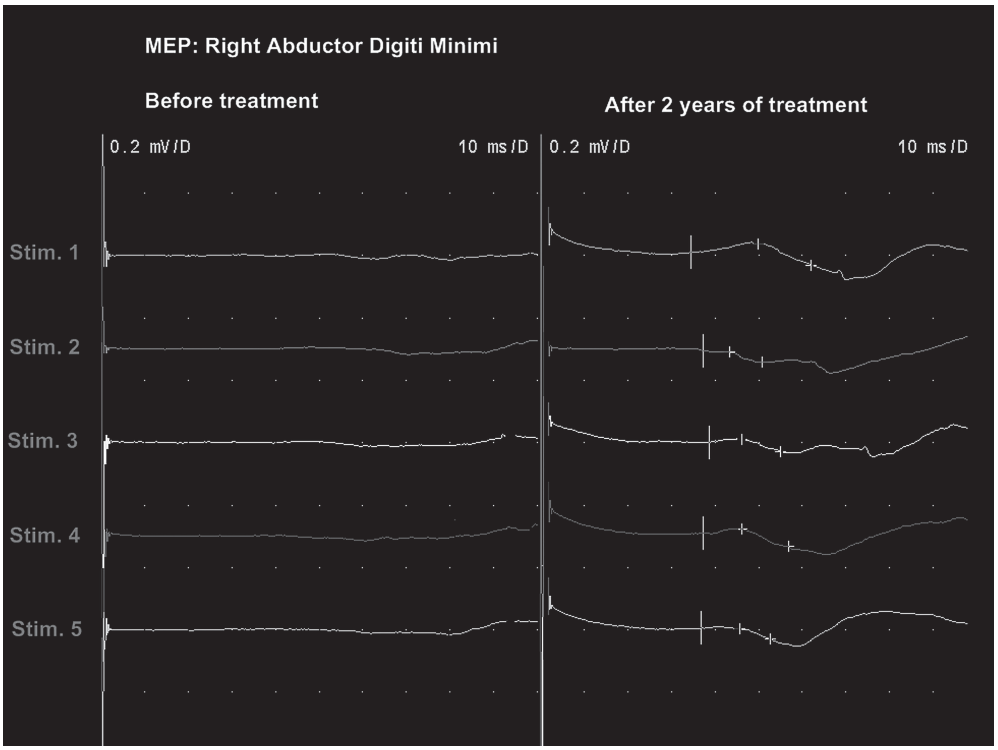

Figure 4. The dynamics of motor evoked potentials (MEPs) elicited in right wrist muscles by transcranial magnetic stimulation of contralateral primary motor cortex (hand area). The stem cell treatment was started at 3 years after injury. MEP in the recording made prior to stem cell transplantations is not identified in 100 ms epoch after the stimulus. The repeated clinical examination revealed motor level lowering from C6 to C7 segments on the same side.

higher than the injury. In tibial SEP the initial recordings revealed the abnormal latency increase in most of the cases (in four of five patients). After 1 year of treatment in some patients the reduction of latency constituted several milliseconds and showed a stable trend with time.

The conduction time, which reflects the conduction velocity, is considered to be a sensitive electrophysiological marker of myelin consistency. The remyelination potential of stem cells is well known. Various stem cell populations were shown to myelinate efficiently after transplantation into damaged neural structures (2,12). The remyelinating potential of bone marrow stem cells was reported by Akiyama et al. (1).

Principally, all of the mentioned effects can be explained by a remyelinating process. The extensive myelin defect can cause the blockage of the impulse propagation. So with myelin restoration the number of effectively conducting fibers will increase. This must result in SEP amplitude increase and SEP restoration. On the other hand, demyelination usually causes dispersion of conduction velocities in nervous fibers. The dispersion of resulting single potentials affects their summation in the compound potential and influences its amplitude.

We have not detected the universal effect of stem cell transplantation in the spinal cord; each of the detected effects was not frequent in the group of patients. We suppose that the variety of the revealed effects can be explained by differences in the initial state (axonal loss, variants of demyelination) of spinal pathways: anterolateral cortico-spinal tract (tested by MEP) and dorsal columns pathway of proprioception (tested by SEP). The observed changes were relatively small in most of the patients. Moreover, the dynamics of SEP parameters was often nonlinear. So serial recordings during the long period of observation (to our opinion at least 1 year) are required to verify the trend. In this sense SEP method is more precise then MEP. Besides, added to clinical assessment of spinal cord functions SEP examination offers important information about pathways of proprioception, the function of which is difficult to assess during clinical examination.

Clinical neurologic assessment before and during the treatment was based on the ASIA scale. We did not observe any clinical neurophysiologic correlations. This can be explained by the variety of the detected effects and variety of clinical manifestations of improvement in

the relatively small trial group. However, we did not publish these data as we cannot exclude another reason—the impotence of the ASIA scale in monitoring chronic SCI treatment effects. The following issues must be mentioned.

1) Most patients in the trial group noted, first of all, improvement in the trunk muscles while ASIA scale allows only examination of extremities. 2) Estimating the maximal force of the muscles the neurologist relies on the maximal voluntary effort of the patient. This circumstance lowers the reliability and precision of the estimates. The most striking case we have observed (not included in the study) is absolute (psychogenic) immobility of lower extremities muscles in chronic SCI patient with full preservation of corticospinal tract confirmed by MRI and MEP. 3) One can argue that strengthening of a muscle can be achieved by physical training, and not by regeneration of spinal cord tract. 4) The changes in sensitivity can hardly result directly from training. However, the ASIA scale is too robust: normal, abnormal, and zero. Precision of ASIA clinical assessment of sensory function cannot be comparable to the precision of SEP assessment. 5) Even if it were, the scale is elaborated only for light touch and pain sensations, while SEP method estimates the proprioception pathways. It is worth saying that none of the clinical tests can differentiate between demyelination and axonal loss. 6) Finally, estimates of sensitivity depend on the patient's report, which is subjective (3,8).

Thus, nowadays there is no standard and universally accepted method for clinical neurologic testing, with reliability and precision that could be compared to neurophysiologic methods of monitoring motor and sensory function in chronic SCI patients. Several methods have been proposed by various authors. However, the issues of new approaches in clinical neurologic testing to heighten the precision of chronic SCI patients monitoring is beyond the frameworks of this article.

In summary, we have not detected the universal effect of stem cell transplantation in the spinal cord—each of the detected effects was not seen universally in the entire group of patients. We confirm that restoration of tibial SEP (4,14) can take place in part of the chronic SCI patients after hematopoietic autologous stem cell transplantation. We have found that changes in median SEP can also take place in cervical cord SCI patients, demonstrating once more the well-known capability of stem cells to migrate and home to the injured region of the CNS. The disappearance of SEP and MEP was not observed in any of the patients.

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