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Efficiency Evaluation of Allogenic Umbilical Cord Blood (UCB) Transfusion in Patients With Autism

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URGENCY OF THE RESEARCH AND LITERATURE REVIEW

Autism Spectrum Disorders (ASDs) are heterogeneous neurodevelopmental diseases. The most common disorder of the autism spectrum is autism characterized by dysfunctions in sympathetic social interaction and communication and the mannerisms. Recent reports indicate a sharp increase in the number of children with autism (57% increase compared to the rate of the year 2002), while the prevalence rate in the United States is approaching 1%. The exact aetiology of autism remains unclear. Therefore, determining effective treatments for autism is particularly difficult.

Although the pathophysiology of autism remains poorly understood, the accumulated data suggest that the aetiology may potentially include immune dysregulation (see Onore et al., 2012). Extensive research points to immune system abnormalities, including active brain neuroinflammation, pro-inflammatory cytokines enhanced profiles, immune cells dysfunction, and the autoimmunity directly associated with an increase in behavioural disorders. Studies have shown an ongoing neuroinflammatory process with pronounced activation of microglia and astroglia in the cerebral cortex, alba and cerebellum of patients with autism. A unique profile of pro-inflammatory cytokines in autistic patients was recorded in the cerebrospinal fluid, including a marked increase in macrophage chemoattractant protein-1 and in peripheral plasma, such as significantly elevated levels of interleukin (IL) -1β, IL -8 and IL-12p40. Additional studies have shown altered function in subsets of immune cells (Ashwood P, Corbett BA, Kantor A, Schulman H, Van de Water J, Amaral DG. In search of cellular immunophenotypes in the blood of children with autism. , Enstrom AM, Lit L, Onore CE, Gregg JP, Hansen RL, Pessah IN, Hertz-Picciotto I, Van de Water JA, Sharp FR, Ashwood P. Altered gene expression and function of peripheral blood natural killer cells in children with autism. Brain Behav Immun. 2009.) That leads to an inappropriate or ineffective immune response in autism. Different "housekeeping" antibodies enter into reaction with proteins found in the central nervous system of the children with autism associated with an autoimmune process. Taken together, these data suggest that immune dysfunction is not only a symptom or associated disease but also an indicant of the underlying pathophysiological process, so targeting this pathology and changing neuroimmune reactions can be effective from a therapeutic point of view. However, only a few clinical research studies of anti-inflammatory drugs are aimed at correcting immune dysregulation or continuing neuroinflammation in autism. Due to its known ability to alter immune response, cord blood mononuclear cells can be a new therapeutic effect in clinical practice to normalize the immune response characteristic of some children with autism.

There are behavioural, educational, medical, health care and optional methods of autism care. However, there is no definitive standard treatment for children with autism. Stem cell therapy

has shown great promise in modern treatment and rehabilitation of patients with autism. Preclinical research studies have reported that transplantation of human cord blood mononuclear cells (CBMNC) in animals in models of cerebral ischemia promotes functional recovery by improving local blood perfusion in damaged areas through angiogenesis (Park DH, Borlongan CV, Willing AE, Eve DJ, Cruz LE, Sanberg CD, Chung YG, Sanberg PR, Cell Transplant, 2009)

Some studies have confirmed that cerebral hypoperfusion is associated with many of leading autism symptoms (Wilcox J, Tsuang MT, Ledger E, Algeo J, Schnurr T. Brain perfusion in autism varies with age. Neuropsychobiology. 2002;). Generalized brain hypoperfusion, achieved in the frontal and prefrontal areas, was observed in children with autism and is associated with cognitive and neuropsychological defects. Besides, a decrease in cerebral perfusion, especially in the temporal-parietal areas, is associated with a cognitive deficit, such as speech deficits, cognitive disorder or object representation or anomalous perception and responses to sensory stimuli. Inadequate perfusion leading to hypoxia of brain tissue had not only caused apoptosis and necrosis of neurons but also led to abnormal brain tissue metabolism and accumulation of pathological doses of neurotransmitters. As a result of the systemic administration of stem cells CDbrain tissue34 + cord blood to overcome ischemia in vitro and in animals, therapeutic angiogenesis has been proven experimentally. Also, it was proved that the endothelial progenitor cell contained in the population of CD34 + cells had the ability to initiate angiogenesis in ischemic tissues. Circulating CD34 + progenitors in the PC with endothelium development potential has turned into new endothelial cells to either restore the damaged endothelium wall or germinate a new vascular structure. Moreover, human CD34 + cells and hematopoietic progenitors could secrete multiple angiogenic factors, such as vascular endothelium growth factor (VEGF), HGF, and insulin-like growth factor-1.

