Cerebral palsy treatment with autologous bone marrow aspirate concentrate challenges for the future

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Abstract

Background

Cerebral Palsy (CP) is a heterogeneous group of conditions that results in permanent motor disability. Recent studies have suggested that cellular transplantation may have functional efficacy in the treatment of CP. We conducted a pilot study of the intrathecal transplantation of autologous bone marrow aspirate (BMAC) concentrate in children with CP to assess the safety of the procedure as well as its potential efficacy in motor and cognitive functions.

Materials and methods

Twenty-four patients with CP received BMAC and were evaluated 12 months. The treatment procedure involves three intrathecal BMAC applications along with neurorehabilitation. We assessed potential efficacy by using Gross motor functional classification system, Ashworth scale, Learning accomplishment profile-diagnostic scale, brain magnetic resonance imaging and electroencephalogram.

Results

The study enrolled 24 CP patients with chronological age from 1-12 years but the current developmental age was from 6 -10 months. After BMAC therapy, immediate improvements were noted within first 7 days, decreased muscle tone, and decreased involuntary limb movements, better head control and decreased salivation. After treatment, 83% patients developed relaxation of the extremities. The cognitive function assessment also revealed significant improvement in 40% patients. The average developmental age of the CP patients after the intervention was 22 months in the field of cognition and, 20 months in the field of locomotor skills, 21 months in the field of speech skills.

Conclusion

Autologous BMAC transplantation seems to be safe and feasible, and can help reduce the degree of impairment of CP patients and, improve the quality of life. The combination of cell therapy and neurorehabilitation can lead to functional restoration that reduces disability in CP.

Background

Several prenatal, perinatal and postnatal factors contribute to the development of a syndrome known as cerebral palsy (CP), with functional brain damage or lesions affecting immature brain [1]. Spastic cerebral palsy is the most common form of CP limiting locomotor functions, children have cognitive impairment, speech and hearing impairment, as well as other pathological disorders of which epilepsy is the most significant [2]. Underlying neuropathology of cerebral palsy is periventricular leukomalacia (PVL). PVL consists of diffuse brain white matter damage, with or without focal necrosis [3] and loss of oligodendrocytes, astrogliosis, and microglial infiltration [4]. The vulnerability of cells depends on the type of cell and age of a child where the damage occurs [5]. Oligodendrocytes develop from myelinating

oligodendrocytes by gradual maturation. If an ischemic lesion of a particular region occurs during this period, the ischemia leads to a lack of mature oligodendrocytes resulting in neural dysfunction [6]. Another mechanism contributing to the pathophysiology of CP is microglial activation after hypoxic-ischemic injury. Microglia secrete various forms of cytokines, such as tumor necrosis factor-alpha (TNF-α), interferon-gamma (INF-γ), interleukin-1 beta (IL-1β), and radical superoxide, and have toxic effects that alter neuronal and oligodendrocyte metabolism [7]. Standard therapy of CP involves individual approach with the aim to improve the functional abilities of a child. This treatment includes medical therapy, physical therapy, occupational therapy, speech therapy and, use of adaptive equipment. But these therapies, applied alone are rarely effective [8], since that treatment is not causative, does not cure impaired neural tissue, but only alleviate its consequences. Currently, autologous cell therapy holds a special place because of the possibility of nerve tissue restoration. Cell diversity, consisting of embryonic stem cells, adult stem cells, umbilical cord blood cells, and induced pluripotent stem cells, are potential therapeutic options for the treatment of neurological disorders, including stroke, Alzheimer's and Parkinson's disease, spinal cord lesions, autism, and cerebral palsy [9,10]. Numerous researchers report, within preclinical studies, the improvement of functional deficits in animal models of cerebral palsy [11,12]. The transplantation of autologous bone marrow aspirate concentrate (BMAC) doesn’t present any ethical or moral problems. Mechanism of BMAC action includes process that involves neuromodulation of neurons, axon growth, triggering of multimodal synapses through activation neurogenesis, neuroregeneration, reparation of damaged neurons, and replacement of neurons [13,14]. Although several therapies are available for the treatment of patients with CP, none therapies have shown satisfactory outcomes [15].

