

Dmytro Maltsev

IMMUNODIAGNOSTICS AND IMMUNOTHERAPY OF NEUROPSYCHIATRIC DISORDERS IN CHILDREN

Monograph

UDC 612:616
M21

Published in 2025
by TECHNOLOGY CENTER PC®
Kharkiv, Ukraine

M21

Author:

Dmytro Maltsev

Immunodiagnosics and immunotherapy of neuropsychiatric disorders in children: monograph / D. Maltsev. – Kharkiv: TECHNOLOGY CENTER PC, 2025. – 208 p.

This scientific monograph presents a review of the current literature and the results of original controlled clinical studies on the immunogenetic, biochemical, microbiological, immunological and rheumatological aspects of the pathogenesis of neuropsychiatric syndromes in children, such as autism spectrum disorders, attention-deficit/hyperactivity disorder and other common mental disorders. The data of this scientific monograph complement and systematize existing approaches to the diagnosis and immunotherapy of immune-dependent lesions of the body of children, leading to psychiatric diseases. The presented scientific work puts forward a folate-centric concept of the pathogenesis of neuropsychiatric syndromes and develops an original approach to clinical patient management under the acronym GBINC, which is an important step forward in the fight against the threatening epidemic of mental disorders in the modern child population. Figures 59, Tables 26, References 207 items.

This book contains information obtained from authentic and highly regarded sources. Reasonable efforts have been made to publish reliable data and information, but the author and publisher cannot assume responsibility for the validity of all materials or the consequences of their use. The authors and publishers have attempted to trace the copyright holders of all material reproduced in this publication and apologize to copyright holders if permission to publish in this form has not been obtained. If any copyright material has not been acknowledged please write and let us know so we may rectify in any future reprint.

The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made.

Trademark Notice: product or corporate names may be trademarks or registered trademarks, and are used only for identification and explanation without intent to infringe.

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

ISBN 978-617-8360-21-4 (on-line)

ISBN 978-617-7319-76-3 (print)

Cite as: Maltsev, D. (2025). Immunodiagnosics and immunotherapy of neuropsychiatric disorders in children: monograph. Kharkiv: TECHNOLOGY CENTER PC, 208. doi: <http://doi.org/10.15587/978-617-8360-21-4>



Copyright © Author(s) 2025
This is an open access paper under
the Creative Commons Attribution 4.0
International License (CC BY 4.0)


AUTHOR

DMYTRO MALTSEV

¹ PhD, Neuroimmunologist, Head of Laboratory
Laboratory of Immunology and Molecular Biology
Research Institute of Experimental and Clinical Medicine

² Associate Professor
Department of Modern Technologies of Medical Diagnostics and
Treatment

Bogomolets National Medical University

 ORCID: <https://orcid.org/0000-0002-6615-3072>

ABSTRACT

This scientific monograph presents a review of the current literature and the results of original controlled clinical studies on the immunogenetic, biochemical, microbiological, immunological and rheumatological aspects of the pathogenesis of neuropsychiatric syndromes in children, such as autism spectrum disorders, attention-deficit/hyperactivity disorder and other common mental disorders. The data of this scientific monograph complement and systematize existing approaches to the diagnosis and immunotherapy of immune-dependent lesions of the body of children, leading to psychiatric diseases. The presented scientific work puts forward a folate-centric concept of the pathogenesis of neuropsychiatric syndromes and develops an original approach to clinical patient management under the acronym GBINC, which is an important step forward in the fight against the threatening epidemic of mental disorders in the modern child population.

CONTENTS

List of Tables	viii
List of Figures.....	ix
Circle of readers and scope of application.....	x
Introduction	1
CHAPTER 1. Immunogenetic aspects of the pathogenesis of disease in children with autism spectrum disorders (review)	2
Association study of genetic deficiency of the folate cycle (GDFC) and ASD.....	2
Oxidative stress in ASD	3
Immune system status in ASD	4
Infectious syndrome in ASD.....	6
Autoimmune syndrome in ASD	6
Allergic syndrome in ASD	9
Immunoinflammatory syndrome in ASD.....	11
Immunodependent treatment approaches for ASD	11
Conclusions to the Section 1.....	13
References	13
CHAPTER 2. Results of a study of biochemical profile indicators in children with autism spectrum disorders associated with genetic deficiency of the folate cycle	19
Materials and methods of the research.....	20
Results and discussion of the research	21
Conclusions to the Section 2	27
References	27
CHAPTER 3. Results of the assessment of immune status in children with ASD: immunodeficiency associated with genetic deficiency of the folate cycle	30
Materials and methods of the research.....	32
Results and discussion of the research	34
Conclusions to the Section 3	45
References	45
CHAPTER 4. Results of the study of the microbial spectrum in children with autism spectrum disorders associated with genetic deficiency of the folate cycle	51

Materials and methods of the research.....	53
Results and discussion of the research	55
Conclusions to the Section 4	60
References.....	60
CHAPTER 5. Results of the search for laboratory signs of autoimmune reactions to brain and extracerebral autoantigens in children with autism spectrum disorders associated with genetic deficiency of the folate cycle	65
Materials and methods of the research.....	67
Results and discussion of the research	68
Conclusions to the Section 5	78
References.....	78
CHAPTER 6. Assessment of markers of inflammation and neuronal damage in patients with autism spectrum disorders associated with genetic deficiency of the folate cycle	83
References.....	89
CHAPTER 7. Neuroradiological signs of encephalopathy in children with autism spectrum disorders associated with genetic deficiency of the folate cycle	92
References.....	106
CHAPTER 8. Results of valacyclovir, valganciclovir, artesunate for the treatment of reactivated EBV-, HHV-6-, HHV-7-infections in children with autism spectrum disorders associated with genetic deficiency of the folate cycle	110
References.....	120
CHAPTER 9. Efficacy of combined immunotherapy with Propes and Inflamafertin for selective NK and NKT cell deficiency in children with autism spectrum disorders associated with genetic deficiency of the folate cycle	124
References.....	130
CHAPTER 10. Efficacy of combined use of transfer factors based on colostrum immune extract in NK and NKT cell deficiency in children with autism spectrum disorders associated with genetic deficiency of the folate cycle	132
References.....	143
CHAPTER 11. Efficacy of infliximab in autism spectrum disorders in children associated with genetic deficiency of the folate cycle	147
References.....	156

CHAPTER 12. Efficacy of rituximab in autism spectrum disorders associated with genetic deficiency of the folate cycle with signs of antineuronal autoimmunity	158
References	167
CHAPTER 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	170
References	184
CONCLUSIONS	190
PRACTICAL RECOMMENDATIONS	192

LIST OF TABLES

2.1	Mean values ($\bar{X} \pm m$) of the studied biochemical profile parameters in patients SG ($n = 225$) and CG ($n = 51$)	22
2.2	Results of the study of the association of disorders of the studied biochemical parameters (OR; 95 % CI) with the combination of identified polymorphisms of folate cycle genes SG ($n = 225$)	23
3.1	Comparative analysis of the average values of the studied immune status indicators in patients of the observation groups	34
3.2	Results of calculations of the chi-square test of immune status indicators when comparing patients SG ($n = 225$) and CG ($n = 51$)	39
3.3	Assessment of the φ criterion and other indicators of the strength of the relationship between GDFC and indicators of immune status in patients with SG ($n = 225$) and CG ($n = 51$)	39
3.4	Results of measuring odds ratio (OR) and 95 % confidence interval (95 % CI) in studying the association of GDFC and immune status indicators among SG ($n = 225$) and CG patients ($n = 51$)	40
3.5	Results of the study of associations (OR; 95 % CI) between biochemical abnormalities and immune factor deficiencies in SG patients ($n = 225$)	41
3.6	Results of measuring odds ratio (OR) and 95 % confidence interval (95 % CI) in studying the association of GDFC and immune status indicators among SG patients ($n = 225$)	43
4.1	Results of the association study (OR; 95 % CI) of immune disorders and identified microorganisms in children with ASD associated with GDFC ($n = 225$)	58
5.1	Results of the study of the association (OR; 95 % CI) of microbial agents and laboratory signs of autoimmune reactions among SG patients ($n = 225$)	75
6.1	Results of the association study (OR; 95 % CI) of the studied indicators of systemic inflammation and indicators of neuronal damage among SG patients ($n = 225$)	88
7.1	Results of the study of associations (OR; 95 % CI) between pathological radiological phenomena and clinical syndromes in children with GDFC associated with ASD ($n = 225$)	103
7.2	Results of the study of associations (OR; 95 % CI) between pathological radiological phenomena and the results of special laboratory tests in children with GDFC associated with ASD ($n = 225$)	104
8.1	Results of the association study (OR; 95 % CI) between antiviral treatment outcomes and serum concentrations of NSE and S-100 protein	116
9.1	Evaluation of Pearson's chi-square (χ^2) and other indicators of association between immunotherapy administration and normalization of immune status indicators in SG patients ($n = 225$)	128
9.2	Evaluation of the φ criterion and other indicators of the strength of the relationship between immunotherapy and normalization of immune status indicators in SG patients ($n = 225$)	128

LIST OF TABLES

9.3	Estimation of the odds ratio (OR) and other indicators of the association between immunotherapy and normalization of immune status indicators in SG patients ($n = 225$)	129
10.1	Results of OR, S and 95 % CI measurements in assessing the association of immunocorrective interventions and normalization of immune status indicators in SG patients ($n = 225$)	140
11.1	ABC scale scores in SG ($n = 225$) and CG patients ($n = 51$)	151
11.2	Results of calculating the Pearson chi-square (χ^2) test in SG ($n = 225$) when studying the association between serum TNF-alpha concentration and clinical outcome according to the ABC scale	154
11.3	Calculation of the strength of the relationship between serum TNF-alpha concentration and hyperactivity and hyperexcitability indicators according to the ABC scale in SG children ($n = 225$)	154
11.4	Differences in study endpoints between SG ($n = 225$) and CG patients ($n = 51$)	155
12.1	Results of the study of the association between the phenomenon of negativity of serological test results and normalization of serum concentrations of NSE and S-100 protein (OR; 95 % CI) in SG ($n = 62$)	165
12.2	ABC score in SG ($n = 62$) and CG ($n = 19$) patients after completion of rituximab immunotherapy	166
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	175
13.2	Differences in study endpoints between SG ($n = 225$) and CG patients ($n = 50$)	183

LIST OF FIGURES

1.1	Distribution of 677C > T rs1801133 and 1298A > C rs1801131 SNPs in CARS (childhood autism rating scale) < 37 and ≥ 37 cases	3
1.2	Forest plots of the effects of glutathione metabolism disorders in ASD, showing the association of GSH(A), GSSG(B), tGSH(C), GSH/GSSG(D), tGSH/GSSG(E) and ASD	4
1.3	Schematic diagram of migration of anti-brain autoantibodies from blood serum to the CNS in children with ASD	7
1.4	Schematic diagram of CNS damage in autoimmune anti-NMDA receptor limbic encephalitis in humans	8
1.5	Schematic diagram of the involvement of the atopic allergic mechanism in the pathogenesis of encephalopathy in ASD in children	10
2.1	Results of structural analyses of biochemical profile indicators among patients SG (n = 225) and CG (n = 51)	21
3.1	Structure of SG (n = 225) by NK and/or NKT cell deficiency	35
3.2	Structure of dysimmunoglobulinemias in SG patients (n = 225) by immunoglobulin class and subclass deficiencies	36
3.3	Structure of SG (n = 225) by detected disorders in immune status	37
3.4	Structure of SG (n = 225) according to the identified immunological phenotypes when assessing immune status	38
3.5	Results of structural analysis of SG (n = 225) and CG (n = 51) by major immune-dependent syndromes	42
4.1	Structure of SG (n = 225) and CG (n = 51) according to the detected microorganisms of the studied spectrum	56
5.1	Structure of SG (n = 225) compared with CG (n = 51) according to laboratory signs of autoimmunity to various autoantigens of the patient's body	69
5.2	Structure of subgroup 1 SG (n = 72) by type of serum autoantibodies to autoantigens of the subcortical ganglia of the CNS	70
5.3	Structure of subgroup 2 SG (n = 81) by type of serum autoantibodies to autoantigens of neurons of the mesolimbic system of the temporal lobes of the cerebral hemispheres	71
5.4	Structure of subgroup 3 SG (n = 97) by type of serum autoantibodies to autoantigens of myelin of the white matter of the cerebral hemispheres	72
5.5	Structure of subgroup 4 SG (n = 119) by type of serum autoantibodies to connective tissue nuclear autoantigens	73
5.6	Structure of subgroup 5 SG (n = 108) by type of serum autoantibodies to lumbar striated muscle autoantigens	74
6.1	Results of studying the structure of SG (n = 225) and CG (n = 51) in cases of elevated serum concentrations of the studied pro-inflammatory mediators	85

LIST OF FIGURES

6.2	Mean values ($X \pm m$) of serum TM2PC concentrations in SG ($n = 225$) and CG ($n = 51$)	87
6.3	Mean values ($X \pm m$) of serum concentrations of TNF-alpha and IL-6 in SG ($n = 225$) and CG ($n = 51$)	87
7.1	SG structure according to radiological phenotypes detected by neuroimaging ($n = 225$)	94
7.2	Heterogeneity of MR manifestations of leukoencephalopathy in SG children ($n = 225$)	95
7.3	Photo of a brain MRI image (left) in the FLAIR mode in the axial projection of a child with ASD associated with GDFC, demonstrating bilateral hyperintensity of the MRI signal in the hippocampal area and signs of atrophy of these structures due to vicarious expansion of local CSF pathways and photo of an EEG (right) of the same patient, showing epileptiform activity (pathological waves are circled) associated with the indicated neuroimaging changes (own observation)	97
7.4	Photo of T2-weighted axial MR images of the brain showing bilateral hypertrophy of the caudate subcortical nuclei with compression of both anterior horns of the lateral ventricles (right; lesions are circled by an oval) and FLAIR coronal images showing unilateral hypertrophy of the caudate nucleus with compression of the ipsilateral anterior horn of the lateral ventricle (left) in children with ASD associated with GDFC (own observations)	98
7.5	EEG signs of excitation in the subcortical ganglia of the cerebral hemispheres in a child with GDFC associated with ASD, who had MRI signs of subcortical ganglia hypertrophy and positive results of the Cunningham panel (PANDAS; pathological complexes are circled) (own observation)	98
7.6	EEG signs of bilateral lateralized synchronous electrical discharges (pathological complexes highlighted) in a child with GDFC associated with ASD, who had MRI signs of subcortical ganglia hypertrophy and positive results of the Cunningham panel (PANDAS; pathological complexes circled) (own observation)	99
7.7	EEG signs of diffuse hypersynchronization of cortical rhythms in a child with GDFC associated with ASD, who had MRI signs of subcortical ganglia hypertrophy and positive results of the Cunningham panel (PANDAS; own observation)	99
7.8	Typical neuroimaging signs of congenital cytomegalovirus neuroinfection in children with ASD associated with GDFC (own observations)	100
7.9	Photo of MR images of the brain in the FLAIR mode in coronal (left) and axial (right) projections in a child with ASD associated with GDFC, demonstrating signs of bilateral atrophy and cystic-gliotic transformation in the temporal lobes of the cerebral hemispheres in the areas of bilateral temporal partial necrotic-hemorrhagic encephalitis of HSV1 etiology suffered in the first year after birth (own observation)	101
7.10	Photo of a FLAIR MRI of the brain in axial projection (right) demonstrating signs of bilateral autoimmune limbic encephalitis caused by autoantibodies to neuronal potassium channels, with asymmetric hyperintensity of the MR signal and manifestations of atrophy of both hippocampi and insula with vicarious expansion of local CSF pathways and EEG data (left) demonstrating epileptiform activity (abnormal waves circled) in a child with ASD associated with GDFC (own observation)	102

8.1	SG structure ($n = 225$) based on genetic testing for GDFC	112
8.2	Dynamics of the proportion of responders to antiviral drugs used among observation groups during the course of therapy for reactivated EBV infection	114
8.3	Dynamics of the proportion of responders to antiviral drugs used among observation groups during the course of therapy for reactivated HHV-6 infection	115
8.4	Dynamics of the proportion of responders to antiviral drugs used among observation groups during the course of therapy for reactivated HHV-7 infection	115
9.1	Dynamics of the number of NKT cells in the blood in the observation groups during the clinical study	127
9.2	Dynamics of the number of NK cells in the blood in the observation groups during the clinical study	127
10.1	Electrophoregram of allele-specific PCR products of the A1298C polymorphism of the MTHFR gene. Pair of lanes 2,6,7 – genotype AA; pair of lanes 1,3,5 – genotype AC; pair of lanes 4 – genotype CC, M – marker 100 bp	133
10.2	Electrophoregram of restriction analysis products of the A66G polymorphism of the MTRR gene. Lanes 3,6 – genotype AA; lanes 2,4,7 – genotype AG; lanes 1,5,8 – genotype GG, M – marker 100 bp	134
10.3	SG structure ($n = 225$) based on genetic testing for GDFC	134
10.4	Example of a flow laser cytofluorimetry protocol for studying the subpopulation composition of blood lymphocytes by CD markers on the Epics XI device at the Institute of Experimental and Clinical Medicine (NDI ECM) of the National Medical University	135
10.5	Structure of SG ($n = 225$) according to NKT and NK cell deficiencies in peripheral blood	136
10.6	Dynamics of the average number of NK cells in the peripheral blood of patients SG ($n = 225$) and CG ($n = 52$) during the observation period	138
10.7	Dynamics of the average number of NKT cells in the peripheral blood of patients SG ($n = 225$) and CG ($n = 52$) during the observation period	139
10.8	Dynamics of the average number of CD3 + T cells in the peripheral blood of patients SG ($n = 225$) and CG ($n = 52$) during the observation period	140
11.1	Dynamics of the main indicators of the ABC scale in SG children ($n = 225$) during a 3-month course of infliximab immunotherapy	152
11.2	Structure of responders to infliximab therapy by indicators of hyperactivity and hyperexcitability according to the ABC scale among SG patients ($n = 225$)	153
11.3	Dynamics of serum TNF-alpha concentration in SG ($n = 225$) and CG ($n = 51$) during the observation period	154
12.1	MRI image of bilateral autoimmune limbic encephalitis with asymmetric hippocampal and insular involvement associated with the production of autoantibodies to neuronal potassium channels in a child with ASD associated with GDFC (FLAIR mode, coronal projection; own observation)	160
12.2	EEG pattern of epileptiform bioelectrical activity associated with GADA autoantibody-induced temporal median sclerosis in a child with ASD associated with GDFC (pathological waves are circled; own observation)	160

LIST OF FIGURES

12.3 Structure of SG ($n = 62$) by type of serum autoantibodies to CNS neuron autoantigens	161
12.4 Comparison of the proportion of cases of elimination of serum antineuronal autoantibodies in patients SG ($n = 62$) and CG2 ($n = 19$) during the course of rituximab immunotherapy	162
12.5 Dynamics of serum concentrations of antineuronal autoantibodies in patients SG ($n = 62$) and CG ($n = 19$) during the course of rituximab immunotherapy	163
12.6 Dynamics of serum antineuronal autoantibodies to GADA and neuronal potassium channels in SG patients ($n = 62$) during a course of rituximab immunotherapy	164
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	174
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	176
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	180
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	181
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)	182

CIRCLE OF READERS AND SCOPE OF APPLICATION

This book is intended for a multidisciplinary audience involved in the diagnosis, treatment, and research of autism spectrum disorder and related immune-metabolic conditions. These include pediatric neurologists, immunologists, psychiatrists, gastroenterologists, clinical pathologists, and biomedical researchers focusing on neuroimmune interactions. The findings presented herein may also be of interest to clinicians developing individualized therapeutic protocols for children with ASD, particularly those exhibiting dietary sensitivities and immune dysfunction. Additionally, this review provides valuable insights for academic institutions, translational medicine specialists, and pharmaceutical developers working on targeted immunomodulatory therapies. By highlighting the complex interplay between gastrointestinal, immune, metabolic, and neurological factors in children with ASD and GDFC, this paper contributes to the growing body of evidence supporting precision medicine in neurodevelopmental disorders.

INTRODUCTION

Autism spectrum disorder (ASD) is a heterogeneous group of neurodevelopmental conditions characterized by persistent deficits in social communication and interaction, as well as restricted, repetitive patterns of behavior, interests, or activities. Although the core symptoms of ASD are well recognized, the disorder is increasingly understood to encompass a wide range of biological, immunological, and metabolic disturbances. Among these, gastrointestinal dysfunction and dietary intolerances, particularly to gluten and casein, have garnered growing interest, especially in a subset of children with ASD who follow gluten- and dairy-free diets (GDFC).

Emerging evidence suggests that this subgroup exhibits distinct clinical and laboratory features that extend beyond behavioral symptoms. These include specific biochemical abnormalities, such as hyperhomocysteinemia, deficiencies in key vitamins and micronutrients, and elevated markers of tissue damage. Furthermore, immune system dysregulation appears to play a central role in the pathophysiology of ASD in children with GDFC. This is evidenced by marked deficiencies in cytotoxic T lymphocytes, natural killer (NK) cells, and phagocyte enzymes, which may predispose these children to opportunistic infections and chronic inflammation.

The interplay between immune dysfunction and microbial factors also appears to influence the development of autoimmune responses targeting both central nervous system structures and peripheral tissues. This, in turn, may contribute to the complex neurological and systemic manifestations observed in this population, which are often detectable through neuroimaging and laboratory studies.

Understanding the immunometabolic and infectious landscape of ASD in the context of GDFC is crucial for advancing diagnostic accuracy and tailoring therapeutic interventions. Recent developments in immunotherapy, including the use of biologics and immunoglobulin infusions, have shown promise in modulating immune dysfunction and improving clinical outcomes in affected children. This monograph aims to explore the multifaceted pathophysiological features associated with ASD in the context of GDFC and to review the evidence supporting current and emerging treatment strategies.

1

**IMMUNOGENETIC ASPECTS OF THE PATHOGENESIS OF DISEASE
IN CHILDREN WITH AUTISM SPECTRUM DISORDERS (REVIEW)****INTRODUCTION**

Preservation of children's mental health is a priority task of modern medicine. A common and severe psychiatric pathology in the child population is the so-called autism spectrum disorders (ASD), the frequency of which has been rapidly increasing over the past decades. This determines the relevance of the problem and justifies the urgent need to develop effective methods of treatment and prevention of this mental illness, which is an unattainable goal without understanding the etiology and pathogenesis of the disease, which still remain insufficiently clarified. However, recent achievements in the field of immunogenetics, neuroimmunology and immunopsychiatry, at least partially, shed light on the mechanisms of encephalopathy development in children with ASD, which allows us to look with cautious optimism at the prospect of overcoming this severe pathology of the childhood psyche in the near future.

ASSOCIATION STUDY OF GENETIC DEFICIENCY OF THE FOLATE CYCLE (GDFC) AND ASD

One of the important achievements in psychiatry in recent years is the discovery of the association of GDFC and ASD in children. The data of the first ever meta-analysis of randomized controlled clinical trials by D. Pu et al. in 2013, which analyzed the results of 8 studies involving 1672 children with ASD and 6760 healthy children, demonstrated that the pathogenic polymorphic variant MTHFR C677T is associated with ASD in children [1]. Further, a meta-analysis of randomized controlled clinical trials by N. S. Mohammad et al. in 2016, which included data from 1361 children with autism spectrum disorders and 6591 healthy children, showed that MTHFR C677T and the associated hyperhomocysteinemia are associated with ASD in children. Additionally, synergism between MTHFR C677T and MTRR A66G in inducing hyperhomocysteinemia and increasing the risk of developing ASD in a carrier has been demonstrated [2]. The results of a subsequent meta-analysis of randomized controlled clinical trials by V. Rai in 2016, which included data from 13 studies involving 1978 children with ASD and 7257 healthy children, established an association between MTHFR C677T and ASD in children among both European and Asian individuals. MTHFR C677T increased the risk of ASD in all 4 genetic models used (ORT vs. C = 1.48; 95 % CI = 1.18-1.86; $p = 0.0007$; ORTT + CT vs. CC = 1.70, 95 % CI = 0.96-2.9, $p = 0.05$; ORTT vs. CC = 1.84, 95 % CI = 1.12-3.02, $p = 0.02$; % CI = 1.2-2.1, $p = 0.003$; ORTT vs. CT + CC = 1.5, 95 % CI = 1.02-2.2, $p = 0.03$) [3]. Data from a recent meta-analysis of randomized controlled clinical trials by T. Sadeghiyeh et al. 2019, which analyzed the results of 25 case-control clinical studies, found an association between MTHFR 677C > T and ASD in the general population and MTHFR 1298A > C and ASD in children only among Europeans. Specifically, MTHFR 677C > T increased the risk of ASD in children in 5 genetic models (T vs. C: OR = 1.483, 95 % CI = 1.188-1.850, $p \leq 0.001$; TT vs. CC: OR = 1.834, 95 % CI = 1.155-2.913, $p = 0.010$; TC vs. CC: OR = 1.512, 95 % CI = 1.101-2.078, $p = 0.011$; TT + TC vs. CC: OR = 1.632, 95 % CI = 1.26; TT vs. TC + CC: OR = 1.427, 95 % CI = 1.002-2.032, $p = 0.049$) [4]. A recent meta-analysis of randomized

controlled clinical trials by Y. Li et al. 2020, covering the results of 15 studies, indicates an association of MTHFR C677T and ASD in children in 5 genetic models (viz, allelic, dominant, recessive, heterozygous, homozygous). Subgroup analysis showed an association of both MTHFR C677T and MTHFR A1298C with ASD in children [5].

The results of a controlled clinical trial by R. Haghiri et al. involving 103 children with ASD and 130 healthy control children showed a strong association of MTR A2756G and ASD in children. A 1.6-fold increased risk of ASD was demonstrated in carriers of MTR A2756G [6].

Thus, all 4 major polymorphic variants of folate cycle enzyme genes are associated with ASD in children, but the current evidence base for such an association is greater for MTHFR C677T and MTHFR A1298C and less for MTR A2756G and MTRR A66G (**Fig. 1.1**).

OXIDATIVE STRESS IN ASD

Biochemical abnormalities, including homocysteine cytotoxicity and disruption of gene censorship mechanisms via DNA methylation, induced by GDFC are thought to lead to a state of persistent oxidative stress in humans. There is now substantial evidence for the development of a state of oxidative stress in children with ASD, as summarized in several meta-analyses and systematic reviews.

The results of a meta-analysis and systematic review of randomized controlled trials by A. Frustaci et al. showed evidence of oxidative stress in children with ASD associated with GDFC. There were decreases in serum concentrations of the antioxidant compounds glutathione (27 %), glutathione peroxidase (18 %), methionine (13 %), and cysteine (14 %) and an abnormal increase in serum concentrations of oxidized glutathione (45 % of normal) [7]. These data indicate a serious disruption of the redox system in children with ASD, which is responsible for regulating the redox balance in the human body.

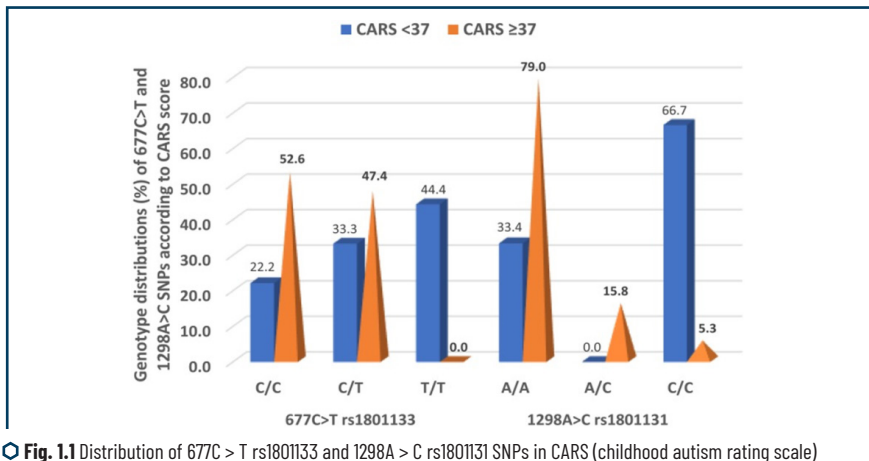


Fig. 1.1 Distribution of 677C > T rs1801133 and 1298A > C rs1801131 SNPs in CARS (childhood autism rating scale) < 37 and ≥ 37 cases. Source: [3]

The results of a meta-analysis of randomized controlled clinical trials conducted by L. Chen et al. in 2021, which included 87 studies involving 4928 children with ASD and 4181 healthy control children, demonstrate that in children with ASD, compared to healthy individuals, the serum concentration of such pro-oxidant agents as oxidized glutathione (GSSG), malondialdehyde, S-adenosylhomocysteine, nitric oxide, and copper is significantly increased, and, conversely, the serum concentration of known antioxidants glutathione (GSH), total glutathione (tgsh), methionine, cysteine, vitamins B9, D, B12, E, and calcium is significantly reduced, as well as the level of such indicators of the antioxidant system of the human body as GSH/GSSG, tgsh/GSSG, and S-Adenosylmethionine/S-Adenosylhomocysteine (**Fig. 1.2**) [8]. These results can be used when planning laboratory examinations of children with ASD to assess the current intensity of oxidative stress in the patient's body to determine the severity of their condition and the individual need for biochemical correction agents.

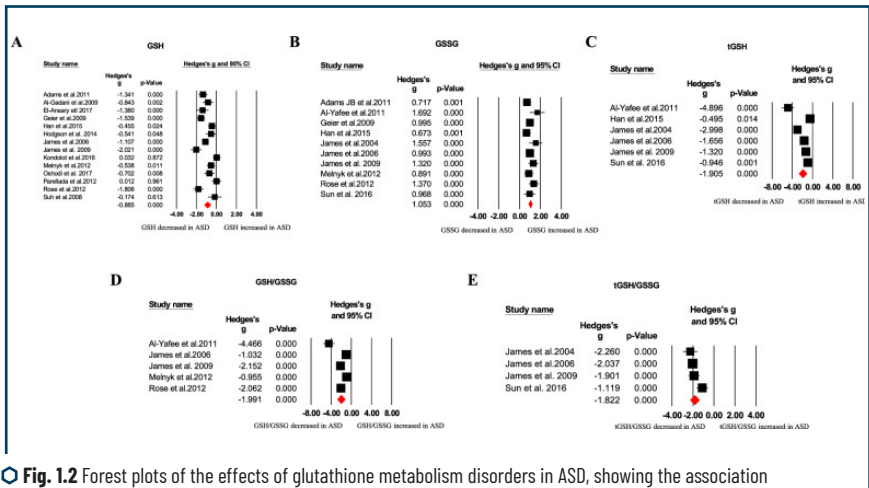


Fig. 1.2 Forest plots of the effects of glutathione metabolism disorders in ASD, showing the association of GSH(A), GSSG(B), tGSH(C), GSH/GSSG(D), tGSH/GSSG(E) and ASD. Source: [8]

IMMUNE SYSTEM STATUS IN ASD

Indeed, there is now accumulating evidence that the immune system plays an important role in the development of the human brain, participating in the regulation of neuronal proliferation and synapse formation, as well as influencing the processes of neuroplasticity, so disruption of its functioning may be important in the formation of encephalopathy in children with ASD.

There are indirect signs of compromised immune systems in children with ASD, including the following: abnormally high frequency of congenital cytomegalovirus infection, high microbial load on the body, frequent episodes of infections and antibiotic use, development of the ASD phenotype after neuroinfectious episodes, hyper-

production, production of anti-brain autoantibodies, association with some loci of the HLA major histocompatibility complex molecules, immunoinflammatory intestinal damage, hypersensitivity to food antigens and other forms of allergic reactions, predisposition to the formation of malignant neoplasms, poor tolerance of vaccines, clinical efficacy of a number of immunomodulatory, anti-inflammatory and immunotherapeutic interventions.

In particular, unclassified hypogammaglobulinemia, deficiency of CD4 + T-helper cells, complement component C4b, NK cells, IgA molecules, neutrophil myeloperoxidase, CD8 + cytotoxic T-lymphocytes, IgG subclasses, specific antibodies, and specific T-lymphocytes to certain infectious agents have been described in children with ASD [9-13].

Autistic features have been described in primary immunodeficiencies such as common variable immunodeficiency [14], type II adhesion molecule deficiency [15], ataxia telangiectasia [16], DiGeorge syndrome [17], CaV1.2 channelopathies [18], and hyper-IgE syndrome [19]. Results of a population-based national study by J. Isung et al. involving 8378 patients showed that humoral immunodeficiencies (common variable immunodeficiency, selective IgG subclass deficiency, and specific antibody deficiency) are associated with an increased risk of any mental disorder (adjusted odds ratio (AOR) = 1.91 = 18; 2.01), and the closest association among other mental illnesses was with ASD in children (AOR = 2.99, 95 % CI = 2.42-3.70) [20].

A systematic review by J. Mead et al. suggests evidence of immune dysregulation in children with ASD, including neuroinflammation, autoantibodies, enhanced T-cell responses, and abnormal natural killer and monocyte activity. These immune aberrations have been associated with worsening clinical features of ASD, including impaired social interaction, stereotyped behavior, and communication deficits. Furthermore, animal models have demonstrated resolution of ASD symptoms following removal of immune factors implicated in aberrant immune responses [21]. D. B. Noriega and H. F. Savelkoul in another systematic review on ASD in children, indicate signs of immune dysregulation in such patients, including hyperproduction of pro- and suppression of anti-inflammatory cytokines, increased permeability of the blood-brain barrier, abnormal synthesis of anti-brain autoantibodies, and modification of the functional activity of natural killer cells [22]. As noted by H. K. Hughes et al. in a recent systematic review on the phenomenon of immune system dysfunction in children with ASD, in such cases, an aberrant cytokine profile, deviations in the absolute and relative number of immunocompetent cells and their subpopulations, signs of neuroinflammation, dysfunction of the adaptive and innate immune systems, and signs of autoimmunity are noted [23].

Experimental and clinical studies have already reported various immune status disorders in patients with both verified GDFC and folic acid deficiency. In particular, van der M. B. van der Weyden et al. established the inhibition of lymphoblast metabolism in folate deficiency, which includes disorders of deoxy nucleotide metabolism and the thymidylate cycle [24]. T. Partearroyo et al. showed that the imbalance of folic acid and vitamin B12, typical of the GDFC phenotype, disrupts NK cell activity, B lymphocyte activity and lymphoproliferation [25]. C. Courtemanche et al. showed that folate deficiency leads to inhibition of proliferation of primary CD8 + cytotoxic T lymphocytes [26]. I. Abe et al. showed that folic acid deficiency leads to a decrease in the number of NK cells, T lymphocytes and B cells, but not basophils and granulocytes [27]. A. M. Troen et al. found that unmetabolized serum folate, which occurs in GDFC, causes suppression of NK cell cytotoxicity in postmenopausal women [28]. Accordingly, N. Bhatnagar et al. described pancytopenia in severe folate deficiency [29].

INFECTIOUS SYNDROME IN ASD

The presence of immune dysfunction implies a decrease in the host's resistance to microbial factors. Indeed, to date, there have been many reports of the abnormal development of opportunistic and conditionally pathogenic infections in children with ASD, which can be explained by the damage to the immune system induced by GDFC. Initially, a number of clinical reports of the development of the ASD phenotype in people after herpesvirus encephalitis were accumulated. Later, T. Binstock first pointed out selectively reduced immunoresistance in children with ASD, identifying a subgroup of patients with the so-called intramonocytic infectious pathogens – measles virus, cytomegalovirus, herpes virus type 6 and *Yersinia enterocolitica* [30]. Such children were characterized by suppression of hematopoiesis, impaired peripheral immunity, increased permeability of the blood-brain barrier and manifestations of demyelination in the white matter of the cerebral hemispheres – signs, as it turned out, typical of GDFC. G. L. Nicolson et al. in a controlled clinical study using blood PCR showed abnormally frequent detection of *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and herpes virus type 6 in children with ASD compared to healthy individuals [31]. A. Sakamoto et al. in a specially designed study found that congenital CMV infection with CNS involvement in children with ASD occurs probably more often (7.4 %) than in the general population (0.31 % of cases) ($p = 0.004$). CMV was identified by real-time PCR of dried newborn blood samples and umbilical cord blood samples obtained immediately after delivery [32]. S. Valayi et al. in a controlled clinical study demonstrated that specific IgM to EBV in the serum of children with ASD occurs probably more often than in healthy individuals ($p < 0.05$) [33]. H. Jyonouchi et al. in a specially designed study showed an association of ASD with a primary deficiency of specific antipolysaccharide antibodies, which may explain the known predisposition to the development of chronic streptococcal infection in such children [34]. H. K. Hughes and P. Ashwood in a controlled clinical study found that sulfur positivity for *Candida albicans* in children with ASD occurs in 36.5 % of cases, while in healthy children it occurs in only 14.3 % of cases (OR = 3.45, 95 % CI = 1.0409–11.4650, $p = 0.041$). *Candida* seropositivity has been shown to be associated with clinical manifestations of gastrointestinal dysfunction in children with ASD [35]. T. Nayeri et al. conducted a meta-analysis of randomized controlled clinical trials, in which they demonstrated the association of ASD with toxoplasmosis, and that the presence of toxoplasmosis infection increases the risk of developing ASD in a child by 1.93 times compared with uninfected individuals (95 % CI = 1.01–3.66) [36]. M. Kuhn et al. reported a series of clinical cases of the combination of chronic active borreliosis and ASD in children and a significant reduction in ASD manifestations following long-term therapy with ampicillin and azithromycin for borreliosis [37].

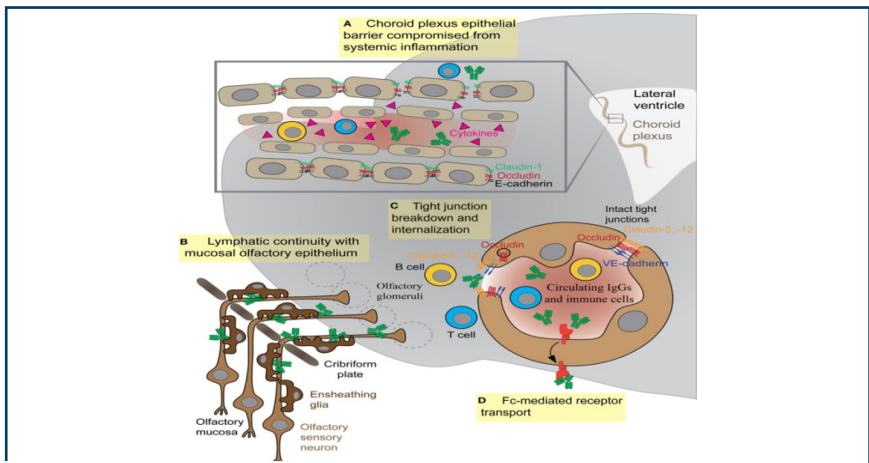
AUTOIMMUNE SYNDROME IN ASD

A special role in the pathogenesis of encephalopathy in children with ASD is attributed to autoimmune mechanisms, which are believed to develop as a result of impaired immune responses to maintain tolerance to self-antigens in conditions of immune dysfunction. Such ideas are based on a number of scientific evidences.

