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RESEARCH ARTICLE

Efficiency of a High-dose Intravenous Immunoglobulin Therapy in Children with Autism Spectrum Disorders Associated with Genetic Deficiency of Folate Cycle Enzymes

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Abstract

Previously, it has been repeatedly reported on the effectiveness of intravenous immunoglobulin therapy in some children with autism spectrum disorders without specifying selection criteria of potential responders to immunotherapy. Objective: to evaluate high-dose immunoglobulin therapy efficacy and safety in children with autism spectrum disorders and genetic deficiency of folate cycle. The studied group consisted of 78 children aged 2 to 10 years who have been on intravenous immunoglobulin at a dose of 2 g/kg per month for 6 months. The control group included children of similar age and gender distribution, which received only non-drug rehabilitation support. The dynamics of psychiatric symptoms were assessed using the Aberrant Behavior Checklist scale. Complete elimination of the phenotype of autism spectrum disorders has been obtained in 21 patients and an improvement was marked in 33 children of study group. In parallel, positive dynamic of other clinical manifestations of folate cycle deficiency phenotype was evaluated: PANDAS (19 out of 21), epilepsy (29 out of 36) and gastrointestinal (in 49 out of 68 children) syndromes. Intravenous immunoglobulin has a complex positive impact on the manifestation of a genetic deficiency of folate cycle, including autism spectrum disorders, extrapyramidal disturbances, bowel syndrome, epileptiform brain activity, immune deficiency, and leukoencephalopathy.

Keywords: *Folate cycle, Intravenous immunoglobulin, Autism spectrum disorder.*

Introduction

At the moment, evidence has been accumulated of the immune mechanisms involvement in the pathogenesis of autism spectrum disorders in children. In this regard, there might be a lot of opportunities for testing the immunotherapeutic interventions in this severe and widespread ailment. Thus, the association of autism spectrum disorders with some histocompatibility loci is shown, being similar to a number of autoimmune and allergic syndromes [1]. In these children, various forms of primary immunodeficiency are described [2, 3]. The studies on some primary immune dysfunctions indicate an increased risk of autism [4, 5]. There are multiple reports on autism in adults and children after episodes of neuroinfections, mainly of opportunistic nature [6, 7]. In children with autism spectrum disorders, various autoantibodies to brain antigens are identified which are not observed in healthy individuals [8, 9]. Furthermore, the results of a number of clinical trials indicate the benefit of immunotherapy application in some patients with autism spectrum disorders [10, 11]. All these convincing arguments make one pay close attention to the role of immune-dependent mechanisms in the pathogenesis of autistic disorders. Nonetheless, the action mechanism of immunoglobulin therapy in autistic spectrum has not been elucidated until now, nor have been established the subgroups of patients who are potential responders to immunotherapy. The author has recently demonstrated a close relation of genetic folate cycle disorders with autistic spectrum in children, which was also reported by other researchers [12]. It is shown that these children have a special form of primary immunodeficiency with a variable phenotype, the core of which is the deficiency of natural killers and natural killer T-lymphocytes (Fig.1). Hence, a selective decrease in the resistance to viral agents and susceptibility to generate autoimmune and allergic complications are predetermined.

It has also been demonstrated that the main form of the CNS involvement is leukoencephalopathy, conceivably related to the delayed myelination/demyelination in the white matter of the cerebral hemispheres and associated with reactivated herpesvirus infections and autoimmunization to brain antigens.

It is reasonable to believe that the cases of autism with the genetic folate cycle deficiency represent exactly the specific subgroup that responds to intravenous immunoglobulin therapy, which should be tested in a specially designed and controlled clinical trial. The objective of the study is to evaluate the efficacy and safety of high-dose immunoglobulin therapy in autistic spectrum disorders in children with the genetic folate cycle deficiency.

Materials and Methods

The prospective, controlled, single center, non-randomized clinical study was conducted. This study involved 78 children diagnosed with autism spectrum disorder and/or cerebral palsy. The diagnosis was made by psychiatrists according to the DSM-IV and ICD-10 criteria. The recruitment of children for the study group (SG) was carried out during the period between 2010 and 2015 included. These were the patients from different regions of Ukraine aged 2 to 10, 47 boys and 31 girls. These children were prescribed with intravenous immunoglobulin at a dose of 2 g/kg per month for 6 consecutive months.

The control group (CG) consisted of 32 children with similar age and gender distribution. These patients did not receive intravenous immunoglobulin therapy, but underwent conventional rehabilitation course only, including work with a developmental pediatrician as well as with specially trained teachers and psychiatrists. The dynamics of mental symptoms throughout the study were assessed by means of the Aberrant Behavior Checklist (ABC) scale [13]. Identification of the folate cycle gene polymorphisms was performed by the method of Polymerase chain reaction (PCR) with restriction enzyme digest analysis in three centers. These centers include Neurological Research Institute (USA), Kharkiv Specialized Medical Genetic Center (Ukraine) and Sinevo Commercial Laboratory (Germany). The replacement of nucleotides MTHFR 677 C>T, MTHFR 1298 A>C, MTRR A/G and MTR A/G in different combinations was identified. All the participants in the study were subjected to a multiple complete immunological investigation at the Institute of Immunology and Allergology of the Bogomolets National Medical University.

This investigation, apart from the general blood count, included also the examination of lymphocytes subset composition by using a laser flow cytofluorometry (a cytofluorometer Epics Xl, USA). Along with a method of indirect immunofluorescence with monoclonal antibodies to CD markers with two or three labels (CD3+, CD3+CD4+, CD3+CD8+, CD3-CD19+, CD3-CD16+ CD56+, CD3+CD16+CD56+) (Beckman Coulter reagents, USA). Phagocytosis was assessed by latex test data with determination of phagocytosis indicator, phagocytic index, the amount of active phagocytes and phagocytic blood capacity. The activity of myeloperoxidase (cytofluorometry) and NADPH oxidase (NBT-test) enzymes were also assessed.

The serum concentrations of immunoglobulins of the main classes (M, G, A) were determined by the results of single radial immunodiffusion according to Mancini. The concentration of classes IgE, IgD and subclasses of IgG (IgG1, IgG2, IgG3, IgG4) in the blood serum was measured by means of a solid-phase enzyme immunoassay (reagents Vector BEST, RF).