The aim of this study was to find out whether intrathecal injecting concentrated autologous BMAC would help 24 patients with CP to improve child’s motor and cognitive functions.

Material And Methods

Patient Enrollment. The study enrolled 24 patients with CP aged between 1 to 12 years. The study was initiated from Mart 2018-Mart 2019. The clinical study protocol was approved by the Institutional Committee of Global Care Surgery Hospital in Novi Sad, Serbia. The Committee evaluated the ethical aspects of the study in accordance with World Medical Association Declaration of Helsinki [16]. The procedure was explained to patients parents and a properly signed the Informed Consent Form (ICF). The procedure was explained to patients parents and a properly filled informed agreement was obtained. The exclusion criteria were hydrocephalus with ventricular drain, coagulation disorders and allergy to anesthetic agents, severe health conditions such as cancer, failure of heart, lung, liver or kidney and, active infections. All patients underwent a detailed physical and neuropediatric examination together with serological, biochemical and hematological tests. Brain magnetic resonance imaging (MRI), as well electroencephalography (EEG) was performed in all patients. The Gross Motor Function Classification System (GMFCS) [17] and Ashworth classification were used to classify the severity of the disease [18]. The Learning Accomplishment Profile-Diagnostic (LAP-D) test were used to evaluates fine locomotor skills, cognitive skills, and speech skills [19].

Procurement of Autologous Bone Marrow Cells. Drilling of bone marrow and BMAC administration have to be done in aseptic and antiseptic conditions. Patient was under total endotracheal anesthesia (ETA). The intervention included 3 intrathecal administrations of autologous bone marrow aspirate concentrate (BMAC). Drilling of bone marrow and BMAC administration has to be done in aseptic and antiseptic conditions. Patient was under total endotracheal anesthesia. Bone marrow was aspirated from the iliac crest using 22G needle after preparing the drilling place and making a small cutting (7 mm) using surgical blade No 11. Bone morrow was anticoagulated using Acid citrate dextrose (ACD) formula A in ratio 7:1. This meant that in two 60 ml syringes we took 8 ml of ACD formula A and filled up with bone marrow up to 60 ml. We placed the puncture needle on the orthopedic drill and drilled the periosteum and placed the needle in spongious part. After drilling, the bone marrow was processed using one of two table top separators (Angel whole blood separation system, Arthrex, USA). Separation of bone marrow with system was fully automated and did not require any assistance during process. After separation we got BMAC, red blood cells (RBCs), platelet poor plasma (PPP). Depending of patient baseline bone marrow count, 1.5-4 ml of BMAC as end volume was obtained.

Stem Cell Isolation. The cytokines measured from the BMAC and cerebrospinal fluid (CSF) samples for all patient were the following: adiponectin, adipsin, RBP4, MCP-1, IL-1β, IP-10, IL-10, IL-8, leptin, IL-6, IFN-γ, resistin, TNF- α. We were measuring the levels of the following: Stro-1, CD133, CD73, CD146, CD105, CD45, CD34, CD90, 7AAD. The BMAC were counted and checked for viability. Hematopoietic stem cells (CD34+) were also counted. The viability of the cells was found to be 98%. After preparing the application place, we injected BMAC intrathecal using 20G spinal needle. Before the administration we took CSF sample. We always were careful to take out the similar volume of CSF and BMAC solution in order to avoid disturbance of CSF circulation. The route of administration was in intrathecal between L_4-L_5 vertebra and, the transplantation lasted for 30 minutes.