The preclinical research study data on the mononuclear core blood cells ability to stimulate neoangiogenesis in ischemic areas allow introducing the method of injecting these cells in cases of brain hypoperfusion or hypoxia to limit and reduce the area of ischemic tissue degeneration.

To assess the therapeutic efficacy of using core blood cells, during the research studies the CARS, CGI and ABC scales were used. CARS provides descriptive information about pathological behaviour and classifies severity in children with autism, while the CGI scale, as a global measure, indicates a noticeable overall treatment effect. However, further research will need to extend the rating scales to standardize the diagnosis and treatment of autism in detail.

In 2011, a Chinese research group from the Shenzhen Beike Bio-Technology Co. conducted a scientific clinical research study of the use of intravenous mononuclear cord blood cells in patients diagnosed with autism. In total, 37 patients aged 3 to 12 years were tested. There were 3 clinical groups: Group 1 consisted of 14 patients receiving the injections of mononuclear cord blood cells

at a dosage of 2 million cells per 1 kg of patient weight (1 intravenous injection, 3 intrathecal with an interval of 5-7 weeks). Group 2 consisting of 9 patients receiving the injections of mononuclear cord blood cells at a dosage of 2 million cells per 1 kg of patient weight and mesenchymal stem cells at a dosage of 1 million cells per 1 kg of patient weight (2 intrathecal and intramuscular injections and 2 intrathecal injections solely). Patients of Groups 1 and 2 received standard rehabilitation therapy. Group 3 consisted of 14 patients receiving standard rehabilitation therapy. The evaluation was conducted 24 weeks after the course of injections. During the treatment, all patients had normal and stable vitals. At the periods of injections or period after them. No allergic or immunological reactions were observed in any patient. There were no deviations from the normal ranges or significant changes in the laboratory indicators of the liver and kidneys functioning, in comparison with the initial level (ALAT, ASAT, bilirubin, urea, and creatinine). Evaluation of the psychological status was made on the CARS scale. The total CARS score was significantly reduced in the Group 2 (a decline of 37.9%); in Group 1, the indicator decreased by 20.0%; in Group 3, i.e. the control group, the indicator decreased by 13.7%. The initial CARS score indicator in each group was at the level of 45. In Groups 1 and 2, there were improvements in behaviour defined for the “Attitude to people”, “Body use”, “Visual response”, “Taste, smell, the reaction on touch and use” and "General Impressions" sub-scales. Those changes were not observed in the control group. The research study proved the safety and effectiveness of the use of cord blood mononuclear cells in the complex treatment of patients. (Transplantation of human cord blood mononuclear cells and umbilical cord-derived mesenchymal stem cells in autism
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CLINICAL PURPOSES

Estimation of the safety and efficiency of the method of transfusion of umbilical blood hemopoetic cells to patients with diagnosed autism spectrum disorder without depending on the degrees of compatibility of donor and recipient.

TASKS

1. Develop an algorithm for using the method of transfusion of hematopoietic umbilical cord blood cells in the treatment of patients with autistic type disorder.
2. To formulate criteria for the selection of patients for this technique.

3. To analyze the efficacy and safety of transfusion of hematopoietic cord blood cells in patients with autistic type disorder using rating scales.
4. Introduce the method of transfusion of cord blood hematopoietic cells to patients with autistic type disorder in the complex therapy of patients with autistic type disorder.