First, the results of a number of controlled clinical studies indicate the abnormal detection in patients with ASD of autoantibodies to CNS neurons, validated as markers of autoimmune encephalitis, which are not observed in healthy children. U. K. Rout et al. found autoantibodies to the brain autoantigen GAD65 (GADA) among children with autism in 15 % of cases, autism spectrum in 27 % of cases and in no healthy control children [38]. These autoantibodies are a recognized laboratory marker of autoimmune anti-GAD65 limbic encephalitis, which leads to the development of a number of severe mental disorders in children and adults. R. E. Frye et al. identified autoantibodies to folic acid receptors of brain neurons in children with ASD, indicating the heterogeneity of manifestations of anti-brain autoimmunity in such cases [39]. M. Cabanlit et al. established an association between ASD and the presence of autoantibodies to neurons of the hypothalamus and thalamus of the brain [40].

Secondly, experimental models have demonstrated the clinical significance of anti-brain autoantibodies in ASD, since the transfer of blood serum from children with ASD into the body of the tested animals led to the development of behavioral disorders similar to ASD in the latter. Thus, M. Gonzalez-Gronow et al. demonstrated that catalytic antibodies IgG and IgA isolated from the blood of patients with ASD disrupt the processes of hippocampal neuroplasticity in rats. The authors also demonstrated the ability of IgA to the myelin basic protein to act as a serine protease, cleaving the specified human autoantigen *in vitro* [41]. As noted by B. Gesundheit et al., experimental studies have shown that after the introduction of autoantibodies obtained from the blood serum of children with ASD, rhesus macaques develop specific behavioral disorders that closely resemble ASD in humans [42].

Third, four main pathways of migration of anti-brain autoantibodies from serum to CNS tissues are shown, including the transolfactory pathway, as well as penetration through the choroid plexus of the ventricles of the brain, damaged tight junctions between cells in the blood-brain barrier, and the endothelium of cerebral vessels via Fc-dependent transport (Fig. 1.3) [43].



○ Fig. 1.3 Schematic diagram of migration of anti-brain autoantibodies from blood serum to the CNS in children with ASD. Source: [43]

Fourth, the molecular mechanisms of CNS tissue damage by anti-brain autoantibodies observed in children with ASD have now been discovered. In particular, both the specific stimulatory and inhibitory effects of autoantibodies on neurotransmitter receptors on the surface of neurons, which causes a clinically significant functional imbalance in the processes of neurotransmission, and the immune reactions of antibody-dependent cell-mediated cytotoxicity with the participation of natural killer cells and macrophages have been described. In particular, the cellular immune reaction leads to apoptotic and/or necrotic death of the attacked neurons with subsequent destruction of the CNS neuronal networks and the formation of aberrant interneuronal connections in the affected areas. These autoimmune anti-cerebral reactions ultimately lead to the development of a specific encephalopathy, which is characterized by disintegrative processes of mental activity, which are clinically manifested as symptoms of ASD (Fig. 1.4) [44].

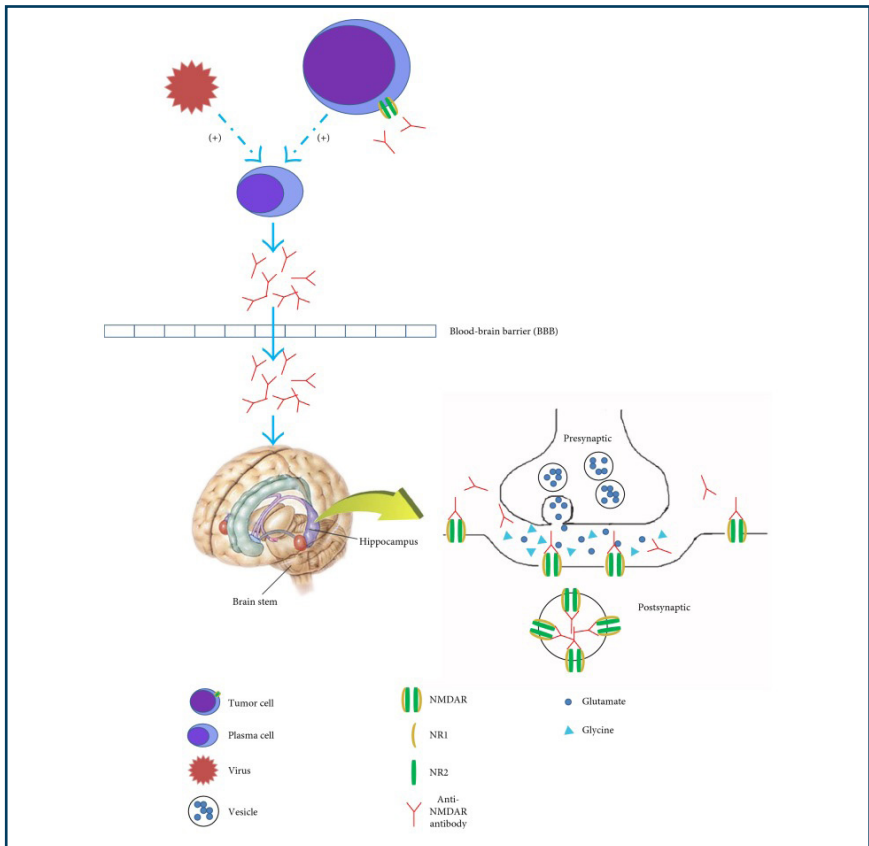


Fig. 1.4 Schematic diagram of CNS damage in autoimmune anti-NMDA receptor limbic encephalitis in humans. Source: [44]

Fifth, there are a number of described cases of the development of clinical manifestations of ASD after the onset of verified autoimmune limbic encephalitis in children and achieving clinical improvement with the treatment of autoimmune CNS disease. Thus, M. C. González-Toro et al. in 2013 reported two cases of autoimmune anti-NMDA limbic encephalitis in children, the clinical manifestations of which were consistent with symptoms of ASD [45]. R. Kiani et al. two years later also reported autistic regression of mental activity in the development of autoimmune anti-NMDA limbic encephalitis in a child [46]. D. U. Menon et al. described subacute autoimmune encephalitis caused by autoantibodies to the 3rd subunit of N-acetylcholine receptors of CNS neurons in a child with a clinical picture of ASD [47]. In all cases, at least partial recovery of mental status occurred after the administration of specific antirheumatic therapy.

Sixth, the results of a systematic review and meta-analysis of randomized controlled clinical trials conducted by S. Wu et al. indicate that a positive family history of autoimmune diseases is associated with a significant increase in the risk of ASD cases in children in the family [48].

And, finally, seventh, several drugs with anti-inflammatory and immunomodulatory therapeutic effects have demonstrated clinical efficacy in ASD, the mechanisms of which are associated with the inhibition of anti-neuronal autoimmunity and the associated intracerebral inflammation that underlies encephalopathy in children with ASD.

In addition to antineuronal, antimyelin autoimmunity has also been described in ASD. Thus, A. Vojdani et al. showed that in children with ASD, *Chlamydia pneumoniae* peptides, streptococcal M protein, and milk butyrophilin induce the production of defective specific antibodies with cross-reactivity, capable of recognizing not only microbial and food antigens, but also some molecules of nervous tissue, in particular, myelin basic protein, myelin-associated glycoprotein, myelin oligodendrocyte protein, neurofilament proteins, and tubulin [49].

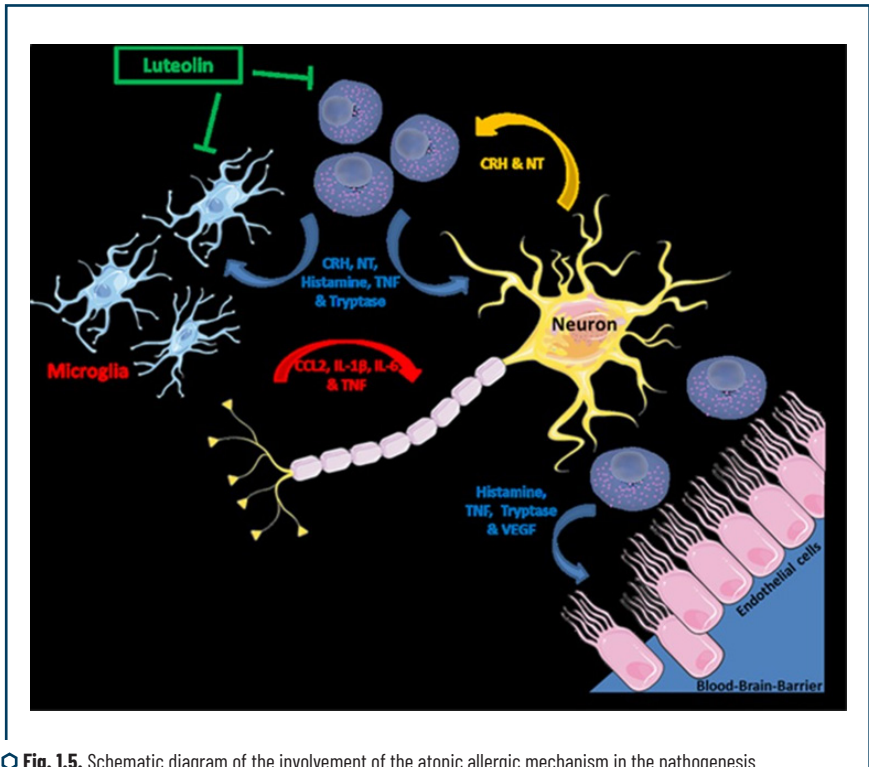
A separate subtype of ASD by the autoimmune mechanism of cerebral damage is the so-called maternal-fetal immune conflict, in connection with which the fetal brain is damaged antenatally by allogeneic anticerebral autoantibodies of the mother, which migrate into the child's body through the fetoplacental barrier [50].

ALLERGIC SYNDROME IN ASD

A large population-based clinical study of 199,520 children by G. Xu et al. showed that food allergy, respiratory allergy, and skin allergy occurred in 11.25 %, 18.73 %, and 16.81 % of children with ASD, respectively, while these disorders were less common in mentally healthy children (4.25 %, 12.08 %, and 9.84 %, respectively) [51]. The odds ratios in children with ASD for different types of allergies were as follows: food allergy - OR = 2.29, 95 % CI 95 % = 1.87-2.81; respiratory allergy - OR = 1.28, 95 % CI 95 % = 1.10-1.50; and skin allergy - OR = 1.50, 95 % CI 95 % = 1.28-1.77.

T. C. Theoharides et al. conducted a systematic review of experimental and clinical studies devoted to the study of the relationship between atopic allergic reactions and ASD in children, proposing a scientific concept that explains the mechanisms of allergy involvement in the pathogenesis of encephalopathy in ASD.

It has now been established that mast cells are in a hyperactivated state in children with ASD due to the presence of comorbid atopy. Activated mast cells have been found in the perivascular spaces of the thalamus and hypothalamus of the brain of patients with ASD. These cells, by releasing histamine and secreting a number of proinflammatory cytokines (IL-1beta, IL-6, IL-17, TNF-alpha), increase the permeability of the blood-brain barrier and cause intracerebral inflammation with predominant activation of Th17, which disrupts the formation of encephalopathy with the clinical picture of ASD (**Fig. 1.5**) [52].



◉ **Fig. 1.5.** Schematic diagram of the involvement of the atopic allergic mechanism in the pathogenesis of encephalopathy in ASD in children. Source: [52]

Thus, children with ASD are characterized by an allergic syndrome, but the real contribution of such immune-dependent disorders to the pathogenesis of encephalopathy in ASD remains unclear. It is necessary to study the relationship between the allergic syndrome and immune dysfunction observed in children with ASD in order to better understand the origin of allergies in such cases and to develop effective methods for assessing the severity of the patient's condition, predicting the further course of the disease, and treating and preventing the development of encephalopathy in ASD.

IMMUNOINFLAMMATORY SYNDROME IN ASD

Evidence for the development of a persistent systemic inflammatory response associated with immune dysregulation in children with ASD is based on the results of 2 meta-analyses of randomized controlled clinical trials. In particular, the first systematic review and meta-analysis of randomized controlled clinical trials showed increased serum concentrations of the pro-inflammatory mediators interleukin-1beta (IL-1beta) ($p < 0.001$), interleukin-6 (IL-6) ($p = 0.03$), interleukin-8 ($p = 0.04$), interferon-gamma (IFN-gamma) ($p = 0.02$), eotaxin ($p = 0.01$), and monocyte chemoattractant factor 1 ($p < 0.05$) and decreased levels of the anti-inflammatory cytokine transforming growth factor beta 1 ($p < 0.001$) in children with ASD ($n = 743$) compared to healthy subjects ($n = 592$) [53]. The results of a meta-analysis of randomized controlled clinical trials prepared by A. Saghazadeh et al., which includes 38 studies involving 2487 children, show a significant increase in serum concentrations of tumor necrosis factor alpha (TNF-alpha), IFN-gamma, IL-1beta and IL-6 in children with ASD compared with healthy individuals [54]. H. Jyonouchi et al. in a specially designed clinical study showed that increased serum concentrations of pro-inflammatory cytokines of monocyte origin, including TNF-alpha and IL-6, are associated with a sharp deterioration in the mental state of a child with ASD, which is explained as a well-known neurotoxic effect of serum pro-inflammatory molecules of the blood-brain barrier, and the associated induction of low-yielding intracerebral inflammation with dysfunction of neuronal networks of the CNS. The authors proposed to identify the immunoinflammatory mechanism as a separate link in the pathogenesis of encephalopathy in ASD, as well as a separate subgroup of children with ASD in whom the immunoinflammatory pathway of cerebral damage predominates [55].

IMMUNODEPENDENT TREATMENT APPROACHES FOR ASD

ASD is currently considered an incurable condition due to the lack of adequate evidence of the effectiveness of drugs in this pathology. Given the above data, therapeutic interventions have been repeatedly attempted to intervene in the biochemical and related immune-dependent mechanisms of encephalopathy formation in children with ASD to normalize the mental status of patients.

A systematic review devoted to the analysis of controlled studies on restrictive diets in autism recommends the use of a gluten-free and casein-free elimination diet only in laboratory-confirmed celiac disease and allergy to cow's milk casein to improve intestinal function and optimize the overall nutritional status of the child. However, no direct effect of the diet on the mental state of children with ASD has been demonstrated [56].

A systematic review of the results of 8 controlled clinical trials on the use of pre/probiotics in children with ASD, conducted by Q. X. Ng et al. in 2019, shows a small improvement in some ASD symptoms with the isolated use of pre/probiotics and a more significant positive dynamics in psychiatric manifestations with the combination of pre/probiotic therapy and an elimination gluten-free/casein-free diet, however, the data accumulated so far are insufficient for the routine use of these treatment strategies in children with ASD [57].

The results of the first open clinical trial conducted by D.-W. Kang et al. in 2017 demonstrated not only a reduction in gastrointestinal dysfunction (diarrhea, constipation, bloating, abdominal pain) but also ASD symptoms in children who received intestinal microbial transfer after a two-week course of antibiotic therapy, which justifies further research in this direction [58].

In 2018, Y.-J. Li et al. conducted a systematic review of the results of randomized controlled clinical trials devoted to the correction of micronutrient deficiencies observed in children with ASD. The results of 7 such studies indicate that vitamin B6 supplementation is ineffective in correcting mental status disorders in children with autism. The data of two other studies showed that the use of methyl forms of vitamin B12 leads to some improvement in mental status indicators in children with ASD. The results of three studies using vitamin D3 preparations indicate the insufficient effectiveness of this approach in correcting mental disorders in children with ASD. The data of another study showed the benefit of prescribing folic acid in ASD in children [59]. The obtained data indicate that the effectiveness of supplementation for correcting biochemical status abnormalities in GDFC in children with ASD is an insufficiently proven treatment strategy for routine use in clinical practice to improve mental functions, although they do not refute the need for further research in this direction.

As noted by J. Marchezan et al. in a systematic review devoted to the analysis of the limited evidence base of clinical studies 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 prescribed for non-immunological indications, but providing additional immunomodulatory properties unrelated to the main mechanism of action, in particular, risperidone, vitamin D, omega-3 polyunsaturated fatty acids, ginkgo biloba, L-carnosine, N-acetylcysteine of intestinal microflora [60].

Positive results from individual clinical studies are not sufficient for the routine use of anti-inflammatory therapy in children with ASD in clinical practice, although these data may serve as the basis for further research in the outlined direction.

A recent controlled clinical trial of infliximab (a monoclonal antibody to TNF-alpha) in children with ASD associated with GDFC who had elevated serum TNF-alpha levels was conducted. Infliximab was shown to reduce hyperactivity and hyperexcitability, but not eye contact and language, by normalizing serum TNF-alpha levels, and to reduce PANDAS symptoms, intestinal dysfunction, and epileptiform activity on the EEG [61].

Another controlled clinical trial demonstrated the ability of rituximab (a monoclonal antibody to the CD20 molecule of B lymphocytes) to reduce ASD symptoms across all ABC scores in children with GDFC who had serological, neuroimaging, and neurophysiological features of autoimmune limbic encephalitis [62].

The results of clinical trials in the field of immunoglobulin therapy for ASD are now summarized in a systematic review and meta-analysis of clinical trials by D. A. Rossignol and R. E. Frye et al. 27 relevant studies 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 intravenous human normal immunoglobulin preparations according to this meta-analysis is improvement in communication, hyperexcitability, hyperactivity, cognition, attention, social interaction, eye contact, echolalia, language, response to commands, drowsiness, decreased activity, and in some cases, complete elimination of ASD symptoms [63]. Currently, intravenous immunoglobulin therapy is the treatment strategy with the largest evidence base of effectiveness among other considered therapeutic approaches for ASD, which indirectly indicates the priority of immune-dependent mechanisms of encephalopathy pathogenesis in children with ASD.

CONCLUSIONS TO THE SECTION 1

The accumulated evidence base indicates that immune dysfunction and the related immune-dependent mechanisms of cerebral damage are undoubtedly important components of the pathways of encephalopathy formation in children with ASD associated with GDFC. Subsequent clinical studies should focus on clarifying and expanding current ideas about the involvement of the immune system in the pathogenesis of ASD in humans. In particular, there is a lack of systematization of the accumulated data and the formulation of a single scientific concept of the scenario of pathological events, starting from the presence of pathogenic polymorphic nucleotide substitutions in the genes of folic acid cycle enzymes and ending with the clinical manifestations of ASD in a child. Such systematization and generalization would not only provide a coherent system of theoretical knowledge on the immune-dependent mechanisms of the pathogenesis of encephalopathy in ASD associated with GDFC for basic science, but would also help to create an effective diagnostic algorithm of medical care for clinical practice. Further clinical trials are also needed to test immunotropic treatments in patients with GDFC-associated ASD, given the encouraging results of previous trials in this direction. The results of recent genetic, biochemical, immunological, immunobiochemical, and neuroimmunological studies indicate new potentially useful points of application of immunotherapeutic interventions for the treatment of encephalopathy in children with ASD. There is reason to believe that the successful testing of such treatment strategies will allow for a breakthrough in the treatment of GDFC-associated ASD in children, which will not only ensure recovery from a severe and currently incurable neuropsychiatric disorder, but also contribute to stopping the large-scale threatening epidemic of autism worldwide.

REFERENCES

1. 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>
2. 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.000000000000152>

3. 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>
4. 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>
5. 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>
6. Haghiri, R., Mashayekhi, F., Bidabadi, E., Salehi, Z. (2016). Analysis of methionine synthase (rs1805087) gene polymorphism in autism patients in Northern Iran. *Acta Neurobiologiae Experimentalis*, 76 (4), 318-323. <https://doi.org/10.21307/ane-2017-030>
7. Frustaci, A., Neri, M., Cesario, A., Adams, J. B., Domenici, E., Dalla Bernardina, B., Bonassi, S. (2012). Oxidative stress-related biomarkers in autism: Systematic review and meta-analyses. *Free Radical Biology and Medicine*, 52 (10), 2128-2141. <https://doi.org/10.1016/j.freeradbiomed.2012.03.011>
8. Chen, L., Shi, X.-J., Liu, H., Mao, X., Gui, L.-N., Wang, H., Cheng, Y. (2021). Oxidative stress marker aberrations in children with autism spectrum disorder: a systematic review and meta-analysis of 87 studies (N=9109). *Translational Psychiatry*, 11 (1). <https://doi.org/10.1038/s41398-020-01135-3>
9. Yonk, L. J., Warren, R. P., Burger, R. A., Cole, P., Odell, J. D., Warren, W. L. et al. (1990). CD4+ helper T cell depression in autism. *Immunology Letters*, 25 (4), 341-345. [https://doi.org/10.1016/0165-2478\(90\)90205-5](https://doi.org/10.1016/0165-2478(90)90205-5)
10. Warren, R. P. (1994). Decreased Plasma Concentrations of the C4B Complement Protein in Autism. *Archives of Pediatrics & Adolescent Medicine*, 148 (2), 180-183. <https://doi.org/10.1001/archpedi.1994.02170020066011>
11. Warren, R. P., Foster, A., Margaretten, N. C. (1987). Reduced Natural Killer Cell Activity in Autism. *Journal of the American Academy of Child & Adolescent Psychiatry*, 26 (3), 333-335. <https://doi.org/10.1097/00004583-198705000-00008>
12. 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>
13. 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>
14. Salehi Sadaghiani, M., Aghamohammadi, A., Ashrafi, M. R., Hosseini, F., Abolhassani, H., Rezaei, N. (2013). Autism in a child with common variable immunodeficiency. *Iranian Journal of Allergy, Asthma and Immunology*, 12 (3), 287-289.
15. Gazit, Y., Mory, A., Etzioni, A., Frydman, M., Scheuerman, O., Gershoni-Baruch, R., Garty, B.-Z. (2010). Leukocyte Adhesion Deficiency Type II: Long-Term Follow-Up and Review of the Literature. *Journal of Clinical Immunology*, 30 (2), 308-313. <https://doi.org/10.1007/s10875-009-9354-0>

16. Vinck, A., Verhagen, M. M. M., Gerven, M. van, de Groot, I. J. M., Weemaes, C. M. R. et al. (2011). Cognitive and speech-language performance in children with ataxia telangiectasia. *Developmental Neurorehabilitation*, 14 (5), 315–322. <https://doi.org/10.3109/17518423.2011.603368>
17. Shin, S., Yu, N., Choi, J. R., Jeong, S., Lee, K.-A. (2015). Routine Chromosomal Microarray Analysis is Necessary in Korean Patients With Unexplained Developmental Delay/Mental Retardation/Autism Spectrum Disorder. *Annals of Laboratory Medicine*, 35 (5), 510–518. <https://doi.org/10.3343/alm.2015.35.5.510>
18. Liao, P., Soong, T. W. (2009). CaV1.2 channelopathies: from arrhythmias to autism, bipolar disorder, and immunodeficiency. *Pflügers Archiv – European Journal of Physiology*, 460 (2), 353–359. <https://doi.org/10.1007/s00424-009-0753-0>
19. Grimbacher, B., Dutra, A. S., Holland, S. M., Fischer, R. E., Pao, M., Gallin, J. I., Puck, J. M. (1999). Analphoid marker chromosome in a patient with hyper-IgE syndrome, autism, and mild mental retardation. *Genetics in Medicine*, 1 (5), 213–218. <https://doi.org/10.1097/00125817-199907000-00008>
20. Isung, J., Williams, K., Isomura, K., Gromark, C., Hesselmark, E., Lichtenstein, P. et al. (2020). Association of Primary Humoral Immunodeficiencies With Psychiatric Disorders and Suicidal Behavior and the Role of Autoimmune Diseases. *JAMA Psychiatry*, 77 (11), 1147–1154. <https://doi.org/10.1001/jamapsychiatry.2020.1260>
21. Mead, J., Ashwood, P. (2015). Evidence supporting an altered immune response in ASD. *Immunology Letters*, 163 (1), 49–55. <https://doi.org/10.1016/j.imlet.2014.11.006>
22. Noriega, D. B., Savelkoul, H. F. J. (2013). Immune dysregulation in autism spectrum disorder. *European Journal of Pediatrics*, 173 (1), 33–43. <https://doi.org/10.1007/s00431-013-2183-4>
23. 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>
24. van der Weyden, M. B., Hayman, R. J., Rose, I. S., Brumley, J. (1991). Folate-deficient human lymphoblasts: Changes in deoxynucleotide metabolism and thymidylate cycle activities. *European Journal of Haematology*, 47 (2), 109–114. <https://doi.org/10.1111/j.1600-0609.1991.tb00131.x>
25. Partearroyo, T., Úbeda, N., Montero, A., Achón, M., Varela-Moreiras, G. (2013). Vitamin B12 and Folic Acid Imbalance Modifies NK Cytotoxicity, Lymphocytes B and Lymphoproliferation in Aged Rats. *Nutrients*, 5 (12), 4836–4848. <https://doi.org/10.3390/nu5124836>
26. Courtemanche, C., Elson-Schwab, I., Mashiyama, S. T., Kerry, N., Ames, B. N. (2004). Folate Deficiency Inhibits the Proliferation of Primary Human CD8+ T Lymphocytes In Vitro. *The Journal of Immunology*, 173 (5), 3186–3192. <https://doi.org/10.4049/jimmunol.173.5.3186>
27. Abe, I., Shirato, K., Hashizume, Y., Mitsuhashi, R., Kobayashi, A., Shiono, C. et al. (2012). Folate-deficiency induced cell-specific changes in the distribution of lymphocytes and granulocytes in rats. *Environmental Health and Preventive Medicine*, 18 (1), 78–84. <https://doi.org/10.1007/s12199-012-0286-6>
28. Troen, A. M., Mitchell, B., Sorensen, B., Wener, M. H., Johnston, A., Wood, B. et al. (2006). Unmetabolized Folic Acid in Plasma Is Associated with Reduced Natural Killer Cell Cytotoxicity among Postmenopausal Women. *The Journal of Nutrition*, 136 (1), 189–194. <https://doi.org/10.1093/jn/136.1.189>

29. Bhatnagar, N., Wechalekar, A., McNamara, C. (2012). Pancytopenia due to severe folate deficiency. *Internal Medicine Journal*, 42 (9), 1063–1064. Portico. <https://doi.org/10.1111/j.1445-5994.2012.02849.x>
 30. Binstock, T. (2001). Intra-monocyte pathogens delineate autism subgroups. *Medical Hypotheses*, 56 (4), 523–531. <https://doi.org/10.1054/mehy.2000.1247>
 31. Nicolson, G. L., Gan, R., Nicolson, N. L., Haier, J. (2007). Evidence for *Mycoplasma ssp.*, *Chlamydia pneumoniae*, 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>
 32. Sakamoto, A., Moriuchi, H., Matsuzaki, J., Motoyama, K., Moriuchi, M. (2015). Retrospective diagnosis of congenital cytomegalovirus infection in children with autism spectrum disorder but no other major neurologic deficit. *Brain and Development*, 37 (2), 200–205. <https://doi.org/10.1016/j.braindev.2014.03.016>
 33. Valayi, S., Eftekharian, M. M., Taheri, M., Alikhani, M. Y. (2018). Evaluation of antibodies to cytomegalovirus and Epstein-Barr virus in patients with autism spectrum disorder. *Human Antibodies*, 26 (3), 165–169. <https://doi.org/10.3233/hab-180335>
 34. 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>
 35. Hughes, H. K., Ashwood, P. (2018). Anti-*Candida albicans* IgG Antibodies in Children With Autism Spectrum Disorders. *Frontiers in Psychiatry*, 9. <https://doi.org/10.3389/fpsy.2018.00627>
 36. Nayeri, T., Sarvi, S., Moosazadeh, M., Hosseini, Z., Sharif, M., Amouei, A., Daryani, A. (2020). Relationship between toxoplasmosis and autism: A systematic review and meta-analysis. *Microbial Pathogenesis*, 147, 104434. <https://doi.org/10.1016/j.micpath.2020.104434>
 37. Kuhn, M., Grave, S., Bransfield, R., Harris, S. (2012). Long term antibiotic therapy may be an effective treatment for children co-morbid with Lyme disease and Autism Spectrum Disorder. *Medical Hypotheses*, 78 (5), 606–615. <https://doi.org/10.1016/j.mehy.2012.01.037>
 38. Rout, U. K., Mungan, N. K., Dhossche, D. M. (2012). Presence of GAD65 autoantibodies in the serum of children with autism or ADHD. *European Child & Adolescent Psychiatry*, 21 (3), 141–147. <https://doi.org/10.1007/s00787-012-0245-1>
 39. Frye, R. E., Sequeira, J. M., Quadros, E. V., James, S. J., Rossignol, D. A. (2012). Cerebral folate receptor autoantibodies in autism spectrum disorder. *Molecular Psychiatry*, 18 (3), 369–381. <https://doi.org/10.1038/mp.2011.175>
 40. 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>
 41. Gonzalez-Gronow, M., Cuchacovich, M., Francos, R., Cuchacovich, S., Blanco, A., Sandoval, R. et al. (2015). Catalytic autoantibodies against myelin basic protein (MBP) isolated from serum of autistic children impair in vitro models of synaptic plasticity in rat hippocampus. *Journal of Neuroimmunology*, 287, 1–8. <https://doi.org/10.1016/j.jneuroim.2015.07.006>
-

42. Gesundheit, B., Rosenzweig, J. P., Naor, D., Lerer, B., Zachor, D. A., Procházka, V. et al. (2013). Immunological and autoimmune considerations of Autism Spectrum Disorders. *Journal of Autoimmunity*, 44, 1-7. <https://doi.org/10.1016/j.jaut.2013.05.005>
43. Platt, M. P., Agalliu, D., Cutforth, T. (2017). Hello from the Other Side: How Autoantibodies Circumvent the Blood-Brain Barrier in Autoimmune Encephalitis. *Frontiers in Immunology*, 8. <https://doi.org/10.3389/fimmu.2017.00442>
44. Liu, C., Zhu, J., Zheng, X.-Y., Ma, C., Wang, X. (2017). Anti-N-Methyl-D-aspartate Receptor Encephalitis: A Severe, Potentially Reversible Autoimmune Encephalitis. *Mediators of Inflammation*, 2017, 1-14. <https://doi.org/10.1155/2017/6361479>
45. González Toro, M. C., Jadraque Rodríguez, R., Sempere Pérez, Á., Martínez Pastor, P., Jover Cerdá, J., Gómez Gosálvez, F. A. (2013). Encefalitis antirreceptor de NMDA: dos casos pediátricos. *Revista de Neurología*, 57 (11), 504-508. <https://doi.org/10.33588/rn.5711.2013272>
46. Kiani, R., Lawden, M., Eames, P., Critchley, P., Bhaumik, S., Odedra, S., Gumber, R. (2015). Anti-NMDA-receptor encephalitis presenting with catatonia and neuroleptic malignant syndrome in patients with intellectual disability and autism. *BJPsych Bulletin*, 39 (1), 32-35. <https://doi.org/10.1192/pb.bp.112.041954>
47. Garg, A., Chedrawi, A., Pardo, C., Johnston, M., Menon, D. (2015). Subacute encephalitis in a child seropositive for alpha-3 subunit of neuronal nicotinic acetylcholine receptors antibody. *Journal of Pediatric Neurology*, 12 (3), 161-166. <https://doi.org/10.3233/jpn-140658>
48. Wu, S., Ding, Y., Wu, F., Li, R., Xie, G., Hou, J., Mao, P. (2015). Family history of autoimmune diseases is associated with an increased risk of autism in children: A systematic review and meta-analysis. *Neuroscience & Biobehavioral Reviews*, 55, 322-332. <https://doi.org/10.1016/j.neubiorev.2015.05.004>
49. Vojdani, A., Mumper, E., Granpeesheh, D., Mielke, L., Traver, D., Bock, K. et al. (2008). Low natural killer cell cytotoxic activity in autism: The role of glutathione, IL-2 and IL-15. *Journal of Neuroimmunology*, 205 (1-2), 148-154. <https://doi.org/10.1016/j.jneuroim.2008.09.005>
50. Ramirez-Celis, A., Becker, M., Nuño, M., Schauer, J., Aghaeepour, N., Van de Water, J. (2021). Risk assessment analysis for maternal autoantibody-related autism (MAR-ASD): a subtype of autism. *Molecular Psychiatry*, 26 (5), 1551-1560. <https://doi.org/10.1038/s41380-020-00998-8>
51. Xu, M., Xu, X., Li, J., Li, F. (2019). Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. *Frontiers in Psychiatry*, 10. <https://doi.org/10.3389/fpsy.2019.00473>
52. Theoharides, T. C., Tsilioni, I., Patel, A. B., Doyle, R. (2016). Atopic diseases and inflammation of the brain in the pathogenesis of autism spectrum disorders. *Translational Psychiatry*, 6 (6), e844-e844. <https://doi.org/10.1038/tp.2016.77>
53. 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>
54. Saghazadeh, A., Ataenia, B., Keynejad, K., Abdolalazadeh, 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>

55. Jyonouchi, H., Geng, L., Davidow, A. L. (2014). Cytokine profiles by peripheral blood monocytes are associated with changes in behavioral symptoms following immune insults in a subset of ASD subjects: an inflammatory subtype? *Journal of Neuroinflammation*, 11 (1). <https://doi.org/10.1186/s12974-014-0187-2>
56. Mari-Bauset, S., Zazpe, I., Mari-Sanchis, A., Llopis-González, A., Morales-Suárez-Varela, M. (2014). Evidence of the Gluten-Free and Casein-Free Diet in Autism Spectrum Disorders. *Journal of Child Neurology*, 29 (12), 1718–1727. <https://doi.org/10.1177/0883073814531330>
57. Ng, Q., Loke, W., Venkatanarayanan, N., Lim, D., Soh, A., Yeo, W. (2019). A Systematic Review of the Role of Prebiotics and Probiotics in Autism Spectrum Disorders. *Medicina*, 55 (5), 129. <https://doi.org/10.3390/medicina5505012>
58. Kang, D.-W., Adams, J. B., Gregory, A. C., Borody, T., Chittick, L., Fasano, A. et al. (2017). Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome*, 5 (1). <https://doi.org/10.1186/s40168-016-0225-7>
59. Li, Y.-J., Li, Y.-M., Xiang, D.-X. (2017). Supplement intervention associated with nutritional deficiencies in autism spectrum disorders: a systematic review. *European Journal of Nutrition*, 57 (7), 2571–2582. <https://doi.org/10.1007/s00394-017-1528-6>
60. 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>
61. 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>
62. 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>
63. Rossignol, D. A., Frye, R. E. (2011). A review of research trends in physiological abnormalities in autism spectrum disorders: immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures. *Molecular Psychiatry*, 17 (4), 389–401. <https://doi.org/10.1038/mp.2011.165>

RESULTS OF A STUDY OF BIOCHEMICAL PROFILE INDICATORS IN CHILDREN WITH AUTISM SPECTRUM DISORDERS ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

INTRODUCTION

Since pathogenic polymorphic nucleotide substitutions in the genes of the folate and methionine cycles, as shown by the results of a number of recent meta-analyses and systematic reviews of randomized controlled clinical trials [1-5], are an associated factor with the ASD phenotype in children, a natural question arises regarding the diagnostic significance of conducting appropriate genetic tests in the routine practice of child clinical psychiatry specialists, as well as which part of the wide spectrum of known biochemical disorders identified in children with ASD is directly and/or indirectly related to genetically determined disorders in the functionally interconnected metabolic cycles of folic acid and methionine. The efforts of a number of research groups are currently focused on solving these key problems, as there is an opportunity to obtain informative and clinically applicable folate-associated laboratory biomarkers for rational prediction, risk assessment, and determination of the severity of the condition of patients with ASD, as well as to provide effective and convenient tools for optimizing family planning processes, preparation for pregnancy and childbirth, prenatal diagnosis, postnatal secondary prevention, and therapy of ASD and a number of related comorbid pathological conditions.

N. S. Mohammad et al., using the ANN (artificial neural network) model in a controlled clinical study involving 138 children with autism spectrum disorders and 138 healthy children, showed that the determination of pathogenic polymorphic variants of the GCPII C1561T, SHMT1 C1420T, MTHFR C677T, MTR A2756G, and MTRR A66G genes for diagnostic purposes allows determining the risk of developing autism spectrum disorders in a carrier with an accuracy of 63.8 % [2].

Various pathogenic polymorphic variants of folate cycle genes can act synergistically, significantly increasing the risk of autism spectrum disorders in children. Synergism between MTHFR C677T and MTRR A66G was discussed in the results of a meta-analysis by N. S. Mohammad et al. [2]. In contrast, A. H. Arab et al. in a controlled clinical study involving 112 children with autism spectrum disorders and 104 healthy children established a synergistic effect of MTHFR C677T and MTHFR A1298C in shaping the risk of autism spectrum disorders in children (**Fig. 1.1**, Chapter 1) [6]. The greater the number of pathogenic polymorphic variants of folate cycle genes in the carrier's genome, the higher the risk of autism spectrum disorders in him.

The results of clinical studies demonstrate that pathogenic polymorphic variants of folate cycle genes can lead to the development of encephalopathy with a clinical picture of autism spectrum disorders in at least three ways:

- a) metabolic, closely related to the phenomenon of hyperhomocysteinemia and the induction of oxidative stress in the CNS tissue [7-9];
- b) immune-dependent, caused by the development of neurotropic opportunistic infections, antineuronal autoimmunity and persistent systemic/intracerebral inflammation [10-12];

c) gene regulatory, mediated by derepression of other pathogenic mutations/polymorphisms in the carrier's genome due to disruption of DNA methylation processes as a universal mechanism of gene censorship [13].

There is an assumption that both direct and immune-dependent mechanisms of encephalopathy development are associated with metabolic disorders, therefore it seems important to study the profile of biochemical disorders in children with genetic deficiency of the folate cycle associated with autism spectrum disorders.

The aim of the work: study of biochemical disorders in children with genetic deficiency of the folate cycle associated with autism spectrum disorders, to understand the mechanism of encephalopathy and immunodeficiency formation, as well as the search for biomarkers - monitoring the condition and targets of further therapeutic interventions to prevent and/or reduce neurotoxicity and immunosuppression.

MATERIALS AND METHODS OF THE RESEARCH

The medical data of 225 children aged 3 to 8 years with genetic deficiency of the folate cycle, who had autism spectrum disorders, were analyzed. 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 were carried out in accordance with contract No. 150221 dated February 15, 2021, and the conclusion of the bioethical examination commission (protocol No. 140 dated December 21, 2020, Bogomolets NMU). The diagnosis of autism spectrum disorders 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 PCR based on the detection of the MTHFR C677T nucleotide substitution in monoform (27 patients), as well as - in combination with other nucleotide substitutions - MTHFR A1298C, MTRR A66G and/or MTR A2756G (111 people). These individuals constituted the study group (SG). The control group (CG) consisted of 51 children (37 boys and 14 girls) of similar age distribution who did not suffer from genetic deficiency of the folate cycle. We analyzed biochemical profile indicators that, according to recent studies, are considered informative biomarkers of genetic deficiency of the folate cycle, in particular, serum concentrations of homocysteine ($N = <5.2 \mu\text{mol/l}$), vitamins B6 ($N = 8.7\text{-}27.2 \mu\text{g/l}$), B12 ($N = 197\text{-}771 \text{pg/ml}$), D3 ($N = 30\text{-}60 \text{ng/ml}$), folic acid, or vitamin B9 ($N = 3.89\text{-}26.8 \text{ng/ml}$), creatinine (1-3 years $N = 21\text{-}36 \mu\text{mol/l}$; 3-5 years $N = 27\text{-}42 \mu\text{mol/l}$; 5-8 years $N = 28\text{-}52 \mu\text{mol/l}$), creatine phosphokinase (total) ($N = 39\text{-}308 \text{U/l}$) and lactate dehydrogenase ($N = 135\text{-}225 \text{U/l}$).

