DOI: 10.15587/978-617-8360-21-4.CH13

13 RESULTS OF A RETROSPECTIVE ANALYSIS OF THE USE OF HIGH-DOSE INTRAVENOUS HUMAN NORMAL IMMUNOGLOBULIN FOR THE TREATMENT OF IMMUNE-DEPENDENT ENCEPHALOPATHY WITH A CLINICAL PICTURE OF AUTISM SPECTRUM DISORDERS IN CHILDREN WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

INTRODUCTION

Autism spectrum disorders (ASD) are a group of heterogeneous neuropsychiatric disorders that are variable in phenotype and are clinically characterized by deficits in social interactions, impaired communication, and stereotyped behavior [18]. Currently, there is a rapid increase in the frequency of this severe pathology in the child population, the reasons for which are still not sufficiently understood. As noted by Hughes H.K. et al. in a systematic review on the problem of ASD, in the USA, for the period from 1972 to 2014, the frequency of registered cases of this neuropsychiatric pathology increased from 1 case per 10 thousand people (0.01 %) to 1 case per 57 children (2 %), that is, 200 times, which cannot be explained only by an increase in the quality of detection of this pathology by modern medicine [25].

There is now accumulating evidence that immune mechanisms are involved in the pathogenesis of ASD in children, which may open the way for testing immunotherapeutic interventions in this severe and common disease. Thus, the association of ASD with certain HLA histocompatibility antigen loci has been demonstrated, as is noted in a number of human autoimmune and allergic syndromes [49]. Various forms of immunodeficiencies have been described in children with ASD [26, 51, 54], and studies devoted to some primary immune dysfunctions indicate an increased risk of autism in such cases [57, 64, 65]. There are frequent reports of the appearance of ASD in adults and children after episodes of neuroinfections, mainly of an opportunistic nature [19, 24, 35]. Children with ASD have been shown to have various autoantibodies to brain autoantigens that are not produced in healthy individuals [8, 40, 61]. Moreover, several clinical trials have suggested the benefit of immunotherapy in selected patients with ASD [14, 21, 42]. All of these compelling arguments call for attention to the role of immune-mediated mechanisms in the pathogenesis of ASD in humans.

An important step in deepening the understanding of the role of immune-related disorders in the development of neuropsychiatric disorders is the elucidation of the association of genetic deficiency of the folate cycle (GDFC) and ASD in children, evidence of which is provided in the results of at least 5 meta-analyses of randomized controlled clinical trials [29, 38, 48, 50, 55]. Specific biochemical disorders caused by GDFC have been characterized [20, 67], which lead to pathological abnormalities in the functioning of the immune system with the formation of a specific immunodeficiency, the core of which is a deficiency of natural killer (NK) and natural killer T lymphocytes (NKT), as well as a reduced activity of neutrophil myeloperoxidase [33]. It seems obvious that this immunodeficiency, through a decrease in immune resistance and induction of a state of immune dysregulation, is responsible for the development of encephalopathy with clinical manifestations of ASD, which most likely has an immune-dependent inflammatory mechanism of development. Currently, at least 3 different immune-mediated mechanisms of encephalopathy formation in GDFC are known, which can cause the formation of the clinical phenotype of ASD.

These are some neurotropic opportunistic and conditionally pathogenic infections [35, 41], autoimmune reactions to autoantigens of neurons, myelin, neuroglia and cerebral vascular walls [8, 61], as well as systemic and associated intracerebral aseptic inflammation [36, 56]. It would be useful to clinically test available agents to suppress these immune-dependent mechanisms of CNS damage, which may open up a currently unavailable prospect of an effective strategy for treating ASD manifestations in children with GDFC [9]. In particular, it is believed that suppression of autoimmunity and CNS neurons and myelin can significantly improve the mental functions of sick children. A number of clinical studies have already been conducted in this direction. In particular, clinical case reports and the results of small trials have shown the benefit of using glucocorticosteroids and some other anti-inflammatory agents in children with ASD, the mechanism of action of which is seen precisely in the implementation of anti-inflammatory action and suppression of anti-brain autoimmunity. As noted by Marchezan J. et al. In a systematic review devoted to the analysis of the limited evidence base of clinical trials of anti-inflammatory drugs in ASD, all drugs approved so far can be divided into two main groups: (a) drugs with primary anti-inflammatory and immunomodulatory effects, which include sulforaphane, celecoxib, lenalidomide, pentoxifylline, spironolactone, flavonoid luteolin, corticosteroids, oral and intravenous immunoglobulin, cell therapy, dialyzed blood lymphocyte extract, minocycline and pioglitazone; (b) other drugs that are prescribed for non-immunological indications, but have additional immunomodulatory effects not related to the main mechanism of action, in particular, risperidone, vitamin D, omega-3 polyunsaturated fatty acids, ginkgo biloba, L-carnosine, N-acetylcysteine and restoration of intestinal microflora [34].

At least 9 clinical trials have been conducted to test the immunomodulatory biological agent normal human immunoglobulin IV in ASD, which is thought to improve patients' mental function by suppressing intracerebral inflammation and autoimmune responses against brain autoantigens [5, 6, 11, 14, 21, 32, 37, 42, 46]. Recently, infliximab, a monoclonal antibody against the pro-inflammatory molecule tumor necrosis factor alpha, has been shown to be effective in suppressing hyperactivity and hyperarousal in children with ASD associated with GDFC in a small controlled clinical trial [30]. Accordingly, in another controlled clinical trial, rituximab, a monoclonal antibody to the CD20 B-lymphocyte molecule, was shown to significantly improve the mental status of children with ASD associated with GDFC by suppressing the autoimmune response against autoantigens in the hippocampus and temporal lobes of the cerebellum [31].

Intravenous human normal immunoglobulin, which has already proven itself in the treatment of various autoimmune and immunoinflammatory human diseases in neurology and rheumatology [7], appears to be the most promising treatment strategy for immune-dependent inflammatory encephalopathy in children with GDFC due to its broad therapeutic spectrum and good tolerability. A small controlled clinical trial of 6 months of high-dose intravenous immunoglobulin therapy in children with ASD associated with GDFC has previously been published, demonstrating significant improvement in all study endpoints at the end of the immunotherapy course [32]. The encouraging results of this pilot study should prompt a larger clinical trial with more participants. The mechanism of action of immunoglobulin therapy in ASD has not yet been clearly defined, as have the subgroups of patients who may potentially respond positively to immunotherapy. There is good reason to believe that patients with ASD associated with GDFC are a specific subgroup that responds well to intravenous immunoglobulin therapy, which needs to be tested in specifically designed controlled clinical trials.

The aim of the reserch: to evaluate the efficacy and safety of a 6-month course of high-dose immunoglobulin therapy for immune-dependent encephalopathy with the clinical picture of ASD in children with GDFC.

Materials and methods. To achieve this goal, we retrospectively analyzed the medical records of 225 children aged 2 to 9 years with GDFC who had clinical manifestations of ASD (183 boys and 42 girls). All of them were patients of the specialized neuroimmunological clinic Vivere (registration file dated 12/22/2018 No. 10/2212–M). Obtaining data for the study and processing the material was carried out in accordance with contract No. 150221 dated 02/15/2021, and the conclusion of the bioethical examination commission (protocol No. 140 dated 12/21/2020, Bogomolets NMU). The clinical diagnosis of ASD was made by child psychiatrists according to the criteria of DSM–IV–TR (Diagnostic and Statistical Manual of mental disorders) and ICD–10 (The International Statistical Classification of Diseases and Related Health Problems).

Pathogenic polymorphic variants of folate cycle genes were determined by restriction PCR (Sinevo, Ukraine) based on the detection of the MTHFR C677T nucleotide substitution in monoform (68 patients), as well as – in combination with other nucleotide substitutions – MTHFR A1298C, MTRR A66G and/or MTR A2756G (157 individuals). These individuals formed the study group (SG). These children, in addition to conventional educational programs, were prescribed intravenous normal human immunoglobulin preparations at a dose of 2 g/kg/month once every 30 days for 6 consecutive months. Intravenous immunoglobulin was administered for 3–5 consecutive days at a rate of 20–25 drops per minute, and the intervals between immunotherapy courses were from 27 to 25 days, respectively.