Post therapy Assessment. After BMAC intrathecal administration, all patients underwent neurorehabilitation. A personalized home rehabilitation program was planned for each patient depending on the patient's condition assessment. It included physiotherapy, occupational therapy, speech therapy, and psychological procedures. All patients were advised to continue neurorehabilitation for at least 12 months. Post therapy follow-up was done using the GMFCS, Ashworth scale, LAP-D scale, MRI and EEG on 1st, 3rd, 6th, and 12th month. Changes in neurological deficits and improvements in function were compared between pre-therapy and post-therapy assessments.

Results

Pre-therapy Observations

The study enrolled 24 patients with CP aged between 1 to 12 years. The majority were males 58%, and the female were 42%. The motor function was hypotonic in 12%, hypertonic in 67%, dyskinetic in 21%. The demographic data of patients with CP are shown in Table 1.

Table 1
Gender and age group, type of CP and number of patients.

Demographic characteristic	Demographic group	No. of patients (N = 24)
Gender	male	14
	female	10
Age	< 3 years	3
	3–8 years	19
	> 8 years	2
Type of CP	hipotonia	3
	hypertonia	16
	dyskintec/ miscellaneous	5

The average volume of aspirated bone marrow was 92 ml. Average volume of intrathecal applied BMAC substrate was 2.26 ml. Quality control assessment showed very high level of viability in the applied BMACs samples, analyzed by multiparameter flowcytometry where 98% of cells showed no staining for 7AAD. There were no serious adverse events or severe complications during or after the transplantation procedure. During measurements, an increase in the number of CD90 positive cells was observed in each subsequent measurement, both as a percentage of the total nucleated cells in the sample and as the absolute number of events recorded on the flow cytometer.

Post-therapy Observations

The adverse events reported were limited to mild headaches (6%), transient fever (3%) and vomiting (2%). All side effects resolved within few days.

Motor Movements. After BMAC therapy, immediate improvements were noted within first 7 days, decreased muscle tone, and decreased involuntary limb movements, better head control, decreased salivation. From 7 days to 12 months after intervention, an improvement in the voluntary control of the limb parts was observed. The results are summarized in Table 2.

Table 2

Number of patients showing improvements based on gender and age of the patients.

	No improvement	Mild improvement	Moderate improvement	Significant improvement
Gender				
Male	3	3	6	2
Female	1	3	4	2
Age				
< 3 years	0	1	2	0
3–8 years	3	2	10	4
> 8 years	0	2	0	0

As a result, the opening and closing the fingers of the hand occurred, as well as hand movements that have not been reported so far. Muscle hypertonia was reduced from mild spasm to major reduction, improving sitting and maintaining balance. Patients cognitive abilities have increased. It was reported that seizures were less common, and in one case those could no longer be registered on EEG. After the one year of BMAC therapy, patients reported improving hand coordination, an improvement in head and gross motor controls. The sitting balance is further enhanced in one patient by moving the body weight while lying and while sitting. The associated movements have been improved due to the increase in trunk control and the gross motor skills. Development of consciousness, reduction of tone and improvement of the function of the antigravity musculature have helped to form a support in patients and maintained them in an upright position while standing; this was reported in one child. The formation of new steps with a walker or with assistance of a therapist has also been observed. Muscle tone and motor function management was improved. The standardized observational tool used to evaluate motor function was the Gross Motor Function Classification System was applied to evaluate muscle function (Table 3). The improvement ranged from 1 to 3 levels on the GMFCS scoring system. The average improvement was 1.3 points with a range of 0 to 3 points of improvement with no patients showing regression.

Table 3
Improvements based on GMFCS levels of the patients 12 months after intervention.

GMFCS levels	PRE-GMFCS	POST-GMFCS Mild improvement	POST-GMFCS Moderate improvement	POST-GMFCS Major improvement
I	1	0	0	0
II	2	1	0	0
III	7	0	3	1
IV	8	1	4	1
V	6	0	0	0

The severity of spasticity measured by the Ashworth Scale was reduced at the end of the study (Table 4). The modified Ashworth score, in which lower scores or reduced muscle tone represent motor function improvement, was applied to evaluate muscle spasticity. The functional and muscle tone assessments were conducted by an experienced physiotherapist at baseline, 3, 6 and 12 months after the first BMAC transplantation.