Statistical processing of the material was carried out by comparative and structural analyses. To determine the probability of differences between indicators in the observation groups, the parametric Student's T-test with the confidence probability indicator p and the nonparametric criterion - the number of signs Z according to Yu. Urbach were used. To study the associations between pathogenic polymorphic variants of folate cycle genes and parameters of the biochemical profile, the odds ratio (OR) and 95 % confidence interval (95 % CI) were used. Microsoft Excel was used to perform statistical calculations.

RESULTS AND DISCUSSION OF THE RESEARCH

As a result of structural and comparative analyses, it was found that the following pattern of biochemical disorders was characteristic of SG patients: hyperhomocysteinemia, decreased serum concentrations of vitamins B6, B12, D3 and folic acid, hypercreatininemia, increased serum concentrations of CPK and LDH, which significantly distinguished them from CG individuals (**Fig. 2.1**). In particular, an increase in the concentration of homocysteine beyond the reference values in the serum of SG children at the time of examination occurred in 88 %, a decrease in the serum concentration of vitamin B6 - in 76 %, vitamin B12 - in 79 %, vitamin D3 - in 72 %, folic acid - in 69 %, hypercreatininemia - in 65 %, increased serum concentrations of CPK - in 57 % and LDH - in 79 % of cases.

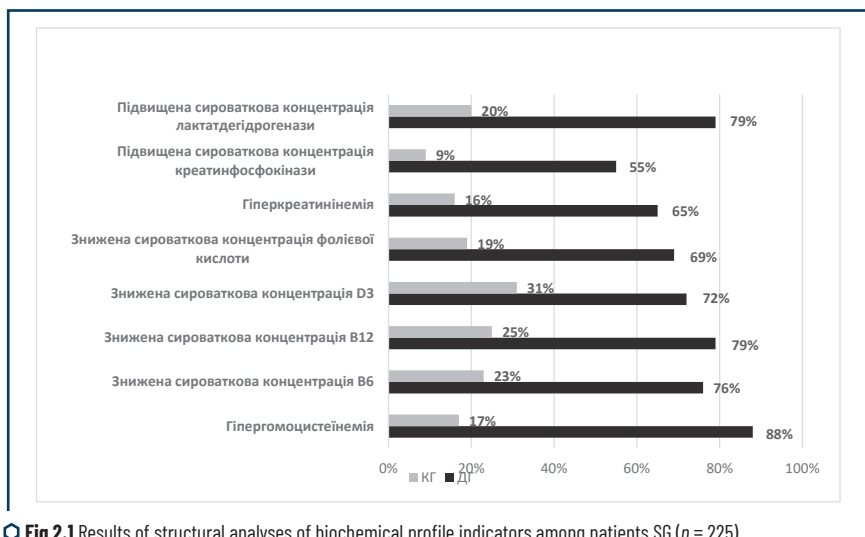


Fig. 2.1 Results of structural analyses of biochemical profile indicators among patients SG ($n = 225$) and CG ($n = 51$)

As can be seen from **Fig. 2.1**, there were significant differences in the structural distribution of patients with the studied biochemical status disorders in SG and CG. As expected, hyperhomocysteinemia was the most typical sign of SG individuals and was 5 times more common than in CG. Reduced serum concentrations of vitamins B6 and B12 occurred three times more often, and low vitamin D3 concentrations were twice as common in SG than in controls. Increased serum CPK concentrations in SG were less common than changes in other studied parameters, but were five times more common than in CG.

The results of the analysis of variance in the observation groups indicate that the studied serum biochemical disorders are typical disorders specifically for children with GDFC associated with ASD, and are not characteristic of healthy children. **Table 2.1** compares the mean values ($\bar{X} \pm m$) of the studied indicators of the biochemical profile of blood serum in SG and CG.

● **Table 2.1** Mean values ($\bar{X} \pm m$) of the studied biochemical profile parameters in patients SG ($n = 225$) and CG ($n = 51$)

Indicator	SG ($\bar{X} \pm m$)	CG ($\bar{X} \pm m$)	T-test	Z
Homocysteine, $\mu\text{mol/L}$	9.63 \pm 0.36	3.42 \pm 0.11	$p < 0.05$	$Z < Z_{0.05}$
Vitamin B12, pg/mL	112.64 \pm 25.12	337.78 \pm 38.47	$p < 0.05$	$Z < Z_{0.05}$
Vitamin B6, $\mu\text{g/L}$	6.32 \pm 0.24	18.11 \pm 1.72	$p < 0.05$	$Z < Z_{0.05}$
Vitamin D3, ng/mL	13.98 \pm 1.37	35.65 \pm 2.94	$p < 0.05$	$Z < Z_{0.05}$
Folic acid, pg/mL	2.97 \pm 0.46	17.19 \pm 2.99	$p < 0.05$	$Z < Z_{0.05}$
Creatine, $\mu\text{mol/L}$	69.13 \pm 4.31	32.36 \pm 2.12	$p < 0.05$	$Z < Z_{0.05}$
Creatine phosphokinase, U/L	314.2 \pm 29.76	52.17 \pm 19.53	$p < 0.05$	$Z < Z_{0.05}$
Lactate dehydrogenase, U/L	378.47 \pm 29.78	178.24 \pm 17.83	$p < 0.05$	$Z < Z_{0.05}$

Thus, serum concentrations of homocysteine, creatinine, CPK and LDH are significantly higher, and serum concentrations of vitamins B6, B12, D3 and folic acid are significantly lower in patients with GDFC associated with ASD than in healthy children of similar age and sex.

Similar data on most of the studied biochemical parameters have already been demonstrated in other clinical studies published in PubMed, but so far no comprehensive analysis of the biochemical profile in such cases has been carried out. The conducted scientific works concerned the study of only individual metabolic parameters without correlation with other indicators, often without specifying the diagnosis of GDFC and only taking into account the clinical manifestations of ASD, which may have a heterogeneous origin. In this work, a comprehensive analysis of key indicators of biochemical status in children with ASD associated with GDFC is performed for the first time, which allows us to recreate a holistic picture of the metabolic profile in such cases.

The question of the pathogenetic significance of the identified biochemical abnormalities in children with ASD associated with GDFC is important. Currently, direct neurotoxic effects are being discussed, such as in homocysteine and creatinine [7]. Vitamin deficiency can disrupt metabolism in the CNS, in particular, affecting the metabolism of key neurotransmitters. As noted by F. Gevi et al., vitamin B6 deficiency slows down the transformation of the excitatory amino acid glutamate into the inhibitory neurotransmitter gamma-aminobutyric acid, which may play an important role in the induction of symptoms of hyperactivity and hyperexcitability in children with ASD [14]. Also, biochemical disorders can negatively affect the development and functioning of the child's immune system, contributing to the formation of a state of immunodeficiency and associated immune dysregulation [56], which can mediate a number of severe immune-dependent complications involved in the pathogenesis of encephalopathy in children with ASD [13, 15].

There is also an important question regarding the origin of the identified biochemical disorders. It has now been established that the mechanism of biochemical imbalance in children with ASD is complex and multicomponent. Thus, some disorders are a direct consequence of the presence of pathogenic

polymorphic nucleotide substitutions in the genes of folic acid cycle enzymes, that is, they are directly related to the dysfunction of the folate cycle. In particular, we are talking about the phenomenon of hyperhomocysteinemia [7]. Other disorders may have an indirect mechanism of development. For example, the deficiency of a number of vitamins, in addition to GDFC, is explained both by impaired absorption of nutrients in the small intestine in connection with the development of persistent enterocolitis in children with ASD, and by behavioral disorders that involve dietary restrictions due to pathological selectivity in food in ASD.

Signs of mitochondrial dysfunction, including increased serum creatinine, LDH, and CPK, are a consequence of oxidative stress, which develops both through the direct effect of homocysteine on enzymes of the antioxidant system and the indirect effect of immune dysregulation caused by biochemical disorders, which is associated with abnormally increased production of prooxidant compounds in the process of persistent systemic inflammation [16].

Thus, with GDFC, children with ASD have a specific pattern of biochemical disorders that differs from the biochemical profile of healthy children and may have significant practical significance, in particular, becoming a cornerstone of the diagnostic algorithm for ASD associated with GDFC.

Important issues are the association of biochemical profile abnormalities with pathogenic polymorphic nucleotide substitutions in genes encoding folic acid cycle enzymes and the differences in associations between different folate cycle deficiency genotypes and certain biochemical disorders. Data on the association of biochemical profile abnormalities (OR; 95 % CI) with genetic testing results among SG patients are presented in **Table 2.2**.

● **Table 2.2** Results of the study of the association of disorders of the studied biochemical parameters (OR; 95 % CI) with the combination of identified polymorphisms of folate cycle genes SG (n = 225)

Genotype	Homo	B12	B6	D3	B9	Creatinin	CPK	LDH
1	2	3	4	5	6	7	8	9
MTHFR C677T	4.094; 1.851– 9.051	2.841; 1.235– 6.533	2.506; 1.110– 5.660	2.402– 1.068– 5.401	2.367; 1.056– 5.304	2.882; 1.257– 6.609	3.267; 1.399– 7.626	2.917; 1.268– 6.707
MTHFR C677T + + MTHFR A1298C	5.444; 2.314– 12.807	5.464; 2.320– 12.872	4.992; 2.132– 11.685	4.958– 2.124– 11.577	4.516– 1.949– 10.463	5.464; 2.320– 12.872	3.857; 1.669– 8.911	6.612; 2.762– 15.831
MTHFR C677T + + MTRR A66G	4.737; 2.080– 10.788	3.111; 1.340– 7.223	2.917; 1.268– 6.707	3.182; 1.362– 7.431	3.598; 1.530– 8.462	3.857; 1.669– 8.911	3.231; 1.384– 7.541	3.947; 1.780– 8.756
MTHFR C677T + + MTR A2756G	4.334; 1.945– 9.660	3.947; 1.780– 8.756	3.750; 1.701– 8.265	4.737; 2.080– 10.788	3.553; 1.623– 7.775	3.947; 1.780– 8.756	3.801; 1.708– 8.461	3.725; 1.672– 8.300
MTHFR C677T + + MTHFR A1298C + + MTRR A66G	6.629; 2.629– 16.718	6.261; 2.500– 15.682	5.392; 2.209– 13.166	7.292; 2.831– 18.782	5.367; 2.179– 13.218	4.911; 1.944– 12.403	5.612; 2.143– 14.698	4.819; 1.917– 12.114

1	2	3	4	5	6	7	8	9
MTHFR C677T + + MTHFR A1298C + + MTR A2756G	6.432; 2.606- 15.870	5.404; 2.380- 12.272	5.765; 2.514- 13.217	6.125; 2.649- 14.163	5.921; 2.554- 13,727	5.224; 2.295- 11.895	4.644; 2.073- 10.401	5.573; 2.424- 12.811
MTHFR C677T + + MTRR A66G + + MTR A2756G	6.176; 2.570- 14.842	4.871; 2.100- 11.297	5.412; 2.315- 12.651	5.828; 2.462- 13.793	5.701; 2.406- 13,508	5.701; 2.406- 13.508	5.729; 2.341- 14.023	3.947; 1.780- 8.756
MTHFR C677T + + MTHFR A1298C + + MTR A2756G + + MTRR A66G	7.206; 3.026- 17.157	7.212; 2.861- 18.177	6.657; 2.677- 16.555	5.641; 2.316- 13.740	6.044; 2.456- 14,873	4.583; 1.942- 10.816	6.509; 2.614- 16.205	6.111; 2.475- 15.091

As shown in **Table 2.2**, all genotypes studied are associated with a characteristic pattern of biochemical changes, which was discussed above, and not only with deviations in the levels of individual biochemical indicators. The expected frequency of certain biochemical disorders in the presence of a certain genotype increases from 2 to 7 times depending on the biochemical indicator and the pathogenic polymorphic variant of nucleotide substitution in the gene of the folic acid cycle enzyme. This indicates a complex and interconnected nature of the detected biochemical disorders in SG patients. For each genotype studied, mostly conjugate deviations in the levels of various biochemical indicators were characteristic, which allows us to speak about characteristic differences in biochemical status depending on the genotype (genotype-associated biochemical profiles). Thus, for the MTHFR C677T genotype, the frequency of biochemical status abnormalities increases at least 2-4 times, while for the MTHFR C677T + MTHFR A1298C genotype, it increases 3-6 times. The unequal influence of different pathogenic polymorphic variants of nucleotide substitutions in the genes of folate cycle enzymes on the severity of the studied biochemical disorders in blood serum was demonstrated. Thus, MTHFR A1298C was characterized by a closer association with deviations in the levels of the studied biochemical parameters compared to MTRR A66G and MTR A2756G. It was also shown that the accumulation of pathogenic polymorphic variants of nucleotide substitutions in the genes of folic acid cycle enzymes in the patient's body is associated with more pronounced changes in the biochemical profile, which indicates a cumulative effect of the identified genotype-associated biochemical profiles in SG. Accordingly, the most severe in terms of violations of the biochemical status of blood serum is the broadest genotype MTHFR C677T + MTHFR A1298C + MTR A2756G + MTRR A66G, which includes all the main pathogenic nucleotide substitutions, and the lightest is the mono-form MTHFR C677T. Homocysteine, among other studied biochemical indicators, was more closely associated with GDFC, and changes in its serum concentration better correspond to differences in the patient's genetic status. Thus, homocysteine is the most representative serum biochemical indicator for GDFC, which should be taken into account in the algorithms of laboratory screening of GDFC among children with ASD. This feature can be attributed to the direct relationship of the phenomenon of hyperhomocysteinemia with the specific metabolic block formed in GDFC, while, for example, serum vitamin concentrations are expected to be influenced by some other factors, such as the quality of absorption in the small intestine and the patient's dietary habits.

Hyperhomocysteinemia is known to be the main biochemical abnormality in genetic deficiency of the folate cycle in humans [7, 17]. A meta-analysis of randomized controlled clinical trials by B. Q. Guo et al. of 31 trials involving 3304 children, including 1641 patients with autism spectrum disorders, demonstrated that hyperhomocysteinemia is associated with autism spectrum disorders (Hedges's $g = 0.56$; 95 % CI = 0.36-0.76, $p < 0.001$) [17]. The neurotoxic effects of homocysteine are now well understood [7], and direct dysmetabolic mechanisms of encephalopathy are associated with them, which usually lead to certain clinical outcomes [17]. According to our observations, the immunotoxic effects of homocysteine and oxidative stress induced by this agent are of greater pathogenetic importance, leading to impaired development of the child's immune system with the formation of a specific form of immunodeficiency [18-20]. This immunodeficiency is responsible for the development of immune dysregulation and the implementation of immune-mediated and immunoinflammatory pathways of neuronal damage with the formation of encephalopathy, which is manifested by a delay in psycho-speech development in a child with manifestations of autism spectrum disorders [10, 12]. This is confirmed by the clinical effectiveness of some immunotherapeutic interventions [11].

The results of a meta-analysis of controlled clinical trials prepared by Z. Wang et al. in 2020, which included the results of 34 trials involving 20,580 children, indicate that reduced serum vitamin D concentrations are a characteristic feature of children with autism spectrum disorders (mean difference (MD): -7.46 ng/mL, 95 % CI: -10.26; -4.66 ng/mL, $p < 0.0001$, $I^2 = 98$ %) [14]. Accordingly, the data of a systematic review and meta-analysis of controlled clinical trials by B. Li et al. in 2020, which includes the results of 5 trials involving 349 people, indicate that vitamin D supplementation for serum vitamin D deficiency significantly reduces the severity of hyperactivity (pooled MD: -3.20; 95 % CI: [-6.06; -0.34]) with low heterogeneity ($I^2 = 10$ %, $p = 0.33$) in children with autism spectrum disorders [21].

The results of a controlled clinical trial by Ç. Yektaş et al. with the participation of 118 children demonstrated a significant increase in serum homocysteine concentrations and a decrease in vitamin B12, but not folic acid, in children with autism spectrum disorders and attention deficit hyperactivity disorder compared with healthy individuals [22]. Data from a controlled clinical study by A. Belardo et al. with the participation of 120 patients indicate a significant decrease in serum concentrations of vitamins B6 and B12, as well as folic acid in children with autism spectrum disorders compared with healthy children [23].

The results of controlled clinical studies by M. Lv et al. [24] and O. A. Al-Mosalem et al. [25], conducted independently of each other, indicate a probable increase in serum concentration and activity of creatine phosphokinase in children with autism spectrum disorders compared with healthy individuals. The data of a controlled clinical study by A. El-Ansary et al. indicate that increased serum concentrations of lactate dehydrogenase and creatine phosphokinase are biomarkers of autism spectrum disorders in children along with some other indicators of the metabolic profile [26].

In 2018, Y. J. Li et al. conducted a systematic review of the results of randomized controlled clinical trials on micronutrient deficiencies observed in children with autism spectrum disorders. The results of 7 such trials indicate that vitamin B6 supplementation is ineffective in correcting mental status disorders in children with autism. Data from two other trials showed that the use of methyl form of vitamin B12 leads to some improvement in mental status indicators in children with autism spectrum disorders. The results of three studies using vitamin D3 preparations indicate insufficient effectiveness of this approach

in correcting mental disorders in children with autism. Data from another trial showed the benefit of prescribing folic acid in autism spectrum disorders in children [27].

The question of the pathogenetic significance of the identified biochemical disorders in children with autism spectrum disorders associated with genetic deficiency of the folate cycle is important. Currently, there is talk of direct neurotoxic effects, such as homocysteine [7]. Vitamin deficiency can disrupt metabolism in the CNS, in particular, affect the metabolism of neurotransmitters. As noted by F. Gevi et al., vitamin B6 deficiency slows down the transformation of the excitatory amino acid glutamate into the inhibitory neurotransmitter gamma-aminobutyric acid, which may play an important role in the induction of symptoms of hyperactivity and hyperexcitability in children with autism spectrum disorders [15]. Also, biochemical disorders can negatively affect the development of the immune system, contributing to the formation of an immunodeficiency state, which can mediate a number of immune-dependent complications involved in the pathogenesis of encephalopathy in children with autism spectrum disorders [10, 12].

There is also an important question regarding the origin of the identified biochemical disorders. It has now been established that the mechanism of biochemical imbalance in children with autism spectrum disorders is complex and multicomponent. Thus, some disorders are a direct consequence of the presence of pathogenic polymorphic variants of the folate cycle genes, that is, they are directly related to the dysfunction of folate cycle enzymes. In particular, we are talking about the phenomenon of hyperhomocysteinemia [17]. Other disorders may have an indirect mechanism of development. For example, the deficiency of a number of vitamins is explained both by impaired absorption of nutrients in the small intestine in connection with the development of persistent enterocolitis in children with autism spectrum disorders, and by behavioral disorders that involve dietary restrictions due to food selectivity in autism spectrum disorders [2]. Signs of mitochondrial dysfunction, including increased serum concentrations of creatinine, lactate dehydrogenase, and creatine phosphokinase, are a consequence of oxidative stress, which develops both through the direct effect of homocysteine on enzymes of the antioxidant system and the indirect effect of immune dysregulation caused by biochemical disorders, which is associated with increased production of prooxidant compounds [8, 12].

Thus, for children with GDFC associated with ASD, a specific pattern of pathological biochemical changes in the blood serum, determined by GDFC, is characteristic, which is not typical for healthy children, and may be an important component of the pathogenesis of immunodeficiency and encephalopathy, which usually occur in such cases. Hyperhomocysteinemia, deficiency of certain vitamins and signs of mitochondrial dysfunction are noted. The mechanism of development of these serum biochemical disorders can be complex and multicomponent, however, all the pathological biochemical abnormalities studied are closely associated with pathogenic polymorphic variants of nucleotide substitutions in the genes of folate cycle enzymes, and their severity depends both on the type of pathogenic polymorphic variant of nucleotide substitution in the gene of the folate acid cycle, and on the number and composition of these pathogenic nucleotide substitutions in the patient's genome. The most favorable in biochemical terms is the MTHFR C677T genotype, which includes only one pathogenic nucleotide substitution, while the most severe is the broadest genotype with a combination of all major polymorphisms of the folate cycle enzyme genes MTHFR C677T + MTHFR A1298C + MTR A2756G + MTRR A66G.

The obtained data allow us to better understand the pathogenesis of the disease in children with ASD associated with GDFC. The identified pattern of laboratory biochemical abnormalities in serum can be used in the diagnostic process both in laboratory screening of GDFC among children with m, and in assessing the severity of the patient's current condition, predicting the further course of the disease and conducting clinical monitoring of children with ASD associated with GDFC. In addition, these GDFC-associated serum biochemical abnormalities can be the object of targeted therapeutic interventions to correct the patient's metabolic status, reduce the manifestations of GDFC-induced immunosuppression and reduce the severity of associated mental disorders in a child with ASD.

CONCLUSIONS TO THE SECTION 2

Children with genetic deficiency of the folate cycle associated with autism spectrum disorders are characterized by a specific pattern of biochemical changes that is not typical for healthy children and may explain the pathogenesis of immunodeficiency and encephalopathy. Hyperhomocysteinemia, deficiency of some B vitamins and signs of mitochondrial dysfunction are noted. The mechanism of development of these biochemical disorders can be complex and multicomponent, however, all studied biochemical abnormalities are closely associated with pathogenic polymorphic variants of the genes of folate cycle enzymes, and their severity depends on both the type of pathogenic polymorphic variant of the gene and the number and composition of pathogenic nucleotide substitutions in the patient's genotype. The most favorable in biochemical terms is the MTHFR C677T genotype, while the most severe is the genotype with the combination of polymorphisms MTHFR C677T + MTHFR A1298C + MTR A2756G + MTRR A66G. The obtained data allow us to better understand the pathogenesis of the disease. The identified pattern of biochemical disorders can be used in the diagnostic process both in screening for genetic deficiency of the folate cycle, and in assessing the severity of the condition and conducting clinical monitoring of children with autism spectrum disorders. In addition, these disorders can be the object of therapeutic interventions in order to correct the biochemical status of the patient and reduce the manifestations of immunosuppression and mental disorders.

REFERENCES

1. 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>
2. 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>
3. 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>

4. 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>
 5. 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>
 6. Arab, A. H., Elhawary, N. A. (2019). Methylenetetrahydrofolate Reductase Gene Variants Confer Potential Vulnerability to Autism Spectrum Disorder in a Saudi Community. *Neuropsychiatric Disease and Treatment*, 15, 3569-3581. <https://doi.org/10.2147/ndt.s230348>
 7. Bhatia, P., Singh, N. (2015). Homocysteine excess: delineating the possible mechanism of neurotoxicity and depression. *Fundamental & Clinical Pharmacology*, 29 (6), 522-528. <https://doi.org/10.1111/fcp.12145>
 8. Chen, L., Shi, X.-J., Liu, H., Mao, X., Gui, L.-N., Wang, H., Cheng, Y. (2021). Oxidative stress marker aberrations in children with autism spectrum disorder: a systematic review and meta-analysis of 87 studies (N=9109). *Translational Psychiatry*, 11 (1). <https://doi.org/10.1038/s41398-020-01135-3>
 9. Frustaci, A., Neri, M., Cesario, A., Adams, J. B., Domenici, E., Dalla Bernardina, B., Bonassi, S. (2012). Oxidative stress-related biomarkers in autism: Systematic review and meta-analyses. *Free Radical Biology and Medicine*, 52 (10), 2128-2141. <https://doi.org/10.1016/j.freeradbiomed.2012.03.011>
 10. Mead, J., Ashwood, P. (2015). Evidence supporting an altered immune response in ASD. *Immunology Letters*, 163 (1), 49-55. <https://doi.org/10.1016/j.imlet.2014.11.006>
 11. 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>
 12. Noriega, D. B., Savelkoul, H. F. J. (2013). Immune dysregulation in autism spectrum disorder. *European Journal of Pediatrics*, 173 (1), 33-43. <https://doi.org/10.1007/s00431-013-2183-4>
 13. Rai, V. (2017). Strong Association of C677T Polymorphism of Methylenetetrahydrofolate Reductase Gene With Nosyndromic Cleft Lip/Palate (nsCL/P). *Indian Journal of Clinical Biochemistry*, 33 (1), 5-15. <https://doi.org/10.1007/s12291-017-0673-2>
 14. Wang, Z., Ding, R., Wang, J. (2020). The Association between Vitamin D Status and Autism Spectrum Disorder (ASD): A Systematic Review and Meta-Analysis. *Nutrients*, 13 (1), 86. <https://doi.org/10.3390/nu13010086>
 15. Gevi, F., Belardo, A., Zolla, L. (2020). A metabolomics approach to investigate urine levels of neurotransmitters and related metabolites in autistic children. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1866 (10), 165859. <https://doi.org/10.1016/j.bbadis.2020.165859>
 16. Haghiri, R., Mashayekhi, F., Bidabadi, E., Salehi, Z. (2016). Analysis of methionine synthase (rs1805087) gene polymorphism in autism patients in Northern Iran. *Acta Neurobiologiae Experimentalis*, 76 (4), 318-323. <https://doi.org/10.21307/ane-2017-030>
 17. 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>
-

18. Maltsev, D. V. (2019). Immunodeficiency due to genetically determined disturbance of folate cycle in children with autism spectrum disorders. *Immunology and Allergology: Science and Practice*, 1, 4-22.
19. Maltsev, D. V. (2018). Evaluation of the immune status in children with autism spectrum disorders associated with genetic folate cycle deficiency. *Likarska Sprava*, 1-2, 11-23. [https://doi.org/10.31640/ljvd.1-2.2018\(02\)](https://doi.org/10.31640/ljvd.1-2.2018(02))
20. Maltsev, D. (2021). Extended clinical and laboratory phenotype in genetically determined folate cycle deficiency in children with autism spectrum disorders. *International Neurological Journal*, 5 (99), 5-19. <https://doi.org/10.22141/2224-0713.5.99.2018.142958>
21. Li, B., Xu, Y., Zhang, X., Zhang, L., Wu, Y., Wang, X., Zhu, C. (2020). The effect of vitamin D supplementation in treatment of children with autism spectrum disorder: a systematic review and meta-analysis of randomized controlled trials. *Nutritional Neuroscience*, 25 (4), 835-845. <https://doi.org/10.1080/1028415x.2020.1815332>
22. 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>
23. Belardo, A., Gevi, F., Zolla, L. (2019). The concomitant lower concentrations of vitamins B6, B9 and B12 may cause methylation deficiency in autistic children. *The Journal of Nutritional Biochemistry*, 70, 38-46. <https://doi.org/10.1016/j.jnutbio.2019.04.004>
24. Lv, M., Zhang, H., Shu, Y., Chen, S., Hu, Y., Zhou, M. (2016). The neonatal levels of TSB, NSE and CK-BB in autism spectrum disorder from Southern China. *Translational Neuroscience*, 7 (1), 6-11. <https://doi.org/10.1515/tnsci-2016-0002>
25. Al-Mosalem, O. A., El-Ansary, A., Attas, O., Al-Ayadhi, L. (2009). Metabolic biomarkers related to energy metabolism in Saudi autistic children. *Clinical Biochemistry*, 42 (10-11), 949-957. <https://doi.org/10.1016/j.clinbiochem.2009.04.006>
26. El-Ansary, A., Hassan, W. M., Daghestani, M., Al-Ayadhi, L., Ben Bacha, A. (2020). Preliminary evaluation of a novel nine-biomarker profile for the prediction of autism spectrum disorder. *PLOS ONE*, 15 (1), e0227626. <https://doi.org/10.1371/journal.pone.0227626>
27. Li, Y.-J., Li, Y.-M., Xiang, D.-X. (2017). Supplement intervention associated with nutritional deficiencies in autism spectrum disorders: a systematic review. *European Journal of Nutrition*, 57 (7), 2571-2582. <https://doi.org/10.1007/s00394-017-1528-6>
28. Wan., Li, Y., Zhang, Z., Sun, Z., He, Y., Li, R. (2018). Methylene tetrahydrofolate reductase and psychiatric diseases. *Translational Psychiatry*, 8 (1). <https://doi.org/10.1038/s41398-018-0276-6>

RESULTS OF THE ASSESSMENT OF IMMUNE STATUS IN CHILDREN WITH ASD: IMMUNODEFICIENCY ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

INTRODUCTION

The results of the last 5 meta-analyses of randomized controlled clinical trials indicate an association between genetically determined deficiency of folate cycle enzyme (GDFC) and autism spectrum disorders (ASD) in children [1-5], however, the mechanism of this relationship, as well as the association with additional clinical manifestations of a broad disease phenotype, has not yet been fully explained. Since there are frequent reports of the association of ASD with immunodeficiency diseases [6, 7] and signs of immune dysregulation [8, 9], and immune-dependent mechanisms of CNS damage in children with ASD have been described, including opportunistic infections [10-12], cerebral autoimmunity [8, 13], and intracerebral inflammation [14], there is reason to assume that impaired immune status is a key link in the pathogenesis of ASD in children with GDFC.

Indeed, there is now accumulating evidence that the immune system plays an important role in brain development, participating in the regulation of neuronal proliferation and synapse formation, as well as influencing neuroplasticity processes, so disruption of its functioning may be important in the formation of encephalopathy in children with ASD [15].

There are indirect signs of compromised immune systems in children with ASD, including the following: an abnormally high frequency of congenital cytomegalovirus infection [16], high microbial burden on the body [17], frequent episodes of infections and antibiotic use [18], hyperproduction of proinflammatory cytokines [14], a state of oxidative stress [19], production of anti-brain autoantibodies [8], association with certain HLA histocompatibility molecule loci [20], immunoinflammatory intestinal lesions [21], hypersensitivity to food antigens and other forms of allergic reactions [9, 22], predisposition to the formation of malignant neoplasms [23], poor tolerance of vaccines [24], and the effectiveness of a number of immunomodulatory, anti-inflammatory, and immunotherapeutic interventions [25].

Various forms of primary immunodeficiencies have been described in children with ASD, and studies of some primary immune dysfunctions indicate an increased risk of developing ASD in immunocompromised individuals.

In particular, unclassified hypogammaglobulinemia [6] and deficiencies of NK cells [26], CD8+ cytotoxic T lymphocytes [60], CD4+ T helper cells [27], neutrophil myeloperoxidase [28], IgA molecules [29, 30], IgG subclasses [6], complement component C4b [31], specific antibodies [7], and specific T lymphocytes [32] to certain infectious agents have been described in children with ASD.

Autistic traits have been described in primary immunodeficiencies such as common variable immunodeficiency [33], type II adhesion molecule deficiency [34], ataxia telangiectasia [35], DiGeorge syndrome [36], CaV1.2 channelopathy [37], and hyper-IgE syndrome [38]. Results of a population-based national study J. Isung et al. involving 8378 patients showed that humoral immunodeficiencies (common variable immunodeficiency, selective IgG subclass deficiency, and specific antibody deficiency) are associated

with an increased risk of any psychiatric disorder (adjusted odds ratio (AOR) = 1.91; 95 % CI = 1.81-2.01), and the closest association among other psychiatric illnesses was with ASD (AOR = 2.99; 95 % CI = 2.42-3.70) [39].

A systematic review by J. Mead et al. suggests evidence of immune dysregulation in children with ASD, including neuroinflammation, autoantibodies, enhanced T-cell responses, and abnormal natural killer and monocyte activity. These immune aberrations have been associated with worsening clinical features of ASD, including impaired social interaction, stereotyped behavior, and communication deficits. Furthermore, animal models have demonstrated resolution of ASD symptoms after removal of immune factors implicated in aberrant immune responses [15]. D. B. Noriega and H. F. J. Savelkoul in a systematic review devoted to ASD in children, indicate signs of immune dysregulation in such patients, including hyperproduction of pro- and suppression of anti-inflammatory cytokines, increased permeability of the blood-brain barrier, abnormal synthesis of anti-brain autoantibodies and modification of the functional activity of natural killer cells [40]. As noted by H. K. Hughes et al. in a systematic review devoted to the phenomenon of immune system dysfunction in children with ASD, in such cases, an aberrant cytokine profile, deviations in the absolute and relative number of immunocompetent cells and their subpopulations, signs of neuroinflammation, dysfunction of the adaptive and innate immune systems, imbalance of immunoglobulins of different classes and signs of autoimmunity are noted [17].

Indirect evidence of compromised immune system in patients with GDFC has been accumulated, since in such cases an association with immune-dependent complications has been demonstrated, which in classical immunology are described as manifestations of immunodeficiency diseases, in particular with autoimmune diseases [41, 42], various types of allergies [43], immunoinflammatory syndromes [44] and oncological lesions [45].

In experimental and clinical studies, various disorders of the immune status have already been reported in patients with both verified GDFC and folic acid deficiency. In particular, van der M. B. Weyden et al. established inhibition of lymphoblast metabolism in folate deficiency, which includes disorders of deoxynucleotide metabolism and the thymidylate cycle [46]. T. Partearroyo et al. showed that folic acid and vitamin B12 imbalances, typical of the GDFC phenotype, disrupt NK cells, B-lymphocyte activity, and lymphoproliferation [47]. C. Courtemanche et al. demonstrated that folate deficiency leads to inhibition of proliferation of primary CD8+ cytotoxic T lymphocytes [48]. I. Abe et al. showed that folic acid deficiency leads to a decrease in the number of NK cells, T lymphocytes and B cells, but not basophils and granulocytes [49]. A. M. Troen et al. found that unmetabolized folic acid in serum, which is noted in GDFC, causes inhibition of NK cell cytotoxicity in postmenopausal women [50]. Accordingly, Bhatnagar N. et al. described pancytopenia in severe folate deficiency [51].

Thus, the current evidence base indicates signs of impaired immune function in both patients with GDFC and children with ASD. Establishing the association of GDFC and ASD should attract additional attention to the problem of studying the immune status in children with autism. Currently, there is a lack of systematization of knowledge, a comprehensive analysis of immune status, a single solid concept of the involvement of the immune system in the pathogenesis of ASD in children with GDFC, which could not only deepen fundamental knowledge in the field of autism neuroimmunology, but also provide informative diagnostic markers and potentially useful targets for immunotherapeutic interventions for clinical practice.

In this regard, we conducted this clinical study, dedicated to an in-depth comprehensive assessment of the immune status in patients with ASD associated with GDFC, taking into account the connections with biochemical profile data and clinical syndromes of the extended phenotype.

The aim of the research: to carry out a comprehensive analysis of immune status indicators in children with ASD associated with GDFC, in connection with specific biochemical disorders and immune-dependent clinical manifestations.

MATERIALS AND METHODS OF THE RESEARCH

Data on the selection of patients for the study and control groups, the principles of clinical diagnosis of ASD, ethical and organizational aspects, diagnostics of pathogenic polymorphic nucleotide substitutions in the genes of folate cycle enzymes, and the laboratory methods used to study associated biochemical disorders are given in the **Section Materials and methods of the research in Chapter 2.**

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 (Ukraine), which, in addition to a general blood test, included the study of the subpopulation composition of lymphocytes using laser flow cytometry (Epics XI cytometer, 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 cytometry) 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, the Russian Federation; MDI Limbach Berlin GmbH, the Federal Republic of Germany).

The results of the diagnosis of reactivated viral infection were evaluated based on the results of quantitative PCR of blood leukocytes with species-specific primers for herpesviruses (herpes simplex viruses 1 and 2 types (HSV-1 and HSV-2), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesviruses 6, 7 and 8 types (HHV-6, HHV-7, HHV-8)), measles and rubella viruses (DNA-Technology reagents, RF). Group A beta-hemolytic streptococcus was detected by bacteriological culture from the oropharyngeal mucosa on a selective nutrient medium or by specific antitoxic immunity in blood serum (antistreptolysin-O, antistreptodornase, antihyaluronidase) (ELISA; MDI Limbach Berlin GmbH, the Federal Republic of Germany). Infection caused by *Candida albicans* was diagnosed based on specific IgM in serum (ELISA; MDI Limbach Berlin GmbH, the Federal Republic of Germany). Infections caused by *Mycoplasma* and *Chlamydia pneumoniae* were detected based on specific IgM in serum (ELISA, Sinevo, Ukraine) [47]. Borreliosis and yersiniosis were identified based on the Western blot analysis with simultaneous detection of IgM and IgG to a number of surface and deep antigens of the indicated pathogens (Sinevo, Ukraine). Toxoplasmosis was diagnosed based on specific IgA in serum (ELISA, Sinevo, Ukraine). Transferred congenital

CMV neuroinfection was identified based on the data of anamnestic studies of the newborn's serum (PCR, Department of Neurobiochemistry of the Institute of Neurosurgery of the National Academy of Medical Sciences of Ukraine and other laboratory centers).

The results of the Cunningham Panel™ (Moleculera Labs, Inc, United States of America) were analyzed to identify autoantibodies to antigens of CNS subcortical ganglia neurons in serum, including measurement of specific IgG to dopamine receptors types 1 and 2, lysoganglioside, and tubulin (ELISA) and assessment of Ca-dependent calmodulin kinase activity in neurons of diagnostic culture after contact with patient serum (cell-based assay; CBA). The results of serological studies of blood serum were evaluated for the detection of specific antineuronal autoantibodies to hippocampal autoantigens, which are currently validated as laboratory markers of autoimmune limbic encephalitis in children and adults, namely autoantibodies to glutamic acid decarboxylase (GADA), neuronal potassium channels, amphiphysin, NMDA-neuronal receptors, GABA, CV2, Yo, Ri, Ma, Hu, AMPAR 1 and 2 (ELISA; MDI Limbach Berlin GmbH, the Federal Republic of Germany). Autoimmunization to myelin was determined by serum concentrations of autoantibodies to myelin basic protein (ELISA) and signs of neurosensitization to myelin by neutrophils and CD8+ cytotoxic T lymphocytes (CBA; Department of Neuroimmunology, Institute of Neurosurgery, NAMS of Ukraine). Autoantibodies to extracerebral autoantigens were measured by Western blotting in the Sinevo laboratory (Ukraine). In particular, the results of the "ANA profile" were analyzed, which included the determination of specific IgG to the autoantigens of the nuclei of connective tissue cells nRMP/Sm, Smith antigen, RNP-70 -A and -C, SS-A, Ro-52, SS-B, Scl-70, PM-Scl, Jo-1, CEN-pB, PCNA, dsDNA, Nucleosomes, Histones, Rib P-protein, AMA-M-2, and the "Myositis profile" with the measurement of specific IgG to the autoantigens of the lumbar striated muscles Mi-2, Ku, PM-Scl, Jo-1, PL-7, PL-12, and Ro-52 in blood serum. Signs of systemic inflammation were assessed by serum concentrations of TNF-alpha (N up to 8.1 pg/ml; ELISA), IL-6 (N up to 7 pg/ml), and tumor M2 pyruvate kinase (N up to 20 U/ml) (Sinevo, Ukraine).