The control group (CG) included 50 children (36 boys and 14 girls) of similar age distribution, who also suffered from GDFC and ASD of corresponding severity. These patients did not receive intravenous immunoglobulin therapy, but underwent only conventional rehabilitation measures, which included work with speech therapists/defectologists, specially trained teachers, psychiatrists and physiotherapists.

The dynamics of mental symptoms of ASD during this clinical study in the observation groups was assessed using the Aberrant Behavior Checklist (ABC) scale [1].

All patients underwent a comprehensive immunological examination at the Institute of Immunology and Allergology of the Bogomolets National Medical University and/or the Sinevo laboratory, which, in addition to a general blood test, included the study of the subpopulation composition of lymphocytes using laser flow cytofluorimetry (Epics XI cytofluorimetry, USA) and the indirect immunofluorescence method 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 using a latex test to determine the phagocytosis index, phagocytic index, number of active phagocytes and phagocytic capacity of the blood, as well as the activity of the enzymes myeloperoxidase (flow cytofluorimetry) and NADPH oxidase (NST test). Serum concentrations of immunoglobulins of the main classes (M, G, A) were determined using the results of simple radial immunodiffusion according to Mancini and solid-phase ELISA. The concentration of IgE, IgD and IgG subclasses (IgG1, IgG2, IgG3, IgG4) in serum was measured using a home-made solid-phase enzyme-linked immunosorbent assay (VectorBEST, Russia; MDI Limbach Berlin GmbH, Germany).

In addition, diagnostics of reactivated viral infection was performed based on the results of quantitative PCR of blood leukocytes with species-specific primers of herpesviruses (herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpes viruses types 6, 7 and 8 (HHV-6, HHV-7, HHV-8)), measles and kansas viruses (DNA-Technology reagents, Russia).

All children underwent control MRI scans of the brain in conventional modes (T1- and T2-weighted, FLAIR) on tomographs with a magnetic induction value of at least 1.5 T, at least twice: before the beginning and after the end of participation in the study. Signs of leukoencephalopathy of varying severity were typical (**Fig. 13.3**). Also in 46 % of cases, an additional MR pattern of temporal mesial sclerosis was observed. Mostly such children suffered from epileptic syndrome and had cognitive disorders. In 17 % of cases, typical signs of congenital cytomegalovirus neuroinfection were noted in the form of ventriculomegaly, periventricular calcified foci, cysts in the poles of the temporal lobes, hypogenesis of the corpus callosum and zones of delayed myelination in the parietal lobes of the cerebral hemispheres. These data are consistent with the results of an 18-year retrospective study by Pinillos-Pisón R. et al. [44]. Such children mainly had symptoms of damage to the pyramidal and cerebellar tracts, in connection with which they were often diagnosed with cerebral palsy, although manifestations of ASD were also observed.

The criteria for inclusion of the patient in this study were the presence of folate cycle gene polymorphisms, NK and/or NKT cell deficiency, reactivated infection caused by lymphotropic herpesviruses, signs of leukoencephalopathy on MRI of the brain, clinical symptoms of ASD according to the ABC scale. The criteria for exclusion of the patient from this study were: refusal of the child's parents to use medical documentation in the study, the presence of additional verified genetic pathology involved in the development of the picture of existing mental disorders, the absence of a phenotype of NK and/or NKT cell deficiency and signs of leukoencephalopathy, as well as the development of side effects of immunotherapy that made it impossible to continue the approved treatment according to the plan. The study endpoints were the main clinical manifestations of ASD disorders according to the ABC scale, brain MRI data in conventional modes, the absolute number of NK and NKT cells in peripheral blood, the current viral load of lymphotropic herpesviruses according to PCR data of blood leukocytes, as well as the dynamics of the main additional clinical manifestations associated with GDFC, including PANS/PITANDS/PANDAS (pediatric acute-onset neuropsychiatric syndrome/pediatric infection-triggered autoimmune neuropsychiatric disorder/pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections), intestinal syndrome (persistent immunoinflammatory enteropathy/colitis), temporal median epilepsy associated with temporal median sclerosis (TME-TMS), and clinical symptoms of damage to the pyramidal and cerebellar motor pathways of the CNS.

Statistical analysis of the obtained information was processed by methods of structural and comparative analysis using the electronic program Microsoft Excel. To study the distribution of the variant in the variation series, the Shapiro-Wilk test was used. To establish the reliability of the differences in the results, the Student's T-test was used to calculate the confidence probability coefficient p (parametric criterion) and the number of signs Z according to Urbach (non-parametric criterion). Differences were considered reliable when $p < 0,05$ and $Z < Z_{0,05}$.

The study was carried out as a fragment of research work commissioned by the Ministry of Health of Ukraine (state registration number 0121U107940).

Results and discussion. High-dose normal human intravenous immunoglobulin was effective in reducing clinical symptoms of ASD according to the ABC scale in 199 out of 225 SG children (88 % of cases; responders to immunotherapy), however, the severity of the achieved positive clinical effect, as well as the stability of the achieved progress in the child's psycho-speech development, varied in different patients (**Table 13.1; Fig. 13.1**). Resistance to the performed immunotherapy from the side of ASD manifestations among SG children was noted in 12 % of cases (non-responders to immunotherapy).

A significant reduction in autistic symptoms (at least 50 % from baseline) with the exposure of a pronounced deficit in knowledge and skills in the child was noted in 61 cases among SG children (27 % of cases; strong responders). Partial regression of achievements in the child's psychospeech development after the abolition of immunotherapy occurred only in five patients from the subgroup of strong responders to IV immunoglobulin (8 % of cases). Other children developed normally and reached the level of peers 3–5 years after the course of immunotherapy under the influence of non-drug treatment, including classes with a speech therapist, general educators, psychiatrists and psychotherapists. Moderate regression of autistic manifestations (by 30–50 % from baseline) was observed in 83 cases among SG children (37 % of cases; among responders), allowing patients to significantly expand the range of current social adaptation. 32 children from the average responders (39 % of cases in this subgroup) continued to demonstrate positive dynamics of reduction of mental disorders after completion of immunoglobulin therapy under the influence of rehabilitation measures. The other children (61 % of cases in this subgroup) retained significant autistic features 2–3 years after immunotherapy. Apparently, the 6-month course of immunotherapy was too short for them, and further positive dynamics of mental disorders could be achieved with repeated similar courses of high-dose intravenous immunoglobulin therapy due to the cumulative effect. However, 55 SG children (24 % of cases; weak responders) responded with only vague positive dynamics in the existing mental disorders of the ASD type (improvement of no more than 20 % from the initial level) after completing the full course of immunotherapy (**Fig. 13.3**). Half of these weak responders had a loss of achievements in psychospeech development already 2–4 months after the completion of intravenous immunoglobulin therapy and, apparently, they required repeated courses of immunotherapy in the future to achieve an adequate clinical result.

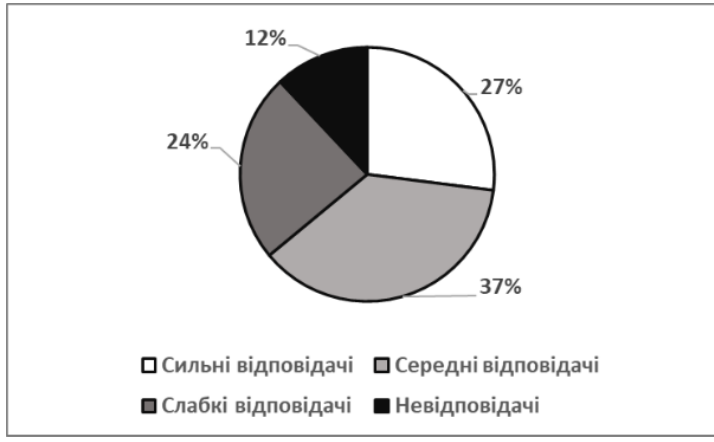

Fig. 13.1. SG structure (n = 225) on the effectiveness of a 6-month course of high-dose intravenous human normal immunoglobulin in relieving ASD symptoms according to the ABC scale

In children with CG, weak or moderate positive dynamics in the main clinical symptoms of ASD on the ABC scale at the end of the observation period occurred in 12 of 51 individuals (24 % of cases), and, apparently, was a reflection of the natural course of the disease and/or the recommended rehabilitation measures taken ($p < 0,05$: $Z < Z_{0,05}$). In no child with CG at the end of the observation period, a reduction in the clinical phenotype of ASD by 50 % or more from the baseline level on the ABC scale was registered during the 6-month observation period ($p < 0,05$: $Z < Z_{0,05}$).