Table 4
Improvements based on the Ashwort scale of the patients 12 months after intervention.

Ashwort score	Mild improvement	Moderate improvement	Major improvement
1	0	0	0
2	1	0	0
3	4	4	2
4	0	3	1
5	0	2	0

The muscle spasticity of the patients was significantly reduced from 3.5 at baseline to 2.3 (t-test, P-value < 0.001) 12 months after the intervention, and clinical examination confirmed that patients movements became smoother. Also, abnormal posture and contracture deformities were corrected at approximately 1–2 scales, and patients were somewhat able to maintain their balance while sitting or standing. Reduction of spasticity in 50% of patients and, the cognitive assessment revealed significant improvement in 40% of patients.

Sensory, Cognitive, Speech Improvements.

The progressive dynamics seen after the treatment was presence of eye contact and, fine hearing developed. Collaborating with the therapist on therapies was better, which made it easier for the therapist to exercise and manipulate patients. Cognition of improvements in awareness, understanding, response time, leads to better communication between parents and child and therapist and child. The study enrolled 24 CP patients with chronological age from 1 to 12 years but the current developmental age was from 6–10 months (Table 5).

Table 5
Pre therapy and post therapy LAP-D scores of the CP patients.

LAP-D scores	No. of patients	Average developmental age of patients (in month)	SD	Minimum	Maximum
Pre therapy	24	8.0	0.603	6	10
Post therapy	24	13.1	7.067	6	30

The cognitive function assessment also revealed significant improvement in 40% patients. Speech has improved in terms of clarity, fluency, and intelligibility. The average developmental age of the CP patients after the intervention was 22 months in the field of cognition and, 20 months in the field of locomotor skills, 21 months in the field of speech skills. Improvements in speech skills shoved a statistically significant difference with a correlation of $r > 0.7$. Details on the changes in skills are shown in Table 6.

Table 6
Improvements based on LAP-D of the patients 12 months after intervention.

LAP-D	Cognitive skills	Locomotor skills	Speech skills	Sitting	Standing
r	0.045	0.073	0.833	0.121	0.136
P	0.844	0.759	0.000	0.602	0.536
AD	22	20	21	21	23

r-Pearson's correlation coefficient ($r > 0,7$)

P-statistical significance ($p = 0.000$)

AD-the average developmental age of patients (in months)

Discussion

Due to the heterogeneous nature of pathophysiology of CP, standard medical procedures have different treatment outcomes. More recently, autologous cellular therapy has evolved as a strategy for treatment of

cerebral palsy [20]. During childhood, brain neuroplasticity is at its maximum, making cell therapy a powerful treatment modality in children [21,22,23]. Various experimental studies have shown that cell transplantation in CP models can lead to survival of neurons, and differentiation of cells into neurons, oligodendrocytes and astrocytes [24,25]. Stem cells stimulate the recovery process by affecting damaged brain cells to regenerate via paracrine signaling [26]. Cell therapy can restore lost myelin by replacing dead oligodendrocytes and their precursor cells. Functional cell survival can be stimulated by introducing another type of cells that will be able to restore the lack of enzymes necessary for brain function [27]. Stem cells can reduce the levels of TNF, IL-1, IL-1 and IL-6 increased due to microglial activation [28]. These cells also secrete neurotropic and growth factors such as connective tissue growth factor, fibroblast growth factors, interleukins, vascular endothelial growth factor, fibroblast growth factor, and basic fibroblast growth factor, which are responsible for proliferation, cytoprotection, and angiogenesis, and stimulate recovery of lost tissue function [29,30]. During measurements, an increase in the number of CD90 positive cells was observed in each subsequent measurement. Mesenchymal cells increase angiogenesis by producing signaling molecules, stimulate tissue remodeling, decrease inflammation and activate the satellite cells. Moraes et al. [31] have hypothesized that CD90 controls the differentiation of MSCs by acting as a barrier in the pathway of differentiation commitment. Our data could indicate that the maintenance of MSC stemness and their paracrine effects rather than their differentiation underlie the good effects of therapy of our CP patients.