OBJECT OF STUDY

The protocol for testing the method will be carried out on 10 patients with autism spectrum disorder by transfusion of hematopoietic cord blood cells. As a control group, 10 patients with a similar pathology will be examined, comparable in age, gender and degree of autistic disorder according to the "paracopy" type against the background of standard therapy.

Type of study: interventional, prospective, non-randomized with a control group.

Patient selection criteria (indications for this type of treatment):

• age of the patient is from 3 to 15 years.
• Diagnosis: autistic disorder.
• Systemic speech underdevelopment
• The presence of attention deficit hyperactivity disorder as a comorbid state
• Cognitive impairment

Patient exclusion criteria (contraindications for this type of treatment):

• The patient's under 3 years, after 15 years;
• The presence of organic pathology of the brain according to CT, MRI
• The presence of the following diseases in the anamnesis: heart failure in the stage of decompensation, a history of stroke less than 1 year ago, anemia and other blood diseases;
• Decompensation of chronic and endocrinological diseases;
• Acute respiratory viral and bacterial infections, period less than 1 month after the acute phase.
• HIV infection, hepatitis B and C.
• Cancer, chemotherapy and history of cancer;
• Tuberculosis.
• Severe form of intellectual disability as a concomitant disease (diagnosis can be ignored, according to the decision of the Medical Committee of the Center);
• Cerebral palsy.
• Increased convulsive readiness, epilepsy.

Indications and contraindications to this type of treatment for each patient are determined at a consultation of doctors with the participation of a paediatrician or a psychologist (psychiatrist), and transfusiologist.

Research methods

Evaluation scales CARS, ATEC (to assess the dynamics). Neuropsychological examination, examination of a speech therapist-pathologist. Assessment of neurological status. Determination of the immune status by flow cytometry (CD3; CD4; CD8; CD19; CD16-56). Determination of the level of cytokines in peripheral blood: interleukin 1 beta, interleukin 6, tumor necrosis factor alpha, interleukin 8, gamma interferon. Determination of the HLA genotype of the subject parents and siblings.

The results will be statistically processed using parametric and non-parametric statistics methods using STATA version 9.0, Statistica for Windows version 6.0 and MS Office Excel 2007. Changes from the start of therapy and after 1, 2, 6, 12 months will be compared using the Wilcoxon criterion, comparing data between the main group and the control group using non-parametric method, using the Mann-Whitney test (data are presented in Me - median format with an indication of 25% (q1) and 75% (q3) quartile.

Treatment protocol

Selection of patients and assessment of their psychological status, transfusion of umbilical cord blood HSC in the conditions of day hospital is carried out by specialists of the State budgetary institution of public health "Samara Regional Children's Clinical Hospital N.N. Ivanova
Laboratory examination, selection of biomaterial will be carried out by the State Budgetary Institution "MC Dynasty".

Stage 1 - selection and examination of the patient, determination of indications and contraindications to this method of treatment is carried out by a child neurologist, pediatrician (pediatrician), medical psychologist. Assessment of autism disorder is carried out according to the assessment scales of CARS, ATEC.

A prerequisite for inclusion:

- diagnosed with autism spectrum disorder by a psychiatrist,
- Dispensary supervision by a child psychiatrist at the place of residence.

Mandatory general clinical trials (clinical and diagnostic laboratory):

a) General clinical blood tests (erythrocytes, hemoglobin, platelets, clotting time, leukocytes + formula, COE) - shelf-life 14 days.

b) general clinical urine test - shelf life 14 days.

c) feces per helminth eggs - shelf life 14 days.

d) blood type and rhesus factor.

A patient who meets the selection criteria and has no contraindications for this type of treatment enters into a protocol and signs an informed consent (or his/her legal representative).

Stage 2 - umbilical cord blood sampling. It is performed by a transfusion doctor of the State Budgetary Institution "MC Dynasty".