The obtained data allow us to state a clear positive modifying effect of high-dose intravenous immunoglobulin therapy on the mental development of children with ASD associated with GDFC (**Fig. 13.2**; **Table 13.1**).

● **Table 13.1.** Comparison of ABC score levels in SG (n = 225) and CG (n = 50) patients at the end of the 6-month follow-up period

№	Subscales	SG (n = 225)	CG (n = 50)
	ABC		
1	Irritability	7.1±0.6*	14.3±0.8
2	Hyperactivity	12.1±0.7*	23.4±1.2
3	Inadequate eye contact	5.4±0.5*	9.7±0.6
4	Inappropriate speech	2.8±0.3*	7.6±0.7
	Symptom checklist		
1	Drowsiness	6.4±0.6*	13.3±1.0
2	Decreased activity	2.7±0.2*	4.8±0.3

Note. * – $p < 0,05$: $Z < Z_{0,05}$

The clinical efficacy of intravenous human normal immunoglobulin preparations in ASD in children is associated with both direct neutralization of anti-brain autoantibodies circulating in the blood of such patients by exogenous IgG molecules of the immunobiological agent, and with an indirect effect due to the suppression of T-cell-mediated activation of autoreactive B lymphocytes committed to the synthesis of anti-brain autoantibodies [9], although in this clinical study we identified additional mechanisms of the positive effect of the immunotherapy used in ASD, which will be discussed below.

The benefits of intravenous human normal immunoglobulin for ASD in children have been previously reported. Different doses of the immunobiological preparation and immunotherapy regimens were used, which, as it now seems obvious, differ significantly in clinical efficacy.

Thus, Plioplys A.V. first conducted a small uncontrolled clinical study involving 10 children (2 girls and 8 boys) aged 4 to 17 years, suffering from ASD. Patients received low-dose immunotherapy with intravenous human normal immunoglobulin (at a dose of 200–400 mg/kg) every 6 weeks in the form of four courses for 5 consecutive months.

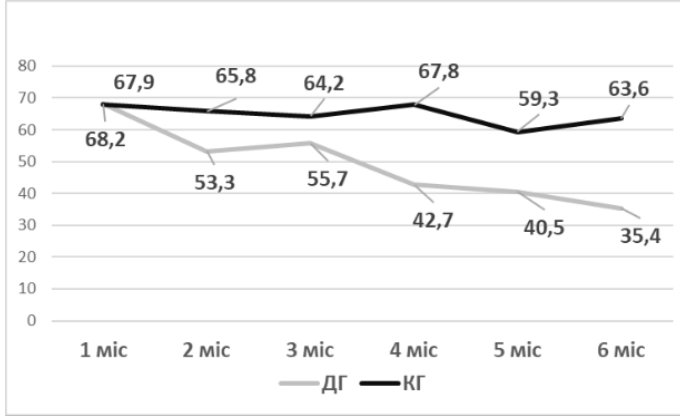

Fig. 13.2. Dynamics of the overall score of the severity of the main clinical manifestations of ASD according to the ABC scale in SG (n = 225) and CG (n = 50) children during the 6-month observation period

Only one child from the observation group showed a pronounced regression of ASD manifestations after a course of immunotherapy. Another 4 patients who received intravenous immunoglobulin showed a slight improvement in mental disorders, but the other 5 children proved resistant to treatment [46]. DelGiudice-Asch G. et al. studied the effectiveness of low-dose immunotherapy in an open pilot study involving 5 children with a clinical picture of ASD. The preparation of normal human immunoglobulin IV was administered at a dose of 400 mg/kg per month for six months. Of the 10 scales used to assess the severity of clinical symptoms of ASD, only the Ritvo–Freeman scale demonstrated positive dynamics of clinical indicators of mental status during the course of immunotherapy [14]. Gupta S. studied the effectiveness of low-dose immunoglobulin therapy in 10 children aged 3–12 years with ASD in an open pilot clinical study. The immunobiological preparation was administered at a dose of 400 mg/kg every 4 weeks for 6 consecutive months. Improvement in ASD symptoms was noted in almost all cases, and was recorded by both the investigator and the behavioral and language disorder specialists, parents, and nurses administering the infusions. Young children responded better to immunotherapy than older patients [21]. Niederhofer N. et al. conducted a small double-blind, placebo-controlled, crossover clinical trial in 12 boys aged 4.2 to 14.9 years with ASD. Patients received low-dose intravenous human normal immunoglobulin therapy (400 mg/kg) once. Improvement was demonstrated in the main criteria of the ABC scale: hyperexcitability, hyperactivity, inadequate eye contact, inappropriate speech [42]. Boris M. et al. conducted a retrospective study of the effectiveness of immunotherapy in 27 children with ASD (21 boys and 6 girls). Patients received normal human immunoglobulin IV at a dose of 400 mg/kg every 4 weeks for 6 consecutive months. The ABC scale was used to monitor the dynamics of mental status indicators. Almost all participants in this study showed significant improvement in the studied indicators

characterizing ASD: hyperactivity, inappropriate speech, hyperexcitability, lethargy and stereotypies. However, 22 of 26 children who responded to immunotherapy had a return of many of the eliminated ASD symptoms 2–4 months after the end of the course of IV immunoglobulin [5].

Thus, the evidence accumulated to date suggests that low-dose immunoglobulin therapy (400 mg/kg/month) produces an inconsistent, moderate, and apparently short-lived positive clinical effect in ASD in children.

The first uncontrolled clinical trial to examine the efficacy of high-dose immunoglobulin therapy was more promising. Thirteen children with ASD, aged 2.7 to 10.9 years (10 boys and 3 girls), received 1.5–2.0 g/kg/month of intravenous human normal immunoglobulin. All participants showed significant improvements in behavior, language, and social interaction, with two children experiencing complete resolution of the autism phenotype. In contrast to the low-dose regimen, there was no loss of psychomotor development after the end of the immunotherapy course [6].

Subsequently, Melamed I.R. et al. conducted an uncontrolled pilot clinical study of high-dose intravenous immunoglobulin therapy (1 g/kg/month) in 14 patients with ASD in the form of 10 courses with an interval of 1 time in 3 weeks, obtaining positive dynamics in inadequate behavior, social interference and communication according to the data of the scales for assessing the severity of clinical manifestations of ASD Children's Communication Checklist (CCC-2), Social Responsiveness Scale (SRS), ABC, Clinical Global Impressions-Severity (CGI-S) and -Improvement (CGI-I), Autism Diagnostic Observation Schedule (ADOS) and Peabody Picture Vocabulary Test (PPVT). In parallel, a decrease in the level of laboratory biomarkers of cerebral inflammation, such as CD154, Toll-like receptor-4, memory B cells, FOXP3 and the results of the lymphocyte stimulation test was noted [37].

Accordingly, Connery K. et al. conducted a controlled clinical study involving 82 patients with ASD with signs of autoimmune encephalopathy according to the results of the Cunningham panel (the presence of autoantibodies to dopamine receptors type 2 and neuronal tubulin). 49 of them additionally received high-dose normal human immunoglobulin IV, and 32 patients received only the recommended educational programs. Improvement in at least one indicator of the ASD severity assessment scales SRS and ABC was noted in 90 % of cases, while improvement in 2 or more indicators was noted in 71 % of cases among SG patients, which significantly differed from the results of the CG. As in the previous study, there was no regression of the acquired skills after the withdrawal of immunotherapy. The immunobiological drug not only reduced the manifestations of ASD, but also led to positive dynamics in other immune-dependent symptoms of the disease [11].

The largest prospective controlled clinical trial to date, testing high-dose intravenous immunoglobulin therapy (2 g/kg/month for 6 consecutive months) in 78 patients with GDFC-associated ASD and 32 matched controls who did not receive normal human intravenous immunoglobulin, demonstrated near-term resolution of ASD symptoms in at least one-third of cases, as well as significant and sustained improvement in ASD symptoms on the ABC scale in 40 % of patients. The results of this study show that proper selection of patients for GDFC and associated clinical and paraclinical manifestations, including leukoencephalopathy, can significantly improve the effectiveness of immunotherapy [32].