With the aim of studying safety, feasibility and efficacy of cell therapy in cerebral palsy syndrome, we present 24 cases with BMAC administration. Studies have shown that the entire bone marrow contains multiple stem cells: they represent a microenvironment around stem cells that enables cellular support and paracrine signaling by regulating also self-renewal and differentiation. Together with the niche, stem cells have better effect compared to single cell fractions [32,33]. BMAC contains different types of cells: platelets, erythrocytes, nucleated cells, progenitor cells, hematopoietic stem cells, mesenchymal stem cells. The aim is to bring hematopoiesis and mesenchymal and progenitor cells to the site of treatment. These cells are injected into the subarachnoid space by intrathecal injection. The procedure is minimally invasive and safe, probably the most effective route of administration. Intracranial transplantation may be considered as a form of treatment, but it involves the risk of surgical damage. In animal models of cerebral ischemia, it has been observed that during intravenous administration, most stem cells have been found in all organs except the brain, such as the lung, spleen, kidney, and intestines [34]. The question arises as to the openness of the hematoencephalic barrier to structures such as the stem cell niche, which does not classify intravenous stem cell administration as necessary administration route for BMAC.

With cell therapy, all patients underwent neurorehabilitation as part of the protocol. Most of them had been in the rehabilitation process before the intervention, but they still had a high degree of residual neurodeficiency. Physical treatment accelerates stem cell mobilization, proliferation and neurogenesis by increasing oxygen flow to the brain [35]. Cell therapy, rehabilitation and neurorehabilitation work synergistically and can together enhance the positive effects of healing.

In CP, damage to the motor control centers in the brain causes increased motor tone, leading to muscle stiffness. We assessed the motor function of the patients before and after cell injection. GMFCS scores had remarkable changes 12 months after transplantation compared with baseline. According to the Ashworth scale, there was a significant reduction in spasticity and, accordingly, patients movements became more flexible and easier. In a report from Lebanon, 17 sequential patients with CP treated with intrathecal administration of 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 [36]. Chahine et al. shows that about 73% of patients with CP may benefit from this treatment. The improvement ranges from 0 to 3 score levels averaging 1.3 points. There is also a good degree of cognitive, functional, and bladder and bowel control as well as improvement of the spasticity [36]. Zali et al. reported a significant improvement 6 months after cell transplantation versus baseline according to GMFM, GMFCS, FIMþFAM and Ashworth Scale [37].

After treatment, 83% our patients developed some relaxation of the extremities. In XCell-Center 66.4% of the 104 treated patients reported improvements [38]. Sharma et al. reported an 85% improvement among cerebral palsy cases, out of which 75% reported improvement in muscle tone and 50% in speech among other symptoms [6]. These improvements suggest that the combination of cell therapy and rehabilitation can lead to functional restoration that reduces disability in CP, thereby improving the quality of life of these patients.

The brain needs training to get the best out of its potential for appropriate functional reorganization. The goal of rehabilitation in CP is to develop coordination, increase flexibility, balance and co-ordination, manage spasticity and maximize independence. Sources, types, numbers of cells managed, frequency and time of transplantation are concerns which need attention imperatively. Exercise enhances the effect of injected stem cells by activating and proliferating the local stem cells, promoting muscle angiogenesis and release of cytokines and nerve growth factors. Stem cells have the capacity of repairing the underlying neural and muscular dysfunction through its neuroregenerative property. Increased enrolment of hematopoietic stem cells to peripheral blood is detected post exercise. The neurorehabilitation promotes and assists neural plasticity [39]. Neurorehabilitation increases angiogenesis and oxygen supply to the brain thereby improving the cognitive function. [40,41]. Exercise and neurorehabilitation has a synergistic effect for the profits of cell transplantation.