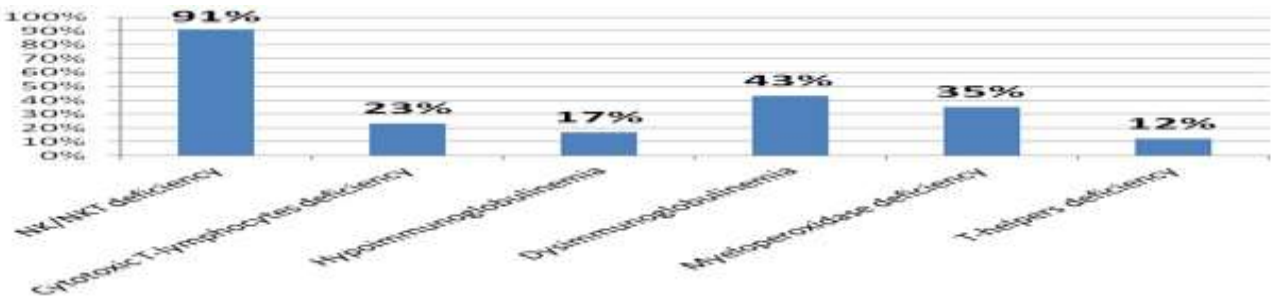

Figure 1: Structure of the immune status disorders in the SG children with genetically determined folate cycle disorders (n=78)

In addition, the diagnosis of a reactivated viral infection was made by the results of a quantitative PCR test of the blood serum with species-specific primers of herpesviruses (herpes simplex viruses, types 1 and 2 (HSV-1 and HSV-2), varicella zoster virus (VZV).Along with Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesviruses of 6, 7 and 8 types (HHV-6, HHV-7, HHV-8), measles and rubella viruses (DNA-Technology reagents, RF). Serologic tests were also performed by conducting a solid-phase enzyme immunoassay to identify virus-specific IgM and IgG in the blood serum (Vector-BEST reagents, RF). The structure of infectious syndrome in SG patients is shown in detail in Fig.2.

Intracellular neurotropic pathogens prevail. Namely, viruses with opportunistic properties, especially- human lymphotropic herpes viruses (EBV, CMV, HHV-6, HHV-7), which is consistent with the typically found NK- and NKT- cell deficiency in such patients. In 21% of cases, the serological tests showed an abnormally intense humoral immune response to the measles virus and, less commonly, to rubella. This phenomenon in children with autism spectrum disorders has been repeatedly reported earlier [14]. Serum concentrations of known biomarkers of the genetic folate cycle deficiency-homocysteine, folic acid, B12 and B6 vitamins, were also evaluated by the available biochemical methods.

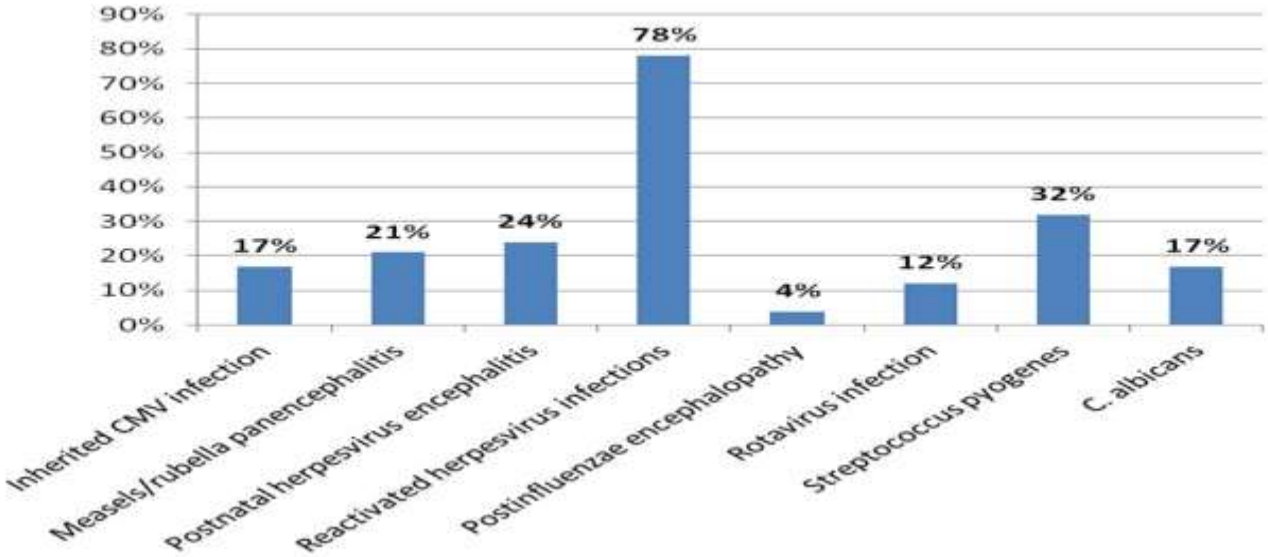

Figure 2: Structure of the infectious syndrome in patients with the genetic folate cycle deficiency (n=78)

All the children underwent control MRI of the brain in conventional regimens (T1- and T2-weighted, FLAIR) on tomographs with magnetic induction of no less than 1.5 T at least twice: prior to and after participation in the study. The typical signs were leukoencephalopathy of various severity (Fig. 3). In 46% of cases, there was also an additional pattern of temporal mesial sclerosis. Usually such children suffered from an epileptic syndrome. In 17% of cases, typical signs of a congenital cytomegalovirus neuroinfection were observed in the form of ventriculomegaly, periventricular foci, cysts in temporal poles, hypogenesis of the corpus callosum and the delayed myelination zones in the parietal lobes. These data comply with the results of the 18-year retrospective study by Pinillos-Pisón R. et al [15]. Such children usually had symptoms of pyramid pathway lesion; for this reason they were often diagnosed with cerebral palsy, although in this case the autistic mental disorders were also observed.

Statistical Analysis

Of the obtained information is processed by the methods of structural and comparative analysis using the Microsoft Excel electronic program. In order to establish accuracy of differences in the results, the Student's T-test was applied with the calculation of confidence coefficient p (parametric criterion) and the number of Z signs according to Urbach (nonparametric criterion). This study is a part of the Ministry of Health of Ukraine's grant to conduct a research study "The improvement in the diagnosis of human immunodeficiency diseases based upon the recording of surface plasmon resonance and the development of modern treatment methods" (State registration No. 0113U000709).