The results of clinical trials in the field of immunoglobulin therapy for ASD are currently summarized in the data of a systematic review and meta-analysis of clinical trials prepared by Rossignol D.A., Frye R.E.

et al. in 2021. 27 relevant trials were analyzed, of which 4 were prospective controlled (one double-blind placebo controlled), 6 were prospective uncontrolled, 2 were retrospective controlled, and 15 were retrospective uncontrolled). The overall clinical outcome of the trial of human normal intravenous immunoglobulin preparations according to this meta-analysis is improvement in communication, hyperexcitability, hyperactivity, cognition, attention, social interaction, eye contact, echolalia, speech, response to commands, drowsiness, reduced activity, and in some cases, complete elimination of ASD symptoms [52].

The data from this study were not included in the aforementioned meta-analysis, but are fully consistent with its results. The results of the presented study are consistent with the data of four previous clinical trials that demonstrated higher efficacy of high-dose intravenous immunoglobulin therapy in children with ASD [6, 11, 32, 37] compared with low-dose intravenous immunoglobulin regimens [5, 14, 21, 42, 46] with preservation of the achieved achievements in the mental development of the child after the completion of the course of immunotherapy.

Regarding other clinical manifestations of GDFC, the elimination or marked suppression of PANS/PITANDS/PANDAS symptoms occurred in 27 % of 32 % of cases in the SG, while in the CG there was no positive dynamics of extrapyramidal disorders and obsessive-compulsive syndrome in all children with manifestations of autoimmune subcortical encephalitis ($p < 0,05$: $Z < Z_{0,05}$). Previously, a double-blind placebo-controlled clinical trial demonstrated the clinical efficacy of high-dose immunoglobulin therapy for PANDAS in children, and the achieved result was consistent with that of plasmapheresis [43].

Improvement in the epileptic syndrome, which consisted in a decrease in the frequency/severity of epileptic seizures and positive dynamics of EEG data, was achieved in 33 % of 43 % of cases of SG patients who had these disorders, and only in 12 % of 40 % of CG children ($p < 0,05$: $Z < Z_{0,05}$). Monge-Galindo L. et al. in a clinical longitudinal study showed a close relationship between ASD and temporal median sclerosis in children [39]. At the same time, the neurotropic opportunistic agent HHV-6, which often undergoes reactivation in children with ASD, was found in biopsies from the hippocampal sclerosis zone in TME-TMS [15], the manifestations of which are often recorded in children with autism. Previously, Plebani A. et al. demonstrated the effectiveness of intravenous immunoglobulin therapy for refractory childhood epilepsy in patients with selective deficiency of IgG subclasses. The clinical effect of immunotherapy was explained by the combined immunoreplacement and immunomodulatory effects of the immunobiological drug [45]. Later, Billiau An.D. et al. demonstrated the clinical effectiveness of intravenous normal human immunoglobulin for refractory epilepsy in children without taking into account the patient's immune status [3].

Positive dynamics of clinical manifestations of intestinal syndrome were registered in 69 % of 82 % of cases in SG, which enhanced the effect of the previously prescribed elimination gluten-free/casein-free diet. At the same time, further improvement of intestinal function was observed only in 25 % of 84 % of cases in CG ($p < 0,05$: $Z < Z_{0,05}$). Previously, Russo A.J. et al. described ileocecal lymphoid nodular hyperplasia in children with ASD, which resembled the well-known lymphocytic nodular hyperplasia of the intestine in patients with primary immunodeficiencies [54]. At the same time, Torrente F. et al. characterized immunoinflammatory small intestinal enteropathy with epithelial deposits of complement proteins and IgG molecules in children with regressive autism [63]. The efficacy of intravenous human

normal immunoglobulin in the treatment of irritable bowel syndrome in children with ASD associated with GDFC can be explained by the known immunomodulatory, antimicrobial, and anti-inflammatory effects of the immunobiological drug, given the established immune-dependent mechanism of intestinal damage in such cases. Previously, oral normal immunoglobulin has shown clinical efficacy in irritable bowel syndrome in children with ASD in a prospective pilot study [58], although a subsequent placebo-controlled clinical trial did not confirm the positive effect obtained [22]. In this scientific work, we demonstrate the clinical efficacy of systemic high-dose immunoglobulin therapy in persistent enteropathy/colitis in children with ASD associated with GDFC.

Motor symptoms decreased in only 7 % of 21 % of SG children and 5 % of 19 % of CG patients ($p < 0,05$: $Z < Z_{0,05}$) (**Table 13.2**), indicating that there was no significant benefit of intravenous human normal immunoglobulin in heavy rain on symptoms of pyramidal and cerebellar tract involvement in children with ASD associated with GDFC. This may be partly explained by the fact that motor symptoms are often residual effects of a previous pathological process, such as congenital cytomegalovirus infection [16], and are not the result of real-time immune-mediated reactions. However, some SG patients showed dramatic improvement in motor symptoms after intravenous human normal immunoglobulin administration, and these patients were able to walk independently after a prolonged period of partial immobilization.

Thus, high-dose human normal immunoglobulin (2 g/kg/month) has a complex polymodal positive effect in children with ASD associated with GDFC, which consists not only in eliminating or reducing ASD-type mental disorders, but also in improving extrapyramidal disorders, obsessive-compulsive syndrome, intestinal disorders and epileptiform brain activity. Such a broad clinical effect of the used immunobiological agent can be explained by similar immune-dependent mechanisms of development of, at first glance, different clinical manifestations of the disease. Previously, a broad clinical phenotype, including epilepsy, intestinal disorders, autoimmune disorders, delayed-type hypersensitivity and deficiency of specific antipolysaccharide antibodies, in ASD in children was reported by Jyonouchi H. et al. in the results of a specially designed study [26].

The presence of multiple reactivated viral infections in SG children can be fully explained by the existing deficiency of NK and/or NKT cells. Previously, Binstock T. identified a specific subgroup of children with ASD who had pathologically reduced resistance to intramonocytic pathogens [4], and Nicolson G.L. et al. found an abnormally high frequency of detection of Mycoplasma ssp., Chlamydia pneumoniae and HHV-6 DNA in the blood of such children [41]. As it seems obvious, we were talking about cases of ASD disorders associated with GDFC, in which a primary deficiency of NK and/or NKT cells is noted. Viral agents can induce delayed myelination/demyelination in the brain, as demonstrated by Kamei A. et al. in the case of primary HHV-6 infection [27], and Pinillos-Pisón R. et al. in the case of CMV reactivation from a latent state [44]. Accordingly, there are a number of descriptions of the development of the autism phenotype after viral encephalitis in previously mentally healthy people [19, 24, 35].

In addition, through the mechanism of molecular mimicry, viruses may be involved in the phenomenon of anti-brain autoantibody production in children with ASD. Thus, Singh V.K. et al. demonstrated a consistent association between the presence of measles virus or HHV-6 in a reactivated state and the production

of autoantibodies to brain antigens in children with ASD, including myelin in the white matter of the cerebral hemispheres [60]. Another study showed cross-reactivity between anti-measles antibodies and autoantibodies to myelin basic protein in children with ASD [59]. In the context of these data, we consider it extremely useful that high-dose intravenous human normal immunoglobulin led to a gradual but steady decrease in the total viral load caused by lymphotropic herpesviruses in blood leukocytes among SG patients (**Fig. 13.3**), which did not occur in CG ($p < 0,05$: $Z < Z_{0,05}$).