The selection criteria for the sample will be the following parameters:

1. Blood group compatibility by system AB0 and Rh

2. Infectious safety (negative HIV test results, hepatitis B and C, syphilis, herpes simplex virus, cytomegalovirus, toxoplasmosis)

3. No bacterial or fungal contamination.

4. Number of hematopoietic cells (mononuclears) in the range of 20-50 million cells per kg of patient weight.

Stage 3 - intravenous injection of hematopoietic cells in umbilical cord blood.

Preliminary examination of the patient and relatives:

a) Determination of immune status by flow cytometry (SD3; SD4; SD8; SD19; SD16-56) - patient.

b) Determination of cytokine levels in peripheral blood: interleukin 1 beta, interleukin 6, tumor necrosis factor alpha, interleukin 8, gamma-interferon - patient.

c) Determination of HLA genotype by loci A, B, DRB1 - patient and parent, sibling.

The sample consists of a suspension of hematopoietic cells diluted in 10 ml 0.9% NaCl + 10 ml 6% hydroxyethyl starch in a disposable syringe labeled by the manufacturer (label).

The patient is injected in drops with the use of infusion. The rate of administration is 60 - 70 ml per hour. Immediately at the beginning of injection, a biological compatibility test should be performed.

Three-fold injection is planned at an interval of 1 month.

Possible area of application

Specialized hospitals.

Data to be obtained during implementation

- correct selection of criteria for selecting patients for treatment of patients with autism type disorder;
- developed algorithm for using the method of transfusion of hematopoietic cells of umbilical cord blood in complex therapy of patients with autism type disorders;
- information on the efficacy and safety of treatment of patients with autism-type disorder by transfusion of hematopoietic cells of umbilical cord blood;
- appropriateness and effectiveness of this method of treatment. To evaluate its impact on treatment tactics and results;
- Association of HLA genes with the development of autism spectrum disorders;
- relationship between immune status and cytokine profile and severity of the disease.

Therapy evaluation criteria

Evaluation of the effect of treatment is carried out 1, 2, 3 and 6 months after treatment.

Evaluation of the effect is carried out using the scales used in the introduction to the protocol, evaluation of the neurological and psychological status of the patient.

Evaluation of immune status indicators is carried out 6 months after the last injection.

Cytokine profile is assessed 1, 2, 3 months after the first injection.

Expected results:

It is assumed that transfusions of hematopoietic cord blood cells in patients with autism spectrum disorders will not cause adverse reactions and complications (safe) and will lead to improved social interaction and communication of patients, increased social adaptation, reduced stereotyping, increased self-service skills, increased cognitive functions.

STATISTICAL CONSIDERATIONS

This study will enroll autistic children age 3 to 15 years of age.

Participants will be carried out on 10 patients with autism spectrum disorder by transfusion of hematopoietic cord blood cells. As a control group, 10 patients with a similar pathology will be examined, comparable in age, gender and degree of autistic disorder according to the “paracopy” type against the background of standard therapy.

This is interventional, prospective, non-randomized with a control group study with three intravenous allogeneic, unrelated CB infusion in children ages 3-15 years with ASD/

The primary outcomes will be assessed 1, 2,3,6 and 12 months after the initial infusion in the sequence. Duration of study participation will be 12 months from the time of baseline infusion.

Accrual

It is estimated that up to 2-3 research participants will be enrolled each week and that approximately 4 weeks of accrual will be necessary to enroll 10 participants.

Study Duration

Research participants will be followed for safety for 12 months after the third study infusion.

Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized for all research participants. Characteristics to be examined include age, sex, and baseline behavioral status (ATEK).

The results will be statistically processed using parametric and non-parametric statistics methods using STATA version 9.0, Statistica for Windows version 6.0 and MS Office Excel 2007. Changes from the start of therapy and after 1, 2, 6, 12 months will be compared using the Wilcoxon criterion, comparing data between the main group and the control group using non-parametric method, using the Mann-Whitney test (data are presented in Me - median format with an indication of 25% (q1) and 75% (q3) quartile.