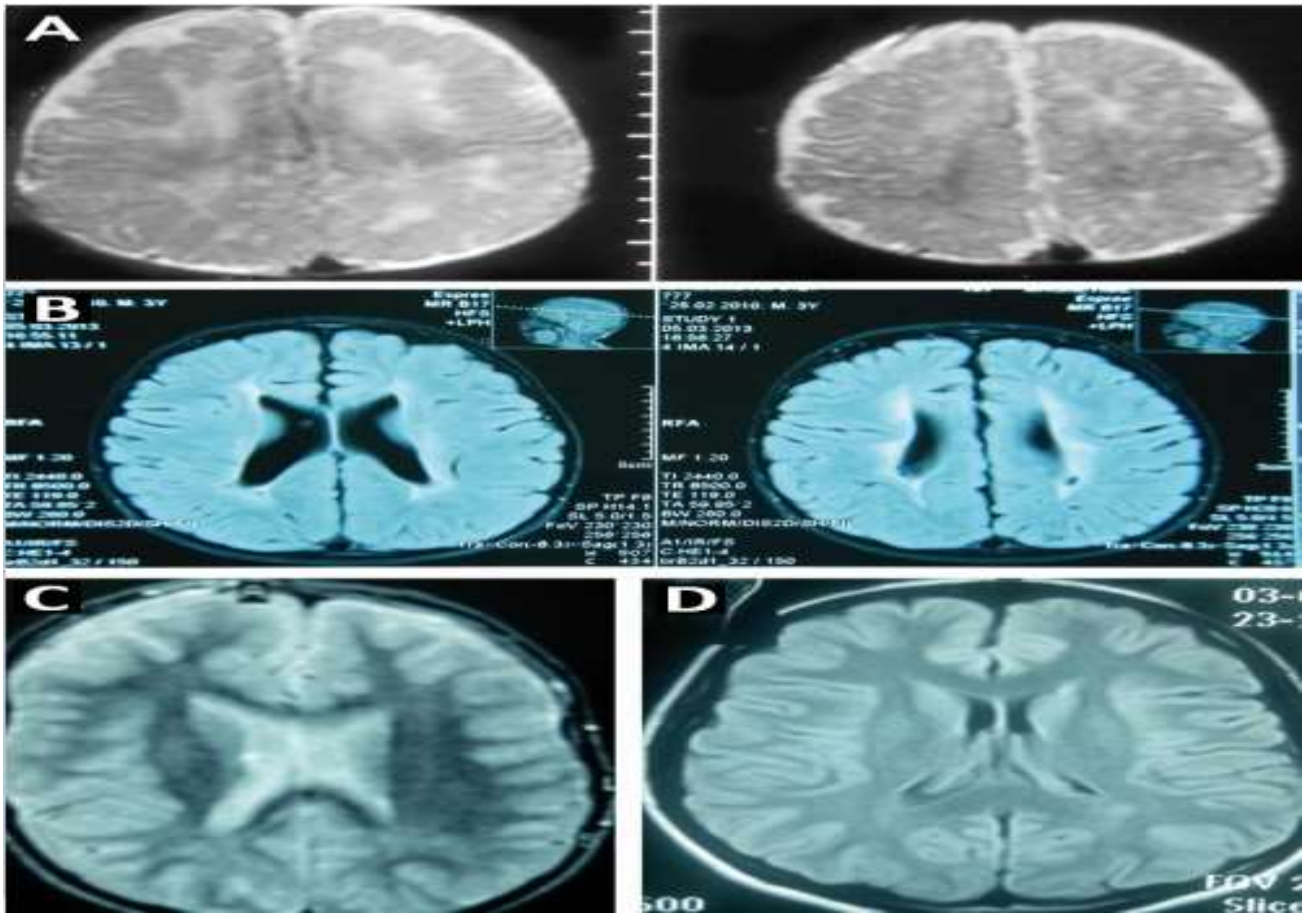

Figure 3: Heterogeneity of the leukoencephalopathy manifestations in children with the genetic folate cycle disorder (A - immaturity of the brain and diffuse myelination disturbances; B - extensive periventricular demyelination, resembling leukodystrophy; C - pronounced bilateral periventricular demyelination in the parietal lobes, the brain dysgenesis, deformation of the ventricular system; D - limited bilateral periventricular myelination disorder in the parietal lobes; author's own observations)

The criteria to enroll a patient in the study were the presence of 2-4 polymorphisms of the folate cycle genes, NK- and/or NKT-cell deficiency, a reactivated infection induced by lymphotropic herpesviruses and/or measles virus. Other criteria include the signs of leukoencephalopathy on the MRI of the brain, and the clinical symptoms of autism spectrum disorders. The criteria to exclude a patient from the study were as follows. Parents' refusal for child's participation in the trial, the presence of an additional genetic pathology involved in the development of mental disorders, the lack of a phenotype of NK- and/or NKT-cell deficiency and of the signs of leukoencephalopathy. Together with the development of immunotherapy adverse effects, which make it impossible to continue the treatment tested. The endpoints of the study were the main clinical manifestations of autism spectrum disorders, the data of the brain MRI, the number of NK- and NKT-cells in the peripheral blood, the current viral load shaped by lymphotropic herpesviruses. Along with the dynamics in major additional clinical manifestations associated with the genetic folate cycle deficiency, including PANDAS, enteropathy, colitis, temporal mesial epilepsy and the symptoms of pyramidal pathway lesion (Fig. 4).

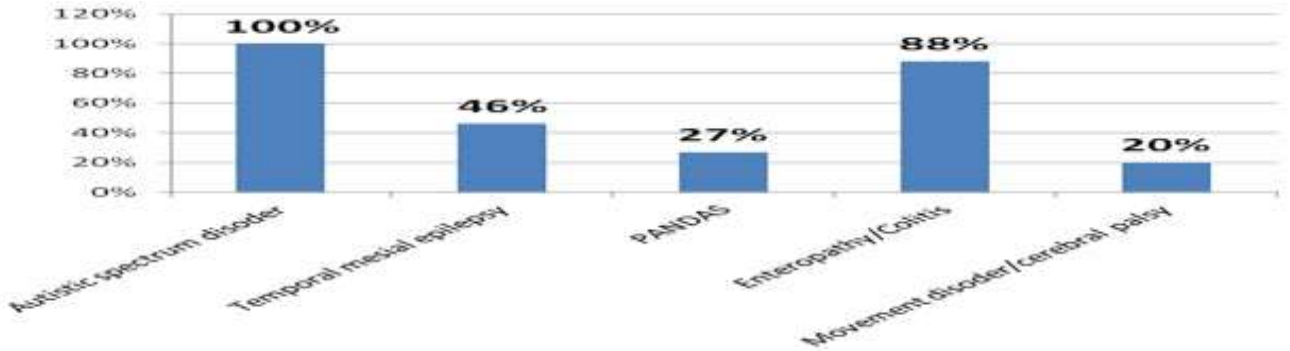

Figure 4: Heterogeneous clinical phenotype in children with the genetic folate cycle disorder (n = 78)