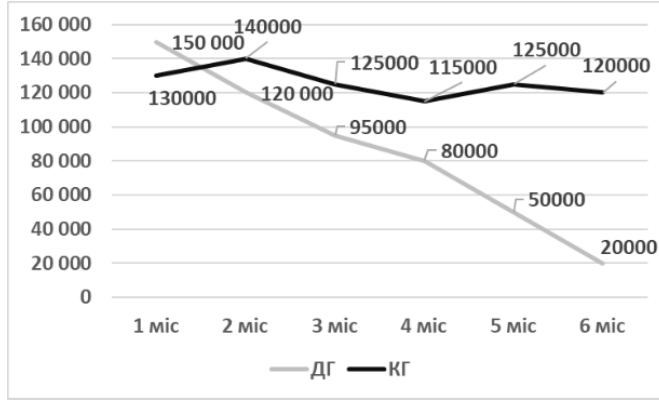

Fig. 13.3. Dynamics of total viral load in blood leukocytes caused by lymphotropic herpesviruses (EBV, CMV, HHV-6, HHV-7), according to PCR data in patients of SG (n = 225) and CG (n = 50) during the observation period

Currently, intravenous human normal immunoglobulin is routinely used for the prevention of reactivated opportunistic viral infections in immunocompromised individuals with a level of evidence A–C depending on the nosology. Thus, Cowan J. et al. recently conducted a systematic review of controlled clinical trials devoted to the study of the effectiveness of immunotherapy for the prevention of viral infections in recipients of allogeneic hematopoietic blood cells, demonstrating a clear benefit from the use of immunotherapy [12].

Also, this study revealed a gradual increase in the previously pathologically reduced absolute number of NK cells in the peripheral blood of SG patients, which was delayed and most pronounced only at 5–6 months of the course of immunotherapy (**Fig. 13.4**). Previously, Finberg R.W. et al. demonstrated that high-dose immunoglobulin therapy promotes an increase in the functional activity of NK cells in humans, most likely through immunomodulation due to the effect on the Fc receptors of these lymphocytes [17]. At the same time, the use of medium- and low-dose immunotherapy regimens (400–800 mg/kg/month) leads, on the contrary, to a decrease in the number and activity of natural killer cells, as demonstrated by Ruiz J.E. et al. in a clinical study involving women with multiple episodes of spontaneous abortions associated with immune dysregulation [53].

In addition, the used normal human intravenous immunoglobulin helped to compensate for hypo- or dysimmunoglobulinemia observed in many SG children, i.e., it implemented an immunoreplacement effect

on the humoral component of the immunodeficiency caused by GDFC. As is known, preparations of normal human intravenous immunoglobulin are now routinely used for replacement purposes in the treatment of primary hypoimmunoglobulinemia in people with a level of evidence B [2]. Previously, Heuer L. et al. established that reduced levels of immunoglobulins in the blood in ASD closely correlate with the severity of clinical manifestations of mental disorders in children [23]. Thus, the used high-dose immunoglobulin therapy helped to compensate or, at least, subcompensate for GDFC-induced specific immunodeficiency in SG children by immunomodulation and immune replacement.

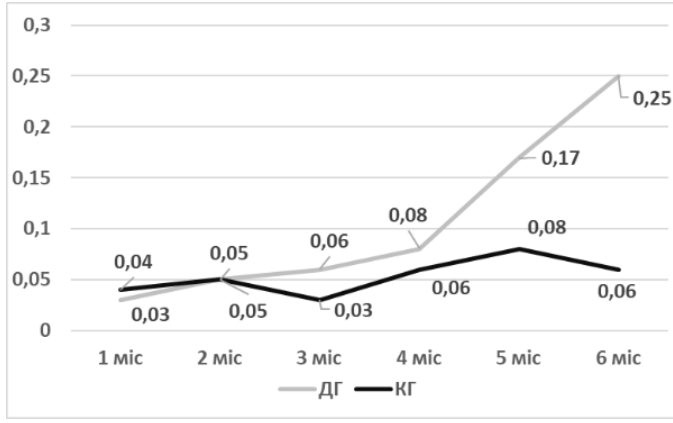

Fig. 13.4. Dynamics of the absolute number of NK cells in the peripheral blood of patients SG (n = 225) and CG (n = 50) during the observation period

Finally, in this clinical study, a clear positive dynamics was obtained in the severity of MR signs of leukoencephalopathy, which was noted in the majority of children with ASD. Previously, Strunk T. et al. described the phenomenon of abnormally facilitated demyelination of the white matter conductors of the cerebral hemispheres in GDFC [62]. Complete or partial elimination of MR signs of pre-existing leukoencephalopathy was observed in 69 % of 88 % of cases in SG (**Fig. 13.5**). The absence of positive changes in MR signs of leukoencephalopathy at the end of the course of immunotherapy, which were registered in 19 % of cases among SG patients, was associated with a slight clinical improvement in the manifestations of mental disorders and a high risk of the return of eliminated ASD symptoms after the cessation of the course of immunotherapy. In CG, moderate positive dynamics in MR signs of leukoencephalopathy were noted only in 15 % of 83 % of cases ($p < 0,05$: $Z < Z_{0,05}$) (**Table 13.2**), which apparently reflected the natural course of the disease. The obtained positive neuroradiological effect of immunotherapy can be explained by the antimicrobial and immunomodulatory properties of normal human intravenous immunoglobulin, in particular, the ability demonstrated above to reduce the abnormally high microbial load formed by lymphotropic herpesviruses, and the ability to suppress autoimmune reactions against CNS myelin autoantigens.

It is known that high-dose intravenous human normal immunoglobulin is able to stimulate remyelination of peripheral nerve fibers in Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy (evidence level A), which is associated with the suppression of the autoimmune reaction under the influence of the immunobiological drug, which is the main pathogenetic link in the development of these diseases [7]. However, Ciric B. et al. described a direct stimulating effect of intravenous human normal immunoglobulin on the process of remyelination of peripheral nerve fibers, independent of immunomodulation, which is believed to be associated with the direct effect of the IgG molecules of the drug on the Fc receptors of Schwann cells, which are myelin producers [10].

In this clinical study, a pronounced potentiating effect of high-dose intravenous immunoglobulin therapy on the process of myelination/remyelination of nerve conductors in the white matter of the cerebral hemispheres in children with ASD associated with GDFC, according to brain MRI in conventional modes, was found. In our opinion, it is this immunotherapy-induced neurorehabilitation phenomenon that can largely explain the positive modifying therapeutic effect of the immunotherapy on the main clinical manifestations of ASD in SG children according to the ABC scale.

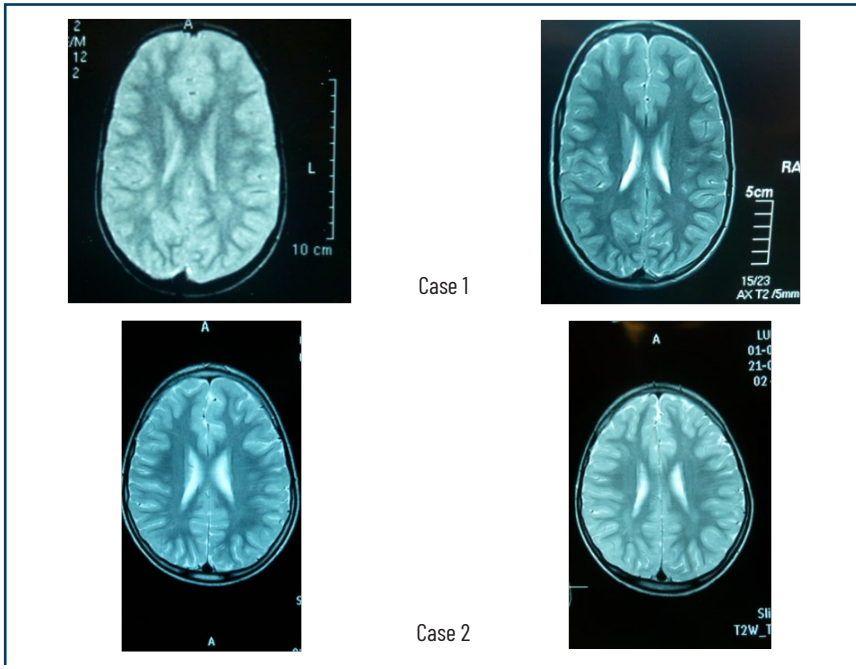

Fig. 13.5. Reduction of periventricular areas of impaired myelination in the parietal lobes of the cerebral hemispheres after a 6-month course of high-dose intravenous immunoglobulin therapy in 2 patients with ASD associated with GDFC (MRI images of the brain in axial projection, T2-weighted mode; 1.5 T; own observations) (left – before immunotherapy, right – after a course of intravenous immunoglobulin; own observations)

The achieved changes in all endpoints of this clinical study in the observation groups are summarized in **Table 13.2**.

