

Autism treatment with stem cells: a case report

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Abstract. – OBJECTIVE: Autism Spectrum Disorder is a complex brain disorder and has multiple causes that occur in diverse combinations. There is a need to classify children with ASD at a very young age so that they can access evidence-based intervention that can significantly improve their outcomes.

CASE REPORT: In this report we present a case of autism, which underwent intrathecal autologous bone marrow mononuclear cells transplantation along with neurorehabilitation. The primary goal of the treatment is to improve the quality of life of the patient. After the procedure, the child started to speak, therefore, the third communication subscale was scored within the GARS-2 assessment instrument. With these three subscales, a score of 91 has been achieved, representing an autism index of 27%, a significant improvement over the previous score.

CONCLUSIONS: Our study demonstrated evidences to support the safety and effectiveness of BMAC transplantation in the management of autism.

Key Words:

Autism, Autologous, Bone marrow, Mononuclear cells, Transplantation, Case report.

Introduction

Autism Spectrum Disorder (ASD) or autism is a complex brain disorder and has multiple causes that occur in diverse combinations. Studies^{1,2} of

ASD-related brain pathologies indicate that autism have a strong genetic component. It is very likely that the answer to what causes autism will not reside solely in genetics or in environment but in a combination of the two³. Brain hypoperfusion in ASD may affect processes ranging from progenitor cell proliferation, neuronal differentiation and migration, axon and dendrite outgrowth and synaptic function. Presence of atypical levels of proinflammatory cytokines in the cerebral spinal fluid⁴, overexpression of immune-related gene networks⁵ may contribute to pathogenesis of immune pathology in the brains of patients with ASD⁶. Extreme microglial activation leading to abnormal nerve connectivity can cause immune regulation or modulation of brain connectivity among ASD patients⁷. There were indications of a chronic up-regulation of inflammatory cytokines in the ASD brain^{8,9}. Buehler et al¹⁰ have described the correlations between pro-inflammatory cytokine levels and autistic symptoms.

There is a need to classify children with ASD at a very young age so that they can access evidence-based intervention that can significantly improve their outcomes¹¹. Some studies¹²⁻¹⁴ have shown promising results suggesting that stem cell therapy could be an additional treatment for autism. Mesenchymal stem cells (MSCs) provide a useful tool for the treatment of several diseases associated with inflammation and subsequent regeneration¹⁵. It is common practice for clinical

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applications, to acquire MSCs from bone marrow aspirates under local anesthesia. There is an absence of uncontrollable growth or tumorigenesis with MSCs, in contrast to the potential problems intrinsic to embryonic stem cells¹⁶. MSCs make no moral/ethical/religious controversies, unlike embryonic or fetal stem cells¹⁷. In this report we present a case of autism, which underwent intrathecal autologous bone marrow mononuclear cells transplantation along with neurorehabilitation. The primary goal of the treatment is to improve the quality of life of the patient.

Case Report

Patient aged 4 years with a confirmed diagnosis of autism according to the diagnostic criteria for autism spectrum disorder in Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition¹⁸. Magnetic resonance imaging of brain showed no significant intracranial abnormality and electroencephalogram was normal in the sleep state. As part of the stem cell implantation procedure, the child was assessed by a special educator using Gillian Autism Rating Scale 2 (GARS-2) assessment instrument. Given that the child was non-verbal prior to implantation, two subscales were scored: the stereotypical behavior subscale and the social interaction subscale. These two subscales achieved a score of 142, representing an autism index >99%. The 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 Helsinki¹⁹. The exclusion criteria were epilepsy, 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. The procedure was explained to patient's parents and a properly filled informed agreement was obtained.

Pre-intervention routine blood tests, urine analysis and chest x-ray were done to rule out active infection and consider ability for anesthesia. 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 marrow 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, Naples, FL, 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.

The cytokines measured from the BMAC and cerebrospinal fluid (CSF) samples for patient were the following: adiponectin, adipisin, RBP4, MCP-1, IL-1 β , IP-10, IL-10, IL-8, Leptin, IL-6, IFN- γ , resistin, TNF- α . Also, 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 the 4th and 5th lumbar vertebrae, and the transplantation lasted for 30 minutes.