Results and Discussion

Intravenous immunoglobulin appeared to be efficient to reduce clinical symptoms of autism spectrum disorders in 77 out of 78 children. However, the intensity of a clinical effect varied greatly in different patients (Table 1; Fig. 5). The complete control of autistic symptoms, revealing a pronounced lack of knowledge and skills in a child, was observed in 21 cases. The regress in achievements after discontinuation of immunotherapy was observed only in one patient from the subgroup of responders. The rest of the children developed normally and reached their peers' level in 3-5 years after the course of immunotherapy under the influence of non-drug treatment. Such treatment included appointments with speech-language pathologists, general educators, psychiatrists and psychotherapists. The pronounced regress of autistic manifestations was observed in 33 cases, allowing patients to significantly expand the range of social adaptation. Twelve children out of these respondents continued to display positive dynamics in mental disorders after the completion of immunoglobulin therapy under the influence of rehabilitation measures. The other children retained some autistic features for 2 to 3 years after the immunotherapy. Presumably, 6-month course of the immunotherapy appeared to be too short for them and further positive dynamics in mental disorders could have been achieved with continued intravenous immunoglobulin therapy. However, 24 children responded with only moderate or weak positive mental disorders-related dynamics after the course of immunotherapy (Fig.5). Half of them showed loss of achievements 2 to 4 months later after the completion of immunoglobulin therapy and they apparently needed further repeated courses of immunotherapy.

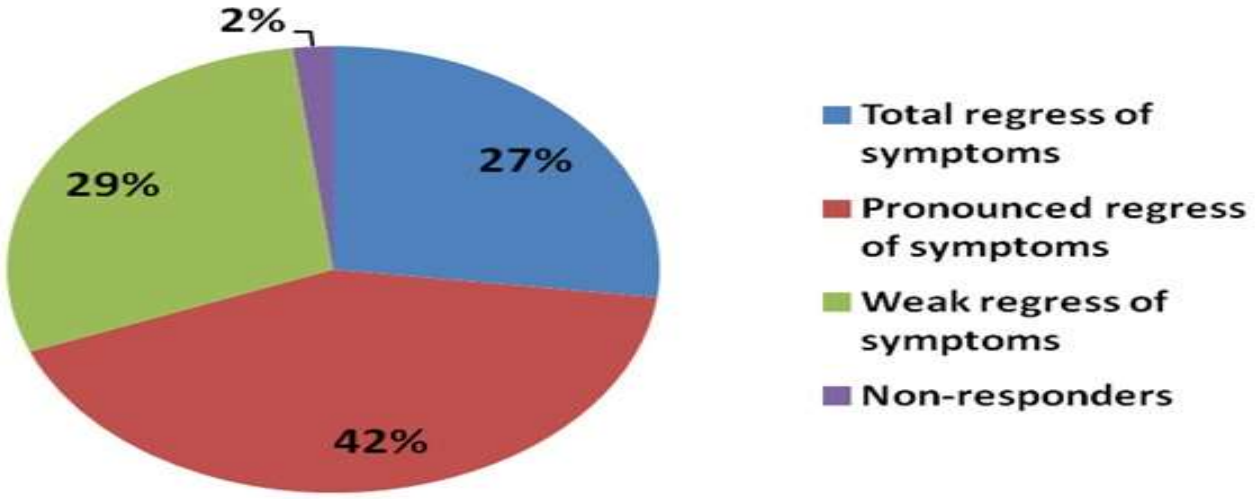

Figure 5: Structure of the SG by the efficacy of intravenous immunoglobulin in relieving the symptoms of autism spectrum disorders (n = 78)

In the CG a moderate or weak positive dynamics occurred in 12 out of 32 children and were the reflection of a natural flow of the disease or of the rehabilitation measures performed ($p<0,05$; $Z<Z_{0,05}$). A complete elimination of the phenotype of autistic disorders or the cases of the pronounced positive dynamics in symptoms of mental disorders were not recorded in CG of children ($p<0, 05$; $Z<Z_{0, 05}$). According to findings the following can be confirmed. A high-dose intravenous immunoglobulin therapy has a modifying effect on the phenotype of mental disorders in autistic spectrum in children with the genetic folate cycle deficiency (Fig. 6).

Table 1: Indicators of the ABC scale in patients of the SG and the CG

№	Subscales	SG (n=78)	CG (n=32)
	ABC		
1	Irritability	6,1±0,9*	14,3±1,2
2	Hyperactivity	11,0±1,2*	24,7±2,4
3	Inadequate eye contact	4,3±0,8*	9,6±1,2
4	Inappropriate speech	1,9±0,6*	8,4±1,1
	Symptom Checklist		
1	Drowsiness	5,4±0,7*	14,5±1,4
2	Decreased activity	1,8±0,6*	4,9±0,4

* - $p < 0, 05; Z<Z_{0, 05}$

The efficiency of intravenous immunoglobulin is usually associated with neutralization of anti-brain autoantibodies and the suppression of T-cell-mediated activation of autoreactive B-lymphocytes. Though, in this study, some additional mechanisms of a positive immunotherapy action were found.