Statistical analysis of the obtained information was processed by methods of structural and comparative analyses using the electronic program Microsoft Excel. To study the distribution of variants in the variation series, the Shapiro-Wilk test was used. The average values of the studied immunity indicators (\bar{X}), standard deviations (σ) and errors of the mean value (m) were calculated. In order to establish the reliability of the differences in the results, the student's T-test was used with the calculation of the confidence probability coefficient p (parametric criterion) and the number of signs Z according to Urbach (non-parametric criterion). Differences were considered reliable at $p < 0.05$ and $Z < Z_{0.05}$.

To study the relationship between folate cycle gene polymorphisms and immune status indicators, Pearson's chi-square (χ^2) test was used, comparing the obtained value with the tabulated value at a given number of degrees of freedom and probability levels $p = 0.05$ and $p = 0.01$. For actual values from 5 to 9, Yates' correction was additionally applied, and for values less than 5, Fisher's exact test was used. The odds ratio and the associated 95 % CI were calculated to clarify the association between folate cycle deficiency and immune status indicators.

To assess the strength of the relationships between the studied phenomena, the φ criterion was used, taking into account that for four-dimensional tables, the φ , Chuprov and Kramer criteria take the same value, as well as the Pearson correlation coefficient (C) and the normalized value of this indicator (C').

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 OF THE RESEARCH

The following pattern was characteristic for the general blood analysis of SG patients: normal leukocyte count or leukopenia ($p > 0.05$; $Z > Z_{0.05}$), neutropenia ($p < 0.05$; $Z < Z_{0.05}$), lymphocytosis ($p < 0.05$; $Z < Z_{0.05}$), eosinophilia ($p < 0.05$; $Z < Z_{0.05}$) and monocytosis ($p < 0.05$; $Z < Z_{0.05}$), normal ESR ($p > 0.05$; $Z > Z_{0.05}$).

As a result of the assessment of the immune status, it was found that almost all children with GDFC were immunocompromised individuals. Although the severity of immunological disorders varied widely, some similar types of immune disorders were noted. The results of the comparative analysis of the mean values of the studied laboratory indicators of immune status in the observation groups are given in **Table 3.1**.

● **Table 3.1** Comparative analysis of the average values of the studied immune status indicators in patients of the observation groups

Cell/factor	X SG	X CG	Statistical significance
NK, $\times 10^9/l$	0.08 \pm 0.004	0.27 \pm 0.09	$p < 0.05$; $Z < Z_{0.05}$
NKT, $\times 10^9/l$	0.03 \pm 0.009	0.19 \pm 0.08	$p < 0.05$; $Z < Z_{0.05}$
CD8+ T lymphocytes, $\times 10^9/l$	0.19 \pm 0.09	0.54 \pm 0.07	$p < 0.05$; $Z < Z_{0.05}$
CD4+ T lymphocytes, $\times 10^9/l$	3.39 \pm 1.21	3.44 \pm 1.82	$p > 0.05$; $Z > Z_{0.05}$
CD19+ B lymphocytes, $\times 10^9/l$	1.78 \pm 0.23	0.35 \pm 0.09	$p > 0.05$; $Z > Z_{0.05}$
Myeloperoxidase, %	54.22 \pm 4.25	89.8 \pm 2.37	$p < 0.05$; $Z < Z_{0.05}$
IgM, g/l	0.90 \pm 0.42	1.22 \pm 0.64	$p > 0.05$; $Z > Z_{0.05}$
IgA, g/l	0.63 \pm 0.24	0.82 \pm 0.73	$p > 0.05$; $Z > Z_{0.05}$
IgG, g/l	8.81 \pm 1.29	11.94 \pm 2.46	$p > 0.05$; $Z > Z_{0.05}$
IgE, IU/ml	27.16 \pm 8.85	38.83 \pm 4.89	$p > 0.05$; $Z > Z_{0.05}$
IgG1, g/l	5.13 \pm 2.29	5.32 \pm 0.57	$p > 0.05$; $Z > Z_{0.05}$
IgG2, g/l	1.81 \pm 0.87	1.69 \pm 0.73	$p > 0.05$; $Z > Z_{0.05}$
IgG3, g/l	0.76 \pm 0.43	0.55 \pm 0.11	$p > 0.05$; $Z > Z_{0.05}$
IgG4, g/l	0.32 \pm 0.12	0.28 \pm 0.07	$p > 0.05$; $Z > Z_{0.05}$

The main finding when assessing the immune status in SG children was a sharply reduced number of lymphocyte subpopulations with the immunophenotype CD3-CD16+CD56+, known as natural killers (NK), immunophenotype CD3+CD16+CD56+, or natural killer T cells (NKT), and immunophenotype CD3+CD8+, or cytotoxic T-lymphocytes, or T killers, in the peripheral blood. The average number of NK, NKT cells and CD8+ cytotoxic T lymphocytes in the peripheral blood of SG children was lower than the reference values and significantly lower than in CG children ($p < 0.05$; $Z < Z_{0.05}$), while the number of CD4+ T-helpers did not significantly differ from that in mentally healthy children ($p > 0.05$; $Z > Z_{0.05}$), and the number of CD3-CD19+

B-lymphocytes was even significantly higher than in CG ($p < 0.05$; $Z < Z_{0.05}$). A significant decrease in myeloperoxidase content in peripheral blood phagocytes was also recorded in SG children compared to CG ($p < 0.05$; $Z < Z_{0.05}$). Although serum concentrations of immunoglobulins of different classes and subclasses were mostly lower in children with autism spectrum disorders, no significant differences were found in peers without ASD ($p > 0.05$; $Z > Z_{0.05}$) due to the large proportion of cases of increased serum antibody concentrations, which most likely reflected the implementation of autoimmune reactions.

It is known that NK cells carry out antiviral and antitumor responses through spontaneous and antibody-dependent cell-mediated cytotoxicity reactions, and NKT cells carry out target selection using an invariant receptor that interacts with individual non-classical histocompatibility molecules, including the CD1 antigen. CD8+ cytotoxic T lymphocytes perform similar functions to NK and NKT cells, but they exert specific immune cytotoxicity after dual recognition of an immunogenic peptide in complex with the HLA I molecule of the target cell. Although these three types of cytotoxic lymphocytes have some overlapping functions, the role of NK cells and CD8+ cytotoxic T lymphocytes in antiviral immunity appears to be much more important than that of NKT cells. At the same time, the latter have a more pronounced immunoregulatory effect, for example, due to the production of the anti-inflammatory cytokine IL-10, which is necessary for the prevention of autoimmunity and allergy.

If we talk about the results of the structural analysis, the deficiency of NK and/or NKT cells was the most frequent finding in the assessment of the immune status and was noted among the SG participants in 82 % of cases, that is, it was a specific feature of children with ASD associated with GDFC, while in CG children signs of a similar immunological phenotype occurred only in 32 % of cases, and usually a slight decrease in the number of cells in the peripheral blood was recorded ($p < 0.05$; $Z < Z_{0.05}$). In general, the deficiency of NKT cells occurred in 73 %, the deficiency of NK lymphocytes in 65 %, and the combined disorder, which included the simultaneous deficiency of both of these lymphocyte subpopulations, in 56 % of cases. Accordingly, isolated NK cell deficiency was noted in 9 %, and isolated NKT lymphocyte deficiency in 17 % of cases (**Fig. 3.1**).

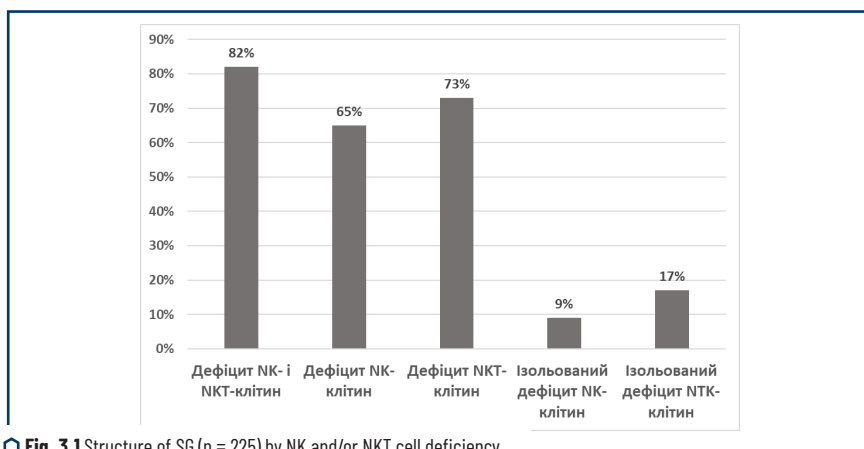


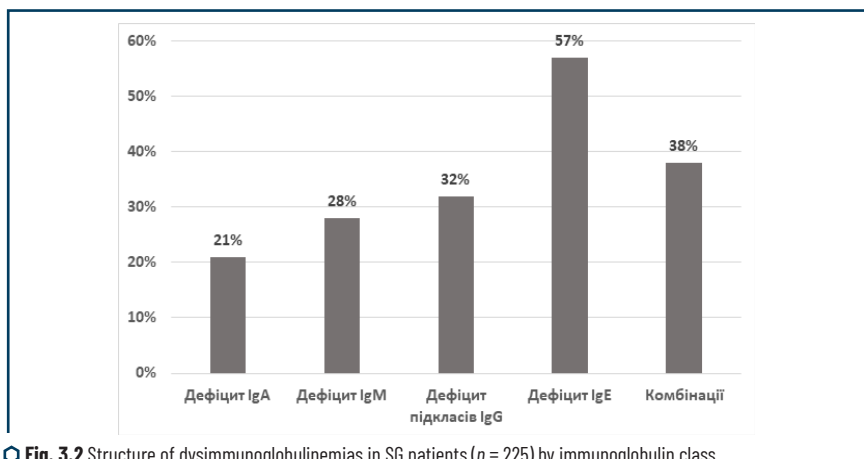
Fig. 3.1 Structure of SG (n = 225) by NK and/or NKT cell deficiency

The decrease in the number of CD8+ T lymphocytes in SG occurred in 49 %, while in CG – only in 18 % of cases ($p < 0.05$; $Z < Z_{0.05}$), and was always combined with a deficiency of NK and/or NKT cells.

Thus, in 49 % of cases there was a total deficiency of all major antiviral subpopulations of effector cytotoxic lymphocytes: T-killers, natural killers and natural killer T-lymphocytes, and it was in such children that a higher microbial load was noted at the time of entry into the study, formed mainly by intracellular pathogens compared to other SG children ($p < 0.05$; $Z < Z_{0.05}$). Combined NK and NKT cell deficiency without CD8+ T cell deficiency was observed in only 7 % of cases, while overall NK cell deficiency without a decrease in CD8+ T cell numbers occurred in 16 %, and corresponding NKT cell deficiency occurred in 24 % of cases.

All three of the effector lymphocyte subpopulations that were found to be reduced in SG patients are minority in number in the peripheral blood, so such cellular immunodeficiency usually did not lead to lymphopenia in the complete blood count. Only every tenth participant in the study group had a total decrease in all studied lymphocyte subpopulations, which was reflected in the form of lymphopenia in the complete blood count. However, a specific form of immunodeficiency with a predominant involvement of cytotoxic lymphocytes of various subpopulations, characteristic of SG, could be easily identified in the complete blood count, recording an abnormally small number of cells with the phenotype of large granular lymphocytes.

Abnormalities in the immunological examination of SG patients were also noted in the humoral link of adaptive immunity. Dysimmunoglobulinemia, which included isolated and combined deficiencies of individual classes and/or subclasses of immunoglobulins without the phenomenon of hypimmunoglobulinemia, was detected in 47 % of cases in children of different ages and usually had a stable nature, reproducing in serial studies during follow-up ($p < 0.05$; $Z < Z_{0.05}$). IgE deficiency was most often noted in SG, which occurred in 57 % of cases. IgG subclass deficiency and combined disorders of antibody genesis were more common than in every third SG participant. IgM and IgA deficiencies were less frequently recorded, occurring in every fourth and fifth child with ASD associated with GDFC, respectively (**Fig. 3.2**).



○ **Fig. 3.2** Structure of dysimmunoglobulinemias in SG patients ($n = 225$) by immunoglobulin class and subclass deficiencies

Hypogammaglobulinemia, which included a simultaneous deficiency of immunoglobulins of all classes in the blood serum with a total concentration of immunoglobulins less than 7 g/l, was registered in SG only in 12 % of cases, mainly in children aged 2–3 years and was often transient. No significant differences with the CG data were found ($p > 0.05$; $Z > Z_{0.05}$).

Deficiency of the microbicidal enzyme myeloperoxidase of peripheral blood phagocytes in SG children was observed in 39 % of cases among patients of different ages and was combined with disorders in other parts of the immune system in a variable form (Fig. 3.3), and significant differences with CG were observed ($p < 0.05$; $Z < Z_{0.05}$). This disorder was mostly persistent, as was the deficiency of killer cells of innate immunity.

In addition, other immune status disorders were observed much less frequently in children with ASD associated with GDFC: deficiencies of CD3+CD4+ T cells (13 %), CD3-CD19+ B lymphocytes (11 % of cases), mainly in children aged 2–3 years, although no significant differences from CG were obtained ($p > 0.05$; $Z > Z_{0.05}$) (Fig. 3.3).

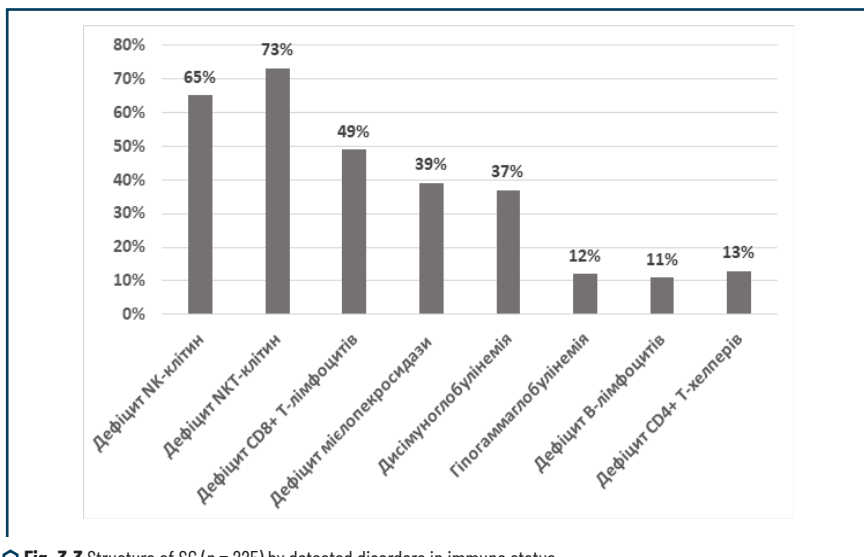
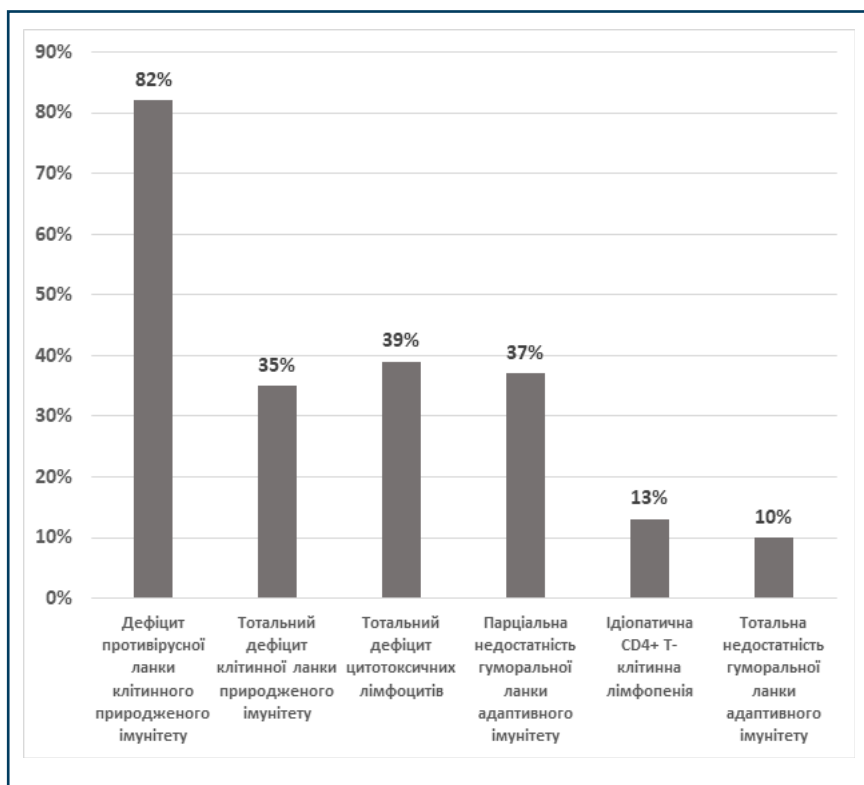


Fig. 3.3 Structure of SG ($n = 225$) by detected disorders in immune status

Based on the data of the immune status analysis, the main immunological phenotypes in children with GDFC can be distinguished. The main immunological phenotype was a deficiency of the antiviral link of cellular innate immunity, which included deficiencies of NK and NKT cells (82 % of cases). A total deficiency of the studied indicators of the cellular link of innate immunity, which included deficiencies of NK, NKT cells and myeloperoxidase of phagocytes, occurred in 35 % of cases, reducing resistance not only to viral, allergens and to a number of bacterial and fungal microbial agents due to impaired phagocytosis.

An immunological phenotype with a total lesion of the studied subpopulations of cytotoxic lymphocytes was also noted, which included deficiencies of all three subpopulations of cytotoxic cells in the peripheral blood (deficiency of large granular lymphocytes, or a combined impairment of the effector link of cellular adaptive and innate immunity), which covered almost half of the cases in SG. Almost as often, the phenomenon of dysimmunoglobulinemia was observed, reflecting a partial deficiency of the humoral component of adaptive immunity and combined with deficiencies of various subpopulations of cytotoxic lymphocytes in a variable manner in almost two-thirds of the registered cases, forming a phenotype of combined immunodeficiency with the involvement of both the cellular component of innate and/or acquired immunity and the humoral component of acquired immunity. Only in 10 % of cases was an immunological phenotype resembling a common variable immunodeficiency with hypogammaglobulinemia and deficiency of certain subpopulations of lymphocytes noted. In 13 % of cases, a phenotype corresponding to idiopathic CD4+ T-cell lymphopenia was noted in SG (Fig. 3.4).



○ Fig. 3.4 Structure of SG ($n = 225$) according to the identified immunological phenotypes when assessing immune status

Thus, the immune status of SG children was significantly different from that of CG children, but it was important to study whether the immunological differences were associated with GDFC. The results of the calculations of the chi-square test to assess the relationship between the presence of folate cycle gene polymorphisms and impaired immune status indicators in SG and CG patients are given in **Table 3.2**.

● **Table 3.2** Results of calculations of the chi-square test of immune status indicators when comparing patients SG ($n = 225$) and CG ($n = 51$)

Cell/factor deficiency	χ^2	χ^2 with Yates' correction	χ^2 with a plausibility adjustment	Statistical significance
NK cells	32.758	30.893	30.876	$p < 0.001$
NKT cells	42.856	40.649	39.238	$p < 0.001$
CD3 + CD8 + T lymphocytes	14.625	13.458	15.540	$p < 0.001$
Myeloperoxidase	10.749	9.743	11.475	$p = 0.002$

As can be seen from the data in **Table 3.2**, the deficiency of NK, NKT cells and CD8+ cytotoxic T-lymphocytes is associated with the presence of polymorphisms in the genes of folate cycle enzymes ($p < 0.001$; $\alpha = 0.05$; **Table 3.2**).

A less pronounced, but statistically significant association of the studied genetic disorders with a deficiency of the microbicidal enzyme of phagocytes, myeloperoxidase, was also demonstrated ($p = 0.002$).

It is important not only to clarify the association between GDFC and disorders in immune status, but also to assess the strength of the relationship between the studied phenomena, the results of which are presented in **Table 3.3**.

● **Table 3.3** Assessment of the φ criterion and other indicators of the strength of the relationship between GDFC and indicators of immune status in patients with SG ($n = 225$) and CG ($n = 51$)

Indicator	NK cells		NKTcells		CD8 + T lymphocytes		Myeloperoxidase	
	value	bond strength	value	bond strength	value	bond strength	value	bond strength
criterion φ	0.345	average	0.395	average	0.230	average	0.197	weak
Pearson's correlation coefficient (C)	0.361	average	0.367	average	0.224	average	0.194	weak
normalized value of Pearson's correlation coefficient (C')	0.461	relatively strong	0.519	relatively strong	0.317	average	0.274	average

As can be seen from the data in **Table 3.3**, there was a medium-strength relationship according to the φ criterion and Pearson's correlation coefficient and a relatively strong relationship according to the normalized value of the Pearson's correlation coefficient between GDFC and NK and NKT cell deficiency in SG children. At the same time, all three studied statistical coefficients demonstrated the average strength of the relationship between GDFC and CD8+ cytotoxic T-lymphocyte deficiency. On the other hand, the φ criterion and Pearson's correlation coefficient indicated a weak relationship between GDFC and phagocyte myeloperoxidase deficiency, but the normalized value of the Pearson's correlation coefficient indicated a medium-strength relationship between the studied indicators. The results obtained are consistent with the data of the structural analysis of SG by the specific gravity of deficiencies of various factors of the immune system and the results of comparing the average values of the studied immune indicators in the observation groups.

Since the obtained data on the presence of specific immune dysfunction are a cornerstone for understanding the pathogenesis of immune-dependent disorders in children with ASD associated with GDFC, an additional verification of the obtained data was carried out using the estimation of the odds ratio and 95 % confidence interval, demonstrating the association of the studied phenomena. (**Table 3.4**).

● **Table 3.4** Results of measuring odds ratio (OR) and 95 % confidence interval (95 % CI) in studying the association of GDFC and immune status indicators among SG ($n = 225$) and CG patients ($n = 51$)

Cell/factor deficiency	OR	95 % CI	S
NK cells	6.011	3.113–11.563*	0.335
NKT cells	7.739	3.975–15.069*	0.340
CD3 + CD8 + T lymphocytes	3.802	1.857–7.784*	0.366
Myeloperoxidase	3.182	1.554–6.516*	0.366

Note: * $\alpha = 0.05$

As can be seen from the data in **Table 3.4**, the presence of ASD associated with GDFC increased the chance of NKT cell deficiency by 7 times, and NK cell deficiency by 6 times, indicating a close association between these phenomena. At the same time, the closeness of the association between ASD associated with GDFC and deficiencies of CD8+ cytotoxic T lymphocytes and myeloperoxidase of phagocytes was half as low, since the appearance of the specified clinical and genetic phenotype increased the chance of detecting the specified disorders of the immune system by 3 times. These results are generally consistent with the data obtained regarding the values of Pearson's chi-square, emphasizing the greater representativeness of NK and NKT cell deficiencies than deficiencies of CD8+ cytotoxic T lymphocytes and myeloperoxidase of phagocytes for characterizing the immunological phenotype in children with ASD associated with GDFC.

Thus, children with ASD associated with GDFC have clear signs of immune dysfunction with specific features that are not characteristic of mentally healthy children without GDFC. The question of the origin of signs of immune dysfunction in children with ASD associated with GDFC is important. In order to determine

whether the identified disorders of the immune status in SG children are a consequence of GDFC itself, an additional study of the associations between the most specific for GDFC violations of the biochemical status and the most characteristic pathological deviations in the indicators of the state of the immune system among SG children was conducted, the results of which are presented in **Table 3.5**.

● **Table 3.5** Results of the study of associations (OR; 95 % CI) between biochemical abnormalities and immune factor deficiencies in SG patients (n = 225)

Indicator	NK cells	NKT cells	CD3 + CD8 + T lymphocytes	Myeloperoxidase
Homocysteine	8.938; 4.388-18.202*	11.375; 5.541-23.353*	7.196; 3.553-14.578*	6.632; 3.279-13.412*
Vit B6	7.727; 3.747-15.934*	7.129; 3.462-14.679*	6.853; 3.331-14.101*	5.402; 2.719-10.735*
Vit B12	7.289; 3.467-15.322*	6.465; 3.145-13.290*	6.372; 3.153-12.877*	5.181; 2.640-10.168*
Vit D	5.702; 2.779-11.702*	5.768; 2.810-11.841*	5.989; 2.916-12.300*	5.877; 2.862-12.068*
Folic acid	4.144; 2.119-8.104*	5.097; 2.531-10.264*	3.764; 1.947-7.275*	4.584; 2.313-9.086*
Creatinine	5.731; 2.881-11.399*	5.846; 2.938-11.633*	4.790; 2.444-9.388*	4.698; 2.398-9.205*
LDH	3.667; 1.897-7.088*	3.694; 1.912-7.139*	3.470; 1.796-6.702*	3.09; 1.714-6.388*
CPK	2.947; 1.527-5.685*	2.296; 1.191-4.427*	2.509; 1.301-4.838*	2.743; 1.422-5.290*

Note: * $\alpha = 0.05$

As can be seen from the data in **Table 3.5**, all the main biochemical disorders characteristic of GDFC are associated with all the identified typical disorders of the immune status observed among children with the indicated genetic pathology. Different biochemical disorders have different closeness of association with the studied immunological abnormalities. Hyperhomocysteinemia turned out to be the most closely associated among other biochemical phenomena with all disorders of the immunological phenotype in SG children, since its presence increased the chance of identifying a deficiency of NKT cells by 11 times, NK cells by at least 9 times, CD8+ cytotoxic T lymphocytes by 7 times, and myeloperoxidase deficiency of phagocytes by at least 6 times. Deficiencies of various vitamins were associated with specific immune status disorders somewhat weaker than hyperhomocysteinemia, increasing the chance of detecting immunological abnormalities by 4–7 times. Laboratory signs of mitochondrial dysfunction were associated with the weakest of all studied indicators of biochemical status with immune status disorders among SG patients, increasing the chance of detecting an immunological abnormality only by 2–5 times. The obtained results are consistent with the data of the structural analysis of SG by the specific weight of various biochemical disorders and deficiencies of immune system factors, as well as the results of comparing the average values of the studied laboratory parameters in the observation groups, indicating a close association of biochemical and immunological disorders in children with ASD associated with GDFC.

Thus, it is shown that children with ASD associated with GDFC not only have immune dysfunction with specific features, but also that the main immunological abnormalities characteristic of the detected immune dysfunction are associated with typical biochemical disorders that represent GDFC as a pathology of metabolism in the human body.

An important question is whether the existing metabolically induced immune dysfunction in children with ASD associated with GDFC leads to clinical manifestations. From the study of immunodeficiency diseases, it is known that immunodeficiencies appear mainly in the form of 5 main syndromes - infectious, autoimmune, allergic, immunoinflammatory and oncological. Accordingly, a structural analysis of SG was performed according to the clinical manifestations of the indicated immune-dependent syndromes in SG patients both at the time of admission and in the anamnesis of the disease. The results of the specified structural analysis are shown in **Fig. 3.5**.

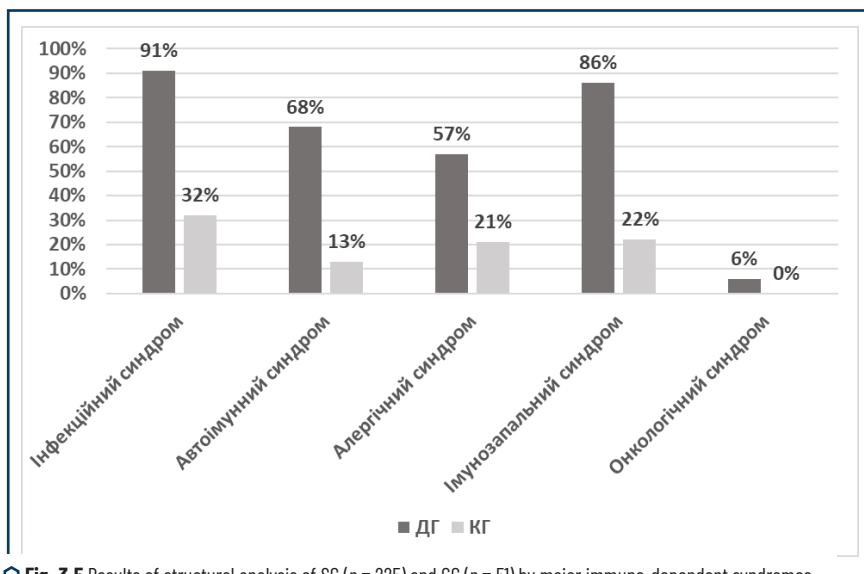


Fig. 3.5 Results of structural analysis of SG ($n = 225$) and CG ($n = 51$) by major immune-dependent syndromes

Comparison of SG results with clinical data of CG patients indicates a significantly higher frequency of all studied immune-dependent clinical syndromes in children with ASD associated with GDFC ($p > 0.05$; $Z > Z_{0.05}$). This is an indirect sign of immunocompromise of SG patients, which is consistent with the previously obtained results of the assessment of immune status. It is important to find out whether the identified immune-dependent clinical syndromes are a consequence of GDFC and the associated biochemical and immunological disorders. For this purpose, a study of associations between immune-dependent clinical syndromes and the main disorders of immune status identified in SG children was conducted, the results of which are presented in **Table 3.6**.

● **Table 3.6** Results of measuring odds ratio (OR) and 95 % confidence interval (95 % CI) in studying the association of GDFC and immune status indicators among SG patients (n = 225)

Indicator	NK cells	NKT cells	CD3+CD8+ T lymphocytes	Myeloperoxidase
Infectious syndrome	2.878; 1.576–5.255*	2.361; 1.547–5.155*	2.162; 1.824–6.163*	3.487; 1.896–6.412*
Autoimmune syndrome	8.400; 4.258–16.571*	7.556; 3.927–14.535*	10.286; 5.254–20.136*	8.816; 4.609–16.865*
Allergic syndrome	4.271; 2.312–7.892*	3.823; 2.078–7.036*	4.457; 2.409–8.244*	3.749; 2.038–6.895*
Immunoinflammatory syndrome	3.429; 1.872–6.281*	3.107; 1.699–5.681*	4.714; 2.551–8.711*	4.408; 2.391–8.128*
Oncological syndrome	1.234; 0.675–2.258	1.957; 0.991–3.862	2.023; 1.025–3.994*	1.796; 0.917–3.518

Note: * $\alpha = 0.05$

As can be seen from **Table 3.6**, almost all identified immune-dependent clinical syndromes were associated with specific disorders of the immune status in SG children, but the strength of the associations differed in different cases. Thus, syndromes associated with immune dysregulation (autoimmune, immunoinflammatory, allergic) were more closely associated with disorders in the immune status than syndromes caused by reduced immune resistance (infectious and oncological). These data indicate that immune dysfunction in SG children has clinical significance, being associated with the induction of a number of immune-dependent complications that may be involved in the pathogenesis of encephalopathy and the development of ASD symptoms.

Previously, fragments of the immunological phenotype identified in this study were reported in patients with ASD. Thus, NK cell deficiency was reported by A. M. Enstrom et al. [52], and cytotoxic T lymphocyte deficiency by R. P. Warren et al. [53], myeloperoxidase deficiency –A. J. Russo et al. [28], and IgA deficiency as a manifestation of dysimmunoglobulinemia –M. L. Santaella et al. [29], however, only in this work was a comprehensive analysis of the immune status carried out, and – with the study of the relationship with biochemical status indicators and clinical syndromes, which allows us to recreate a holistic picture of immunopathological changes in ASD patients associated with GDFC.

NK and NKT cells are minority lymphocyte subpopulations that are extremely important for the implementation of antiviral and antitumor immunity, which can largely explain the selective impairment of antiviral resistance in SG children, as well as the increased susceptibility to the development of neoplasia, mainly virus-induced forms of oncological pathology, in patients with GDFC [45] and ASD [23]. In addition, the deficiency of NK and NKT cells is associated with an increased susceptibility to the development of autoimmune complications [54, 55] and delayed-type hypersensitivity [56], which is consistent with the repeatedly recorded phenomenon of abnormally increased production of autoantibodies to brain antigens [8, 51] and intolerance to many food antigens [57] in children with ASD.

For the first time, a deficiency of natural killer cells in children with ASD was reported by R. P. Warren et al. in 1987, and already in 1990, a selective deficiency of cytotoxic T lymphocytes in this cohort of patients was reported. In particular, a deficiency of natural killer cells was noted in 12 of 31 participants in a clinical study [26]. Recently, A. Vojdani et al. found reduced NK cell activity in 45 % of cases among 1027 children with ASD, and functional disorders were often combined with quantitative ones [58]. Later, A. M. Enstrom et al. characterized in more detail the disorders of the functional activity of natural killer cells in children with ASD. There was an abnormally increased spontaneous production of perforin, granzyme B and proinflammatory cytokines, including interferon-gamma ($p < 0.01$), but there was a sharply reduced cytotoxicity against K562 cells compared with CG ($p < 0.001$) [59]. A. R. Torres et al. in a controlled clinical study showed an abnormally increased expression of the killing-activating receptor of natural killers 2DS1 and the associated ligand HLA-C2 in children with ASD, which deepened the understanding of the mechanism of increased proinflammatory potential of NK cells in such cases [59]. However, according to the results of the work of P. Ashwood et al. in children with the ASD phenotype with severe neuropsychiatric manifestations, the number of NK cells was 40 % higher than in CG [60].

A. J. Russo et al. in a controlled clinical study showed that neutrophil myeloperoxidase deficiency is a specific feature in children with ASD, which is associated with persistent gastrointestinal symptoms related to immunoinflammatory damage to the intestine [28]. Primary myeloperoxidase deficiency occurs in the population with a frequency of 1 case per 2000–4000 inhabitants, and candidal infection is the most frequent clinical manifestation of this immunodeficiency [56]. Accordingly, H. K. Hughes and P. E. Ashwood in a controlled clinical study not only showed an abnormally high frequency of candidal infection in children with ASD, but also the fact that candidiasis was the leading factor in the damage to the gastrointestinal tract [61].

GDFC has previously been associated with several autoimmune diseases, the pathogenesis of which is dominated by cellular immunopathological reactions, including multiple sclerosis [62] and rheumatoid arthritis [41]. It is important to note that these autoimmune lesions are also prevalent in patients with primary NK and NKT cell deficiencies [54, 55]. In addition, polymorphisms of folate cycle genes [45], as well as autism itself [23], have been associated with malignant neoplasms, which are known to be characteristic manifestations of primary NK and NKT cell deficiencies due to reduced antitumor immune surveillance. Thus, the data obtained allow us to find a missing link in the pathogenesis of infectious, autoimmune, allergic and neoplastic lesions in patients with GDFC, which is a primary immunodeficiency associated with a predominant lesion of killer cells of innate and acquired immunity.

We have shown the heterogeneous impact of biochemical disorders caused by GDFC on various factors of the immune status of children with ASD. At the moment, another immunodeficiency is known, in which the metabolic defect is present in all cells of the body, but a clinically significant disorder is formed only in certain subpopulations of lymphocytes, which determines the development of immune dysfunction, and not a classic metabolic genetic disease. We are talking about primary adenosine deaminase deficiency, in which a selective deficiency of T lymphocytes develops, although the mutant gene is expressed in many cells of the human body [63].

In addition, a primary immunodeficiency is already known and well characterized, caused not by classic Mendelian mutations, but by pathogenic polymorphic substitutions in the gene encoding a component of the immune system. Thus, in hereditary mannose-binding lectin deficiency, there are combinations of polymorphisms

of structural genes and the promoter region, which cause abnormally low production of this immune factor. The clinical picture of this immunodeficiency is also dominated by various infectious, allergic, autoimmune, immunoinflammatory, oncological and neuropsychiatric lesions, which closely resemble those in GDFC [64].

Thus, similar forms of primary immunodeficiencies have already been described and studied in clinical immunology, which makes it easier to understand the nature of a new immunodeficiency disease associated with a genetically determined violation of the activity of folate cycle enzymes.

It should be emphasized that some other mental disorders are now associated with GDFC, including major depression, bipolar disorder, and schizophrenia. The data we obtained suggest an immune-dependent component of pathogenesis in such cases.

CONCLUSIONS TO THE SECTION 3

All these data allow us to conclude that GDFC leads to the development of a special form of primary immunodeficiency with a variable immunological phenotype, with the predominant involvement of NK, NKT cells, CD8+ cytotoxic T lymphocytes, myeloperoxidase phagocytes, which are often combined with dysimmunoglobulinemia in an arbitrary manner. This immunodeficiency has a dysmetabolic nature, since its main components are associated with specific biochemical disorders characteristic of GDFC, and most likely determines a sharp decrease in resistance to intracellular microorganisms and tumors, signs of systemic inflammation, autoimmune and allergic reactions, which are typical of children with ASD. We propose to call this new primary immunodeficiency as *immunodeficiency associated with a genetic disorder of the folate cycle*.

Immunodeficiency allows us to combine into a common phenotype seemingly disparate clinical syndromes that often develop sequentially or simultaneously in children with ASD, including various infectious lesions, immune-mediated leukoencephalopathy, PANS/PANDAS, temporal median epilepsy associated with herpesvirus infections, allergies and immunoinflammatory intestinal disorders, autoimmune connective tissue lesions, etc. Although there are obviously also direct metabolic effects, the various clinical neuropsychiatric manifestations observed in children with GDFC are mostly associated not with the direct toxic effects of homocysteine and other harmful metabolic products directly related to methylation disorders on nerve and glial cells, but with the development of immune-dependent complications mediated by the combined (cellular, humoral and phagocytic) immunodeficiency in GDFC, including neuroinfectious lesions, systemic and intracerebral inflammation, cerebral histamine-mediated processes and induction of autoimmune reactions to antigens of nervous tissue and cerebral vessels.