One of the major limitations of this study is that it was a non-randomized open-label study and did not have an adequate placebo control group to compare the results. There is also a disadvantage of a rehabilitation-only group that can substantiate the effect of individual intervention of an autologous stem cell transfer. Disadvantage is also short follow-up period, since longer follow-up period may lead to more accurate data on the effectiveness of the intervention. The results however, should be confirmed in large studies.

This study shows that autologous BMAC transfer in combination with rehabilitation is a safe procedure, easily feasible and to some extent effective for the patients condition. This can help reduce the degree of

impairment within cerebral palsy syndrome and improve the patient's quality of life. The ability of cell migration and differentiation should be assessed by determining a safe tracking procedure.

Conclusions

Autologous BMAC transplantation seems to be safe and feasible, and can help reduce the degree of impairment of CP patients and, improve the quality of life. The autologous BMAC treatment has to be complemented by physical therapy and cognitive stimulation. The combination of cell therapy and neurorehabilitation can lead to functional restoration that reduces disability in CP. The brain needs training to get the best out of its potential for appropriate functional reorganization.

Abbrevations

CP: Cerebral pulsy

BMAC: Autologous bone marrow aspirate

PVL: Periventricular leukomalacia

TNF: Tumor necrosis factor-alpha

INF: Interferon-gamma

IL-1: Interleukin-1 beta

MRI: Magnetic resonance imaging

EEG: Electroencephalography

GMFCS: Gross Motor Function Classification System

LAP-D: Learning Accomplishment Profile-Diagnostic

ETA: Endotracheal anesthesia

ACD: Acid citrate dextrose

ml: Milliliter

RBCs: Red blood cells

PPP: Platelet poor plasma

CSF: Cerebrospinal fluid

RBP4: Retinol binding protein 4

MCP-1: Monocyte chemoattractant protein-1

IL-1β: Interleukin-1-beta

IP-10: Anti-IP-10 antibodies

IL-10: Anti-Il-10 antibodies

IL-8: Anti-Il-8 antibodies

IL-6: Anti-Il-6 antibodies

IFN-γ: Interferon-gama

T NF- α: Tumor necrosis factor- α

Stro-1: Invitrogen monoclonal antibody Stro-1

CD133: Cluster of differentiation 133

CD73: Cluster of differentiation 73

CD146: Cluster of differentiation 146

CD105: Cluster of differentiation 105

CD45: Cluster of differentiation 45

CD34: Cluster of differentiation 34

CD90: Cluster of differentiation 90

7AAD: 7-amino-actinomycin-D

GMFM: Gross motor function measure

FIMþFAM: Functional independence measure and functional assessment measure score

Declarations

Ethics declarations

The clinical study protocol was approved by the Institutional Committee of Global Care Surgery Hospital in Novi Sad, Serbia under reference number 7/2018/GCSH. The Institutional committee evaluated the ethical aspects of the study in accordance with the World Medical Association Declaration of Helsinki. All parents received a written consent form, a cover letter and a clear explanation of the safety issues, potential risks and benefits, and the procedure involved.

Consent for publication

Parental written informed consent was obtained well before patient enrollment in every case. This consent included their agreement about publishing the indirect identifiers of the patients such as age and gender.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests

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Contribution

Dusan M. Maric, Dzihan Abazovic, Vladimir Papic and Mihajlo Radomir were the patients surgeons, and helped supervise the project, Danilo Vojvodic and Ivan Stanojevic performed the measurements and analyzed the data, and supervised the findings of this work. Zoran Milankov designed and supervised the study, Ivana Sokolovac and Kristina Milosavljevic contributed to the interpretation of the results, and reviewed the literature, Dusica L. Maric wrote the paper with input from all authors. All authors read and approved the final manuscript.

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