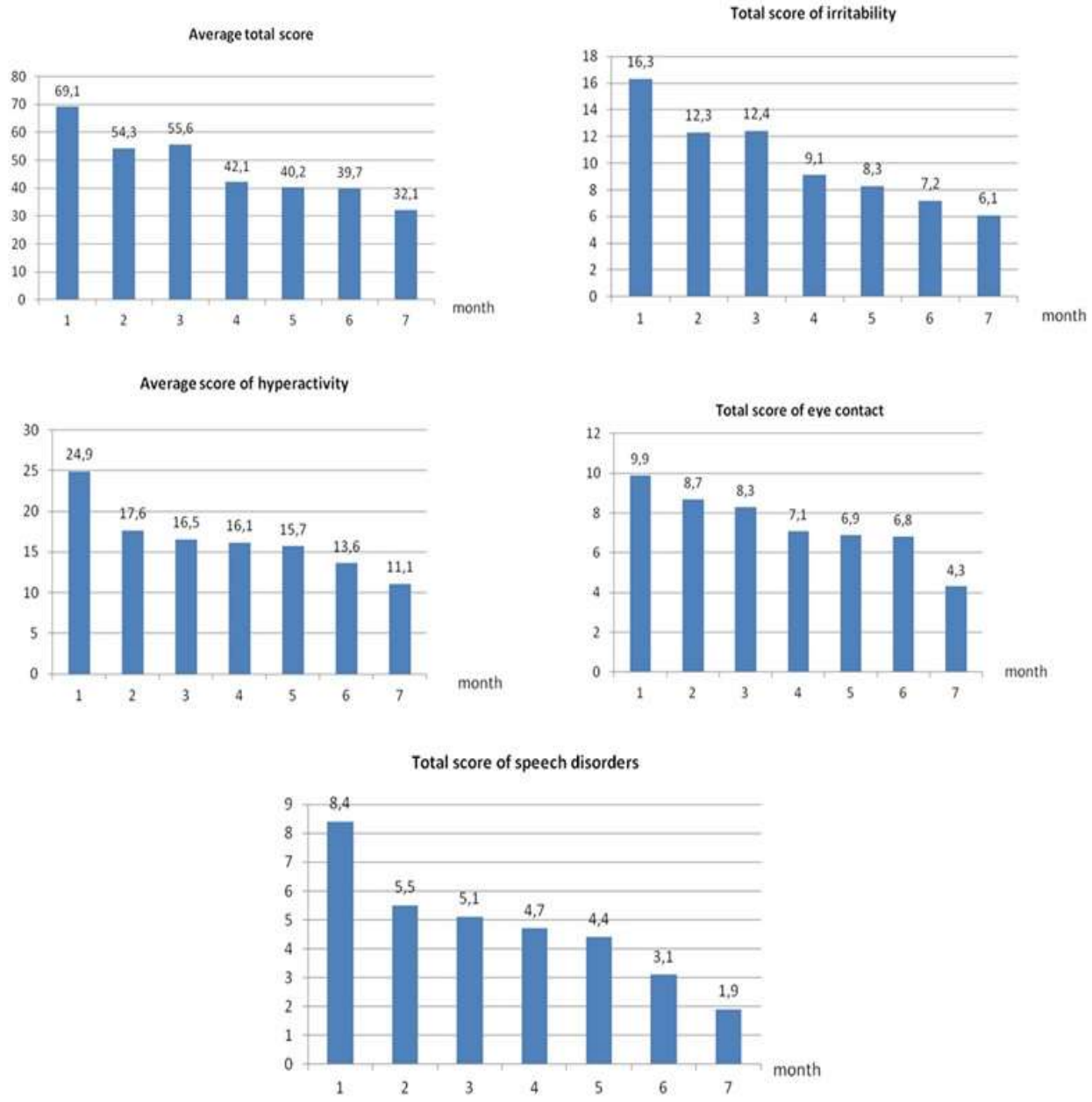

Figure 6: Dynamics of the main indicators of ABC scale in the SG children for up to (1) and for 6-month course of immunotherapy (2-7) (n=78)

It was previously reported of the benefit to use intravenous immunoglobulin in autistic spectrum disorders in children. Plioplys A.V. conducted a small uncontrolled study involving 10 children (2 girls and 8 boys) aged 4 to 17 years suffering from autistic spectrum disorders. The patients received a low-dose therapy with intravenous immunoglobulin at a dose of 200-400 mg/kg every 6 weeks four times. Only one child showed a pronounced regress of autistic manifestations after the course of immunotherapy. Four other patients had a slight mental disorders-related improvement, but 5 children appeared to be non-responsive to the treatment at all [16].

Del Giudice-Asch G. et al [17].Investigated the efficacy of immunotherapy in an open pilot study involving 5 children with autistic disorders. Intravenous immunoglobulin preparation was administered at a dose of 400 mg/kg per month for half a year. The Ritvo-Freeman scale alone showed positive clinical dynamics (out of the 10 scales used) [17]. Gupta S. investigated the effectiveness of the low-dose immunoglobulin therapy in 10 children aged 3 to 12 years with autism spectrum disorders in an open pilot study.

The improvement was marked in almost all the cases, being recorded by both the researcher and the behavioral and speech disorders experts, parents and nurses who administered the drug infusion. Younger children responded better to the immunotherapy [10]. Niederhofer N. et al. conducted a small double-blind, placebo-controlled, cross-over study involving 12 boys aged 4.2 to 14.9 years with autism spectrum disorders.

The patients received a single low-dose intravenous immunoglobulin therapy (400 mg/kg). Improvements in the main criteria of the ABC scale such as: irritability, hyperactivity, inadequate eye contact and inappropriate speech were shown [11]. Boris M. et al. conducted a retrospective study of the efficacy of immunotherapy in 27 children with autistic spectrum disorders (21 boys and 6 girls). Patients received intravenous immunoglobulin at a dose of 400 mg/kg every 4 weeks for 6 consecutive months.

ABC scale was used for control. Almost all the participants showed a significant improvement in the indicators studied: hyperactivity, inappropriate speech, excitability, flaccidness and stereotypy. However, 22 out of the 26 children who responded to immunotherapy showed a recurrence of autism symptoms 2-4 months later after completing the intravenous immunoglobulin course [18]. Thus, a low-dose immunoglobulin therapy exerts an impermanent, moderate and, apparently, short-lived positive clinical effect in children with autism spectrum disorders. Only one study that investigated the efficacy of a high-dose immunoglobulin therapy showed more promising results.

Thirteen children with autism spectrum disorders at the age of 2.7 to 10.9 years (10 boys and 3 girls) received intravenous immunoglobulin at a dose of 1.5-2.0 g/kg per month. There was a pronounced improvement in the behavior, speech and the function of social interference in all the participants with two children having a complete elimination of the autism phenotype. In contrast to the low-dose regimen, no loss of achievements after the completion of the immunotherapy course was observed [19]. A high-dose immunoglobulin therapy has also been performed and it can be stated that there is an opportunity to completely eliminate clinical manifestations of autistic spectrum in the near future .At least in one third of cases, as well as to achieve a pronounced and stable improvement in the majority of patients.