REFERENCES

1. 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>

2. 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>
 3. 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>
 4. 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>
 5. 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>
 6. 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>
 7. 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>
 8. Frye, R. E., Sequeira, J. M., Quadros, E. V., James, S. J., Rossignol, D. A. (2012). Cerebral folate receptor autoantibodies in autism spectrum disorder. *Molecular Psychiatry*, 18 (3), 369-381. <https://doi.org/10.1038/mp.2011.175>
 9. Lau, N. M., Green, P. H. R., Taylor, A. K., Hellberg, D., Ajamian, M., Tan, C. Z. et al. (2013). Markers of Celiac Disease and Gluten Sensitivity in Children with Autism. *PLoS ONE*, 8 (6), e66155. <https://doi.org/10.1371/journal.pone.0066155>
 10. Binstock, T. (2001). Intra-monocyte pathogens delineate autism subgroups. *Medical Hypotheses*, 56 (4), 523-531. <https://doi.org/10.1054/mehy.2000.1247>
 11. Nayeri, T., Sarvi, S., Moosazadeh, M., Hosseiniyjad, Z., Sharif, M., Amouei, A., Daryani, A. (2020). Relationship between toxoplasmosis and autism: A systematic review and meta-analysis. *Microbial Pathogenesis*, 147, 104434. <https://doi.org/10.1016/j.micpath.2020.104434>
 12. Nicolson, G. L., Gan, R., Nicolson, N. L., Haier, J. (2007). Evidence for *Mycoplasma ssp.*, *Chlamydia pneumoniae*, 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>
 13. Brimberg, L., Sadiq, A., Gregersen, P. K., Diamond, B. (2013). Brain-reactive IgG correlates with autoimmunity in mothers of a child with an autism spectrum disorder. *Molecular Psychiatry*, 18 (11), 1171-1177. <https://doi.org/10.1038/mp.2013.101>
 14. Saghazadeh, A., Ataenia, 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>
-

15. Mead, J., Ashwood, P. (2015). Evidence supporting an altered immune response in ASD. *Immunology Letters*, 163 (1), 49–55. <https://doi.org/10.1016/j.imlet.2014.11.006>
 16. Sakamoto, A., Moriuchi, H., Matsuzaki, J., Motoyama, K., Moriuchi, M. (2015). Retrospective diagnosis of congenital cytomegalovirus infection in children with autism spectrum disorder but no other major neurologic deficit. *Brain and Development*, 37 (2), 200–205. <https://doi.org/10.1016/j.brain-dev.2014.03.016>
 17. 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>
 18. Matarazzo, E. B. (2002). Treatment of Late Onset Autism as a Consequence of Probable Autoimmune Processes Related to Chronic Bacterial Infection. *The World Journal of Biological Psychiatry*, 3 (3), 162–166. <https://doi.org/10.3109/15622970209150618>
 19. Chen, L., Shi, X.-J., Liu, H., Mao, X., Gui, L.-N., Wang, H., Cheng, Y. (2021). Oxidative stress marker aberrations in children with autism spectrum disorder: a systematic review and meta-analysis of 87 studies (N=9109). *Translational Psychiatry*, 11 (1). <https://doi.org/10.1038/s41398-020-01135-3>
 20. Puangpetch, A., Suwannarat, P., Chamnanphol, M., Koomdee, N., Ngamsamut, N., Limsila, P., Sukasem, C. (2015). Significant Association of HLA-B*Alleles 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>
 21. Furlano, R. I., Anthony, A., Day, R., Brown, A., McGarvey, L., Thomson, M. A. et al. (2001). Colonic CD8 and $\gamma\delta$ T-cell infiltration with epithelial damage in children with autism. *The Journal of Pediatrics*, 138 (3), 366–372. <https://doi.org/10.1067/mpd.2001.111323>
 22. Xu, G., Snetselaar, L. G., Jing, J., Liu, B., Strathearn, L., Bao, W. (2018). Association of Food Allergy and Other Allergic Conditions With Autism Spectrum Disorder in Children. *JAMA Network Open*, 1 (2), e180279. <https://doi.org/10.1001/jamanetworkopen.2018.0279>
 23. Crawley, J. N., Heyer, W.-D., LaSalle, J. M. (2016). Autism and Cancer Share Risk Genes, Pathways, and Drug Targets. *Trends in Genetics*, 32 (3), 139–146. <https://doi.org/10.1016/j.tig.2016.01.001>
 24. Jefferson, T., Price, D., Demicheli, V., Bianco, E. (2003). Unintended events following immunization with MMR: a systematic review. *Vaccine*, 21 (25–26), 3954–3960. [https://doi.org/10.1016/s0264-410x\(03\)00271-8](https://doi.org/10.1016/s0264-410x(03)00271-8)
 25. 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>
 26. Warren, R. P., Foster, A., Margaretten, N. C. (1987). Reduced Natural Killer Cell Activity in Autism. *Journal of the American Academy of Child & Adolescent Psychiatry*, 26 (3), 333–335. <https://doi.org/10.1097/00004583-198705000-00008>
 27. Yonk, L. J., Warren, R. P., Burger, R. A., Cole, P., Odell, J. D., Warren, W. L. et al. (1990). CD4+ helper T cell depression in autism. *Immunology Letters*, 25 (4), 341–345. [https://doi.org/10.1016/0165-2478\(90\)90205-5](https://doi.org/10.1016/0165-2478(90)90205-5)
-

28. 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>
 29. 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.
 30. Wasilewska, J., Kaczmarek, 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>
 31. Warren, R. P. (1994). Decreased Plasma Concentrations of the C4B Complement Protein in Autism. *Archives of Pediatrics & Adolescent Medicine*, 148 (2), 180–183. <https://doi.org/10.1001/archpedi.1994.02170020066011>
 32. Reinert, P., Moulías, 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.
 33. Salehi Sadaghiani, M., Aghamohammadi, A., Ashrafi, M. R., Hosseini, F., Abolhassani, H., Rezaei, N. (2013). Autism in a child with common variable immunodeficiency. *Iranian Journal of Allergy, Asthma and Immunology*, 12 (3), 287–289.
 34. Gazit, Y., Mory, A., Etzioni, A., Frydman, M., Scheuerman, O., Gershoni-Baruch, R., Garty, B.-Z. (2010). Leukocyte Adhesion Deficiency Type II: Long-Term Follow-Up and Review of the Literature. *Journal of Clinical Immunology*, 30 (2), 308–313. <https://doi.org/10.1007/s10875-009-9354-0>
 35. Vinck, A., Verhagen, M. M. M., Gerven, M. van, de Groot, I. J. M., Weemaes, C. M. R. et al. (2011). Cognitive and speech-language performance in children with ataxia telangiectasia. *Developmental Neurorehabilitation*, 14 (5), 315–322. <https://doi.org/10.3109/17518423.2011.603368>
 36. Shin, S., Yu, N., Choi, J. R., Jeong, S., Lee, K.-A. (2015). Routine Chromosomal Microarray Analysis is Necessary in Korean Patients With Unexplained Developmental Delay/Mental Retardation/Autism Spectrum Disorder. *Annals of Laboratory Medicine*, 35 (5), 510–518. <https://doi.org/10.3343/alm.2015.35.5.510>
 37. Liao, P., Soong, T. W. (2009). CaV1.2 channelopathies: from arrhythmias to autism, bipolar disorder, and immunodeficiency. *Pflügers Archiv – European Journal of Physiology*, 460 (2), 353–359. <https://doi.org/10.1007/s00424-009-0753-0>
 38. Grimbacher, B., Dutra, A. S., Holland, S. M., Fischer, R. E., Pao, M., Gallin, J. I., Puck, J. M. (1999). Analphoid marker chromosome in a patient with hyper-IgE syndrome, autism, and mild mental retardation. *Genetics in Medicine*, 1 (5), 213–218. <https://doi.org/10.1097/00125817-199907000-00008>
 39. Isung, J., Williams, K., Isomura, K., Gromark, C., Hesselmark, E., Lichtenstein, P. et al. (2020). Association of Primary Humoral Immunodeficiencies With Psychiatric Disorders and Suicidal Behavior and the Role of Autoimmune Diseases. *JAMA Psychiatry*, 77 (11), 1147–1154. <https://doi.org/10.1001/jamapsychiatry.2020.1260>
-

40. Noriega, D. B., Savelkoul, H. F. J. (2013). Immune dysregulation in autism spectrum disorder. *European Journal of Pediatrics*, 173 (1), 33–43. <https://doi.org/10.1007/s00431-013-2183-4>
 41. Bagheri-Hosseinabadi, Z., Imani, D., Yousefi, H., Abbasifard, M. (2020). MTHFR gene polymorphisms and susceptibility to rheumatoid arthritis: a meta-analysis based on 16 studies. *Clinical Rheumatology*, 39 (8), 2267–2279. <https://doi.org/10.1007/s10067-020-05031-5>
 42. Dimitroulas, T., Sandoo, A., Hodson, J., Smith, J., Douglas, K., Kitas, G. (2015). Associations between asymmetric dimethylarginine, homocysteine, and the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism (rs1801133) in rheumatoid arthritis. *Scandinavian Journal of Rheumatology*, 45 (4), 267–273. <https://doi.org/10.3109/03009742.2015.1086433>
 43. Li, M., Tang, Y., Zhao, E. Y., Chen, C.-H., Dong, L.-L. et al. (2021). Relationship between MTHFR gene polymorphism and susceptibility to bronchial asthma and glucocorticoid efficacy in children. *Zhongguo Dang Dai Er Ke Za Zhi*, 23 (8), 802–808. <https://doi.org/10.7499/j.issn.1008-8830.2105035>
 44. Mao, N., Chen, J., Wang, J., Xie, X., Li, F., Liu, Y. et al. (2020). Correlations of Methylenetetrahydrofolate Reductase Gene c and Genomic DNA Hypomethylation Level with Ankylosing Spondylitis. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao*, 42 (3), 307–312. <https://doi.org/10.3881/j.issn.1000-503X.11516>
 45. Chen, F., Wen, T., Lv, Q., Liu, F. (2019). Associations between Folate Metabolism Enzyme Polymorphisms and Lung Cancer: A Meta-Analysis. *Nutrition and Cancer*, 72 (7), 1211–1218. <https://doi.org/10.1080/01635581.2019.1677924>
 46. van der Weyden, M. B., Hayman, R. J., Rose, I. S., Brumley, J. (1991). Folate-deficient human lymphoblasts: Changes in deoxynucleotide metabolism and thymidylate cycle activities. *European Journal of Haematology*, 47 (2), 109–114. <https://doi.org/10.1111/j.1600-0609.1991.tb00131.x>
 47. Partearroyo, T., Úbeda, N., Montero, A., Achón, M., Varela-Moreiras, G. (2013). Vitamin B12 and Folic Acid Imbalance Modifies NK Cytotoxicity, Lymphocytes B and Lymphoproliferation in Aged Rats. *Nutrients*, 5 (12), 4836–4848. <https://doi.org/10.3390/nu5124836>
 48. Courtemanche, C., Elson-Schwab, I., Mashiyama, S. T., Kerry, N., Ames, B. N. (2004). Folate Deficiency Inhibits the Proliferation of Primary Human CD8+ T Lymphocytes In Vitro. *The Journal of Immunology*, 173 (5), 3186–3192. <https://doi.org/10.4049/jimmunol.173.5.3186>
 49. Abe, I., Shirato, K., Hashizume, Y., Mitsuhashi, R., Kobayashi, A., Shiono, C. et al. (2012). Folate-deficiency induced cell-specific changes in the distribution of lymphocytes and granulocytes in rats. *Environmental Health and Preventive Medicine*, 18 (1), 78–84. <https://doi.org/10.1007/s12199-012-0286-6>
 50. Troen, A. M., Mitchell, B., Sorensen, B., Wener, M. H., Johnston, A., Wood, B. et al. (2006). Unmetabolized Folic Acid in Plasma Is Associated with Reduced Natural Killer Cell Cytotoxicity among Postmenopausal Women. *The Journal of Nutrition*, 136 (1), 189–194. <https://doi.org/10.1093/jn/136.1.189>
 51. Bhatnagar, N., Wechalekar, A., McNamara, C. (2012). Pancytopenia due to severe folate deficiency. *Internal Medicine Journal*, 42 (9), 1063–1064. Portico. <https://doi.org/10.1111/j.1445-5994.2012.02849.x>
 52. Enstrom, A. M., Lit, L., Onore, C. E., Gregg, J. P., Hansen, R. L., Pessah, I. N. et al. (2009). Altered gene expression and function of peripheral blood natural killer cells in children with autism. *Brain, Behavior, and Immunity*, 23 (1), 124–133. <https://doi.org/10.1016/j.bbi.2008.08.001>
-

53. Warren, R. P., Yonk, L. J., Burger, R. A., Cole, P., Odell, J. D., Warren, W. L. et al. (1990). Deficiency of Suppressor-Inducer (Cd4+Cd45ra+) T Cells in Autism. *Immunological Investigations*, 19 (3), 245–251. <https://doi.org/10.3109/08820139009041839>
54. O’Keeffe, J., Gately, C. M., Counihan, T., Hennessy, M., Leahy, T., Moran, A. P., Hogan, E. L. (2008). T-cells expressing natural killer (NK) receptors are altered in multiple sclerosis and responses to α -galactosylceramide are impaired. *Journal of the Neurological Sciences*, 275 (1-2), 22–28. <https://doi.org/10.1016/j.jns.2008.07.007>
55. Villanueva, J., Lee, S., Giannini, E. H., Graham, T. B., Passo, M. H., Filipovich, A., Grom, A. A. (2004). Natural killer cell dysfunction is a distinguishing feature of systemic onset juvenile rheumatoid arthritis and macrophage activation syndrome. *Arthritis Res Ther*, 7 (1). <https://doi.org/10.1186/ar1453>
56. Nauseef, W. M. (2014). *Diagnostic Assays for Myeloperoxidase and Myeloperoxidase Deficiency*. *Neutrophil Methods and Protocols*. Totowa: Humana Press, 537–546. https://doi.org/10.1007/978-1-62703-845-4_32
57. de Magistris, L., Picardi, A., Siniscalco, D., Riccio, M. P., Sapone, A., Cariello, R. et al. (2013). Antibodies against Food Antigens in Patients with Autistic Spectrum Disorders. *BioMed Research International*, 2013, 1–11. <https://doi.org/10.1155/2013/729349>
58. Vojdani, A., Mumper, E., Granpeesheh, D., Mielke, L., Traver, D., Bock, K. et al. (2008). Low natural killer cell cytotoxic activity in autism: The role of glutathione, IL-2 and IL-15. *Journal of Neuroimmunology*, 205 (1-2), 148–154. <https://doi.org/10.1016/j.jneuroim.2008.09.005>
59. Torres, A. R., Westover, J. B., Gibbons, C., Johnson, R. C., Ward, D. C. (2012). Activating killer-cell immunoglobulin-like receptors (KIR) and their cognate HLA ligands are significantly increased in autism. *Brain, Behavior, and Immunity*, 26 (7), 1122–1127. <https://doi.org/10.1016/j.bbi.2012.07.014>
60. Ashwood, P., Corbett, B. A., Kantor, A., Schulman, H., Van de Water, J., Amaral, D. G. (2011). In Search of Cellular Immunophenotypes in the Blood of Children with Autism. *PLoS ONE*, 6 (5), e19299. <https://doi.org/10.1371/journal.pone.0019299>
61. Hughes, H. K., Ashwood, P. (2018). Anti-Candida albicans IgG Antibodies in Children With Autism Spectrum Disorders. *Frontiers in Psychiatry*, 9. <https://doi.org/10.3389/fpsy.2018.00627>
62. Naghibalhossaini, F., Ehyakonandeh, H., Nikseresht, A., Kamali, E. (2015). Association Between MTHFR Genetic Variants and Multiple Sclerosis in a Southern Iranian Population. *International Journal of Molecular and Cellular Medicine*, 4 (2), 87–93.
63. Watanabe, N., Gao, S., Wu, Z., Batchu, S., Kajigaya, S., Diamond, C. et al. (2021). Analysis of deficiency of adenosine deaminase 2 pathogenesis based on single-cell RNA sequencing of monocytes. *Journal of Leukocyte Biology*, 110 (3), 409–424. <https://doi.org/10.1002/jlb.3hi0220-119rr>
64. Hayes, B., Stanley, J., Peppers, B. P. (2021). COVID-19 Recurrence Without Seroconversion in a Patient With Mannose-Binding Lectin Deficiency. *Allergy & Rhinology*, 12. <https://doi.org/10.1177/21526567211024140>

RESULTS OF THE STUDY OF THE MICROBIAL SPECTRUM IN CHILDREN WITH AUTISM SPECTRUM DISORDERS ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

INTRODUCTION

Evidence from a number of recent independent meta-analyses and systematic reviews of randomized controlled trials, published in peer-reviewed medical journals indexed in the PubMed (MEDLINE) abstract bibliographic electronic database, indicates that genetic deficiency of the folate cycle (GDFC) is associated with the clinical phenotype of autism spectrum disorders (ASD) in children [1-5]. Results of a meta-analysis of randomized controlled trials by B. Q. Guo et al. in 2020, which included 31 trials involving 3304 children, including 1641 patients with ASD, without genetic clarification of the diagnosis, demonstrated that hyperhomocysteinemia, a phenomenon specific to GDFC, is associated with ASD and is a class feature of such children (Hedges's $g = 0.56$; 95 % CI = 0.36-0.76, $p < 0.001$) [6]. N. S. Mohammad et al., using the ANN (artificial neural network) model in a controlled clinical study involving 138 children with ASD and 138 healthy individuals, showed that the determination of pathogenic polymorphic variants of the genes GCPII C1561T, SHMT1 C1420T, MTHFR C677T, MTR A2756G, and MTRR A66G for diagnostic purposes allows determining the risk of developing ASD in a carrier with an accuracy of 63.8 % [2]. These data allow us to consider GDFC as the main factor in the genetic predisposition to the development of ASD in children.

Currently, the pathways of damage to the nervous system in children with GDFC have been discovered, which are implemented in the pathogenesis of encephalopathy in ASD. It has been shown that biochemical disorders caused by GDFC lead to damage to the immune system with the induction of immunodeficiency and associated immune dysregulation [7]. H. K. Hughes et al. in a systematic review of immune system dysfunction in patients with ASD demonstrated a number of characteristic pathological changes in immune status that may have pathogenetic significance and be targets of therapeutic interventions, including an abnormal cytokine profile with increased concentrations of pro- and decreased levels of anti-inflammatory cytokines, various changes in the absolute and relative number of lymphocytes and their subpopulations, laboratory signs of systemic and intracerebral inflammation, defects in the functioning of the adaptive and innate immune systems, deviations in serum concentrations of immunoglobulins of different classes, and serological signs of autoimmunity to both connective tissue autoantigens and neurons and glia [8].

The presence of immune dysfunction predicts a decrease in the body's resistance to microbial factors. Indeed, many reports have accumulated to date about the abnormal development of opportunistic and conditionally pathogenic infections in children with ASD, which can be explained by the damage to the immune system induced by GDFC. Binstock T. first pointed out the selectively reduced immunoresistance in children with ASD, identifying a subgroup of patients with the so-called intramonozytic pathogens - measles virus, cytomegalovirus, herpes virus type 6 and *Yersinia enterocolitica* [9]. Such children were characterized by suppression of hematopoiesis, impaired peripheral immunity, increased permeability of the blood-brain barrier and manifestations of demyelination in the white matter of the cerebral hemispheres - signs, as it is now known, typical of GDFC [10]. G. L. Nicolson et al. in a controlled clinical study using blood PCR showed

abnormally frequent detection of *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and herpes virus type 6 in children with ASD compared with healthy people [11]. A. Sakamoto et al. in a specially designed study found that congenital CMV infection with CNS involvement in children with ASD occurs significantly more often (7.4 %) than in the general population (0.31 % of cases) ($p = 0.004$). CMV was identified by real-time PCR of dried neonatal blood samples and cord blood samples obtained immediately after delivery [12]. S. Valayi et al. in a controlled clinical study demonstrated that specific IgM to EBV in the serum of children with ASD were significantly more common than in healthy individuals ($p < 0.05$) [13]. H. Jyonouchi et al. in a specially designed study showed an association of ASD with a primary deficiency of specific antipolysaccharide antibodies, which may explain the known predisposition to the development of chronic streptococcal infection in such children [14]. H. K. Hughe and P. Ashwood in a controlled clinical study found that seropositivity to *Candida albicans* in children with ASD occurs in 36.5 % of cases, while in healthy children it occurs in only 14.3 % of cases (OR = 3.45; 95 % CI = 1.0409–11.4650; $p = 0.041$). An association of seropositivity to *Candida* with manifestations of gastrointestinal dysfunction was shown [15]. T. Nayeri et al. conducted a meta-analysis of randomized controlled clinical trials, which demonstrated the association of ASD with toxoplasmosis, and that the presence of toxoplasmosis infection increases the risk of developing ASD in a child by 1.93 times (95 % CI = 1.01–3.66) [16]. M. Kuhn et al. reported a series of clinical cases of the combination of borreliosis and ASD in children and a significant reduction in the manifestations of ASD as a result of long-term therapy with ampicillin and azithromycin for borreliosis [17].

As can be seen from the above data, the spectrum of microorganisms that are atypically common in children with ASD compared to healthy people has expanded significantly since the publication of T. Binstock, however, the principle highlighted by the author still remains unchanged, since intracellular/intramonocytic pathogens predominate. X. Kong et al. justify the identification of subtypes of children with ASD depending on lesions of the oral and intestinal microbiota, given the important role of opportunistic and conditionally pathogenic microorganisms in the pathogenesis of mental illness [18].

It has now been established that infectious agents are active components of the pathogenesis of encephalopathy in children with ASD associated with GDFC. Microorganisms are capable of exerting both direct damaging effects on the brain parenchyma and being involved in indirect immune-mediated mechanisms of cerebral damage by inducing systemic inflammation and anti-brain autoimmunity. The direct damaging effect of infectious agents can consist in the induction of encephalitis and neurodegenerative processes. Thus, a number of cases of the development of autism symptoms after temporal partial necrotic-hemorrhagic encephalitis of HSV-1 etiology have been described [19–21]. This is one of the examples of the direct damaging effect of infectious agents on the CNS tissue in patients with ASD. In parallel, it has been established that HHV-6 carries out transolfactory migration to the brain [22] and thereby affects the structures of the mesolimbic system of the temporal lobes, inducing a neurodegenerative process called temporal mesial sclerosis [23], the clinical and radiological features of which are noted in many children with ASD [24]. The association of HHV-6 and temporal mesial sclerosis in humans is confirmed by the results of the latest meta-analysis of randomized controlled clinical trials performed on brain biopsies obtained from areas of neurodegenerative damage [25]. This is a second, neurodegenerative, form of direct damage to the CNS by microorganisms, which may be an important component of the pathogenesis of encephalopathy in children with ASD associated with GDFC.

If we talk about the indirect effects of infectious agents on the CNS tissue in children with ASD, then at least two ways of such damage should be distinguished. First, microorganisms can be triggers of anti-brain autoimmunity to myelin [26] and neurons [27] of the brain. Thus, cases of the development of autism symptoms in children with autoimmune limbic encephalitis [28, 29] with a positive response to immunomodulatory therapy [30] have been described, and viruses of the herpes family [31], as well as *Toxoplasma* [32] and *Mycoplasma* [31], can provoke a breakdown of immune tolerance to autoantigens of hippocampal neurons in such cases. The role of group A beta-hemolytic streptococcus, which is frequently found in children with ASD, in inducing autoimmune subcortical encephalitis, some of the symptoms of which may resemble ASD, is now well known and characterized [33, 34]. On the other hand, infectious agents in the context of immune dysregulation caused by GDFC can induce a state of systemic inflammation with the induction of hypercytokinemia with neurotoxic effects. The phenomenon of systemic hypercytokinemia with a pro-inflammatory profile in children with ASD is supported by the results of two recent meta-analyses of randomized controlled clinical trials [35, 36]. It has been shown that herpes virus type 6, which is often reactivated in autistic disorders, can induce a state of hyperactivation of macrophages with the development of hypercytokinemia, similar in nature to that observed in children with ASD [37]. In addition, when discussing the inflammatory mechanism of microbe-induced cerebral damage, it is worth mentioning the possibility of involvement of the functional microbiota-gut-brain axis [33, 38]. By enhancing inflammation in the intestine, infectious agents can induce further intracerebral inflammation in children with ASD by abnormal spread of the inflammatory process from the intestinal compartment through the blood and pathologically permeable blood-brain barrier to the brain parenchyma [39]. The role of the functional microbiota-gut-brain axis in the pathogenesis of encephalopathy in children with ASD is currently being discussed in a number of systematic reviews [33, 38] and the results of clinical trials [39].

Currently, there is a lack of systematization of knowledge on the microbial spectrum in patients with ASD, which appears to be quite specific and sharply different from that in healthy children. The relationship between microbial load and the state of the immune system requires significant clarification, and the role of microorganisms in the induction of cerebral damage and other complications in children with ASD remains poorly understood. Therefore, conducting specially planned studies in the outlined direction is an urgent task of modern neuroimmunology and immunopsychiatry.

The aim of the research: to study the structure of the microbial spectrum in children with ASD associated with GDFC, according to the evidence base accumulated to date and to study the association of the identified microorganisms with indicators of immune status to improve understanding of the pathogenesis of encephalopathy and improve diagnostic, monitoring and treatment algorithms.

MATERIALS AND METHODS OF THE RESEARCH

Data on the selection of patients for the study and control groups, the principles of making a clinical diagnosis of ASD, ethical and organizational aspects, the diagnosis of pathogenic polymorphic nucleotide substitutions in the genes of folate cycle enzymes, and the laboratory methods used to study associated biochemical disorders are given in the **Section Materials and methods in Chapter 2**. Data on the principles

and approaches to laboratory assessment of the immune status of patients in the observation groups are contained in the **Section Materials and methods in Chapter 3**.

Pathogenic polymorphic variants of folate cycle genes were determined by restriction polymerase chain reaction (PCR) 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 constituted the study group (SG).

The control group (CG) included 51 clinically healthy children (37 boys and 14 girls) of similar age distribution who did not suffer from GDFC.

Special laboratory paraclinical examination of children in the observation groups was performed taking into account modern ideas about the microbial spectrum in patients with ASD. Thus, the diagnosis of reactivated herpesvirus infections and TTV infection was performed by PCR of blood leukocytes (Department of Neurobiochemistry of the Institute of Neurosurgery of the National Academy of Medical Sciences of Ukraine) according to the data of the study by G. L. Nicolson et al. [11]. Detection of beta-hemolytic streptococcus group A was performed by bacteriological culture from the oropharyngeal mucosa on a selective nutrient medium or by specific antitoxic immunity in blood serum (antistreptolysin-O, antistreptodornase, antihyaluronidase) (ELISA; MDI Limbach Berlin GmbH, the Federal Republic of Germany), as stated in the systematic review by D. Dop et al. [34]. Infection caused by *Candida albicans* was diagnosed based on specific IgM in serum (ELISA; MDI Limbach Berlin GmbH, the Federal Republic of Germany) according to the results of the study by H. K. Hughes and P. Ashwood [15]. Infections caused by *Mycoplasma* and *Chlamydia pneumoniae* were detected based on specific IgM in serum (ELISA, Sinevo, Ukraine) [47]. Borreliosis and yersiniosis were identified based on the Versten blot analysis with simultaneous detection of IgM and IgG to a number of surface and deep antigens of the indicated pathogens (Sinevo, Ukraine) according to the data of M. Kuhn et al. [17] and T. Binstock [9]. Toxoplasmosis was diagnosed based on specific IgA in serum (ELISA, Sinevo, Ukraine), as shown by T. Nayeri et al. in the corresponding meta-analysis of randomized controlled clinical trials [16]. Transferred congenital CMV neuroinfection was identified based on the data of anamnestic studies of the newborn's serum (PCR, Department of Neurobiochemistry of the Institute of Neurosurgery of the National Academy of Medical Sciences of Ukraine and other laboratory centers) according to the data of A. Sakamoto et al. [12] and a specific neuroimaging pattern, which was interpreted according to the results of an 18-year longitudinal clinical study by R. Pinillos-Pisón et al. [40].

Thus, SG patients underwent a comprehensive assessment of the microbial spectrum in accordance with the evidence accumulated to date, however, the specificity of the approach consisted precisely in the simultaneous search for all microbial agents, which could provide a holistic picture of the child's current infection, since the scientific articles published so far usually dealt with the diagnosis of only some pathogens from a known list, which does not allow for a comprehensive analysis of the microbial load and an adequate assessment of the immune-microbiological connections and their place in the pathogenesis of the disease.

Thus, the analysis of the microbial spectrum was carried out by taking into account possible connections with the immune status of the child, which is a specific feature of the approach to data analysis, which was not previously used in children with ASD according to the data of available publications in PubMed and Em-base. Such an approach can allow not only to study the microbial load in SG children, but also to explain the

reasons for the formation of a specific pattern of microbial load and to investigate the clinical significance of infectious agents with the identification of potential mechanisms of involvement of microorganisms in the pathogenesis of encephalopathy in children with ASD associated with GDFC.

Statistical processing of the material was carried out by comparative and structural analyses. To determine the probability of differences between the indicators in the observation groups, the parametric student's T-test with a confidence interval of p and the nonparametric criterion - the number of signs Z according to Yu. Urbach. Differences were considered probable at $p < 0.05$ and $Z < Z_{0.05}$. To study the associations between the studied indicators, the odds ratio (OR) and 95 % confidence interval (95 % CI) were used.

Microsoft Excel was used for statistical calculations.

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 OF THE RESEARCH

The results of the applied laboratory tests for the comprehensive identification of opportunistic and conditionally pathogenic microorganisms in SG children are generally consistent with the data of previous separate clinical studies in this area. Positive test results were obtained for all potential microbes that are believed to be involved in the pathogenesis of encephalopathy in ASD. The results of studying the microbial spectrum in SG children significantly differ from the data in CG for all studied pathogens. Therefore, the obtained data are consistent with the ideas formed in recent years about the specific microbial spectrum in children with ASD, which does not correspond to that in healthy individuals. Although all infectious agents currently characterized as associated pathogens in autistic disorders are detected in children with ASD associated with GDFC, the proportion of positive cases differs sharply among different microorganisms, which suggests a heterogeneity of their distribution in the studied cohort of patients and, as a result, a different significance in the development of encephalopathy in the entire group. These differences should be taken into account both when planning laboratory tests to study the microbial load in patients with ASD associated with GDFC, and when conducting clinical and laboratory monitoring and determining the prerogative of certain therapeutic interventions.

Data on the structure of SG compared to CG by the detected opportunistic and conditionally pathogenic microorganisms are shown in **Fig. 4.1**.

The data in **Fig. 4.1** demonstrate the unequal prevalence of various studied microorganisms in SG patients and a significant difference in SG and CG in the specific gravity of detection of all studied infectious agents ($p < 0.05$; $Z < Z_{0.05}$). The results obtained indicate the predominance of viral agents over bacterial, fungal and protozoan. Therefore, opportunistic viral infections in a state of reactivation are the most frequent finding when assessing the microbial load in children with ASD associated with GDFC. Viral agents in such cases are detected at least three times more often than all studied bacterial, fungal and protozoan agents taken together. Therefore, the study of viral opportunistic agents should receive maximum attention when planning diagnostic algorithms for assessing the microbial spectrum in children with ASD associated with GDFC, and the pathogenetic pathways of virus-induced CNS damage should be a potential primary object of clinical research in this area.

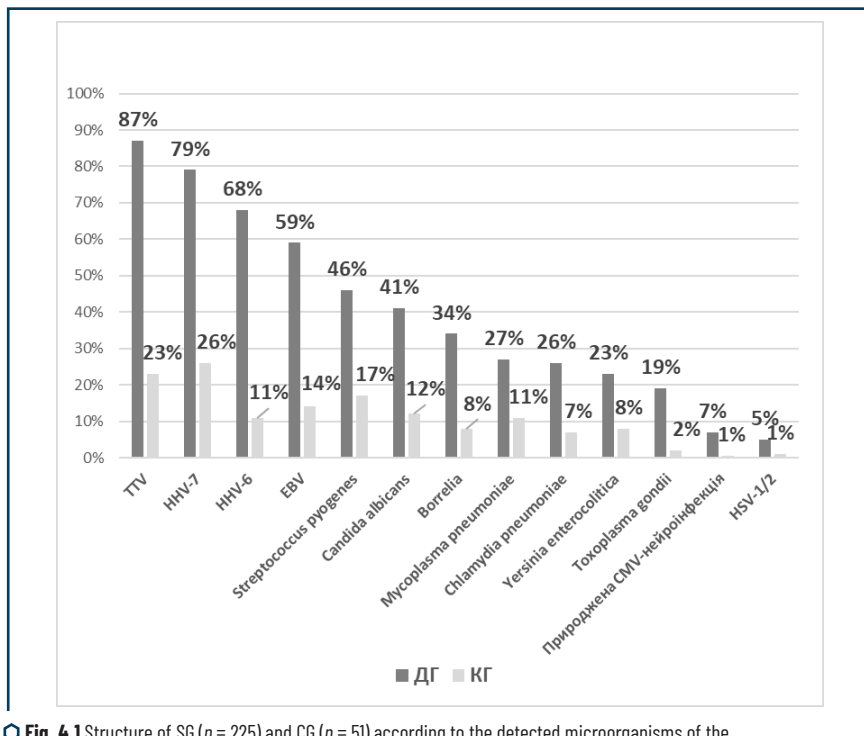


Fig. 4.1 Structure of SG ($n = 225$) and CG ($n = 51$) according to the detected microorganisms of the studied spectrum

Among the viral agents, TTV is most often detected in children with ASD associated with GDFC – in almost 9 out of 10 examined children, which is at least four times more than in healthy children of the CG ($p < 0.05$; $Z < Z_{0.05}$). HHV-7 and HHV-6 are found in 7 and 6 out of 10 examined SG patients, respectively, which is 3 and 6 times less common than in the CG ($p < 0.05$; $Z < Z_{0.05}$). As the results of this study show, TTV, HHV-7 and HHV-6 are the most frequent pathogens in children with ASD associated with GDFC. While the reactivation of herpesviruses in such cases has been reported previously [11], we did not find any relevant reports regarding TTV in the available scientific literature, therefore we believe that we have discovered the indicated association for the first time in the world.

EBV, Streptococcus pyogenes, Candida albicans, and Borrelia were found in children with ASD associated with GDFC at a moderate frequency. The proportion of cases of identification of these pathogens in an active state in SG ranged from 59 % to 34 %, while in CG – from 17 % to 8 % ($p < 0.05$; $Z < Z_{0.05}$).

Mycoplasma pneumoniae, Chlamydia pneumoniae, Yersinia enterocolitica were detected in 27-23 % of SG children and in 11-8 % of CG patients ($p < 0.05$; $Z < Z_{0.05}$). We propose to designate these microorganisms as pathogens with a low frequency of distribution in children with ASD associated with GDFC.

Congenital CMV infection and reactivated HSV-1/2 infection were pathogens with an extremely low proportion in SG (7-5 % vs. 1 % in CG; ($p < 0.05$; $Z < Z_{0.05}$).

The division of the identified microorganisms into four groups according to their specific gravity of distribution can be useful for rational planning and organization of diagnostic studies and therapeutic interventions, taking into account the economic and technical aspects of the problem. We propose a stepwise approach to assessing the microbial spectrum and a stepwise approach to prescribing antimicrobial drugs, taking into account the identified four groups of microorganisms with different specific gravity of distribution. Initially, it seems appropriate to identify microbes with a high specific gravity, and in subsequent stages - with a consistently lower specific gravity of distribution. Accordingly, as we believe, antimicrobial therapy should be carried out.

Of particular note is the identification of the association of EBV, *Streptococcus pyogenes*, and *Candida albicans*, which were simultaneously detected in the same patient in at least 80 % of cases of identification of such microorganisms. The relationship between EBV and *Streptococcus pyogenes* is now well known. Infectious mononucleosis, the primary form of EBV infection, often begins with streptococcal angina. The association of EBV and rheumatoid streptococcus has several aspects. The relationship is known at the level of intermicrobial interaction and at the level of the characteristics of human immune status disorders. If we talk about intermicrobial interaction, it has been established that streptococcus peptidoglycans, through their effect on TLR-2, activate EBV from a latent state in infected cells of the lymphoblastoid lineage of the tonsils, and EBV, by disrupting antibody genesis and inducing neutropenia, promotes the growth of streptococcus [41]. The immunological aspect of the interaction between these microbes lies in the typical associations of genetic disorders of antibody formation in humans. It was found that primary total deficiency of IgG3 (G3(g) allotype), which contributes to EBV reactivation, is associated with primary selective deficiency of specific IgG2 to streptococcal polysaccharides with preservation of the general IgG2 pool (G2(n) negative allotype) [42]. Due to its synergistic effect, coinfection of EBV and *Streptococcus pyogenes* promotes the accelerated development of associated autoimmune complications, as shown by T. Watanabe et al. on the example of acute glomerulonephritis [43]. The combination with candidiasis, in our opinion, can be explained not only by the peculiarities of the immune status, but also by the use of antibiotics for streptococcal infection, which, as is known, promotes the growth of candida.

It is important to establish why a specific microbial spectrum is formed in children with ASD associated with GDFC, which involves abnormally high susceptibility to some microorganisms and normal resistance to others. One of the explanations may relate to the features of the immune status in these patients, since the structure of the specified microbial spectrum is dominated by opportunistic and conditionally pathogenic microorganisms with low virulence, the reactivation of which usually occurs under conditions of immunosuppression. As noted above, various laboratory manifestations of immunodeficiency and related immune dysregulation have been described in patients with ASD [7, 8], therefore it seems appropriate to study the associations of certain disorders of the immune status and certain microorganisms that have undergone activation in the patient's body. The results of the study of such immuno-microbiological associations in SG children are given in **Table 4.1**.

● **Table 4.1** Results of the association study (OR; 95 % CI) of immune disorders and identified microorganisms in children with ASD associated with GDFC (n = 225)

Indicator	NK	NKT	CD8+ T lymphocytes	CD4+ T lymphocytes	ΔIG	ΓIG	ΜΠΟ	Combined violations
TTV	2.3767; 1.1772– 4.7983*	2.2713; 1.1259– 4.5818*	1.8452; 0.9231– 3.6885	1.7642; 0.8831– 3.5246	0.3950; 0.1865– 0.8366	0.4493; 0.2147– 0.9403	0.3152; 0.1485– 0.6690	0.6483; 0.3201– 1.3131
HHV-6	3.7316; 1.8118– 7.6856*	2.1711; 1.077– 4.3767*	3.9184; 1.8989– 8.0857*	0.7756; 0.3835– 1.5684	0.6957; 0.3465– 1.3967	0.4487; 0.2197– 0.9165	0.5269; 0.2605– 1.0659	0.3374; 0.1601– 0.7111
HHV-7	3.8864; 1.8758– 8.052*	2.8095; 1.3893– 5.6814*	3.5561; 1.7296– 7.3116*	0.6625; 0.3293– 1.3327	0.5546; 0.2749– 1.190	0.7018; 0.3455– 1.4256	0.6754; 0.3375– 1.3514	0.8546; 0.4242– 1.7218
EBV	3.7059; 1.7914– 7.6663*	2.4879; 1.2311– 5.0276*	2.8607; 1.4103– 5.8026*	0.8144; 0.4035– 1.6437	0.9214; 0.4627– 1.8348	0.8526; 0.4288– 1.6951	0.7381; 0.3642– 1.4958	0.7300; 0.3643– 1.4630
Str.	0.5068; 0.2445– 1.0504	0.8922; 0.4491– 1.7723	0.5833; 0.2898– 1.1741	0.6130; 0.3052– 1.2312	6.6667; 3.0563– 14.5419*	4.5588; 2.1939– 9.4728*	5.0679; 2.4247– 10.5927*	0.7018; 0.3455– 1.4256
Candida	0.8024; 0.4016– 1.6032	0.5997; 0.2927– 1.2288	0.2936; 0.1373– 0.6280	0.6483; 0.3201– 1.3131	0.5404; 0.2659– 1.0981	0.5844; 0.2869– 1.1902	3.9184; 1.8989– 8.0857*	0.9263; 0.4580– 1.8732
Toxoplasma	0.5678; 0.2761– 1.1675	0.8802; 0.4416– 1.7545	0.7083; 0.3546– 1.4150	9.4286; 4.2272– 21.0303*	0.4242; 0.2069– 0.8698	0.3837; 0.1843– 0.7990	0.6327; 0.3098– 1.2924	7.4582; 3.4436– 16.153*
CMV-infection	0.7655; 0.3826– 1.5317	0.9333; 0.4702– 1.8527	0.4242; 0.2069– 0.8690	0.5678; 0.2761– 1.1670	0.3122; 0.1484– 0.6570	0.3775; 0.1825– 0.7809	0.1970; 0.0884– 0.4392	9.7101; 4.3616– 21.6175*

Note: * $\alpha = 0.05$

It should be noted that *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Borrelia*, *Yersinia enterocolitica* were not associated with any of the identified disorders in the immune status of SG patients, therefore these data were not included in **Table 4.1**. The lack of associations in these cases can be explained by the properties of these microorganisms, which have a higher virulence than other studied microbes, and can usually affect the body of an immunocompetent person. In contrast, other microbes that have demonstrated certain associations with immune disorders have pronounced opportunistic or conditionally pathogenic properties, are usually characterized by low virulence and undergo reactivation from a latent or persistent state mostly in an immunocompromised organism. Analysis of associations for HSV-1/2 was not performed due to the small number of observations.