Based on the total number of cells in the BMAC sample and the percentage of CD90 positive cells we have calculated the absolute number of these cells expressed per milliliter of the BMAC sample, and also determined an increase in their numbers in the subsequent measurements. When these three sets of data: percentage of total nucleated cells in the sample (Figure 1), the absolute number of events recorded on the flowcytometer (Figure 2), and the absolute number of these cells expressed per milliliter of BMAC sample (Figure 3) were processed by Friedman's test for paired samples, we also obtained a statistically significant confirmation of this observation ($p = 0.0278$).

Based on the minimal criteria for identifying mesenchymal stem cells proposed by Dominici et al²⁰, we decided to monitor several surface markers, included in the above criteria in our BMAC

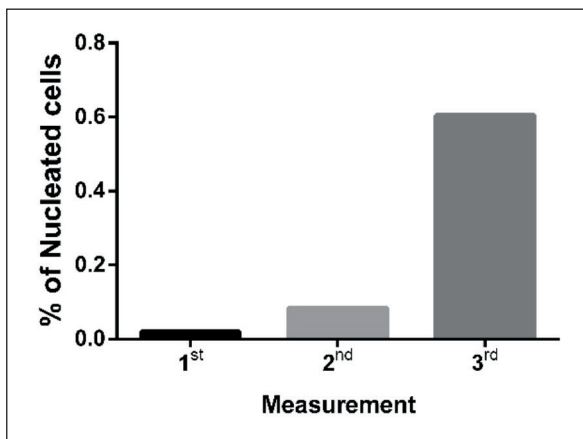


Figure 1. Percentage of total nucleated cells in the sample.

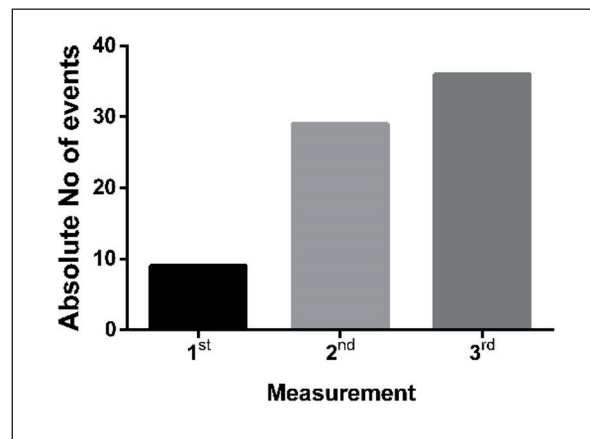


Figure 3. The absolute number of cells expressed per milliliter of BMAC sample.

samples, namely CD34, CD45, CD73, CD90, CD105 and CD271, in different combinations. During successive 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 flowcytometer.

Patient goes through neurorehabilitation which included occupational and psychological therapy, behavior analysis, sensory integration and speech therapy. Clinical examinations were performed by a certified and experienced psychologist and a pediatric neurologist at baseline, 3 months and 6 months. Following the stem cell implantation procedure, the child shows great progress in social interactions, while stereotypical behavior and motor mannerisms diminish. Also, after the

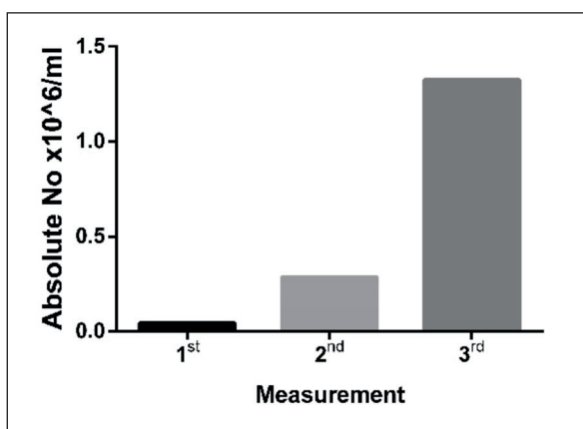


Figure 2. The absolute number of events recorded on the flow cytometer.

procedure, the child started to speak, therefore, the third communication subscale was scored within the GARS-2 assessment instrument. With these three subscales, a score of 91 has been achieved, representing an autism index of 27%, a significant improvement over the previous score (142 > 99%).

Before implementation of the stem cells, the child expressed a definite difference in sensory processing in all the areas: tactile processing (25/35), taste and smell processing (9/20), movement sensitivity (6/15), under responsive/seeking stimulus (10/35), auditive filtering (17/30), low energy/muscle tone (10/30), and visual and auditive processing (12/25). The instrument of assessment that was used was the Short sensory profile²¹.