Table 13.2 Differences in study endpoints between SG (n = 225) and CG patients (n = 50)

End point	SG, %		CG, %		T-test (parametric)	Number of characters Z (non-parametric)
	+	-	+	-		
ASD	69	31	37	64	$p < 0.05$*	$Z < Z0.05$*
PANDAS	27	5	0	100	$p < 0.05$*	$Z < Z0.05$*
Epileptic syndrome	33	10	12	28	$p < 0.05$*	$Z < Z0.05$*
Intestinal syndrome	69	13	25	59	$p < 0.05$*	$Z < Z0.05$*
Motion disorders	7	14	5	14	$p > 0.05$	$Z > Z0.05$
Herpesvirus load in blood leukocytes	61	21	19	56	$p < 0.05$*	$Z < Z0.05$*
Number of NK cells in blood	72	16	15	71	$p < 0.05$*	$Z < Z0.05$*
MRI signs of leukoencephalopathy	69	19	15	58	$p < 0.05$*	$Z < Z0.05$*

*Note. * – reliable differences*

In this clinical study, human normal immunoglobulin IV has proven to be a safe drug with satisfactory tolerance. Transient flu-like syndrome during infusions of this immunobiological agent was noted in only 74 of 225 SG patients (33 % of cases). Single episodes of vomiting occurred in 29 SG children (13 % of cases) shortly after administering the IV immunoglobulin. These mild side effects were not an obstacle to continuing the course of immunotherapy. No other adverse events were recorded in SG children during the use of human normal immunoglobulin IV.

Previously, Price C.S. et al. in a specially designed clinical study demonstrated that preparations of normal human intravenous immunoglobulin are safe and do not contribute to the development of autism in children [47]. Accordingly, Croen L.A. et al. showed that the use of anti-Rhesus immunoglobulin for the prevention of hemolytic disease of the fetus also does not increase the risk of developing autistic disorders in children [13]. As indicated by Wynn J.L. et al., the use of high-dose intravenous immunoglobulin therapy not only does not suppress the development of the child's immune system, but also promotes accelerated maturation of the immature immune system in premature children [66].

Conclusions. Thus, the fact of high clinical efficacy and adequate safety of intravenous immunoglobulin therapy at a dose of 2 g/kg/month in children with ASD associated with GDFC has been established. Such treatment leads not only to the elimination or at least to the weakening of existing mental disorders, but also to the improvement of additional extrapyramidal, epileptic and intestinal disorders. The polymodal positive clinical effect of intravenous normal human immunoglobulin is apparently associated with the well-known immunoreplacement, immunomodulatory, antimicrobial and anti-inflammatory effects of the

drug and is associated with a sharp decrease in the viral load in the blood, an increase in the previously critically reduced absolute number of natural killer cells and the elimination of radiological manifestations of leukoencephalopathy in such children. It has been previously noted that GDFC patients with ASD have a specific primary immunodeficiency that appears to be the direct cause of a broad clinical phenotype of immune-related manifestations, including psychiatric, extrapyramidal, epileptic, motor, cognitive, intestinal, infectious, autoimmune, and allergic syndromes, as well as leukoencephalopathy. High-dose intravenous human normal immunoglobulin has a complex (polymodal) positive effect on most components of the broad clinical phenotype in children with GDFC. This treatment approach contributes to the compensation or, at least, subcompensation of the diverse immune-related cerebral and extracerebral clinical manifestations of the primary immunodeficiency associated with GDFC, and not only reduces the symptoms of ASD.

Current clinical guidelines do not support the use of immunoglobulin therapy in children with ASD [28], although we strongly believe that such a therapeutic strategy can be tried in many patients with resistance to other treatment approaches, especially in children with GDFC in the case of positive clinical, laboratory and instrumental biomarkers of immune-dependent inflammatory encephalopathy. Given the encouraging results of this retrospective analysis of clinical cases, it is advisable to continue research in this direction with a larger number of participants and a more refined design.

REFERENCES

1. Amman, M. G., Singh, N. N., Stewart, A. W., Field, C. J. (1985). Psychometric characteristics of the aberrant behavior checklist. Journal of Mental Deficiency, 89, 492–502.
2. Baris, S., Ercan, H., Cagan, H. H. (2011). Efficacy of intravenous immunoglobulin treatment in children. with common variable immunodeficiency. Immunol, 21 (7), 514–521.
3. Billiau, A. D., Witters, P., Ceulemans, B., Kasran, A., Wouters, C., & Lagae, L. (2007). Intravenous Immunoglobulins in Refractory Childhood-Onset Epilepsy: Effects on Seizure Frequency, EEG Activity, and Cerebrospinal Fluid Cytokine Profile. Epilepsia, 48 (9), 1739–1749. https://doi.org/10.1111/j.1528-1167.2007.01134.x
4. Binstock, T. (2001). Intra-monocyte pathogens delineate autism subgroups. Medical Hypotheses, 56 (4), 523–531. https://doi.org/10.1054/mehy.2000.1247
5. Boris, M., Goldblatt, A., Edelson, S. M. (2005). Improvement in children with autism treated with intravenous gamma globulin. Journal of Nutritional & Environmental Medicine, 15 (4), 169–176. https://doi.org/10.1080/13590840600681827
6. Bradstreet, J., Singh, V. K., El-Dahr, J. (1999). High dose intravenous immunoglobulin improves symptoms in children with autism. The international symposium on autism. Atnhem.
7. Buchwald, B., Ahangari, R., Weishaupt, A., Toyka, K. V. (2002). Intravenous immunoglobulins neutralize blocking antibodies in Guillain-Barré syndrome. Annals of Neurology, 51 (6), 673–680. https://doi.org/10.1002/ana.10205
8. Cabanlit, M., Wills, S., Goines, P., Ashwood, P., Van de Water, J. (2007). Brain-Specific Autoantibodies in the Plasma of Subjects with Autistic Spectrum Disorder. Annals of the New York Academy of Sciences, 1107 (1), 92–103. https://doi.org/10.1196/annals.1381.010

9. Chez, M. G., Guido-Estrada, N. (2010). Immune Therapy in Autism: Historical Experience and Future Directions with Immunomodulatory Therapy. Neurotherapeutics, 7 (3), 293–301. https://doi.org/10.1016/j.nurt.2010.05.008
10. Ciric, B., Van Keulen, V., Paz Soldan, M., Rodriguez, M., Pease, L. R. (2004). Antibody-mediated remyelination operates through mechanism independent of immunomodulation. Journal of Neuroimmunology, 146 (1-2), 153–161. https://doi.org/10.1016/j.jneuroim.2003.11.002
11. Connery, K., Tippett, M., Delhey, L. M., Rose, S., Slattery, J. C., Kahler, S. G. et al. (2018). Intravenous immunoglobulin for the treatment of autoimmune encephalopathy in children with autism. Translational Psychiatry, 8 (1). https://doi.org/10.1038/s41398-018-0214-7
12. Cowan, J., Cameron, D. W., Knoll, G., Tay, J. (2015). Protocol for updating a systematic review of randomised controlled trials on the prophylactic use of intravenous immunoglobulin for patients undergoing haematopoietic stem cell transplantation. BMJ Open, 5 (8), e008316. https://doi.org/10.1136/bmjopen-2015-008316
13. Croen, L. A., Matevia, M., Yoshida, C. K., Grether, J. K. (2008). Maternal Rh D status, anti-D immune globulin exposure during pregnancy, and risk of autism spectrum disorders. American Journal of Obstetrics and Gynecology, 199 (3), 234.e1-234.e6. https://doi.org/10.1016/j.ajog.2008.04.044
14. DelGiudice-Asch, G., Simon, L., Schmeidler, J., Cunningham-Rundles, C., Hollander, E. (1999). Brief report: a pilot open clinical trial of intravenous immunoglobulin in childhood autism. Journal of Autism and Developmental Disorders, 29 (2), 157–160. https://doi.org/10.1023/a:1023096728131
15. Donati, D., Akhyani, N., Fogdell-Hahn, A., Cermelli, C., Cassiani-Ingoni, R., Vortmeyer, A. et al. (2003). Detection of human herpesvirus-6 in mesial temporal lobe epilepsy surgical brain resections. Neurology, 61 (10), 1405–1411. https://doi.org/10.1212/01.wnl.0000094357.10782.f9
16. Engman, M., Sundin, M., Miniscalco, C., Westerlund, J., Lewensohn-Fuchs, I., Gillberg, C., Fernell, E. (2015). Prenatal acquired cytomegalovirus infection should be considered in children with autism. Acta Paediatrica, 104 (8), 792–795. Portico. https://doi.org/10.1111/apa.13032
17. Finberg, R. W., Newburger, J. W., Mikati, M. A., Heller, A. H., Burns, J. C. (1992). Effect of high doses of intravenously administered immune globulin on natural killer cell activity in peripheral blood. The Journal of Pediatrics, 120 (3), 376–380. https://doi.org/10.1016/s0022-3476(05)80900-x
18. Frye, R. E. (2015). Metabolic and mitochondrial disorders associated with epilepsy in children with autism spectrum disorder. Epilepsy & Behavior, 47, 147–157. https://doi.org/10.1016/j.yebeh.2014.08.134
19. Ghaziuddin, M., Al-Khouri, I., Ghaziuddin, N. (2002). Autistic symptoms following herpes encephalitis. European Child & Adolescent Psychiatry, 11 (3), 142–146. https://doi.org/10.1007/s00787-002-0271-5
20. Guo, B.-Q., Li, H.-B., Ding, S.-B. (2020). Blood homocysteine levels in children with autism spectrum disorder: An updated systematic review and meta-analysis. Psychiatry Research, 291, 113283. https://doi.org/10.1016/j.psychres.2020.113283
21. Gupta, S. (1999). Treatment of children with autism with intravenous immunoglobulin. Journal of Child Neurology, 14 (3), 203–205. https://doi.org/10.1177/088307389901400314