As the trial results show, a proper selection of patients by the presence of the genetic folate cycle deficiency and the associated clinical and paraclinic manifestations, leukoencephalopathy included, allows one to remarkably increase the efficacy of the immunotherapy performed. As for other clinical manifestations, the elimination or pronounced suppression of PANDAS symptoms occurred in 19 out of 21 SG patients, while in the CG there was no positive dynamics in extrapyramidal and mental disorders among all 7 children with PANDAS manifestations ($p < 0.05$; $Z < Z_{0,05}$). Previously, in a double-blind, placebo-controlled study the clinical efficacy of high-dose immunoglobulin therapy with PANDAS in children was demonstrated, with the result obtained being consistent with that of plasmapheresis [20].

The epileptic syndrome relief, i.e. a decreased frequency/severity of seizures and a positive dynamics of electroencephalography (EEG) data. Such epileptic syndrome relief was observed in 29 out of 36 SG patients having such disorders, and only in 2 out of 14 children of the CG ($p < 0.05$; $Z < Z_{0,05}$). Monge-Galindo L. et al. in a clinical longitudinal study showed a close relationship between autism spectrum disorders and temporal mesial sclerosis in children [21]. At the same time, HHV-6 is found out in biopsy samples taken from a sclerosis area in temporal mesial epilepsy. Earlier, Plebani A. et al. demonstrated the efficiency of intravenous immunoglobulin therapy in childhood refractory epilepsy in patients with a selective IgG-subclass deficiency.

The clinical effect was explained by the combined immuno-substitutive and immunomodulatory effect of the preparation [22]. Later, Billiau An.D.et al. showed the efficacy of intravenous immunoglobulin in refractory epilepsy in children without taking into account the data of the immune status, which appears to indicate a direct antiepileptic effect of immunotherapy in such cases [23]. Positive dynamics in the persistent enteropathy/colitis clinical manifestations was recorded in 49 out of 68

SG patients, which potentiated the effect of the previously administered gluten-free/casein-free diet. At the same time, further improvement in intestinal function was observed only in 5 out of 27 children of the CG ($p < 0.05$; $Z < Z_{0,05}$).

Earlier, Russo A.J. et al. described ileocecal lymphoid nodular hyperplasia in children with autistic spectrum disorders, resembling the lymphocytic nodular hyperplasia of intestine in patients with primary immunodeficiencies [2]. Along with that, Torrente F. et al. characterized small intestine enteropathy with epithelial deposits of complement and IgG in children with regressive autism [24]. The efficacy of intravenous immunoglobulin as related to intestinal syndrome in children with autistic spectrum may be explained by the immunomodulatory effect of a drug, with regard to the immune-dependent mechanism of intestine lesion in such cases.

Previously, the oral immunoglobulin drug showed efficacy in the intestinal syndrome in children with autism in a prospective pilot study. However, a further placebo-controlled trial did not confirm the obtained clinical effect. The efficiency of a systemic high-dose immunoglobulin therapy is demonstrated for enteropathy/colitis. The motor manifestations reduced only in 3 out of 16 children of the SG and in 2 out of 6 patients of the CG ($p > 0.05$; $Z > Z_{0,05}$), which indicates that there was no intravenous immunoglobulin action on the symptoms of pyramidal tract lesion in children with autistic spectrum disorders.

Partially, it can be explained by the fact that these pyramidal symptoms often represent the residual events of a preceding pathological process, for example, a congenital cytomegalovirus infection. These symptoms didn't appear as a consequence of the real-time immune-mediated reactions. Nonetheless one girl from the SG had a motor disorders - related dramatic positive effect after the administration of intravenous immunoglobulin, and the patient was able to walk independently after a long period of immobilization.

Thus, intravenous immunoglobulin has a complex positive effect in children with autistic spectrum associated with the genetic folate cycle deficiency, which lies not only in the eliminating or suppressing of mental disorders, but also in improvements in the area of extrapyramidal disturbances, intestinal syndrome and the epileptiform brain activity. Such an effect can be explained by the similar immune-dependent mechanisms for the development of seemingly discreet clinical manifestations of the disease. Earlier, Jyonouchi H. et al. conducted a specially designed study, in which reported about broad clinical phenotype covering epilepsy, intestinal disturbances, autoimmune disorders, delayed hypersensitivity and a deficiency of specific anti-polysaccharide antibodies in autistic spectrum disorders in children [3].

The presence of multiple reactivated viral infections in the SG children can be fully explained by the existing deficiency of NK and/or NKT cells. Earlier, Binstock T. singled out a special subgroup of children with autistic spectrum, wherein there was a pathologically lowered resistance to intramonocytic pathogens [25]. While Nicolson G.L. et al. identified in such children an abnormally increased presence of DNA of Mycoplasma ssp., Chlamydia pneunomiae and HHV-6 in blood [26].

Those were exactly the cases of autism spectrum disorders associated with the genetic folate cycle deficiency, wherein a primary NK- and/or NKT-cells deficiency was present. Viral agents may induce a delay myelination/demyelination as was shown by Kamei A. et al. in the case of primary HHV-6 infection [27], and Pinillos-Pisón R. et al. In the case of the CMV reactivation [15].Accordingly, there are a number of descriptions of autism being developed after the preceding viral encephalitis [6, 7].

In addition to that, viruses through the mechanism of a molecular mimicry may be involved in a phenomenon of production of anti-brain auto-antibodies in children with autistic spectrum. Thus, a close association is demonstrated between the presence of measles virus or HHV-6 in a reactivated state and the production of auto-antibodies to brain antigens in children with autism spectrum disorders.

Another study showed cross-reactivity between anti-measles antibodies and auto-antibodies to the main myelin protein in children with autistic syndrome [14]. Within the context of these data, it is considered extremely helpful that the intravenous immunoglobulin leads to a gradual but

steady reduction in the total viral load due to lymphotropic herpesviruses in the blood serum of the SG patients (Fig. 7).