As shown in **Table 4.1**, viral infections were associated with disorders in the effector chain of adaptive and innate cellular immunity, which corresponds to the classical postulates of clinical immunology about the prerogative of cellular mechanisms of immune surveillance during the reactivation of viral agents in the human body [44]. HHV-6, HHV-7 and EBV were associated with deficiencies of NK, NKT and CD8+ cytotoxic T lymphocytes, which, according to the evidence accumulated so far, play an important role in the implementation of the immune response to viral pathogens [44, 45]. In particular, NK and NKT lymphocytes, using different mechanisms of recognition of virus-infected cells, participate in spontaneous and antibody-dependent cell-mediated cytotoxicity reactions and exert a number of regulatory effects aimed mainly at potentiation of effector mechanisms of cellular immunity [45, 46]. Instead, CD8+ cytotoxic T lymphocytes eliminate the virus *in situ* by implementing a specific immune cytotoxicity response [44]. It should be noted that TTV has also been associated with NK and NKT cell deficiencies, but not with CD8+ cytotoxic T cell deficiencies. The data obtained are fully consistent with the results of a systematic review by J. S. Orange on the clinical manifestations of primary NK cell deficiency in humans [46].

Unlike viral agents, streptococcal infection developed mainly in cases of disorders of the humoral component of adaptive immunity – hypo- and dysimmunoglobulinemia, which corresponds to the classical ideas about the predominant role of immunoglobulins in protection against streptococci and the well-known clinical picture of humoral immunodeficiencies, for example, Bruton's disease or common variable immunodeficiency, in which lesions caused by pyogenic opportunistic cocci predominate [47, 48]. Antibodies exert both direct and indirect antimicrobial effects in streptococcal infection in humans. The direct effects include the effects of agglutination, precipitation and neutralization [33]. Indirectly, antibodies help destroy streptococcal cells by opsonization and induction of immune phagocytosis, activation of the complement system by the classical pathway, and by promoting antibody-dependent cell-mediated cytotoxicity reactions, in which macrophages play the role of effector cells [34]. Previously, the association of streptococcal infection with humoral immunodeficiency in children with ASD was reported by H. Jyonouchi et al. [14]. Streptococcus was also associated with a deficiency of phagocyte myeloperoxidase. The role of phagocytosis in the neutralization of bacterial agents in the human body is now well known [49]. Selective abnormal susceptibility to streptococcal infection in primary neutrophil myeloperoxidase deficiency was first reported by P. Cocchi et al. back in 1973 [50].

In contrast, *Candida albicans* was associated only with neutrophil myeloperoxidase deficiency, which is consistent with current understanding of the key role of the myeloperoxidase-mediated microbicidal system of phagocytes in controlling candidal infection in humans [49] and with the results of a systematic review by W. M. Nausee on the problem of primary phagocyte myeloperoxidase deficiency in humans, which noted candidiasis as the leading clinical manifestation of this immunodeficiency [51].

Toxoplasmosis was associated with CD4+ T lymphocyte deficiency and combined immune disorders, in which both cellular and humoral immunity were involved simultaneously. The crucial role of CD4+ T lymphocytes in the control of *Toxoplasma* infection is well known due to numerous observations of severe *Toxoplasma* reactivation in AIDS of HIV etiology and idiopathic CD4+ T-cell lymphopenia [52].

Congenital CMV neuroinfection was associated only with combined immune disorders, which is consistent with the classical notion of the need for deep immunosuppression to reactivate this opportunistic agent in the human body [44].

Thus, it was established that different microorganisms are associated with different disorders in the immune status of patients with ASD associated with GDFC. We can talk about specific immuno-microbiological relationships, which are consistent with the classical notion of differences in the mechanisms of immune surveillance of opportunistic and conditionally pathogenic microbial agents of different nature in the human body. These data allow us to assume that it is the immunodeficiency caused by GDFC that is the cause of the formation of a specific microbial spectrum in children with ASD associated with GDFC. The identified connections allow, by assessing the immune status, to predict which microbes are most likely to multiply in the child's body, properly adjusting the direction of the diagnostic microbiological search and the program of further antimicrobial treatment. Conversely, the prevalence of certain microorganisms may indicate specific disorders in the immune system, which should be taken into account when planning the patient's immunological examination and selecting immunotherapy to correct immune status disorders. It is possible to distinguish subgroups of children with ASD associated with GDFC according to the prevailing microorganisms - viral, bacterial, fungal or protozoan - given the obvious differences in rational algorithms for diagnostic search and programs of antimicrobial and immunotropic treatment of such patients.

CONCLUSIONS TO THE SECTION 4

Children with ASD associated with GDFC are characterized by a specific microbial spectrum with a predominance of intracellular opportunistic and conditionally pathogenic microorganisms, which is determined by the features of immune status disorders provoked by GDFC, which should be taken into account when implementing the algorithm of rational microbiological search, assessment of immune status, and antimicrobial and immunotropic treatment in children with ASD.

REFERENCES

1. 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>
2. 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>
3. 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>
4. 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>

5. 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>
6. 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>
7. Mead, J., Ashwood, P. (2015). Evidence supporting an altered immune response in ASD. *Immunology Letters*, 163 (1), 49–55. <https://doi.org/10.1016/j.imlet.2014.11.006>
8. 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>
9. Binstock, T. (2001). Intra-monocyte pathogens delineate autism subgroups. *Medical Hypotheses*, 56 (4), 523–531. <https://doi.org/10.1054/mehy.2000.1247>
10. Marseglia, L. M., Nicotera, A., Salpietro, V., Giaimo, E., Cardile, G., Bonsignore, M. et al. (2015). Hyperhomocysteinemia and MTHFR Polymorphisms as Antenatal Risk Factors of White Matter Abnormalities in Two Cohorts of Late Preterm and Full Term Newborns. *Oxidative Medicine and Cellular Longevity*, 2015, 1–8. <https://doi.org/10.1155/2015/543134>
11. Nicolson, G. L., Gan, R., Nicolson, N. L., Haier, J. (2007). Evidence for *Mycoplasma ssp.*, *Chlamydia pneumoniae*, 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>
12. Sakamoto, A., Moriuchi, H., Matsuzaki, J., Motoyama, K., Moriuchi, M. (2015). Retrospective diagnosis of congenital cytomegalovirus infection in children with autism spectrum disorder but no other major neurologic deficit. *Brain and Development*, 37 (2), 200–205. <https://doi.org/10.1016/j.braindev.2014.03.016>
13. Valayi, S., Eftekharian, M. M., Taheri, M., Alikhani, M. Y. (2018). Evaluation of antibodies to cytomegalovirus and Epstein-Barr virus in patients with autism spectrum disorder. *Human Antibodies*, 26 (3), 165–169. <https://doi.org/10.3233/hab-180335>
14. 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>
15. Hughes, H. K., Ashwood, P. (2018). Anti-*Candida albicans* IgG Antibodies in Children With Autism Spectrum Disorders. *Frontiers in Psychiatry*, 9. <https://doi.org/10.3389/fpsy.2018.00627>
16. Nayeri, T., Sarvi, S., Moosazadeh, M., Hosseinijad, Z., Sharif, M., Amouei, A., Daryani, A. (2020). Relationship between toxoplasmosis and autism: A systematic review and meta-analysis. *Microbial Pathogenesis*, 147, 104434. <https://doi.org/10.1016/j.micpath.2020.104434>
17. Kuhn, M., Grave, S., Bransfield, R., Harris, S. (2012). Long term antibiotic therapy may be an effective treatment for children co-morbid with Lyme disease and Autism Spectrum Disorder. *Medical Hypotheses*, 78 (5), 606–615. <https://doi.org/10.1016/j.mehy.2012.01.037>

18. Kong, X., Liu, J., Cetinbas, M., Sadreyev, R., Koh, M., Huang, H. et al. (2019). New and Preliminary Evidence on Altered Oral and Gut Microbiota in Individuals with Autism Spectrum Disorder (ASD): Implications for ASD Diagnosis and Subtyping Based on Microbial Biomarkers. *Nutrients*, 11 (9), 2128. <https://doi.org/10.3390/nu11092128>
 19. DeLong, G. R., Bean, S. C., Brown, F. R. (1981). Acquired Reversible Autistic Syndrome in Acute Encephalopathic Illness in Children. *Archives of Neurology*, 38 (3), 191-194. <https://doi.org/10.1001/archneur.1981.00510030085013>
 20. Ghaziuddin, M., Tsai, L. Y., Eilers, L., Ghaziuddin, N. (1992). Brief report: Autism and herpes simplex encephalitis. *Journal of Autism and Developmental Disorders*, 22 (1), 107-113. <https://doi.org/10.1007/bf01046406>
 21. Gillberg, I. C. (1991). Autistic Syndrome with Onset at Age 31 Years: Herpes Encephalitis as a Possible Model for Childhood Autism. *Developmental Medicine & Child Neurology*, 33 (10), 920-924. <https://doi.org/10.1111/j.1469-8749.1991.tb14804.x>
 22. Harberts, E., Yao, K., Wohler, J. E., Maric, D., Ohayon, J., Henkin, R., Jacobson, S. (2011). Human herpesvirus-6 entry into the central nervous system through the olfactory pathway. *Proceedings of the National Academy of Sciences*, 108 (33), 13734-13739. <https://doi.org/10.1073/pnas.1105143108>
 23. Millichap, J. J., Millichap, J. G. (2015). Role of HHV-6B Infection in Mesial Temporal Lobe Epilepsy. *Pediatric Neurology Briefs*, 29 (5), 40. <https://doi.org/10.15844/pedneurbriefs-29-5-7>
 24. Monge Galindo, L., Pérez Delgado, R., López Pisón, J., Lafuente Hidalgo, M., Ruiz del Olmo Izuzquiza, L., 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>
 25. Wipfler, P., Dunn, N., Beiki, O., Trinka, E., Fogdell-Hahn, A. (2018). The Viral Hypothesis of Mesial Temporal Lobe Epilepsy – Is Human Herpes Virus-6 the Missing Link? A systematic review and meta-analysis. *Seizure*, 54, 33-40. <https://doi.org/10.1016/j.seizure.2017.11.015>
 26. 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>
 27. 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>
 28. González Toro, M. C., Jdraque Rodríguez, R., Sempere Pérez, Á., Martínez Pastor, P., Jover Cerdá, J., Gómez Gosálvez, F. A. (2013). Encefalitis antirreceptor de NMDA: dos casos pediátricos. *Revista de Neurología*, 57 (11), 504-508. <https://doi.org/10.33588/rn.5711.2013272>
 29. Kiani, R., Lawden, M., Eames, P., Critchley, P., Bhaumik, S., Odedra, S., Gumber, R. (2015). Anti-NMDA-receptor encephalitis presenting with catatonia and neuroleptic malignant syndrome in patients with intellectual disability and autism. *BJPsych Bulletin*, 39 (1), 32-35. <https://doi.org/10.1192/pb.bp.112.041954>
 30. Nepal, G., Shing, Y. K., Yadav, J. K., Rehrig, J. H., Ojha, R., Huang, D. Y., Gajurel, B. P. (2020). Efficacy and safety of rituximab in autoimmune encephalitis: A meta-analysis. *Acta Neurologica Scandinavica*, 142 (5), 449-459. <https://doi.org/10.1111/ane.13291>
-

31. Venâncio, P., Brito, M. J., Pereira, G., Vieira, J. P. (2014). Anti-N-methyl-D-aspartate Receptor Encephalitis with Positive Serum Antithyroid Antibodies, IgM Antibodies Against *Mycoplasma pneumoniae* and Human Herpesvirus 7 PCR in the CSF. *Pediatric Infectious Disease Journal*, 33 (8), 882–883. <https://doi.org/10.1097/inf.0000000000000408>
32. Li, Y., Viscidi, R. P., Kannan, G., McFarland, R., Pletnikov, M. V., Severance, E. G. et al. (2018). Chronic *Toxoplasma gondii* Infection Induces Anti-N-Methyl-d-Aspartate Receptor Autoantibodies and Associated Behavioral Changes and Neuropathology. *Infection and Immunity*, 86 (10). <https://doi.org/10.1128/iai.00398-18>
33. Baj, J., Sitarz, E., Forma, A., Wróblewska, K., Karakuła-Juchnowicz, H. (2020). Alterations in the Nervous System and Gut Microbiota after β -Hemolytic *Streptococcus* Group A Infection-Characteristics and Diagnostic Criteria of PANDAS Recognition. *International Journal of Molecular Sciences*, 21 (4), 1476. <https://doi.org/10.3390/ijms21041476>
34. Dop, D., Marcu, I., Padureanu, R., Niculescu, C., Padureanu, V. (2020). Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (Review). *Experimental and Therapeutic Medicine*, 21 (1). <https://doi.org/10.3892/etm.2020.9526>
35. 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>
36. Saghazadeh, A., Ataenia, B., Keynejad, K., Abdolalazadeh, 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.jpsy-chires.2019.05.019>
37. Lecointe, D., Fabre, M., Habes, D., Mielot, F., Bernard, O., Nordmann, P. (2000). Macrophage activation syndrome in primary human herpes virus-6 infection: a rare condition after liver transplantation in infants. *Gastroentérologie Clinique et Biologique*, 24 (12), 1227–1228.
38. Azhari, A., Azizan, F., Esposito, G. (2018). A systematic review of gut-immune-brain mechanisms in Autism Spectrum Disorder. *Developmental Psychobiology*, 61 (5), 752–771. <https://doi.org/10.1002/dev.21803>
39. Santocchi, E., Guiducci, L., Fulceri, F., Billeci, L., Buzzigoli, E., Apicella, F. et al. (2016). Gut to brain interaction in Autism Spectrum Disorders: a randomized controlled trial on the role of probiotics on clinical, biochemical and neurophysiological parameters. *BMC Psychiatry*, 16 (1). <https://doi.org/10.1186/s12888-016-0887-5>
40. 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>
41. Ueda, S., Uchiyama, S., Azzi, T., Gysin, C., Berger, C., Bernasconi, M. et al. (2013). Oropharyngeal Group A *Streptococcal* Colonization Disrupts Latent Epstein-Barr Virus Infection. *The Journal of Infectious Diseases*, 209 (2), 255–264. <https://doi.org/10.1093/infdis/jit428>

42. Linde, A., Söderström, R., Edvard Smith, C. I., Sällberg, M., Dahl, H., Grubb, R. et al. (1992). Herpesvirus serology, aberrant specific immunoglobulin G2 and G3 subclass patterns and Gm allotypes in individuals with low levels of IgG3. *Clinical and Experimental Immunology*, 90 (2), 199-203. <https://doi.org/10.1111/j.1365-2249.1992.tb07928.x>
43. Watanabe, T., Sugawara, H., Tamura, H., Ishii, A., Matsubayashi, H., Kakei, M., Momomura, S. (2012). Co-infection with Group A Streptococci and Epstein-Barr Virus Presenting with Acute Glomerulonephritis and Acute Left Ventricular Dysfunction. *Internal Medicine*, 51 (18), 2639-2643. <https://doi.org/10.2169/internalmedicine.51.7761>
44. J. Heath, J., D. Grant, M. (2020). The Immune Response Against Human Cytomegalovirus Links Cellular to Systemic Senescence. *Cells*, 9 (3), 766. <https://doi.org/10.3390/cells9030766>
45. Zuo, W., Zhao, X. (2021). Natural killer cells play an important role in virus infection control: Antiviral mechanism, subset expansion and clinical application. *Clinical Immunology*, 227, 108727. <https://doi.org/10.1016/j.clim.2021.108727>
46. Orange, J. S. (2012). Unraveling human natural killer cell deficiency. *Journal of Clinical Investigation*, 122 (3), 798-801. <https://doi.org/10.1172/jci62620>
47. Cirillo, E., Giardino, G., Ricci, S., Moschese, V., Lougaris, V., Conti, F. et al. (2020). Consensus of the Italian Primary Immunodeficiency Network on transition management from pediatric to adult care in patients affected with childhood-onset inborn errors of immunity. *Journal of Allergy and Clinical Immunology*, 146 (5), 967-983. <https://doi.org/10.1016/j.jaci.2020.08.010>
48. Więsik-Szewczyk, E., Jahnz-Różyk, K. (2020). From infections to autoimmunity: Diagnostic challenges in common variable immunodeficiency. *World Journal of Clinical Cases*, 8 (18), 3942-3955. <https://doi.org/10.12998/wjcc.v8.i18.3942>
49. Klebanoff, S. J., Kettle, A. J., Rosen, H., Winterbourn, C. C., Nauseef, W. M. (2013). Myeloperoxidase: a front-line defender against phagocytosed microorganisms. *Journal of Leukocyte Biology*, 93 (2), 185-198. <https://doi.org/10.1189/jlb.0712349>
50. Cocchi, P., Mori, S., Ravina, A. (1973). Myeloperoxidase-deficient leucocytes in streptococcal infections. *Helvetica Paediatrica Acta*, 28 (1), 79-85
51. Nauseef, W. M. (2014). *Diagnostic Assays for Myeloperoxidase and Myeloperoxidase Deficiency*. *Neutrophil Methods and Protocols*. Totowa: Humana Press, 537-546. https://doi.org/10.1007/978-1-62703-845-4_32
52. Zhao, X.-Y., Ewald, S. E. (2020). The molecular biology and immune control of chronic *Toxoplasma gondii* infection. *Journal of Clinical Investigation*, 130 (7), 3370-3380. <https://doi.org/10.1172/jci136226>

RESULTS OF THE SEARCH FOR LABORATORY SIGNS OF AUTOIMMUNE REACTIONS TO BRAIN AND EXTRACEREBRAL AUTOANTIGENS IN CHILDREN WITH AUTISM SPECTRUM DISORDERS ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

INTRODUCTION

Genetic deficiency of the folate cycle (GDFC) is an important associated factor in autism spectrum disorders (ASD) in children, as evidenced by the accumulated evidence base from meta-analyses of randomized controlled trials [1-5]. Biochemical abnormalities caused by GDFC have been shown to lead to immune system damage with induction of immunodeficiency and associated immune dysregulation [6]. Data from a systematic review by H. K. Hughes et al. clearly outline a range of representative pathological changes in the immune status in children with ASD, in particular, a pronounced cytokine imbalance with a predominance of pro-inflammatory mediators, aberrant subpopulation composition of blood lymphocytes, increased serum and cerebrospinal fluid concentrations of laboratory markers of neuroinflammation, multidirectional abnormal deviations in the functioning of the adaptive and innate immune systems, impaired ratios of immunoglobulins of different classes and subclasses in blood serum, and autoimmune reactions to a number of cerebral and extracerebral autoantigens [7].

At least 3 independent immune-mediated mechanisms of CNS damage in GDFC are currently known, caused by persistent immune dysfunction, which significantly contribute to the formation of encephalopathy with the clinical picture of ASD. These include the development of neurotropic opportunistic and conditionally pathogenic infections [8], autoimmune reactions to neurons and myelin of the cerebral hemispheres [9-12], and systemic and associated intracerebral aseptic inflammation caused by immune dysregulation [13, 14]. Inhibition or elimination of immune-dependent mechanisms of CNS damage appears to be a promising strategy for the treatment of ASD in children with GDFC [15].

A special role in the pathogenesis of encephalopathy in children with ASD is assigned to autoimmune mechanisms. Such ideas are based on a number of scientific evidence.

First, the results of a number of controlled clinical studies indicate the abnormal detection in patients with ASD of autoantibodies to CNS neurons, previously validated as markers of autoimmune encephalitis, which are not observed in healthy children [16]. Thus, U. K. Rout et al. found autoantibodies to the brain antigen GAD65 (GADA) among children with autism in 15 % of cases, autism spectrum disorders in 27 % of cases and in no healthy child in the control group [17]. These autoantibodies are a recognized laboratory marker of the so-called autoimmune anti-GAD65 limbic encephalitis, which leads to the development of a number of severe mental disorders in children and adults [16, 18]. At the same time, R. E. Frye et al. identified antibodies to folic acid receptors of brain neurons in children with ASD, indicating the heterogeneity of manifestations of anti-brain autoimmunity in such cases [10]. M. Cabanlit et al. established an association of ASD and the presence of autoantibodies to autoantigens of hypothalamic and thalamic neurons [9].

Second, there are several descriptions of the acute development of clinical manifestations of ASD after the onset of verified acute autoimmune limbic encephalitis in children and the achievement of clinical

improvement as a result of specific treatment of the autoimmune disease. Thus, M. C. González-Toro et al. reported two cases of autoimmune anti-NMDA limbic encephalitis in children, the clinical manifestations of which were consistent with ASD symptoms [19]. R. Kiani et al. also reported rapid autistic regression in the development of autoimmune anti-NMDA limbic encephalitis in a child [20].

Third, animal experiments have shown that anti-brain autoantibodies found in children with ASD can damage the brain in experimental rats, rabbits, and monkeys, inducing behavioral disorders that resemble the manifestations of ASD in humans. Thus, M. Gonzalez-Gronow et al. showed that catalytic IgG and IgA autoantibodies isolated from the blood of patients with autism disrupt the processes of hippocampal neuroplasticity in rats, inducing a pathological pathomorphological phenomenon similar to mesial temporal sclerosis [21], which is observed in many children with ASD according to the results of a clinical study by L. Monge-Galindo et al. [22]. Other studies have shown that after the introduction of autoantibodies obtained from the blood of children with ASD, rhesus macaques develop pronounced behavioral disorders that closely resemble those in autism in humans [23]. G. A. Mostafa and L. Y. Al-Ayadhi not only found an increased titer of autoantibodies to ganglioside M1 of nervous tissue in children with ASD, but also demonstrated a correlation between the titer of these autoantibodies and the severity of mental disorders in patients [24].

Fourth, several drugs with proven anti-inflammatory and immunomodulatory effects have demonstrated clinical efficacy in ASD, the data on which are summarized in a systematic review by J. Marchezan et al. [15], including infliximab [25] and human normal intravenous immunoglobulin [26], the mechanism of their therapeutic effect is associated precisely with the suppression of antineuronal autoimmunity and the associated intracerebral inflammation in the patient's body.

Opportunistic and conditionally pathogenic that are reactivated in the context of GDFC-induced immunodeficiency [6] may be involved in the induction of anti-brain autoimmunity in children with ASD through the phenomenon of molecular mimicry [27]. M. Mora et al. in a controlled study found abnormally high titers of antibodies to herpes simplex virus type 2 in children with ASD, which were associated with autoantibodies to brain antigens (77 % - anti-amygdala, 70 % - anti-caudate nucleus, 47.5 % - anti-cerebellum and brainstem, 45 % - anti-hippocampus, 40 % - anti-corpus callosum and 17.5 % - anti-cortex) [28]. V. K. Singh et al. established an association between high seropositivity to measles virus and human herpesvirus type 6 and abnormally high titers of autoantibodies to myelin basic protein and axonal filament protein of CNS neurons in children with ASD [12]. In another study, the authors showed cross-reactivity between anti-corrosion and anti-worm antibodies and autoantibodies against the myelin basic protein of the cerebral hemispheres in children with autism syndrome [11]. Induction of cross-reactive antibodies can be provoked by various superantigens of microorganisms in conditions of immunodeficiency caused by GDFC [6]. A. Vojdani et al. showed that in children with ASD, Chlamydia pneumoniae peptides, M-protein of streptococci and milk butyrophyllin lead to the production of defective specific antibodies with cross-reactivity, capable of recognizing not only microbial and food antigens, but also some molecules of nervous tissue, in particular - myelin basic protein, myelin-associated glycoprotein, myelin oligodendrocyte protein, proteins of neurofilaments and tubulin [29].

Therefore, the search for signs of autoimmunity in children with ASD associated with GDFC is an important task of modern neuroimmunology, the solution of which can provide useful information about immune-dependent pathways of CNS damage with the formation of the clinical phenotype of ASD and rational

approaches to immunomodulatory treatment to suppress anti-brain autoimmune reactions with potential neuroprotective effects in these patients.

The aim of the research: to study the structure of autoimmune reactions in children with ASD associated with GDHC, according to the evidence base accumulated to date, and to study the association of signs of autoimmunity with identified microorganisms to improve understanding of the pathogenesis of encephalopathy and improve diagnostic, monitoring, and treatment algorithms.

MATERIALS AND METHODS OF THE RESEARCH

Data on the selection of patients for the study and control groups, the principles of making a clinical diagnosis of ASD, ethical and organizational aspects, the diagnosis of pathogenic polymorphic nucleotide substitutions in the genes of folate cycle enzymes, and the laboratory methods used to study associated biochemical disorders are given in the **Section Materials and methods of the research in Chapter 2**. Data on the principles and approaches to laboratory assessment of the immune status of patients in the observation groups are given in the **Section Materials and methods of the research Section Materials and methods of the research**. The methods used to assess the microbial profile in individuals participating in the study are given in the **Section Materials and methods of the research in Chapter 4**.

We evaluated the known mechanisms of immune-mediated CNS damage in children with ASD according to the evidence accumulated to date in controlled clinical trials published in peer-reviewed medical journals cited in the electronic scientometric databases PubMed and Embase. Accordingly, the results of the Cunningham Panel™ (Moleculara Labs, Inc, United States of America) were analyzed to identify autoantibodies to antigens of CNS subcortical ganglia neurons in serum, including measurement of specific IgG to dopamine receptors types 1 and 2, lysoganglioside, and tubulin (ELISA) and assessment of Ca-dependent calmodulin kinase activity in neurons of diagnostic culture after contact with patient serum (cell-based assay; CBA), which meets modern requirements for laboratory diagnostics of PANS/PITANDS/PANDAS (pediatric acute-onset neuropsychiatric syndrome / pediatric infection-triggered autoimmune neuropsychiatric disorder / pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections) in children according to the review data of D. Dop et al. [30]. The results of a recent controlled clinical study by C. Shimasaki et al. prove the relevance of the results of the Cunningham panel in the diagnosis of autoimmune subcortical encephalitis in children [31]. The results of serological studies of blood serum were evaluated for the detection of specific antineuronal autoantibodies to hippocampal autoantigens, which are currently validated as laboratory markers of autoimmune limbic encephalitis in children and adults, namely, autoantibodies to glutamic acid decarboxylase (GADA), neuronal potassium channels, amphiphysin, neuronal NMDA receptors, GABA, CV2, Yo, Ri, Ma, Hu, AMPAR 1 and 2 (ELISA; MDI Limbach Berlin GmbH, Germany), which corresponds to modern approaches to the diagnosis of autoimmune limbic encephalitis in humans [18]. In particular, such diagnostics were performed according to the data of a systematic review by A. Budhram et al., devoted to a comprehensive analysis of the informativeness of validated methods of paraclinical diagnostics of autoimmune limbic encephalitis in the modern population [16].

Autoimmunization to myelin was determined by serum concentrations of autoantibodies to myelin basic protein (ELISA) and signs of neurosensitization to myelin by neutrophils and CD8+ cytotoxic T lymphocytes (CBA; Department of Neuroimmunology, Institute of Neurosurgery, NAMS of Ukraine), as recommended by V. K. Singh et al. [11, 12]. Autoantibodies to extracerebral autoantigens were measured by Western blotting in the Sinevo laboratory (Ukraine), which is in accordance with generally accepted approaches in modern rheumatology [32, 33]. In particular, the results of the "ANA profile" were analyzed, which included the determination of specific IgG to the autoantigens of the connective tissue cell nuclei nRMP/Sm, Smith antigen, RNP-70 -A and -C, SS-A, Ro-52, SS-B, Scl-70, PM-Scl, Jo-1, CEN-pB, PCNA, dsDNA, Nucleosomes, Histones, Rib P-protein, AMA-M-2 and the "Myositis profile" with the measurement of specific IgG to the autoantigens of the lumbar striated muscles Mi-2, Ku, PM-Scl, Jo-1, PL-7, PL-12 and Ro-52 in blood serum. Signs of systemic inflammation were assessed by the serum concentration of TNF-alpha (N up to 8.1 pg/ml; ELISA; Sinevo, Ukraine) according to the data of the systematic review by A. Masi et al. [13].

Statistical processing of the material was carried out by comparative and structural analyses. To determine the probability of differences between indicators in the observation groups, the parametric student's T-test with the confidence probability indicator p and the non-parametric criterion - the number of signs Z according to Yu. Urbach were used. Differences were considered probable at $p < 0.05$ and $Z < Z_{0.05}$. To study the associations between the studied indicators, the odds ratio (OR) and 95 % confidence interval (95 % CI) were used. Microsoft Excel was used to perform statistical calculations.

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 OF THE RESEARCH

In SG patients, laboratory signs of all five types of autoimmunity studied to cerebral and extracerebral autoantigens with different mechanisms of development and targets of autoaggression were identified, which were previously reported in the results of separate and unsystematic clinical studies evaluating autoimmunity in both children with ASD and patients with GDFC.

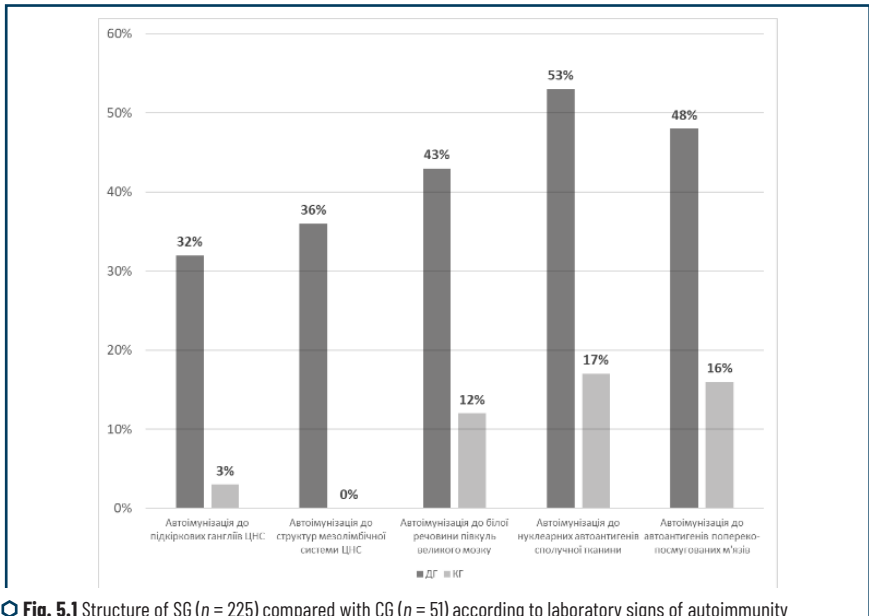
Laboratory signs of autoimmunity were detected among SG children in 68 % of cases, with at least two-thirds of them having a combination of several different autoimmune reactions, and in one-third - a combination of three or four different manifestations of autoimmunity. These data differ from similar results in CG, where signs of autoimmune reactions were detected in only 13 % of cases ($p < 0.05$; $Z < Z_{0.05}$), and combinations of different autoimmune reactions - in 3 % of cases ($p < 0.05$; $Z < Z_{0.05}$). Thus, children with ASD associated with GDFC generally exhibit laboratory signs of autoimmunity to the studied brain and extracerebral autoantigens, which are not typical for healthy children of similar gender and age. We can speak of an abnormal syndrome of impaired maintenance of immune tolerance to self-antigens in SG patients.

Although the reported cases of laboratory signs of autoimmunity to extracerebral autoantigens in SG were somewhat more frequent than to cerebral autoantigens, there was no significant difference between the specific gravity of these types of autoimmunity ($p > 0.05$; $Z > Z_{0.05}$).

The distribution of SG patients ($n = 225$) compared with CG ($n = 51$) according to the detected laboratory signs of autoimmunity to various studied autoantigens of the patient's body is shown in **Fig. 5.1**. According to the detected 5 types of autoimmunity, SG was divided into 5 subgroups. The 1st subgroup of SG included patients with laboratory signs of autoimmunity to subcortical ganglia ($n = 72$), the 2nd - neurons of the mesolimbic system ($n = 81$), the 3rd - myelin of the cerebral hemispheres ($n = 97$), the 4th - nuclei of connective tissue cells ($n = 119$), and the 5th - lumbar striated muscles ($n = 108$).

The appearance of signs of autoimmune reactions can be explained by a state of immune dysregulation caused by immunodeficiency, which, as previously shown, is observed in children with ASD associated with GDFC [6]. These data are consistent with numerous reports and results of controlled clinical studies on the detection of laboratory signs of immunization to various autoantigens in children with ASD [9-12], as well as with the data of a recent systematic review, which summarized the accumulated data on autoimmunity among this category of patients [7]. At the same time, the obtained results are consistent with the current evidence base on the association of GDFC with an increased risk of developing a number of autoimmune diseases during ontogenesis, including autoimmunity to CNS [34] and connective tissue autoantigens [35].

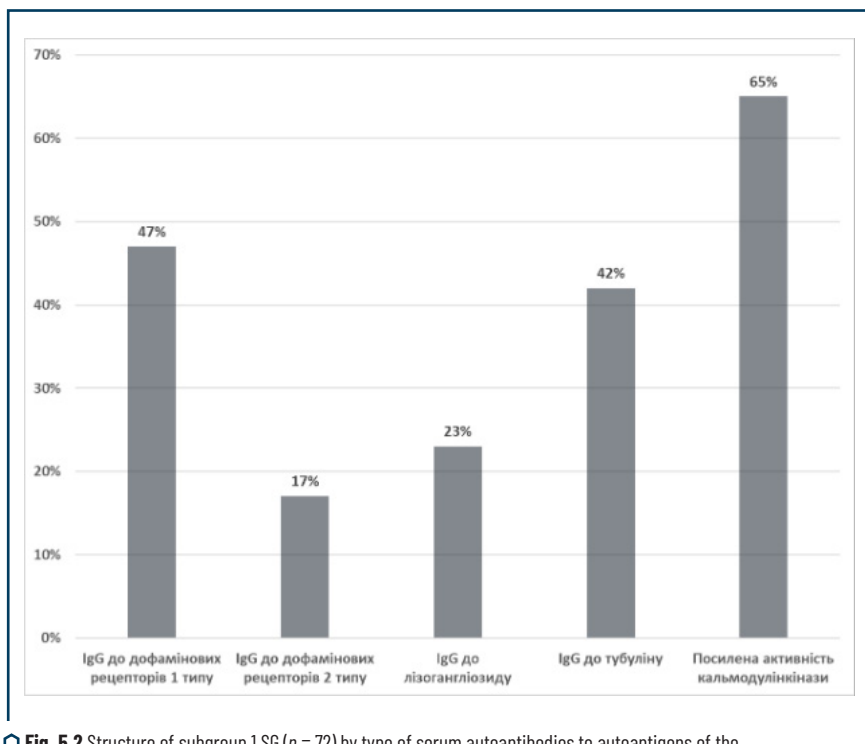
Thus, according to the results of separate analysis accumulated so far, both ASD and GDFC are separately associated with signs of autoimmunity to brain and extracerebral autoantigens, therefore, the detection in this study of a similar association in the combined analysis in children with ASD associated with GDFC seems logical and consistent with the current evidence base of the results of randomized controlled clinical trials in this area.



○ **Fig. 5.1** Structure of SG ($n = 225$) compared with CG ($n = 51$) according to laboratory signs of autoimmunity to various autoantigens of the patient's body

Positive results of the Cunningham panel occurred in 32 % of cases among SG children ($p < 0.05$; $Z < Z_{0.05}$). Positive results of measuring calmodulin kinase activity prevailed (65 % of cases in this subgroup), autoantibodies to dopamine receptors type 1 and tubulin were less common (47 % and 42 %, respectively). Autoantibodies to lysoganglioside and dopamine receptors type 2 were detected only in 23 % and 17 % of children in this subgroup. Combinations of different autoantibodies to subcortical ganglia autoantigens were noted in almost all cases (94 % of cases in this subgroup) (Fig. 5.2).

The presence of anti-antibodies to dopamine receptors of types 1 and 2 in the blood serum was associated with signs of pronounced hyperactivity and hyperexcitability, to tubulin – with manifestations of obsessive-compulsive syndrome, to lysoganglioside – with hyperkinesia in the form of tics, myoclonus and/or dystonia, and abnormally increased activity of calmodulin kinase – with clinical signs of activation of the sympathetic autonomic nervous system ($p < 0.05$; $Z < Z_{0.05}$), which indicates the unequal influence of different autoantibodies to autoantigens of the subcortical ganglia of the CNS on the clinical symptoms of mental illness in SG children and can be used in the diagnostic and prognostic plan in the clinical management of such children.



○ Fig. 5.2 Structure of subgroup 1 SG ($n = 72$) by type of serum autoantibodies to autoantigens of the subcortical ganglia of the CNS

Autoantibodies to autoantigens of neurons of the mesolimbic system of the temporal lobes of the cerebral hemispheres in the blood serum were noted in 81 of 225 SG patients (36 % of cases), while in the CG such autoantibodies were not identified in any case ($p < 0.05$; $Z < Z_{0.05}$). Autoantibodies to GADA occurred in SG in 48 % of cases among children of this subgroup, to potassium channels of neurons - in 39 % of cases. Autoantibodies to amphiphysin (3 individuals, 5 %), NMDA-receptors of neurons (3 individuals, 5 %) and the CV2 molecule (2 individuals, 3 % of cases) were also rarely found (Fig. 5.3). There was no patient in SG who had a combination of these autoantibodies, which is different from the results of the study of autoantibodies to autoantigens of the subcortical ganglia of the CNS, where combinations of autoantibodies to different autoantigens of subcortical neurons were characteristic ($p < 0.05$; $Z < Z_{0.05}$).