22. Handen, B. L., Melmed, R. D., Hansen, R. L., Aman, M. G., Burnham, D. L., Bruss, J. B., McDougle, C. J. (2009). A Double-Blind, Placebo-Controlled Trial of Oral Human Immunoglobulin for Gastrointestinal Dysfunction in Children with Autistic Disorder. Journal of Autism and Developmental Disorders, 39 (5), 796–805. https://doi.org/10.1007/s10803-008-0687-y
23. Heuer, L., Ashwood, P., Schauer, J., Goines, P., Krakowiak, P., Hertz-Picciotto, I. et al. (2008). Reduced levels of immunoglobulin in children with autism correlates with behavioral symptoms. Autism Research, 1 (5), 275–283. https://doi.org/10.1002/aur.42
24. Hiroshi, H., Seiji, K., Toshihiro, K., Nobuo, K. (2003). An adult case suspected of recurrent measles encephalitis with psychiatric symptoms. Seishin Shinkeigaku Zasshi, 105 (10), 1239–1246.
25. Hughes, H. K., Mills Ko, E., Rose, D., Ashwood, P. (2018). Immune Dysfunction and Autoimmunity as Pathological Mechanisms in Autism Spectrum Disorders. Frontiers in Cellular Neuroscience, 12. https://doi.org/10.3389/fncel.2018.00405
26. Jyonouchi, H., Geng, L., Streck, D. L., Toruner, G. A. (2012). Immunological characterization and transcription profiling of peripheral blood (PB) monocytes in children with autism spectrum disorders (ASD) and specific polysaccharide antibody deficiency (SPAD): case study. Journal of Neuroinflammation, 9 (1). https://doi.org/10.1186/1742-2094-9-4
27. Kamei, A., Ichinohe, S., Onuma, R., Hiraga, S., Fujiwara, T. (1997). Acute disseminated demyelination due to primary human herpesvirus-6 infection. European Journal of Pediatrics, 156 (9), 709–712. https://doi.org/10.1007/s004310050695
28. Kooij, S. J., Bejerot, S., Blackwell, A., Caci, H., Casas-Brugué, M., Carpentier, P. J. et al. (2010). European consensus statement on diagnosis and treatment of adult ADHD: The European Network Adult ADHD. BMC Psychiatry, 10 (1). https://doi.org/10.1186/1471-244x-10-67
29. Li, Y., Qiu, S., Shi, J., Guo, Y., Li, Z., Cheng, Y., Liu, Y. (2020). Association between MTHFR C677T/A1298C and susceptibility to autism spectrum disorders: a meta-analysis. BMC Pediatrics, 20 (1). https://doi.org/10.1186/s12887-020-02330-3
30. Maltsev, D., Natrus, L. (2020). The Effectiveness of Infliximab in Autism Spectrum Disorders Associated with Folate Cycle Genetic Deficiency. Psychiatry, Psychotherapy and Clinical Psychology, 3, 583–594. https://doi.org/10.34883/pi.2020.11.3.015
31. Maltsev, D. (2021). Efficacy of Rituximab in Autism Spectrum Disorders Associated with Genetic Folate Cycle Deficiency with Signs of Antineuronal Autoimmunity. Psychiatry, Psychotherapy and Clinical Psychology, 3, 472–486. https://doi.org/10.34883/pi.2021.12.3.010
32. Maltsev D.V. Efficiency of a high dose of intravenous immunoglobulin in children with autistic spectrum disorders associated with genetic deficiency of folate cycle enzymes // Journal of global pharma technology. – 2019. – Vol. 11(05). – P. 597–609.
33. Maltsev, D. (2020). Features of folate cycle disorders in children with ASD. Bangladesh Journal of Medical Science, 19 (4), 737–742. https://doi.org/10.3329/bjms.v19i4.46634
34. Marchezan, J., Winkler dos Santos, E. G. A., Deckmann, I., Riesgo, R. dos S. (2018). Immunological Dysfunction in Autism Spectrum Disorder: A Potential Target for Therapy. Neuroimmunomodulation, 25 (5-6), 300–319. https://doi.org/10.1159/000492225

35. Marques, F., Brito, M. J., Conde, M., Pinto, M., Moreira, A. (2014). Autism Spectrum Disorder Secondary to Enterovirus Encephalitis. Journal of Child Neurology, 29 (5), 708–714. https://doi.org/10.1177/0883073813508314
36. Masi, A., Quintana, D. S., Glozier, N., Lloyd, A. R., Hickie, I. B., Guastella, A. J. (2014). Cytokine aberrations in autism spectrum disorder: a systematic review and meta-analysis. Molecular Psychiatry, 20 (4), 440–446. https://doi.org/10.1038/mp.2014.59
37. Melamed, I. R., Heffron, M., Testori, A., Lipe, K. (2018). A pilot study of high-dose intravenous immunoglobulin 5 % for autism: Impact on autism spectrum and markers of neuroinflammation. Autism Research, 11 (3), 421–433. https://doi.org/10.1002/aur.1906
38. Shaik Mohammad, N., Sai Shruti, P., Bharathi, V., Krishna Prasad, C., Hussain, T., Alrokayan, S. A. et al. (2016). Clinical utility of folate pathway genetic polymorphisms in the diagnosis of autism spectrum disorders. Psychiatric Genetics, 26 (6), 281–286. https://doi.org/10.1097/ypg.0000000000000152
39. Monge Galindo, L., Pérez Delgado, R., López Pisón, J., Lafuente Hidalgo, M., Ruiz del Olmo Izuzquiza, I., Peña Segura, J. L. (2010). Mesial temporal sclerosis in paediatrics: its clinical spectrum. Our experience gained over a 19-year period. Revista de Neurología, 50 (6), 341–348. https://doi.org/10.33588/rn.5006.2009448
40. Mostafa, G. A., AL-ayadhi, L. Y. (2011). Increased serum levels of anti-ganglioside M1 auto-antibodies in autistic children: relation to the disease severity. Journal of Neuroinflammation, 8 (1), 39. https://doi.org/10.1186/1742-2094-8-39
41. Nicolson, G. L., Gan, R., Nicolson, N. L., Haier, J. (2007). Evidence for Mycoplasma ssp., Chlamydia pneunomiae, and human herpes virus-6 coinfections in the blood of patients with autistic spectrum disorders. Journal of Neuroscience Research, 85 (5), 1143–1148. https://doi.org/10.1002/jnr.21203
42. Niederhofer, H., Staffen, W., Mair, A. (2002). Immunoglobulins as an Alternative Strategy of Psychopharmacological Treatment of Children with Autistic Disorder. Neuropsychopharmacology, 28 (5), 1014–1015. https://doi.org/10.1038/sj.npp.1300130
43. Perlmutter, S. J., Leitman, S. F., Garvey, M. A., Hamburger, S., Feldman, E., Leonard, H. L., Swedo, S. E. (1999). Therapeutic plasma exchange and intravenous immunoglobulin for obsessive-compulsive disorder and tic disorders in childhood. The Lancet, 354 (9185), 1153–1158. https://doi.org/10.1016/s0140-6736(98)12297-3
44. Pinillos Pisón, R., Llorente Cereza, M. T., López Pisón, J., Pérez Delgado, R., Lafuente Hidalgo, M., Martínez Sapiñá, A., Peña Segura, J. L. (2009). Congenital infection by cytomegalovirus. A review of our 18 years' experience of diagnoses. Revista de Neurología, 48 (7), 349–353. https://doi.org/10.33588/rn.4807.2008391
45. Plebani, A., Duse, M., Tiberti, S., Avanzini, M. A., Monafo, V., Menegati, E. et al. (1988). Intravenous gamma-globulin therapy and serum IgG subclass levels in intractable childhood epilepsy. Monogr. Allergy, 23, 204–215.
46. Plioplys, A. V. (1998). Intravenous Immunoglobulin Treatment of Children With Autism. Journal of Child Neurology, 13 (2), 79–82. https://doi.org/10.1177/088307389801300207