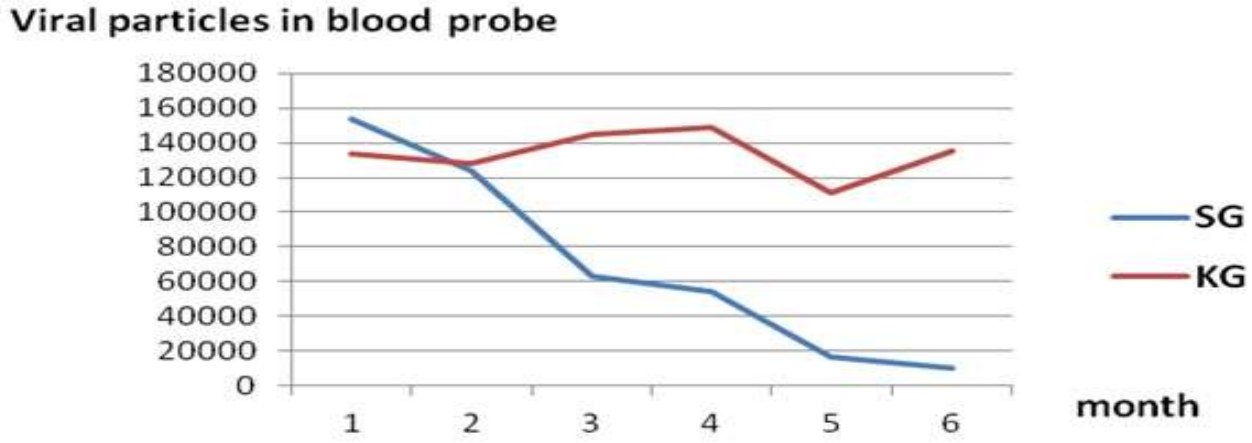

Figure7: Dynamics in the total blood viral load due to lymphotropic herpesviruses (EBV, CMV, HHV-6, HHV-7) according to PCR test in patients of the SG (n=78) and the CG (n=32)

At the moment, intravenous immunoglobulin is used to prevent reactivated opportunistic viral infections in immunocompromised individuals. Thus, Cowan, J. et al. have recently conducted a systematic review of controlled trials on the efficacy of immunotherapy to prevent viral infections in the recipients of allogenic hemopoietic blood cells, having demonstrated the evident benefits of immunotherapy.

The gradual augmentation of a number of NK-cells was also found out in the peripheral blood in the SG patients, which appeared to be delayed and was most strongly manifested by the 5th to 6th month of immunotherapy (Fig.8). High-dose immunoglobulin therapy promotes the growth of NK cells functional activity. At the same time, the use of a low-dose immunotherapy regimen (400 mg/kg per month) leads, on the contrary, to a reduction in a number and activity of natural killers, as was demonstrated by Ruiz J.E. et al. in a clinical trial involving women with multiple episodes of spontaneous abortions [28].

Besides that, intravenous immunoglobulin contributed to compensation of hypo- or dysimmunoglobulinemia, which was observed in some SG children. As it is known, intravenous immunoglobulin is used to treat primary hypoimmunoglobulinemia in humans. The reduced levels of immunoglobulins in the blood correlate with the severity of mental autistic disorders in children. Thus, the applied high-dose immunoglobulin therapy contributed to compensation, or at least, to sub compensation of the underlying primary immunodeficiency in the SG children.

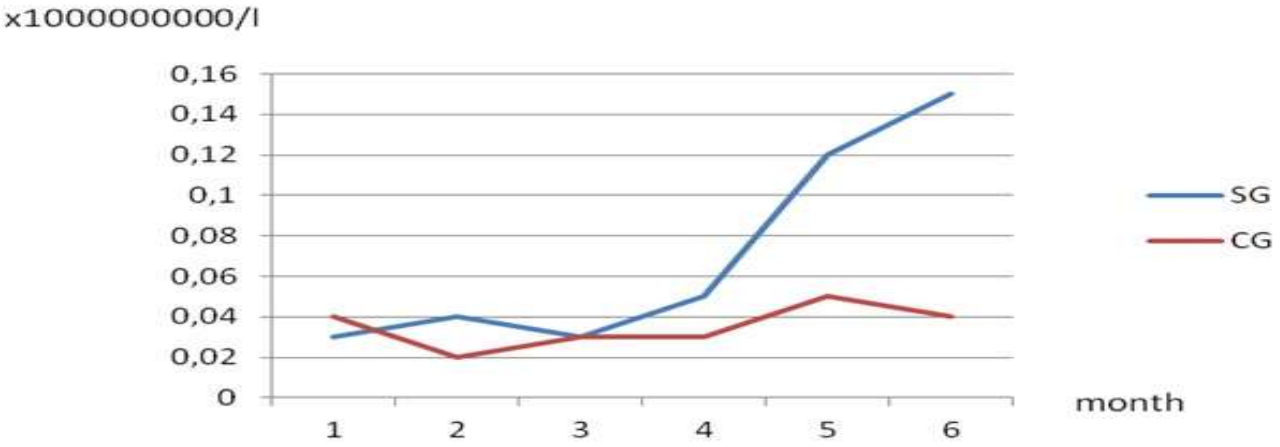

Figure 8: Dynamics in the number of NK-cells in the peripheral blood in patients of the SG (n=78) and the CG (n=32)

Finally, a positive leukoencephalopathy-related dynamics were obtained, being observed in almost all the children of the SG. Complete or almost complete elimination of MR-signs of leukoencephalopathy was observed in 29 out of 78 patients, and a pronounced regress of the delayed myelination/demyelination foci was identified in 24 other children (Fig. 9).

However, minor changes were recorded in 25 individuals, and usually these were the children with slight clinical mental disorders-related improvement and a high risk of recurrent symptoms following the discontinued course of immunotherapy. In the CG, moderate positive leukoencephalopathy-related dynamics were observed in 5 out of 32 children only ($p < 0.05$; $Z < Z_{0,\ 05}$) (Table 2). Intravenous immunoglobulin is known to be able to stimulate remyelination of peripheral nerve fibers in Guillain-Barre syndrome, which is associated with inhibition of an underlying autoimmune reaction under the action of the preparation. Nonetheless, there is a direct stimulating effect of intravenous immunoglobulin on the process of the peripheral nerve fibers remyelination, which was independent of immunomodulation.

The pronounced potentiating effect of a high-dose intravenous immunoglobulin therapy is observed on the process of myelination/remyelination in the white matter of the cerebral hemispheres in children with autistic spectrum associated with the genetic folate cycle deficiency. This phenomenon to a considerable extent can explain the modifying effect of immunotherapy on autistic spectrum disorders. The changes in the study endpoints are summarized in Table 2.