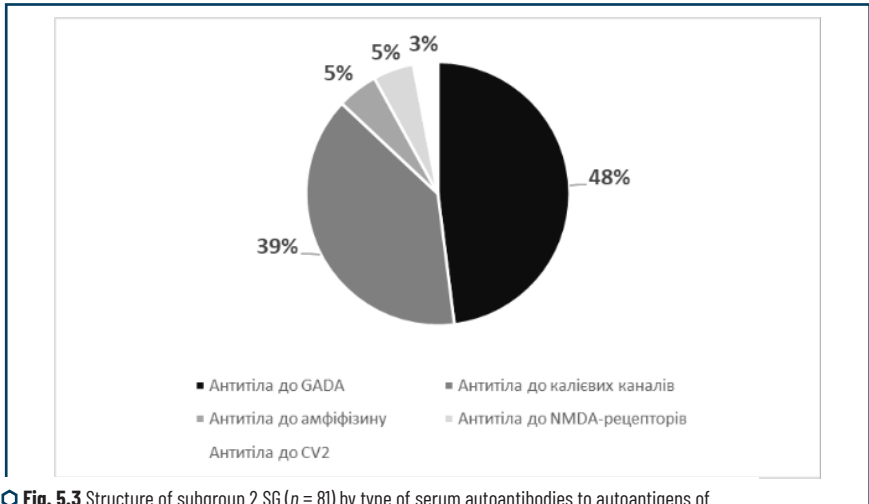


Fig. 5.3 Structure of subgroup 2 SG ($n = 81$) by type of serum autoantibodies to autoantigens of neurons of the mesolimbic system of the temporal lobes of the cerebral hemispheres

The presence of such autoantibodies in the blood serum was associated with more pronounced manifestations of hyperactivity and hyperexcitability in the child at the time of presentation, anamnestic indications of episodes of psychosis, the presence of an epileptic syndrome, and a deeper cognitive decline in the individual ($p < 0.05$; $Z < Z_{0.05}$).

Laboratory signs of autoimmunity to autoantigens of the white matter of the cerebral hemispheres were noted in 43 % of cases among SG children, which was significantly more common than in CG ($p < 0.05$; $Z < Z_{0.05}$). Autoantibodies to the basic myelin protein were most common (69 % of cases in this subgroup), which are currently considered the leading factor in damage to the white matter of the hemispheres in patients with multiple sclerosis [36]. Positive results of the assessment of cellular mechanisms of autoaggression to myelin autoantigens of the white matter of the cerebral hemispheres were less common - signs of sensitization of CD8+ cytotoxic T-lymphocytes to myelin and neurosensitization of neutrophils (24 % and 32 % of cases, respectively) (Fig. 5.4).

Combinations of various autoimmune reactions occurred in every fourth patient of this subgroup, which is probably less than in patients with signs of autoimmunity to autoantigens of the subcortical ganglia of the CNS ($p < 0.05$; $Z < Z_{0.05}$), but more than in children from the subgroup of autoimmunity to autoantigens of the mesolimbic system of the temporal lobes of the cerebral hemispheres ($p < 0.05$; $Z < Z_{0.05}$).

The presence of such autoantibodies in the blood serum was associated with more pronounced MR manifestations of leukoencephalopathy and physical signs of damage to the pyramidal and cerebellar conduction pathways, which clinically manifested themselves as symptoms of central paresis and/or pyramidal insufficiency and static-dynamic cerebellar ataxia, respectively ($p < 0.05$; $Z < Z_{0.05}$).

The obtained data are consistent with the results of controlled clinical studies indicating the association of GDFC with an increased risk of developing multiple sclerosis associated with autoimmunity to myelin autoantigens of the cerebral hemispheres [34], as well as with the data of scientific works on the abnormally frequent detection of laboratory manifestations of antimyelin autoimmunity in children with ASD [11, 12].

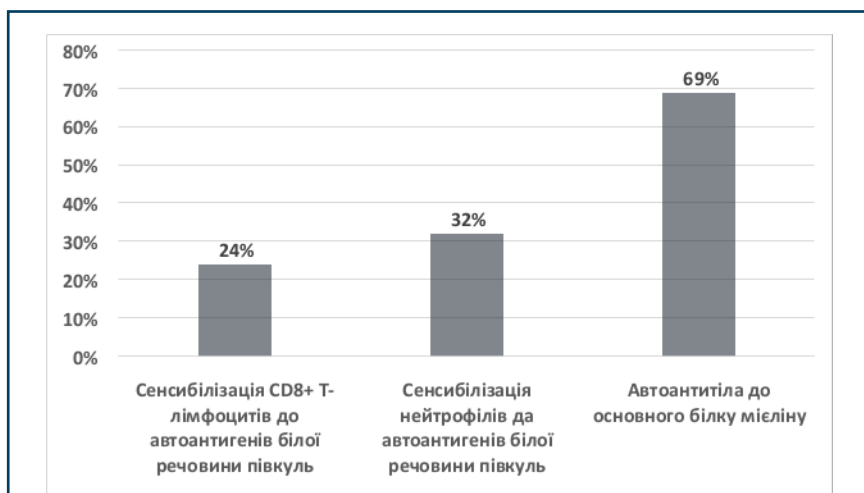


Fig. 5.4 Structure of subgroup 3 SG ($n = 97$) by type of serum autoantibodies to autoantigens of myelin of the white matter of the cerebral hemispheres

Determining different types of autoantibodies to nuclear proteins showed laboratory signs of autoimmunity to connective tissue autoantigens in 53 % of cases among SG children ($p < 0.05$; $Z < Z_{0.05}$). Positive results of autoantibodies to RNP-70 -A and -C, PM-Scl, PCNA and AMA-M-2 prevailed, which occurred in most cases among patients in this subgroup ($p < 0.05$; $Z < Z_{0.05}$). Autoantibodies to nRMP/Sm, Smith antigen, Ro-52, SS-B, Scl-70, Jo-1, CEN-pB, dsDNA, Nucleosomes, Histones, Rib P-protein were less common, each of which was registered in no more than a third of cases among patients in this subgroup (Fig. 5.5). Combinations of different autoantibodies to connective tissue nuclear autoantigens were almost always present in the same patient.

These serum autoantibodies were associated with anamnestic evidence of past arthritis and/or persistent arthralgias and myalgias in SG children ($p < 0.05$; $Z < Z_{0.05}$). It was among patients in this subgroup that all 11 cases of MR signs of diffuse small cerebral artery vasculopathy that were noted in SG were registered.

The obtained data are consistent with the results of randomized controlled clinical trials on the association of GDFC with an increased risk of developing rheumatic diseases, including systemic lupus erythematosus, which is characterized by the production of antinuclear autoantibodies [35].

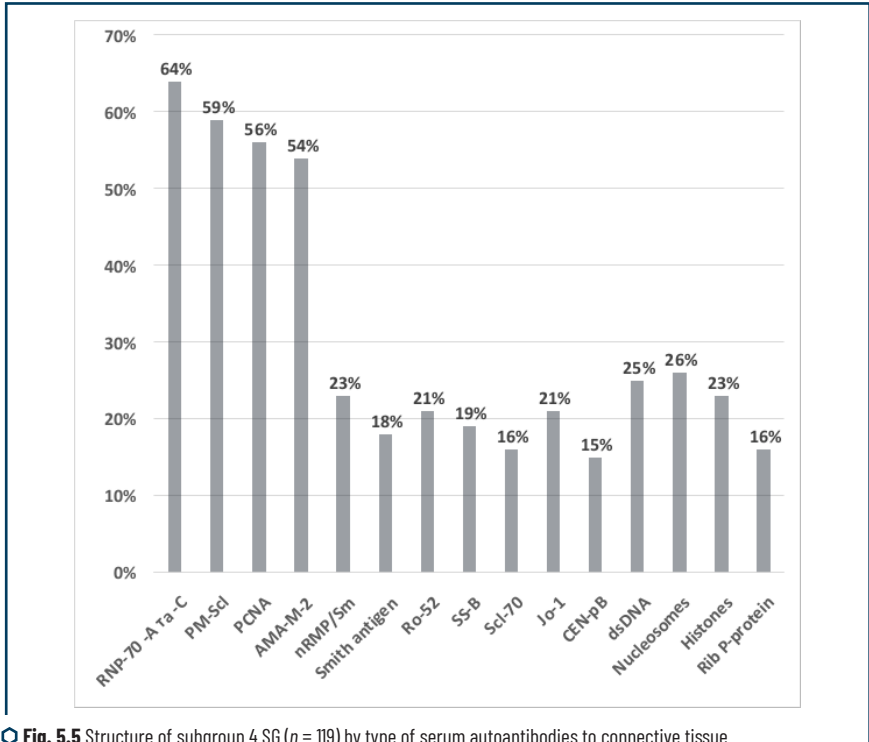


Fig. 5.5 Structure of subgroup 4 SG ($n = 119$) by type of serum autoantibodies to connective tissue nuclear autoantigens

Laboratory signs of autoimmunity to autoantigens of lumbar striated muscles occurred in 48 % of cases among SG patients, almost always in the form of combinations of different antimuscle autoantibodies, while in CG positive results of the assessment of autoimmunity to muscles were registered only in 15 % of cases, mostly in the form of single positive results ($p < 0.05$; $Z < Z_{0.05}$). Serum autoantibodies to Mi-2, Ku and PM-Scl prevailed, while autoantibodies to Jo-1, PL-7, PL-12 and Ro-52 were found almost twice as rarely in SG ($p < 0.05$; $Z < Z_{0.05}$) (Fig. 5.6).

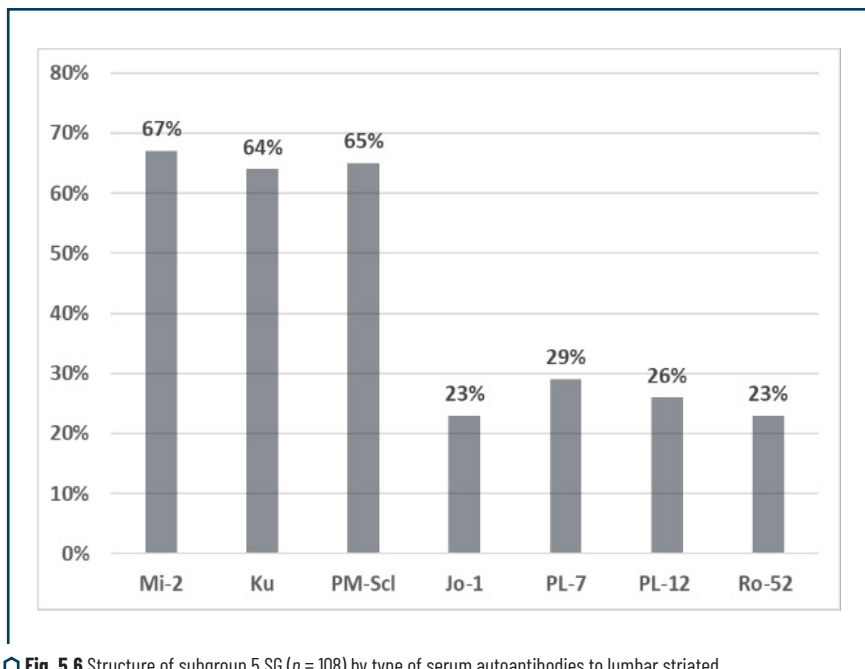


Fig. 5.6 Structure of subgroup 5 SG ($n = 108$) by type of serum autoantibodies to lumbar striated muscle autoantigens

The appearance of such autoantibodies in the blood serum was associated with anamnestic indications of transient periods of lethargy and limitation of mobility and/or manifestations of myositis of various groups of lumbar striated muscles, as well as with deeper disorders of the development of fine motor skills of the hands in SG children ($p < 0.05$; $Z < Z_{0.05}$). In this subgroup, there was a more pronounced decrease in skeletal muscle strength in individuals with signs of damage to the pyramidal tracts of the CNS ($p < 0.05$; $Z < Z_{0.05}$).

If we talk about the mechanism of autoimmune reactions in patients with ASD associated with GDFC, then according to the evidence accumulated so far, it is believed that the triggers of autoimmunity are mainly some opportunistic and conditionally pathogenic microbial agents, the control over which is abnormally weakened due to the presence of immune dysfunction caused by GDFC [6]. There are reasons to believe that different microorganisms have different effects on the development of autoimmune reactions, and the identification of relationships between the type of microorganism and the type of associated autoimmune lesion may be useful for rational planning of laboratory and instrumental paraclinical examinations, monitoring and prognosis, as well as the selection of adequate therapy. The results of the study of associations between microbial agents observed in children with ASD associated with GDFC and registered laboratory signs of autoimmunity are presented in **Table 5.1**.

● **Table 5.1** Results of the study of the association (OR; 95 % CI) of microbial agents and laboratory signs of autoimmune reactions among SG patients ($n = 225$)

Indicator	Autoimmunization to subcortical ganglia	Ат до мезолімбічної системи мозку	Autoimmunization to the white matter of the hemispheres	Ат до нуклеарних антигенів сполучної тканини	Ат до попереково-позвоночних м'язів
EBV	1.0643; 0.5112-2.2156	2.3061; 1.2038-4.4177*	5.1506; 2.4717- 10.7329*	3.1157; 1.6156- 6.0087*	3.0172; 1.5664-5.8118*
HHV-6	0.9903; 0.4773-2.0546	3.2356; 1.6637-6.2926*	3.1130; 1.5662- 6.1873*	2.9229; 1.5192-5.6237*	2.4596; 1.2851-4.7076*
HHV-7	1.1922; 0.5693-2.4968	2.6248; 1.3633-5.0535*	2.8632; 1.449-5.6576*	2.5750; 1.3417-4.9421*	2.3861; 1.2478-4.5628*
Streptococcus	13.2407; 6.2118-28.223*	1.7667; 0.911-3.4262	1.6045; 0.8461- 3.0426	1.7961; 0.9453- 3.4125	1.7855; 0.9398-3.3923
Borrelia	5.9325; 2.8312-12.4308*	1.9146; 0.9902-3.7021	2.5071; 1.3093- 4.8007*	3.3750; 1.7376-6.5555*	4.7884; 2.4216-9.4686*
Toxoplasma	1.7113; 0.8933- 3.2785	2.2475; 1.1773-4.2907*	1.3977; 0.7383- 2.6462	1.7457; 0.9193- 3.3150	1.9436; 1.0215-3.6982
TTV	1.8214; 0.9487-3.4967	2.1202; 1.0915-4.1184*	2.1656; 1.1350- 4.1321*	2.0165; 1.0586- 3.8412*	2.1821; 1.1439-4.1627*

Note: * $\alpha = 0.05$

As can be seen from the data in **Table 5.1**, different microorganisms were differently associated with laboratory signs of certain autoimmune reactions among SG children. No associations were found for *Candida albicans*, *Yersinia enterocolitica*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*, so these data were not included in the table. EBV, among other studied microbial agents, was most closely associated with the development of autoimmune reactions in SG children. Thus, EBV was associated with laboratory signs of autoimmune reactions to autoantigens of the mesolimbic system of the cerebral hemispheres, white matter of the cerebral hemispheres, nuclei of connective tissue cells, and lumbar striated muscles. In particular, identification of EBV increases the chances of detecting positive laboratory signs of autoimmunity to myelin of the white matter of the cerebral hemispheres by at least 5 times, to nuclear and muscle autoantigens by 3 times, and to proteins of neurons of the mesolimbic system of the brain by 2 times. This is consistent with the evidence accumulated so far regarding the participation of this virus as a trigger in the development of autoimmunity in the human body. The leading role of this virus can be associated with a wider arsenal of mechanisms for inducing the breakdown of immune tolerance to the antigens of the body's own body. In particular, EBV uses not only the mechanism of molecular mimicry, like most other studied microorganisms, but also polyclonal activation of B lymphocytes [36].

The results of a meta-analysis of randomized controlled clinical trials by Z. X. Li et al. indicate that EBV is a trigger for the development of an autoimmune reaction in systemic lupus erythematosus in humans, the laboratory marker of which is antinuclear autoantibodies, which, according to the results of this study, are observed in the majority of children with ASD associated with GDHC [37]. The data of a meta-analysis and systematic review of randomized controlled clinical trials by Y. H. Almohmeed et al. confirm that EBV is associated with the development of an autoimmune reaction in multiple sclerosis, when laboratory signs of autoimmunity to autoantigens of myelin of the white matter of the cerebral hemispheres are noted, which correspond to the results of the assessment of the corresponding autoimmunity in SG children [36]. Controlled clinical studies have implicated EBV as a trigger for autoimmune reactions in autoimmune limbic encephalitis [38] and dermatomyositis [33], in which positive autoantibody tests for autoantigens of CNS mesolimbic neurons and serum antimuscle autoantibodies have been identified in SG patients. These data fully explain the observed pattern of association of EBV with laboratory signs of autoimmunity in SG children.

Similar evidence has been accumulated regarding the associations of other studied microorganisms and the specific microbial spectrum characteristic of GDHC-associated immunodeficiency with autoimmune reactions to cerebral and extracerebral autoantigens.

Thus, HHV-6 and HHV-7 had a similar distribution of associations with laboratory signs of autoimmunity to autoantigens to EBV, which can be explained by the biological affinity of these microbial agents, but the chances of detecting certain autoimmune disorders with positive results of virological tests for the identification of HHV-6 and HHV-7 were lower than in the case of EBV reactivation. The presence of these viruses in a reactivated state increased the chances of detecting serological laboratory signs of autoimmunity to cerebral autoantigens at least 3 times, and to extracerebral autoantigens – 2-2.5 times. Accordingly, the results of a meta-analysis of randomized controlled clinical trials by A. Pormohammad et al. indicate that HHV-6 is a trigger for the breakdown of immune tolerance to autoantigens of the white matter of the cerebral hemispheres in multiple sclerosis, in which laboratory markers of autoimmunity to CNS myelin are similar to those observed among SG children [37]. Broccoli F. et al., having discovered selective reactivation of HHV-6 among patients with autoimmune diseases of connective tissue and muscles, which are characterized by abnormal synthesis of antinuclear and antimuscle autoantibodies, respectively, substantiated the role of this as a trigger for the development of an autoimmune reaction in these cases [39]. It has been established that transolfactory migration of HHV-6 to hippocampal neurons [40] and subsequent expression of viral proteins on these cells contributes to autoimmunization to autoantigens of neurons of the mesolimbic system of the temporal lobes of the cerebral hemispheres with the development of autoimmune limbic encephalitis [41], although HHV-6-induced forms of infectious limbic encephalitis in humans have been described [42]. J. J. Linnoila et al. in a specially designed clinical study indicated the simultaneous detection of HHV-6 DNA and autoantibodies to NMDA and GABA receptors of hippocampal neurons in cerebrospinal fluid in patients with signs of limbic encephalitis, indicating a mixed mechanism of CNS damage in many patients [43]. Accordingly, P. Venâncio et al. described an illustrative clinical case of autoimmune limbic encephalitis, in which the trigger for the development of an autoimmune reaction to hippocampal neurons was reactivated HHV-7 infection [44].

Streptococcus pyogenes was associated only with laboratory signs of autoimmune subcortical encephalitis, with a narrow range of associations among other pathogens studied, but the association found

was the strongest of all those found in this clinical study. In particular, the identification of *Streptococcus pyogenes* was associated with an increase in the odds of detecting autoantibodies to neurons of the subcortical nuclei of the cerebral hemispheres in the serum of the SG patient by at least 13 times. These data are consistent with the current concept of PANDAS in children [30].

Borrelia showed a combined pattern of associations, showing some common features with herpesviruses, increasing the odds of identifying signs of autoimmune reactions to autoantigens of myelin white matter of the cerebral hemispheres, connective tissue cell nuclei and proteins of lumbar striated muscles by 2, 3 and 4 times, respectively. However, unlike herpesviruses, there was no association with laboratory signs of autoimmune limbic encephalitis. At the same time, *Borrelia*, like *Streptococcus pyogenes*, showed an association with laboratory signs of PANS/PITANDS/PANDAS, increasing the odds of detecting positive Cunningham panel results by at least 5 times. These data are consistent with the results of a recent systematic review prepared by H. Rhee et al., in which borreliosis is positioned as the second most frequent trigger of the development of antineuronal autoimmune reaction in subcortical autoimmune encephalitides in humans, designated by the acronyms PANS/PITANDS/PANDAS [45].

Toxoplasma gondii had the narrowest and weakest association pattern with the studied signs of autoimmunity, increasing the chances of detecting autoantibodies to CNS hippocampal autoantigens in the serum of the SG patient by at least 2 times. Accordingly, X. Cai et al. reported the development of acute limbic anti-NMDA encephalitis with impaired mental activity during reactivation of toxoplasma infection, and complete resolution of clinical symptoms of autoimmune disease occurred after a 10-day course of azithromycin to suppress *Toxoplasma* reproduction without the use of immunosuppressive therapy for the autoimmune reaction against CNS neurons [46].

TTV demonstrated a distribution of associations similar to that of herpesviruses, which was associated with the simultaneous detection of these viruses in one patient in most cases and, possibly, some interaction between these infectious agents. Thus, S. S. Borkosky et al. showed that EBV stimulates the reproductive activity of TTV, which contributes to a stronger effect of this virus on the disruption of immune tolerance to myelin of the white matter of the cerebral hemispheres in multiple sclerosis [47]. However, the detected associations of TTV with laboratory signs of autoimmunity were weak – at the level of a two-fold increase in the risk of developing autoimmunity upon reactivation of this opportunistic agent from a persistent state. While the role of TTV as a trigger in multiple sclerosis [48] and autoimmune limbic encephalitis [41] has been previously reported, we consider the data on the association of this virus with autoimmunity to extracerebral autoantigens – connective tissue cell nuclei and lumbar striated muscle proteins – to be new, as we did not find relevant information in the available scientific literature.

Thus, serological signs of autoimmunity to autoantigens of the subcortical ganglia of the cerebral hemispheres were associated only with *Streptococcus pyogenes* and *Borrelia*, demonstrating close links between the phenomena under study, while laboratory signs of other autoimmune reactions had a broader pattern of associations with microbes of the studied spectrum, which were generally weaker than with positive results of the Cunningham panel. Thus, laboratory signs of autoimmune limbic encephalitis were associated with EBV, HHV-6, HHV-7, *Toxoplasma* and TTV, and autoimmune demyelination in the white matter of the cerebral hemispheres – with EBV, HHV-6, HHV-7, *Borrelia* and TTV. Laboratory signs of autoimmunity

to extracerebral autoantigens generally demonstrated a broader pattern of associations with the studied microorganisms compared to brain autoantigens, but the closeness of such associations was less. Thus, autoantibodies to autoantigens of connective tissue cell nuclei and lumbar striated muscle proteins were associated with EBV, HHV-6, HHV-7, Borrelia, and TTV, but not with *Toxoplasma gondii* and *Streptococcus pyogenes*.

CONCLUSIONS TO THE SECTION 5

The obtained data suggest the presence of laboratory signs of a syndrome of impaired immune tolerance in children with ASD associated with GDFC, which can be explained by the known state of immune dysregulation in such cases [6]. Five different types of autoimmunity with distinct mechanisms and targets of autoaggression have been identified, three of which are directed against autoantigens of the gray and white matter of the CNS, including antigens of the hippocampus, subcortical ganglia and myelin of the cerebral hemispheres, and two – against extracerebral proteins of the nuclei of connective tissue cells and lumbar striated muscles. In the development of encephalopathy in children with ASD associated with GDFC, not only autoimmune reactions to cerebral autoantigens, but also extracerebral autoimmunity may contribute. In particular, immunization to nuclear autoantigens of connective tissue can lead to the development of cerebral artery vasculopathy, and autoimmune damage to the lumbar striated muscles can exacerbate the manifestations of pyramidal insufficiency. Each of the identified types of autoimmunity is associated with distinct clinical and radiological symptoms, most likely resulting in the level of physical and mental health of children. Association studies shed light on potential mechanisms of autoimmunity in children with ASD associated with GDFC, demonstrating links between certain types of autoimmunity and reactivated opportunistic and conditionally pathogenic microorganisms of a specific spectrum with known properties to disrupt immune tolerance to self-antigens in conditions of immunosuppression. The identified signs of autoimmune reactions may be the object of effective therapeutic interventions aimed at achieving progress in the mental and physical development of children with ASD associated with GDFC by achieving neuroprotection by suppressing anti-brain autoimmunity, which should be tested in specially designed clinical trials.

REFERENCES

1. 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>
2. 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>

3. 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>
4. 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>
5. 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>
6. Mead, J., Ashwood, P. (2015). Evidence supporting an altered immune response in ASD. *Immunology Letters*, 163 (1), 49–55. <https://doi.org/10.1016/j.imlet.2014.11.006>
7. 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>
8. Nicolson, G. L., Gan, R., Nicolson, N. L., Haier, J. (2007). Evidence for Mycoplasma ssp., Chlamydia pneumoniae, 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>
9. 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>
10. Frye, R. E., Sequeira, J. M., Quadros, E. V., James, S. J., Rossignol, D. A. (2012). Cerebral folate receptor autoantibodies in autism spectrum disorder. *Molecular Psychiatry*, 18 (3), 369–381. <https://doi.org/10.1038/mp.2011.175>
11. 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>
12. 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>
13. 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>
14. Saghazadeh, A., Ataieina, B., Keynejad, K., Abdolalazadeh, 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>
15. 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>

16. Budhram, A., Leung, A., Nicolle, M. W., Burneo, J. G. (2019). Diagnosing autoimmune limbic encephalitis. *Canadian Medical Association Journal*, 191 (19), E529–E534. <https://doi.org/10.1503/cmaj.181548>
 17. Rout, U. K., Mungan, N. K., Dhossche, D. M. (2012). Presence of GAD65 autoantibodies in the serum of children with autism or ADHD. *European Child & Adolescent Psychiatry*, 21 (3), 141–147. <https://doi.org/10.1007/s00787-012-0245-1>
 18. Nepal, G., Shing, Y. K., Yadav, J. K., Rehrig, J. H., Ojha, R., Huang, D. Y., Gajurel, B. P. (2020). Efficacy and safety of rituximab in autoimmune encephalitis: A meta-analysis. *Acta Neurologica Scandinavica*, 142 (5), 449–459. <https://doi.org/10.1111/ane.13291>
 19. González Toro, M. C., Jadraque Rodríguez, R., Sempere Pérez, Á., Martínez Pastor, P., Jover Cerdá, J., Gómez Gosálvez, F. A. (2013). Encefalitis antirreceptor de NMDA: dos casos pediátricos. *Revista de Neurología*, 57 (11), 504–508. <https://doi.org/10.33588/rn.5711.2013272>
 20. Kiani, R., Lawden, M., Eames, P., Critchley, P., Bhaumik, S., Odedra, S., Gumber, R. (2015). Anti-NMDA-receptor encephalitis presenting with catatonia and neuroleptic malignant syndrome in patients with intellectual disability and autism. *BJPsych Bulletin*, 39 (1), 32–35. <https://doi.org/10.1192/pb.bp.112.041954>
 21. Gonzalez-Gronow, M., Cuchacovich, M., Francos, R., Cuchacovich, S., Blanco, A., Sandoval, R. et al. (2015). Catalytic autoantibodies against myelin basic protein (MBP) isolated from serum of autistic children impair in vitro models of synaptic plasticity in rat hippocampus. *Journal of Neuroimmunology*, 287, 1–8. <https://doi.org/10.1016/j.jneuroim.2015.07.006>
 22. 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>
 23. Gesundheit, B., Rosenzweig, J. P., Naor, D., Lerer, B., Zachor, D. A., Procházka, V. et al. (2013). Immunological and autoimmune considerations of Autism Spectrum Disorders. *Journal of Autoimmunity*, 44, 1–7. <https://doi.org/10.1016/j.jaut.2013.05.005>
 24. 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>
 25. 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>
 26. Maltsev, D. V. (2016). High-dose intravenous immunoglobulin therapy efficiency in children with autism spectrum disorders associated with genetic deficiency of folate cycle enzymes. *Psychiatry, Psychotherapy and Clinical Psychology*, 7 (2), 207–224. Available at: https://recipe.by/wp-content/uploads/woocommerce_uploads/2016/10/2_2016_Psikhiatriya.indd_.pdf
 27. Fujinami, R. S. (2002). Molecular mimicry that primes for autoimmunity which is triggered by infection. *Molecular Psychiatry*, 7 (S2), S32–S33. <https://doi.org/10.1038/sj.mp.4001173>
 28. Mora, M., Quintero, L., Cardenas, R. (2009). Association between HSV-2 infection and serum anti-rat brain antibodies in patients with autism. *Invest. Clin.*, 50 (3), 315–326.
-

29. Vojdani, A., Campbell, A. W., Anyanwu, E., Kashanian, A., Bock, K., Vojdani, E. (2002). Antibodies to neuron-specific antigens in children with autism: possible cross-reaction with encephalitogenic proteins from milk, *Chlamydia pneumoniae* and *Streptococcus* group A. *Journal of Neuroimmunology*, 129 (1-2), 168-177. [https://doi.org/10.1016/s0165-5728\(02\)00180-7](https://doi.org/10.1016/s0165-5728(02)00180-7)
30. Dop, D., Marcu, I., Padureanu, R., Niculescu, C., Padureanu, V. (2020). Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (Review). *Experimental and Therapeutic Medicine*, 21 (1). <https://doi.org/10.3892/etm.2020.9526>
31. Shimasaki, C., Frye, R. E., Trifiletti, R., Cooperstock, M., Kaplan, G., Melamed, I. et al. (2020). Evaluation of the Cunningham Panel™ in pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection (PANDAS) and pediatric acute-onset neuropsychiatric syndrome (PANS): Changes in antineuronal antibody titers parallel changes in patient symptoms. *Journal of Neuroimmunology*, 339, 577138. <https://doi.org/10.1016/j.jneuroim.2019.577138>
32. Li, Z.-X., Zeng, S., Wu, H.-X., Zhou, Y. (2018). The risk of systemic lupus erythematosus associated with Epstein-Barr virus infection: a systematic review and meta-analysis. *Clinical and Experimental Medicine*, 19 (1), 23-36. <https://doi.org/10.1007/s10238-018-0535-0>
33. Zheng, Q., Zhu, K., Gao, C.-N., Xu, Y.-P., Lu, M.-P. (2019). Prevalence of Epstein-Barr virus infection and characteristics of lymphocyte subsets in newly onset juvenile dermatomyositis. *World Journal of Pediatrics*, 17 (2), 205-209. <https://doi.org/10.1007/s12519-019-00314-7>
34. Naghibalhosseini, F., Ehyakondeh, H., Nikseresht, A., Kamali, E. (2015). Association Between MTHFR Genetic Variants and Multiple Sclerosis in a Southern Iranian Population. *International Journal of Molecular and Cellular Medicine*, 4 (2), 87-93.
35. Salimi, S., Keshavarzi, F., Mohammadpour-Gharehbagh, A., Moodi, M., Mousavi, M., Karimian, M., Sandoughi, M. (2017). Polymorphisms of the folate metabolizing enzymes: Association with SLE susceptibility and in silico analysis. *Gene*, 637, 161-172. <https://doi.org/10.1016/j.gene.2017.09.037>
36. Almohmeed, Y. H., Avenell, A., Aucott, L., Vickers, M. A. (2013). Systematic Review and Meta-Analysis of the Sero-Epidemiological Association between Epstein Barr Virus and Multiple Sclerosis. *PLoS ONE*, 8 (4), e61110. <https://doi.org/10.1371/journal.pone.0061110>
37. Pormohammad, A., Azimi, T., Falah, F., Faghihloo, E. (2017). Relationship of human herpes virus 6 and multiple sclerosis: A systematic review and meta-analysis. *Journal of Cellular Physiology*, 233 (4), 2850-2862. <https://doi.org/10.1002/jcp.26000>
38. Schwenkenbecher, P., Skripuletz, T., Lange, P., Dürr, M., Konen, F. F., Möhn, N. et al. (2021). Intrathecal Antibody Production Against Epstein-Barr, Herpes Simplex, and Other Neurotropic Viruses in Autoimmune Encephalitis. *Neurology Neuroimmunology & Neuroinflammation*, 8 (6). <https://doi.org/10.1212/ncx.0000000000001062>
39. Broccolo, F., Drago, F., Cassina, G., Fava, A., Fusetti, L., Matteoli, B. et al. (2013). Selective reactivation of human herpesvirus 6 in patients with autoimmune connective tissue diseases. *Journal of Medical Virology*, 85 (11), 1925-1934. <https://doi.org/10.1002/jmv.23670>
40. Harberts, E., Yao, K., Wohler, J. E., Maric, D., Ohayon, J., Henkin, R., Jacobson, S. (2011). Human herpesvirus-6 entry into the central nervous system through the olfactory pathway. *Proceedings of the National Academy of Sciences*, 108 (33), 13734-13739. <https://doi.org/10.1073/pnas.1105143108>

41. Perlejewski, K., Pawełczyk, A., Bukowska-Oško, I., Rydzanicz, M., Dzieciak tkowski, T., Paciorek, M. et al. (2020). Search for Viral Infections in Cerebrospinal Fluid From Patients With Autoimmune Encephalitis. *Open Forum Infectious Diseases*, 7 (11). <https://doi.org/10.1093/ofid/ofaa468>
42. Santoro, J. D., Hemond, C. C. (2018). Human herpesvirus 6 associated post-transplant acute limbic encephalitis: Clinical observations of biomarkers for risk of seizure in a pediatric population. *Transplant Infectious Disease*, 21 (1). <https://doi.org/10.1111/tid.13003>
43. Linnoila, J. J., Binnicker, M. J., Majed, M., Klein, C. J., McKeon, A. (2016). CSF herpes virus and autoantibody profiles in the evaluation of encephalitis. *Neurology Neuroimmunology & Neuroinflammation*, 3 (4). <https://doi.org/10.1212/nxi.0000000000000245>
44. Venâncio, P., Brito, M. J., Pereira, G., Vieira, J. P. (2014). Anti-N-methyl-D-aspartate Receptor Encephalitis with Positive Serum Antithyroid Antibodies, IgM Antibodies Against Mycoplasma pneumoniae and Human Herpesvirus 7 PCR in the CSF. *Pediatric Infectious Disease Journal*, 33 (8), 882–883. <https://doi.org/10.1097/inf.0000000000000408>
45. Rhee, H., Cameron, D. (2012). Lyme disease and pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS): an overview. *International Journal of General Medicine*, 5, 163–174. <https://doi.org/10.2147/ijgm.s24212>
46. Cai, X., Zhou, H., Xie, Y., Yu, D., Wang, Z., Ren, H. (2018). Anti-N-methyl-D-aspartate receptor encephalitis associated with acute *Toxoplasma gondii* infection. *Medicine*, 97 (7), e9924. <https://doi.org/10.1097/md.00000000000009924>
47. Borkosky, S. S., Whitley, C., Kopp-Schneider, A., zur Hausen, H., deVilliers, E.-M. (2012). Epstein-Barr Virus Stimulates Torque Teno Virus Replication: A Possible Relationship to Multiple Sclerosis. *PLoS ONE*, 7 (2), e32160. <https://doi.org/10.1371/journal.pone.0032160>
48. Saberi, A., Akhondzadeh, S., Kazemi, S. (2018). Infectious agents and different course of multiple sclerosis: a systematic review. *Acta Neurologica Belgica*, 118 (3), 361–377. <https://doi.org/10.1007/s13760-018-0976-y>

ASSESSMENT OF MARKERS OF INFLAMMATION AND NEURONAL DAMAGE IN PATIENTS WITH AUTISM SPECTRUM DISORDERS ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

JUSTIFICATION

Autism spectrum disorders (ASD) currently occur in at least 1 % of children in the modern population, and the trend towards an increase in the frequency of this psychiatric pathology among the pediatric population continues [6]. Although more than 100 genetic causes of the ASD phenotype in children have been proposed, most of them are rare and do not significantly affect either the high prevalence of the disease or the trend towards an increase in the frequency of these disorders in the population. Genetic deficiency of the folate cycle (GDFC) is a fairly common pathology of the human genome, which may explain the current epidemiological features of ASD. The evidence base for the association of GDFC and ASD in children is based on the results of 5 meta-analyses of randomized controlled clinical trials conducted from 2013 to 2020 and covering the results of 8 to 25 trials involving 1361 to more than 3000 children with ASD and 6591 to 7257 healthy children [13, 17, 20, 21, 22]. It has been established that GDFC leads to the development of a number of pathological biochemical changes in the body [14, 25, 26], including hyperhomocysteinemia [11], which predispose to the development of oxidative stress [6, 8]. The results of a meta-analysis of randomized controlled clinical trials by Guo B.O. et al. 2020, which included 31 trials involving 3304 children, including 1641 patients with ASD without specifying the genetic nature of the disease, demonstrated that hyperhomocysteinemia is closely associated with ASD in children, being a typical feature of this heterogeneous cohort of patients as a whole (Hedges's $g = 0.56$; 95 % CI = 0.36–0.76, $P < 0.001$) [11]. Since hyperhomocysteinemia is a specific biochemical phenomenon for GDFC, the results of this meta-analysis indicate that GDFC, among other genetic abnormalities, is currently the leading factor in the genetic predisposition to the development of ASD in children. The evidence for the development of oxidative stress with an excess of prooxidant and a deficiency of antioxidant molecules in ASD is based on the results of two meta-analyses and systematic reviews of randomized controlled clinical trials, covering 87 trials involving 9109 patients [6, 8]. It is the pathological changes in the biochemical profile and the associated oxidative stress that are considered to be the cause of impaired immune system development in children with ASD associated with GDFC [16, 19]. Such patients develop a special form of immunodeficiency [1], which leads to the development of a number of immune-dependent complications, in particular encephalopathy, which, in fact, leads to the clinical picture of ASD [3], an infectious syndrome with a predominance of intracellular opportunistic and conditionally pathogenic microbes [4, 18], an immunoinflammatory syndrome, including persistent immune-mediated enterocolitis [10, 24], an allergic syndrome [12], an autoimmune syndrome [5, 9], and an increased tendency to develop neoplasia [7]. Actually, encephalopathy in GDFC, in addition to the direct metabolic mechanism of development, mediated, in particular, by the neurotoxic effect of homocysteine [3], is mostly due to the influence of immune-dependent mechanisms - infectious factors [4, 18], autoimmune reactions to neurons and myelin of the cerebral hemispheres [5, 9], systemic/intracerebral inflammation [15, 23].

Evidence for the development of a persistent systemic inflammatory response in children with ASD is based on the results of 2 meta-analyses of randomized controlled clinical trials. In particular, data from the first systematic review and meta-analysis of randomized controlled clinical trials show increased serum concentrations of the pro-inflammatory mediators interleukin-1beta (IL-1beta) ($p < 0.001$), interleukin-6 (IL-6) ($p = 0.03$), interleukin-8 ($p = 0.04$), interferon-gamma (IFN-gamma) ($p = 0.02$), eotaxin ($p = 0.01$), and monocyte chemoattractant factor 1 ($p < 0.05$) and decreased levels of the anti-inflammatory cytokine transforming growth factor beta 1 ($p < 0.001$) in children with ASD ($n = 743$) compared to healthy subjects ($n = 592$) [15]. The results of a meta-analysis of randomized controlled clinical trials prepared by Saghazadeh A. et al., which includes 38 trials involving 2487 children, show a significant increase in serum concentrations of tumor necrosis factor alpha (TNF-alpha), IFN-gamma, IL-1beta, and IL-6 in children with ASD compared to healthy individuals [23].

It is important to further study the indicators of systemic inflammation in children with ASD to determine the most informative biomarkers of inflammation intensity that could be useful for clinical practice. The clinical significance of the phenomenon of persistent systemic inflammation in children with ASD needs to be clarified. It is advisable to study the relationship between increased serum concentrations of certain pro-inflammatory mediators and indicators of neuronal damage, which would provide additional evidence of the contribution of systemic inflammation to the development of encephalopathy and would allow us to propose new pharmacological methods of neuroprotection in children with ASD associated with GDFC for testing.