After the procedure, the child still expressed probable or definite difference in sensory processing in all the areas: tactile processing (24/35), taste and smell processing (11/20), movement sensitivity (6/15), under responsive/seeking stimulus (16/35), auditive filtering (17/30), low energy/muscle tone (16/30), and visual and auditive processing (17/25). The biggest achievement in sensory processing was made in the areas of low energy/muscle tone where the child now expresses more strength that is closer to his chronological age and in the area of visual and auditive processing where the child is now less sensitive to other sounds in his surroundings.

Discussion

An increasing body of evidence has suggested that there is active inflammation in the

CNS in ASD patients. There are evidences that the blood brain barrier function is different in ASD children due to neurological inflammation, immune dysregulation and increased inflammatory cytokines in the brain. Neuroglia responses are activated toward pro-inflammatory processes involving astroglia and microglia^{22,23}. Astrocytes are able to modulate several key events in synaptic processes: neurotransmitter homeostasis, synaptic remodeling, and plasticity. Monocytes are the precursors of macrophages and dendritic cells and regulators of the immune responses. Abnormal responses of monocytic cells could trigger long-term immune alterations in ASD²². Mast cells are induced to release IL-6 and TNF, causing focal brain inflammation²³, while microglia are stimulated to activation and proliferation, leading to disruption of neuronal plasticity^{24,25}, and probably, in this way trigger social interaction, communication, and behavior impairments²⁶. Microglia is considered the primary immune cells or resident macrophages in the CNS. It has been observed that there is increased expression IL-1 β , IL-6, IL-17 and TNF- α in the autistic brain²⁷. Mesenchymal cells improve angiogenesis by producing signaling molecules, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF2), and they stimulate tissue remodeling, decrease inflammation and activate the satellite cells. The precise role of CD90 on MSC has not yet been elucidated, but there is evidence that points to this marker as a good MSC identifier. Moraes²⁸ have hypothesized that CD90 controls the differentiation of MSCs by acting as a barrier in the pathway of differentiation commitment. Same authors²⁹ also link the expression of CD90 with the differentiation status of MSCs. Our data could indicate that the maintenance of MSC stemness and their paracrine effects rather than their differentiation underlie the good effects of our patient therapy¹³. We preferred autologous BMACs in this study because stem cells were administered intrathecally so it was minimally invasive while assuring efficient delivery to the brain. The approach to transplant autologous BMACs intrathecally was also applied in other studies^{13,30}. In our study intrathecal transplantation of autologous BMAC was apparently safe with no severe adverse events recorded during and after the procedure. This research shows that autologous BMAC transfer in combination with rehabilitation is a safe procedure, easily feasible and to some extent effective for the

patient's condition. This can help reduce the degree of impairment of autism and improve the patient's quality of life. Future studies should explore the optimal doses of stem cells and frequency of transplantation.

Major improvement in our patient was observed in body use, intellectual response, visual response score, taste, smell, touch score and fear or nervous score. In the study of Crivelli et al³⁰, improvement was also observed in body use, visual response, taste, smell, and touch score. It was hard to evaluate the changes in autism in our study and the assessment of Sharma et al³¹ because they used Indian Scale for Assessment of Autism.

Neurorehabilitation aims at restoration of functions that have been lost due to destructions caused by disease of nervous system making the patient functionally independent. The neurorehabilitation promotes and facilitates neural plasticity³². Of note, exercise enhances the effect of injected stem cells by inducing mobility of the cells, 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 neuro-regenerative property. The brain needs training to get the best out of its potential for appropriate functional reorganization.

Conclusions

Our study demonstrated evidences to support the safety and effectiveness of BMAC transplantation in the management of autism. This can help reduce the degree of impairment of autism and improve the patient's quality of life. After the procedure, the child started to speak, therefore, the Third communication subscale was scored within the GARS-2 assessment instrument. With these three subscales, a score of 91 has been achieved, representing an autism index of 27%, a significant improvement over the previous score (142 > 99%). The complex decision of whether or not to use stem cells can only be made by parents.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

D.M.M., Dz.A., V.P. and M.R. were the patient's surgeons, and helped supervise the project, I.S. performed the measurements and analyzed the data, and supervised the findings of this work. I.S. and K. M. contributed to the interpretation of the results, and reviewed the literature, D.L.M. wrote the paper with input from all authors. All authors read and approved the final manuscript.

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