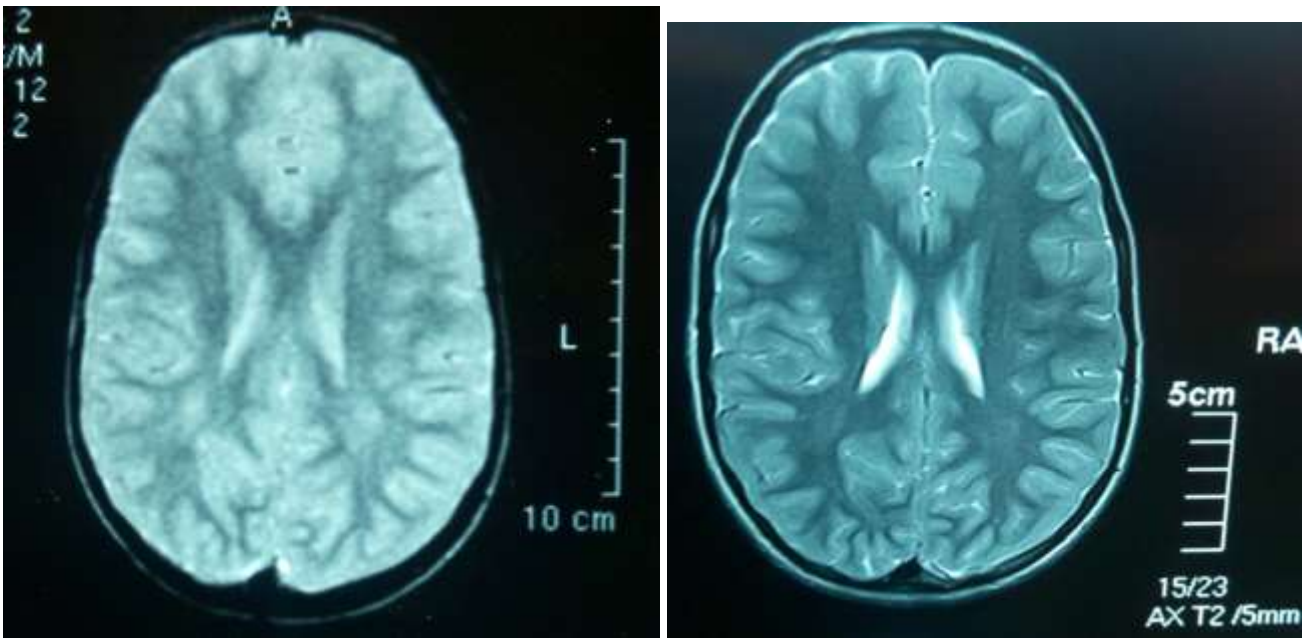

Figure 9: Reduction of periventricular zones of impaired myelination in the parietal lobes of the cerebral hemispheres after a 6 month course of intravenous immunoglobulin therapy in a patient with autistic spectrum associated with the genetic folate cycle deficiency (left – prior to immunotherapy, right - following a course of intravenous immunoglobulin, author's own observation)

Immunoglobulin has proved to be a safe preparation in the course of the study. A flu-like syndrome was observed only in 24 out of the 78 SG patients. In 10 children, single episodes of vomiting occurred shortly after the immunoglobulin injection.

These moderate adverse effects were not the obstacles for the course of immunotherapy to be continued. No other undesirable events in the setting of intravenous immunoglobulin use were recorded.

Table 2: Differences in the end points of the study in patients of the SG and of the CG

End point	SG,%		CG,%		T-criteria (parametrical)	Number of sings Z (non-parametrical)
	+	-	+	-		
Autistic spectrum	69	31	37	64	$p<0,05$*	$Z<Z_{0,05}$*
PANDAS	90	10	0	100	$p<0,05$*	$Z<Z_{0,05}$*
Epilepsy syndrome	81	19	14	86	$p<0,05$*	$Z<Z_{0,05}$*
Bowel syndrome	72	28	56	44	$p<0,05$*	$Z<Z_{0,05}$*
Pyramidal symptoms	19	81	33	77	$p>0,05$	$Z>Z_{0,05}$

Viral load	81	19	29	71	$p<0,05$*	$Z<Z_{0,05}$*
Number of NK-cells	77	33	28	71	$p<0,05$*	$Z<Z_{0,05}$*
Leucoencephalopathy	68	32	16	84	$p<0,05$*	$Z<Z_{0,05}$*

* - significant differences

Intravenous immunoglobulin is safe and does not contribute to the development of autism in children. In compliance with this, Croen L.A. et al. showed that the use of anti-rhesus immunoglobulin to prevent hemolytic disease of a fetus does not increase the risk of developing the autistic disorders either [29]. As was pointed out by Wynn J.L. et al., the use of a high-dose immunoglobulin therapy does not inhibit the development of a child's immune system; it contributes to the maturation of the immature immune system in premature infants [30].

Conclusion

Thus, the fact can be stated of a high efficiency and proper safety of the intravenous immunoglobulin therapy at a dose of 2 g/kg per month in children with autistic spectrum disorders associated with the genetic folate cycle deficiency. Such a treatment results not only in elimination or suppression of mental disorders, but also in the alleviation of extrapyramidal, epileptic and intestinal disorders. The polymodal clinical effect of immunoglobulin appears to be associated with a sharp decrease in the blood viral load, an increase in the earlier critically reduced number of natural killers and the elimination of leukoencephalopathy manifestations in such children.

In the previous publications, it was noted that the patients with a genetically determined folate cycle deficiency have a primary immunodeficiency which appears to be exactly the direct cause for the broad clinical phenotype covering immune-dependent, mental, extrapyramidal, epileptic, motor, intestinal, infectious, autoimmune and allergic manifestations, as well as leukoencephalopathy. Intravenous immunoglobulin has a complex positive effect on most of the components of a broad clinical phenotype in children with the genetic folate cycle deficiency.

This treatment approach promotes compensation or, at least sub compensation of manifestations of the underlying primary immunodeficiency associated with genetic disturbances in the folic acid cycle. The acting currently recommendations do not support the use of immunoglobulin therapy in autism spectrum disorders in children. However, such a therapeutic strategy can be tried in some patients being non-responsive to other therapeutic approaches. With regard to the promising results obtained in this clinical controlled trial, it is expedient to continue the research in this direction with a larger number of participants involved and with a better study design.

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