47. Price, C. S., Thompson, W. W., Goodson, B., Weintraub, E. S., Croen, L. A., Hinrichsen, V. L. et al. (2010). Prenatal and Infant Exposure to Thimerosal From Vaccines and Immunoglobulins and Risk of Autism. Pediatrics, 126 (4), 656–664. https://doi.org/10.1542/peds.2010-0309
48. Pu, D., Shen, Y., Wu, J. (2013). Association between MTHFR Gene Polymorphisms and the Risk of Autism Spectrum Disorders: A Meta-Analysis. Autism Research, 6 (5), 384–392. https://doi.org/10.1002/aur.1300
49. Puangpetch, A., Suwannarat, P., Chamnanphol, M., Koomdee, N., Ngamsamut, N., Limsila, P., Sukasem, C. (2015). Significant Association of HLA-BAlleles and Genotypes in Thai Children with Autism Spectrum Disorders: A Case-Control Study. Disease Markers, 2015, 1–7. https://doi.org/10.1155/2015/724935
50. Rai, V. (2016). Association of methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism with autism: evidence of genetic susceptibility. Metabolic Brain Disease, 31 (4), 727–735. https://doi.org/10.1007/s11011-016-9815-0
51. Reinert, P., Moulias, R., Goust, J. M. (1972). Demonstration of cellular immunity deficiency limited to measles virus in 20 cases of subacute sclerosing leukoencephalitis. Archives Francaises de Pediatrie, 29 (6), 655–665.
52. Rossignol, D. A., Frye, R. E. (2021). A Systematic Review and Meta-Analysis of Immunoglobulin G Abnormalities and the Therapeutic Use of Intravenous Immunoglobulins (IVIG) in Autism Spectrum Disorder. Journal of Personalized Medicine, 11 (6), 488. https://doi.org/10.3390/jpm11060488
53. Ruiz, J. E., Kwak, J. Y. H., Baum, L., Gilman-Sachs, A., Beaman, K. D., Kim, Y. B., Beer, A. E. (1996). Intravenous Immunoglobulin Inhibits Natural Killer Cell Activity In Vivo in Women With Recurrent Spontaneous Abortion. American Journal of Reproductive Immunology, 35 (4), 370–375. https://doi.org/10.1111/j.1600-0897.1996.tb00496.x
54. Russo, A., Krigsman, A., Jepson, B., Wakefield, A. (2009). Low serum myeloperoxidase in autistic children with gastrointestinal disease. Clinical and Experimental Gastroenterology, 2, 85–94. https://doi.org/10.2147/ceg.s6051
55. Sadeghiyeh, T., Dastgheib, S. A., Mirzaee-Khoramabadi, K., Morovati-Sharifabad, M., Akbarian-Bafghi, M. J., Poursharif, Z. et al. (2019). Association of MTHFR 677C > T and 1298A > C polymorphisms with susceptibility to autism: A systematic review and meta-analysis. Asian Journal of Psychiatry, 46, 54–61. https://doi.org/10.1016/j.ajp.2019.09.016
56. Saghazadeh, A., Ataeinia, B., Keynejad, K., Abdolalizadeh, A., Hirbod-Mobarakeh, A., Rezaei, N. (2019). A meta-analysis of pro-inflammatory cytokines in autism spectrum disorders: Effects of age, gender, and latitude. Journal of Psychiatric Research, 115, 90–102. https://doi.org/10.1016/j.jpsychires.2019.05.019
57. Santaella, M. L., Varela, Y., Linares, N., Disdier, O. M. (2008). Prevalence of autism spectrum disorders in relatives of patients with selective immunoglobulin A deficiency. Puerto Rico Health Sciences Journal, 27 (3), 204–208.
58. Schneider, C. K., Melmed, R. D., Barstow, L. E., Enriquez, F. J., Ranger-Moore, J., Ostrem, J. A. (2006). Oral Human Immunoglobulin for Children with Autism and Gastrointestinal Dysfunction: A Prospective, Open-Label Study. Journal of Autism and Developmental Disorders, 36 (8), 1053–1064. https://doi.org/10.1007/s10803-006-0141-y

59. Singh, V. K., Lin, S. X., Newell, E., Nelson, C. (2002). Abnormal measles-mumps-rubella antibodies and CNS autoimmunity in children with autism. Journal of Biomedical Science, 9 (4), 359–364. https://doi.org/10.1007/bf02256592
60. Singh, V. K., Lin, S. X., Yang, V. C. (1998). Serological Association of Measles Virus and Human Herpesvirus-6 with Brain Autoantibodies in Autism. Clinical Immunology and Immunopathology, 89 (1), 105–108. https://doi.org/10.1006/clin.1998.4588
61. Singh, V. K., Warren, R. P., Odell, J. D., Warren, W. L., Cole, P. (1993). Antibodies to Myelin Basic Protein in Children with Autistic Behavior. Brain, Behavior, and Immunity, 7 (1), 97–103. https://doi.org/10.1006/brbi.1993.1010
62. Strunk, T., Gottschalk, S., Goepel, W., Bucsky, P., Schultz, C. (2002). Subacute leukencephalopathy after low-dose intrathecal methotrexate in an adolescent heterozygous for the MTHFR C677T polymorphism. Medical and Pediatric Oncology, 40 (1), 48–50. https://doi.org/10.1002/mpo.10192
63. Torrente, F., Ashwood, P., Day, R., Machado, N., Furlano, R. I., Anthony, A. et al. (2002). Small intestinal enteropathy with epithelial IgG and complement deposition in children with regressive autism. Molecular Psychiatry, 7 (4), 375–382. https://doi.org/10.1038/sj.mp.4001077
64. Warren, R. P., Odell, J. D., Warren, W. L., Burger, R. A., Maciulis, A., Daniels, W. W., Torres, A. R. (1997). Brief report: immunoglobulin A deficiency in a subset of autistic subject. Journal of Autism and Developmental Disorders, 27(2), 187–192. https://doi.org/10.1023/a:1025895925178
65. Wasilewska, J., Kaczmarski, M., Stasiak-Barmuta, A., Tobolczyk, J., Kowalewska, E. (2012). Low serum IgA and increased expression of CD23 on B lymphocytes in peripheral blood in children with regressive autism aged 3-6 years old. Archives of Medical Science, 2, 324–331. https://doi.org/10.5114/aoms.2012.28561
66. Wynn, J. L., Seed, P. C., Cotten, C. M. (2010). Does IVIg administration yield improved immune function in very premature neonates? Journal of Perinatology, 30 (10), 635–642. https://doi.org/10.1038/jp.2009.197
67. Yektaş, Ç., Alpay, M., Tufan, A. E. (2019). Comparison of serum B12, folate and homocysteine concentrations in children with autism spectrum disorder or attention deficit hyperactivity disorder and healthy controls. Neuropsychiatric Disease and Treatment, 15, 2213–2219. https://doi.org/10.2147/ndt.s212361