The aim of the research: to study serum concentrations of typical pro-inflammatory mediators TNF-alpha, IL-6, and tumor M2 pyruvate kinase (TM2PK) in children with ASD associated with GDFC, with clarification of their relationship with serum concentrations of marker molecules of CNS neuronal damage neuron-specific enolase (NSE) and S-100 protein to expand scientific ideas about the influence of the systemic inflammatory response on the development of encephalopathy in this pathology and to discover new points of application of neuroprotective treatment.

Materials and methods. The medical data of 225 children aged 2 to 9 years with GDFC, who were diagnosed with ASD, were analyzed. All of them were patients of the specialized neuroimmunological clinic Vivere (registration dossier dated 12/22/2018 No. 10/2212-M). Data for the study and processing of the material were 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 of the Bogomolets National Medical University). 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 PCR based on the detection of the nucleotide substitution MTHFR C677T in monoform, as well as - in combination with other nucleotide substitutions - MTHFR A1298C, MTRR A66G and/or MTR A2756G (111 individuals). These individuals constituted the study group (SG). The control group (CG) included 51 children (37 boys and 14 girls) of similar age distribution who did not suffer from GDFC.

In patients of both observation groups, the results of the study of serum concentrations of three typical pro-inflammatory mediators characterizing the intensity of systemic inflammation in the human body were analyzed, in particular - TNF-alpha (N up to 8.1 pg/ml), IL-6 (N up to 7 pg/ml) and TM2PC (N up to 20 U/ml). In parallel, in both observation groups, the data from measuring the concentration in the blood serum of two

characteristic indicators of neuronal damage were evaluated, namely – NSE (N up to 16.5 ng/ml) and S-100 protein (N up to 0.105 µg/l). At the time of laboratory studies, patients of both observation groups were not taking any medications that could affect the results of the tests.

Statistical processing of the obtained material was carried out by comparative and structural analyses. To determine the probability of differences between the studied laboratory parameters in the observation groups, the parametric Student's T-test with the confidence probability indicator p and the non-parametric criterion – the number of signs Z according to Urbach V.Yu. Differences were considered probable at $p < 0.05$ and $Z < Z_{0.05}$. To study the associations between indicators of systemic inflammation and indicators of damage to CNS neurons, the odds ratio (OR) and 95 % confidence interval (95 % CI) were used. For the purpose of statistical calculations, Microsoft Excel was used.

This clinical study was performed as a fragment of research work commissioned by the Ministry of Health of Ukraine (state registration number 012IU107940).

Research results and their discussion. The structural analysis of the results of the study of laboratory indicators of systemic inflammation in the observation groups demonstrates that the most frequent disorder detected was an increase in the serum concentration of the pro-inflammatory mediator TM2PK, which was noted in 193 of 225 SG patients (86 %) and only in 11 of 51 CG children (22 % of cases). The serum concentration of the pro-inflammatory cytokine TNF-alpha was high in 139 of 225 SG children (62 %) and only in 7 of 51 CG children (14 % of cases). The serum concentration of another pro-inflammatory cytokine IL-6 was elevated in 69 of 225 SG children (31 %) and only in 3 of 51 CG children (6 % of cases) (Fig. 6.1).

The data from the comparative analyses indicate that there was a significant difference in the studied indicators of systemic inflammation in the observation groups due to a significantly greater proportion of increased serum concentrations of all three studied laboratory indicators of systemic inflammation among children with ASD associated with GDFC, compared with healthy children in the control group ($p < 0,05$; $Z < Z_{0,05}$).

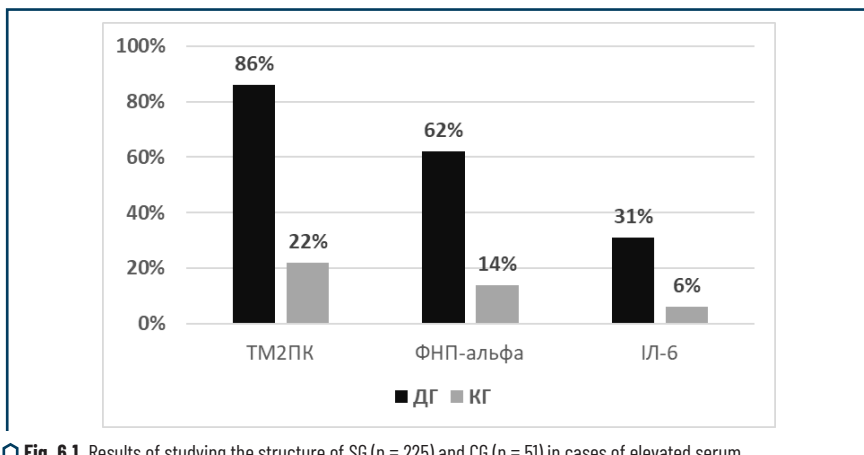


Fig. 6.1. Results of studying the structure of SG (n = 225) and CG (n = 51) in cases of elevated serum concentrations of the studied pro-inflammatory mediators

TM2PK was the most representative biomarker of systemic inflammation in the CG, since an increase in the serum concentration of this pro-inflammatory mediator was noted in almost all patients. However, a relatively large number of false-positive results (22 % of cases among healthy children) somewhat reduces the informativeness of this indicator in assessing the intensity of systemic inflammation in children with ASD associated with GDFC. Probably, in controversial cases, this indicator should be taken into account only with additional confirmation of the results of studying other indicators of inflammation with a smaller number of false-positive results. The concentration of TNF-alpha was increased in most children in the CG, but the number of positive results was almost a third less than in TM2PK. The small number of false-positive results (14 %) allows us to consider TNF-alpha as an informative indicator of the inflammatory reaction, which, however, does not characterize the group as a whole due to a relatively large number of negative results (38 % of cases). Serum IL-6 concentration was elevated only in one third of cases among children of the CG, which does not allow us to consider this biomarker representative of all patients with ASD associated with GDFC, for whom the development of persistent systemic inflammation in the body is characteristic. However, the smallest number of pseudo-negative results of this indicator allows us to consider it the most reliable among other indicators studied in this study. In particular, serum concentrations of TNF-alpha and IL-6 can be used to verify the results of measuring the concentration of TM2PC, for which pseudo-positive results are characteristic in every fifth patient.

The mean serum concentration of TM2PC in SG was high and equaled 63.1 ± 3.74 U/ml, significantly exceeding the similar indicator in CG (23.2 ± 0.91 U/ml) ($p < 0.05$; $Z < Z_{0.05}$) (**Fig. 6.2**). The level of the mean serum concentration of TM2PC exceeded the upper limit of reference values three times, which indicates a large range of fluctuation of its measurement results. In contrast, the mean concentration of TNF-alpha in SG was 13.7 ± 0.65 pg/ml, only 60 % exceeding the upper limit of the normal range, demonstrating a smaller range of fluctuation of measurement results. Comparison with similar data from the CG ($X = 5.3 \pm 0.38$ pg/ml) indicates a significant increase in serum TNF-alpha concentration in children with ASD associated with GDFC compared to healthy children ($p < 0.05$; $Z < Z_{0.05}$). The average serum concentration of IL-6 in SG was elevated and was 8.7 ± 0.57 pg/ml, which was only 20 % higher than the upper reference level, indicating a narrow range of fluctuation in the measurement results of this indicator. There was a significant difference in this indicator in SG and CG ($X = 3.6 \pm 0.27$ pg/ml) ($p < 0.05$; $Z < Z_{0.05}$) (**Fig. 6.3**). The greater the range of fluctuation in the measurement results of the indicator, the easier it is for the clinician to assess the intensity of inflammation and compare the results of studies in different patients. Therefore, TM2PC better than TNF-alpha and IL-6 characterized the intensity of inflammation, due to the large range of fluctuation of the results more fully demonstrating the gradation of the intensity of the systemic inflammatory reaction in different patients.

Thus, the mean serum concentrations of all three studied parameters were significantly increased in the SG compared with the CG, indicating the presence of laboratory signs of systemic inflammation in children with ASD associated with GDFC, and is consistent with the results of relevant meta-analyses of randomized clinical trials [15, 23]. However, significant differences were noted both in the proportion of cases with increased serum concentrations of a particular studied parameter, and in the levels of mean serum concentrations and the range of fluctuation of measurement results for each indicator.

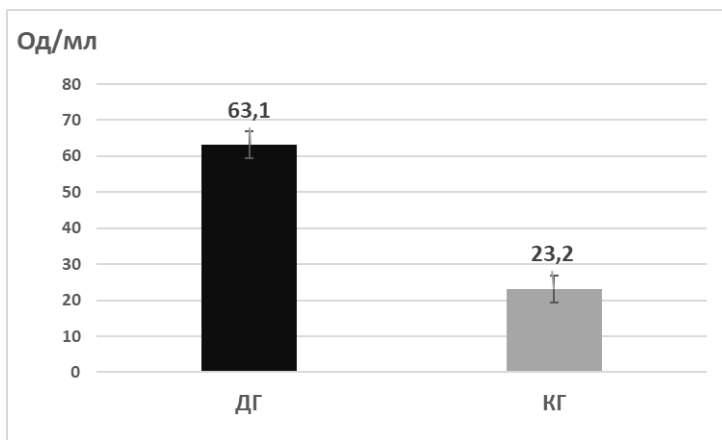


Fig. 6.2. Mean values ($\pm m$) of serum TM2PC concentrations in SG (n = 225) and CG (n = 51)

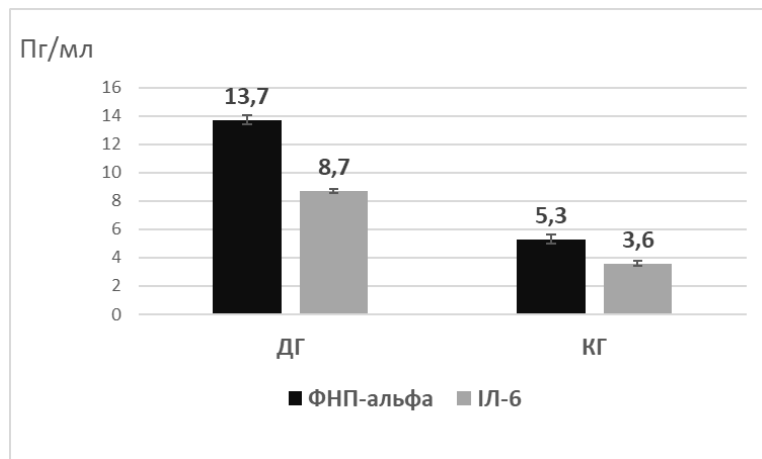


Fig. 6.3. Mean values ($\pm m$) of serum concentrations of TNF-alpha and IL-6 in SG (n = 225) and CG (n = 51)

TM2PC was the most sensitive and labile laboratory indicator of systemic inflammation among other studied indicators in children with ASD associated with GDFC, which, however, had the lowest specificity, giving false-positive results in at least every fifth patient. In contrast, IL-6 was the least sensitive and labile

indicator of systemic inflammation in SG, which, however, was characterized by high specificity, giving false-positive results only in every twentieth patient, i.e. at least 4 times less often than TM2PC. TNF-alpha was characterized by average sensitivity and lability with a relatively small number of false-positive results, therefore, this indicator among the three studied laboratory indicators can be considered the most informative for assessing the intensity of inflammation in children with ASD associated with GDFC. A comprehensive analysis of the results of measuring all three studied indicators of systemic inflammation is important, since each of them has certain advantages and disadvantages compared to the others.

The question of the clinical significance of systemic inflammation observed in children with ASD associated with GDFC is fundamental. In particular, it is necessary to clarify the contribution of systemic inflammation to the development of encephalopathy in such children and to find the most informative biomarkers for assessing the inflammatory mechanism of cerebral damage. To do this, we analyzed the associations of the studied indicators of systemic inflammation with serum concentrations of indicators of CNS neuronal damage NSE and S-100 protein (Table 1). The choice of the latter is not accidental, since their informativeness for assessing the severity of encephalopathy in children with ASD has been demonstrated in relevant clinical studies. Zheng Z. et al. conducted a meta-analysis of randomized clinical trials to study the informativeness of the use of serum concentrations of neurotropic calcium-dependent protein S-100 in children with ASD. The results of 10 trials involving 822 participants were analyzed. It has been shown that the concentration of S-100 protein in serum is significantly higher in children with ASD compared to healthy individuals and can be used as a biomarker of neuronal damage in such cases (standardized mean difference (SMD) = 0.97, 95 % CI = 0.41-1.53; $p < 0.001$) [27]. Accordingly, Lv M.N. et al. conducted a specially designed controlled clinical study involving 80 patients with ASD, demonstrating significantly increased concentration of NSE in serum in children with this mental disorder compared to healthy individuals [14].

● **Table 6.1.** Results of the association study (OR; 95 % CI) of the studied indicators of systemic inflammation and indicators of neuronal damage among SG patients (n = 225)

Indicator	TM2PC	TNF-alpha	IL-6
NSE	6,667; 1,668- 26,639	11,667; 2,064- 65,945	26,667; 1,843- 385,793
S-100 protein	7,570; 1,888- 30,351	10,000; 1,784- 56,060	15,200; 1,157- 199,642

As can be seen from the data in **Table 6.1**, all three studied indicators of systemic inflammation are associated with an increase in serum concentrations of laboratory indicators of neuronal damage NSE and protein S-100, which indicates a connection between the systemic inflammatory response and the development of encephalopathy in children with ASD associated with GDFC. The determination of increased serum concentrations of the studied indicators of systemic inflammation in the results of laboratory examinations of the patient sharply and reliably increases the risk of identifying high levels in the blood serum of such markers of CNS neuronal damage as NSE and protein S-100, which can be used in clinical practice when planning and organizing paraclinical examinations of the patient.

The strongest association was found for IL-6 and markers of neuronal damage, but the clinical application of this phenomenon may be limited by the low sensitivity of serum IL-6 concentration as an indicator of systemic inflammatory response in the defined group of patients. TM2PK demonstrated the weakest association with serum NSE and S-100 protein concentrations among the other studied indicators, which can be explained by the relatively large number of false-positive results when measuring this laboratory indicator. TNF-alpha occupied an average position in terms of the closeness of association with indicators of neuronal damage compared to TM2PK and IL-6. In general, the rule was true: the more specific the laboratory indicator was in assessing the intensity of the systemic inflammatory response in children with ASD associated with GDFC, the closer the association it demonstrated with the studied indicators of neuronal damage and better characterized the neurotoxic effect of systemic inflammation on the brain of patients.

The data revealed in this study not only expand the current understanding of the development of systemic inflammation and its impact on the formation of encephalopathy in children with ASD associated with GDFC, but also open the way to testing new therapeutic anti-inflammatory strategies for neuroprotection by targeting neutralizing pro-inflammatory molecules. The success of infliximab, a monoclonal antibody drug to the TNF-alpha molecule, in children with ASD could prompt more decisive action to plan and implement further dedicated clinical trials to study the efficacy and safety of modern anti-inflammatory treatment approaches in children with ASD associated with GDFC.

Conclusions. In patients with ASD associated with GDFC, serum concentrations of proinflammatory markers such as TM2PC, TNF-alpha, and IL-6 are elevated, indicating a state of systemic inflammation in the body of these children. If we talk about the clinical significance of the studied indicators of systemic inflammation, the latter differ significantly in sensitivity, lability, and specificity. TM2PC is the most sensitive and labile indicator with a relatively large number of false-positive results, while TNF-alpha occupies an intermediate position, and IL-6 is characterized by the lowest sensitivity and lability, but the highest specificity. None of the studied indicators can be considered ideal for assessing the state of systemic inflammation in children with ASD associated with GDFC, which implies the need for comprehensive data analysis. All studied indicators of systemic inflammation are associated with an increase in serum levels of neuronal damage indicators NSE and S-100 protein, which confirms the established notions about the role of systemic inflammation in the induction of encephalopathy in children with ASD associated with GDFC, and opens the way to testing new therapeutic strategies for anti-inflammatory neuroprotective therapy.

REFERENCES

1. Maltsev, D. V. (2018). Evaluation of the immune status in children with autism spectrum disorders associated with genetic folate cycle deficiency. *Likarska Sprava*, 1-2, 11-23. [https://doi.org/10.31640/jvd.1-2.2018\(02\)](https://doi.org/10.31640/jvd.1-2.2018(02))
2. Maltcev, D. V., Natrus, L. V. (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>

3. Bhatia, P., Singh, N. (2015). Homocysteine excess: delineating the possible mechanism of neurotoxicity and depression. *Fundamental & Clinical Pharmacology*, 29 (6), 522–528. <https://doi.org/10.1111/fcp.12145>
 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. 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>
 6. Chen, L., Shi, X.-J., Liu, H., Mao, X., Gui, L.-N., Wang, H., Cheng, Y. (2021). Oxidative stress marker aberrations in children with autism spectrum disorder: a systematic review and meta-analysis of 87 studies (N = 9109). *Translational Psychiatry*, 11 (1). <https://doi.org/10.1038/s41398-020-01135-3>
 7. Crawley, J. N., Heyer, W.-D., LaSalle, J. M. (2016). Autism and Cancer Share Risk Genes, Pathways, and Drug Targets. *Trends in Genetics*, 32 (3), 139–146. <https://doi.org/10.1016/j.tig.2016.01.001>
 8. Frustaci, A., Neri, M., Cesario, A., Adams, J. B., Domenici, E., Dalla Bernardina, B., Bonassi, S. (2012). Oxidative stress-related biomarkers in autism: Systematic review and meta-analyses. *Free Radical Biology and Medicine*, 52 (10), 2128–2141. <https://doi.org/10.1016/j.freeradbiomed.2012.03.011>
 9. Frye, R. E., Sequeira, J. M., Quadros, E. V., James, S. J., Rossignol, D. A. (2012). Cerebral folate receptor autoantibodies in autism spectrum disorder. *Molecular Psychiatry*, 18 (3), 369–381. <https://doi.org/10.1038/mp.2011.175>
 10. Furlano, R. I., Anthony, A., Day, R., Brown, A., McGarvey, L., Thomson, M. A. et al. (2001). Colonic CD8 and $\gamma\delta$ T-cell infiltration with epithelial damage in children with autism. *The Journal of Pediatrics*, 138 (3), 366–372. <https://doi.org/10.1067/mpd.2001.111323>
 11. 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>
 12. Li, H., Liu, H., Chen, X., Zhang, J., Tong, G., Sun, Y. (2020). Association of food hypersensitivity in children with the risk of autism spectrum disorder: a meta-analysis. *European Journal of Pediatrics*, 180 (4), 999–1008. <https://doi.org/10.1007/s00431-020-03826-x>
 13. 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>
 14. Lv, M., Zhang, H., Shu, Y., Chen, S., Hu, Y., Zhou, M. (2016). The neonatal levels of TSB, NSE and CK-BB in autism spectrum disorder from Southern China. *Translational Neuroscience*, 7 (1), 6–11. <https://doi.org/10.1515/tnsci-2016-0002>
 15. 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>
 16. Mead, J., Ashwood, P. (2015). Evidence supporting an altered immune response in ASD. *Immunology Letters*, 163 (1), 49–55. <https://doi.org/10.1016/j.imlet.2014.11.006>
-

17. 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>
18. Nicolson, G. L., Gan, R., Nicolson, N. L., Haier, J. (2007). Evidence for *Mycoplasma ssp.*, *Chlamydia pneumoniae*, 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>
19. Noriega, D. B., Savelkoul, H. F. J. (2013). Immune dysregulation in autism spectrum disorder. *European Journal of Pediatrics*, 173 (1), 33-43. <https://doi.org/10.1007/s00431-013-2183-4>
20. 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>
21. 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>
22. 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>
23. Saghazadeh, A., Ataenia, 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.jpsy-chires.2019.05.019>
24. 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>
25. Wang, Z., Ding, R., Wang, J. (2020). The Association between Vitamin D Status and Autism Spectrum Disorder (ASD): A Systematic Review and Meta-Analysis. *Nutrients*, 13 (1), 86. <https://doi.org/10.3390/nu13010086>
26. 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>
27. Zheng, Z., Zheng, P., Zou, X. (2020). Peripheral Blood S100B Levels in Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. *Journal of Autism and Developmental Disorders*, 51 (8), 2569-2577. <https://doi.org/10.1007/s10803-020-04710-1>

NEURORADIOLOGICAL SIGNS OF ENCEPHALOPATHY IN CHILDREN WITH AUTISM SPECTRUM DISORDERS ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

JUSTIFICATION

One of the important advances in psychiatry and neurology in recent years is the elucidation of the association between genetic deficiency of the folate cycle (GDFC) and autism spectrum disorders (ASD) in children. The evidence for such an association is based on the results of at least five meta-analyses of randomized controlled clinical trials [15, 20, 27, 28, 30] and a number of additional randomized controlled trials [11], the results of which are still not systematized. It has been shown that GDFC leads to the development of a number of typical biochemical disorders [10], which cause a state of a special form of immunodeficiency [19] and associated persistent oxidative stress [4], systemic inflammation, including hyperproduction of tumor necrosis factor alpha (TNF-alpha) and other pro-inflammatory cytokines with neurotoxic effects [18], opportunistic neurotropic infections, including those caused by herpes viruses 6 (HHV-6) and 7 types (HHV-7) [23], and anti-brain autoimmune reactions to neuronal autoantigens [2] and myelin [33]. It seems obvious that these three currently known immune-dependent mechanisms of cerebral damage are important in the development of encephalopathy in GDFC, one of the clinical manifestations of which is ASD, but the general concept of the pathogenesis of the disease is still not properly formulated. Since most of the studied pathways of CNS damage in GDFC are immune-mediated, they suggest a specific violation of the neuroimmune interface as a model for forming encephalopathy in such cases, which can be used in planning and conducting further clinical studies in the outlined direction.

It would be useful for clinical practice to describe typical radiological signs of such encephalopathy, which would improve the detection of the disease and optimize the assessment of the severity of the patient's condition and the effectiveness of the applied therapeutic interventions. Accordingly, Hegarty J.P. et al. in a specially designed clinical study recently showed that radiological data can be potentially informative for predicting the results of rehabilitation of children with ASD [13].

Given the multicomponent pathogenesis of encephalopathy in GDFC, one should expect the detection of a whole complex of heterogeneous neuroimaging signs, which theoretically should correlate with the implementation of certain mechanisms of cerebral damage that are the cause of their development, and the appearance of certain clinical symptoms that are a consequence of their occurrence.

The aim of the study: to describe typical neuroimaging signs of encephalopathy in children with GDFC suffering from ASD, and to search for correlations between clinical signs, mechanisms of nervous system damage and neuroimaging data to optimize the algorithm for diagnosis, monitoring and treatment.

Materials and methods. To achieve the goal, the medical data of 225 children aged 2 to 9 years with GDFC, who had clinical manifestations of ASD type (183 boys and 42 girls), were retrospectively analyzed. All of them were patients of the specialized neuroimmunological clinic Vivere (registration dossier 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 February 15, 2021, and the conclusion of the bioethical

examination commission (protocol No. 140 dated December 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 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 people). These individuals constituted the study group (SG). The control group (CG) included 51 clinically healthy children (37 boys and 14 girls) of similar age distribution who did not suffer from GDFC.

The severity of clinical symptoms of ASD among SG patients was assessed according to the specialized Aberrant Behavior Checklist (ABC) scale.

Special laboratory paraclinical examination of children in the observation groups was carried out taking into account modern ideas about the mechanisms of CNS damage in ASD associated with GDFC. Thus, the diagnosis of reactivated herpesvirus infections was carried out by PCR of blood leukocytes (Department of Neurobiochemistry of the Institute of Neurosurgery of the National Academy of Medical Sciences of Ukraine) according to the data of the study by Nicolson G.L. et al. [23]. Detection of beta-hemolytic streptococcus group A was carried out by bacteriological culture from the oropharyngeal mucosa on a selective nutrient medium or by specific antitoxic immunity in blood serum (antistreptolysin-O, antistreptodornase, antihyaluronidase) (ELISA; MDI Limbach Berlin GmbH, Germany), as stated in the systematic review by Dop D. et al., devoted to autoimmune subcortical encephalitis in children [6]. Accordingly, the Cunningham Panel™ was additionally performed to identify autoantibodies to neurons of the subcortical ganglia (ELISA, cell-based assay; Moleculera Labs, Inc, USA). The results of serological studies of blood serum were evaluated for the detection of specific antineuronal autoantibodies that are validated as markers of autoimmune limbic encephalitis in children and adults, namely autoantibodies to glutamic acid decarboxylase (GADA), neuronal potassium channels, amphiphysin, neuronal NMDA receptors, GABA, CV2, Yo, Ro, Hu, AMPAR 1 and 2 (enzyme-linked immunosorbent assay; MDI Limbach Berlin GmbH, Germany), which corresponds to modern approaches to the diagnosis of autoimmune limbic encephalitis [22]. Serum TNF-alpha concentration was also measured by enzyme immunoassay (N up to 8.1 pg/ml) (Sinevo, Ukraine).

Neuroimaging was performed by MRI of the brain in conventional modes (T1- and T2-weighted, FLAIR) on tomographs with a magnetic induction value of the coil of 1.5 T. Video-EEG monitoring was carried out by 30-minute recording of the bioelectric activity of the child's cerebral cortex in standard leads with photo-stimulation and hyperventilation tests.

Statistical processing of the material was carried out by comparative and structural analyses. To determine the probability of differences between the indicators in the observation groups, the parametric Student's T-test with a confidence probability indicator p and the non-parametric criterion – the number of signs Z according to Urbach Yu.V. Differences were considered probable at $p < 0.05$ and $Z < Z_{0.05}$. To study the associations between the studied indicators, the odds ratio (OR) and 95 % confidence interval (95 % CI) were used.

Microsoft Excel was used to perform statistical calculations.

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 their discussion. Analysis of MR images in children with ASD associated with GDFC, as expected, revealed a number of characteristic neuroimaging signs that were typical for this category of patients and were not observed in healthy children. All detected neuroradiological signs can be combined into at least 5 heterogeneous groups according to their nature. First, manifestations of leukoencephalopathy of varying severity were noted with a predominant violation of myelination in the white matter of the parietal lobes of the cerebral hemispheres periventricularly – in the so-called peritrigonal zones, areas of terminal myelination (89 % of cases in SG and only 17 % of cases in CG; $p < 0,05$; $Z < Z_{0,05}$). Since radiological manifestations of leukoencephalopathy were noted in almost all SG patients, we can speak of these changes as a class feature for children with ASD associated with GDFC. Secondly, there were manifestations of temporal mesial sclerosis with lesions of the hippocampi, parahippocampal gyri, amygdalae and insulae (67 % of cases in SG and only 12 % of cases in CG; $p < 0,05$; $Z < Z_{0,05}$). Thirdly, symptoms of hypertrophy of the subcortical ganglia of the cerebral hemispheres, mainly the caudate nuclei, with compression of the anterior horns of the lateral ventricles were detected (39 % of cases in SG and only 7 % of cases in CG; $p < 0,05$; $Z < Z_{0,05}$). Fourth, neuroradiological signs of congenital CMV neuroinfection were diagnosed (7 % of cases in SG and only 2 % of cases in CG; $p < 0,05$; $Z < Z_{0,05}$) and residual phenomena of postnatally transferred viral encephalitis (16 % of cases and no case in CG; $p < 0,05$; $Z < Z_{0,05}$). And, finally, fifth, manifestations of the so-called small anomalies of brain development were identified (48 % of cases in SG and only 22 % of cases in CG; $p < 0,05$; $Z < Z_{0,05}$) (**Fig. 7.1**).

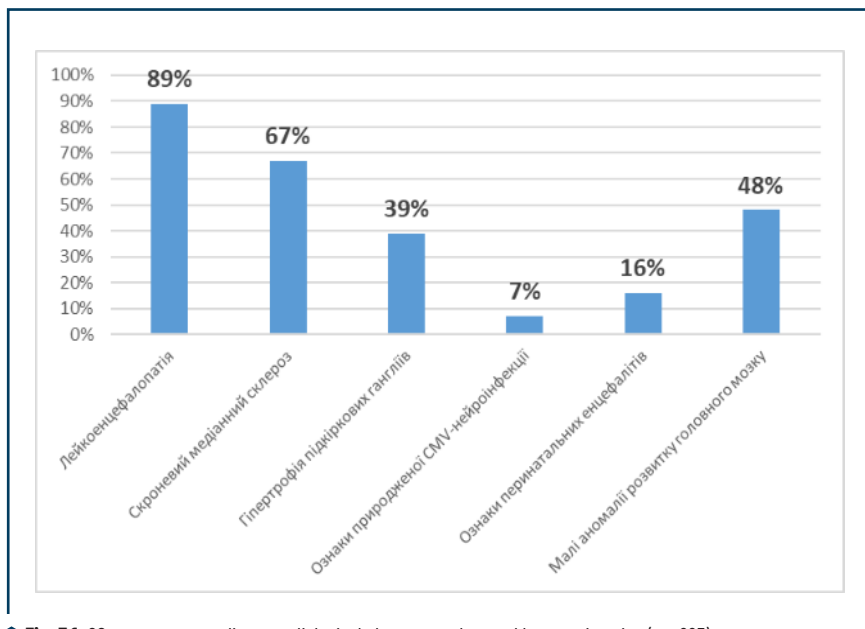
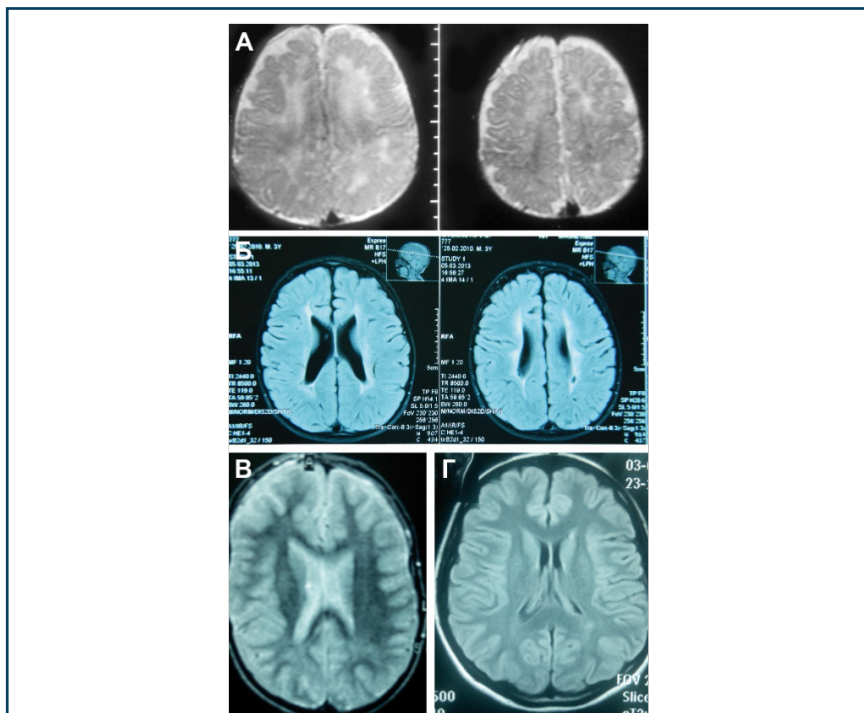


Fig. 7.1. SG structure according to radiological phenotypes detected by neuroimaging (n = 225)

The combination of 4-5 groups of neuroradiological signs of encephalopathy was considered as complete, while the presence of only 1-3 groups of instrumental signs of brain damage was considered as an incomplete neuroimaging phenotype of the disease. The complete phenotype was noted in at least 40 % of cases, and the incomplete phenotype in 60 % of cases. It should be noted that the complete neuroradiological phenotype of encephalopathy was associated with a more severe clinical condition of the patient on the ABC scale compared to the patient with an incomplete phenotype ($p < 0,05$; $Z < Z_{0,05}$).

Leukoencephalopathy, which appeared on MR images as patchy or mosaically unevenly distributed hyperintense signal of moderate or weak intensity with fuzzy contours in T2-weighted and FLAIR modes, varied in severity and prevalence in different SG patients (**Fig. 7.2**).



○ **Fig. 7.2.** Heterogeneity of MR manifestations of leukoencephalopathy in SG children (n = 225)

A - immaturity of the brain and diffuse myelination disorder in the white matter of the cerebral hemispheres (T2-weighted mode, axial projection);

B - a large zone of periventricular demyelination, resembling signs of leukodystrophy (FLAIR mode, axial projection);

C - pronounced bilateral myelination disorder in the parietal lobes periventricularly, brain dysgenesis, deformation of the ventricular system (T2-weighted mode, axial projection),

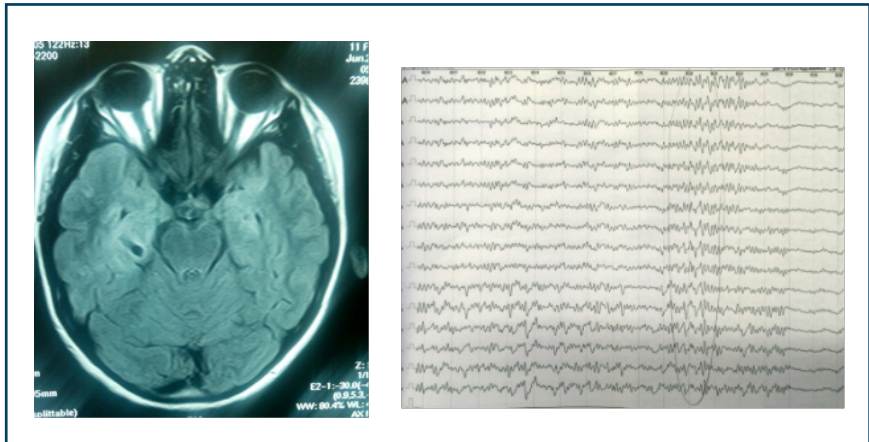
D - limited in size bilateral myelination disorder of the white matter in the parietal lobes periventricularly in the zone of terminal myelination (FLAIR mode, axial projection) (own observations).

It was possible to determine a diffuse form of leukoencephalopathy, in which myelination disorders were almost evenly distributed between different lobes of the cerebral hemispheres (22 % of cases). Focal forms of damage to the white matter of the hemispheres were more often noted, with the parietal lobe being most often involved, especially in the periventricular zone, where, as is known, myelin maturation during ontogenesis occurs the latest. Frontotemporoparietal lesions of the white matter of the cerebral hemispheres occurred in 13 %, temporoparietal lesions in 16 % of cases, frontoparietal lesions in 11 %, frontotemporal lesions in 3 %, and isolated lesions of the white matter of the parietal lobes in 15 % of cases. There were less pronounced radiological manifestations of leukoencephalopathy in children of the older age group (over 4 years) compared to children aged 2–4 years ($p < 0.05$; $Z < Z_{0.05}$), which probably reflects the process of delayed myelin maturation in children with ASD associated with GDFC.

Myelinopathy in children with GDFC has been reported previously. Strunk T et al. reported the phenomenon of facilitated demyelination in the white matter of the cerebral hemispheres in GDFC, describing subacute leukoencephalopathy in a patient with a heterozygous pathological polymorphic nucleotide substitution MTHFR C677T when using methotrexate at a low therapeutic dose [34]. Accordingly, Marseglia L.M. et al. in a controlled clinical study showed the association of the MTHFR A1298C and A1298C/C677T genotypes with the appearance of pathological hyperintense MR signal in the T2-weighted and FLAIR modes in the white matter of the cerebral hemispheres in full-term newborns, namely periventricular demyelination and loss of white matter volume around the ventricles with the development of vicarious ventriculomegaly [17]. Hardan A.Y. et al. conducted a specially designed clinical study to study the state of the white matter of the cerebral hemispheres in children with ASD based on the analysis of the results of brain MR spectroscopy ((1)H-MRS) with the acquisition of multivoxel echo-temporal *in vivo* (1)H-MRS data. Proton MR spectroscopy demonstrated a specific pattern of metabolic disorders, including an abnormal decrease in the N-acetylaspartate/creatine ratio, which indicated extensive multifocal unevenly distributed myelin damage and abnormal axonal development in the white matter of the cerebral hemispheres in children with ASD [12].

Temporal median sclerosis (**Fig. 7.3**), which appeared on MRI in the form of a hyperintense signal from the main structures of the mesolimbic system of the temporal lobes of the cerebral hemispheres in the T2-weighted and FLAIR modes, in SG children also differed in the severity and prevalence of pathological neuroimaging changes in different patients. The total form of the lesion with the involvement of all four main structures of the mesolimbic system occurred in 43 %, while partial forms with the involvement of only 1–3 of these structures occurred in 57 % of cases. Bilateral lesions (76 %) prevailed over unilateral ones (34 % of cases). In partial lesions of the mesolimbic system structures, the hippocampus was predominantly involved (64 % of cases). Hyperintense MR signal from the insula was less common (53 % of cases), and even less common – from the parahippocampal gyri (47 % of cases) and amygdalae (36 % of cases). Signs of atrophy of the mesolimbic system structures were recorded in the majority of SG patients (76 %), and the manifestations of atrophy and the prevalence of hyperintense signal were greater in patients of the older age group (over 4 years) compared to children aged 2–4 years ($p < 0.05$; $Z < Z_{0.05}$), which probably reflected the slowly progressive nature of the course of temporal median sclerosis in children with ASD associated with GDFC. In children with radiological signs of temporal median sclerosis, epileptiform bioelectric activity

was more often recorded during EEG, mainly in frontotemporal leads ($p < 0.05$; $Z < Z_{0.05}$), characteristic of mesial temporal lobe epilepsy associated with hippocampal sclerosis (MTLE-HS) [36].



○ **Fig. 7.3.** Photo of a brain MRI image (left) in the FLAIR mode in the axial projection of a child with ASD associated with GDFC, demonstrating bilateral hyperintensity of the MRI signal in the hippocampal area and signs of atrophy of these structures due to vicarious expansion of local CSF pathways and photo of an EEG (right) of the same patient, showing epileptiform activity (pathological waves are circled) associated with the indicated neuroimaging changes (own observation)

The obtained data correspond to the results of a specially planned retrospective clinical study by Monge-Galindo L. et al. The authors presented the experience of diagnosing temporal median sclerosis in one clinical center in children with symptoms of impaired development over the past 19 years. The causes of the specified cerebral lesion were herpesvirus infection, cytomegalovirus, prenatal cerebral pathology. In 5 patients, there was an isolated epileptic syndrome, in 1 – a delay in psychomotor and intellectual development, in 1 – ASD, in 3 – an epileptic syndrome with a delay in psychospeech development, in another 1 – ASD with an epileptic syndrome, in 2 – ASD with a delay in psychospeech development, in 2 – ASD with an epileptic syndrome and a delay in psychospeech development, and finally, in another 1 – severe migraine cephalgic paroxysms [21].

Hypertrophy of the subcortical ganglia of the cerebral hemispheres was mostly bilateral and almost symmetrical (67 % of cases) (**Fig. 7.4**). Unilateral lesions were found in only 33 % of cases. The caudate nuclei were mainly involved (89 % of cases) as an isolated radiological syndrome or in combination with lesions of other subcortical ganglia (putamen, pallidum and lenticular nuclei; 27 % of cases). Complete compression of the anterior horns of the lateral ventricles due to an increase in the size of the caudate nuclei occurred in 47 %, while partial compression occurred in 53 % of cases. There were no differences in the neuroimaging manifestations of hypertrophy of the subcortical nodes in patients of different age groups ($p > 0.05$; $Z > Z_{0.05}$).

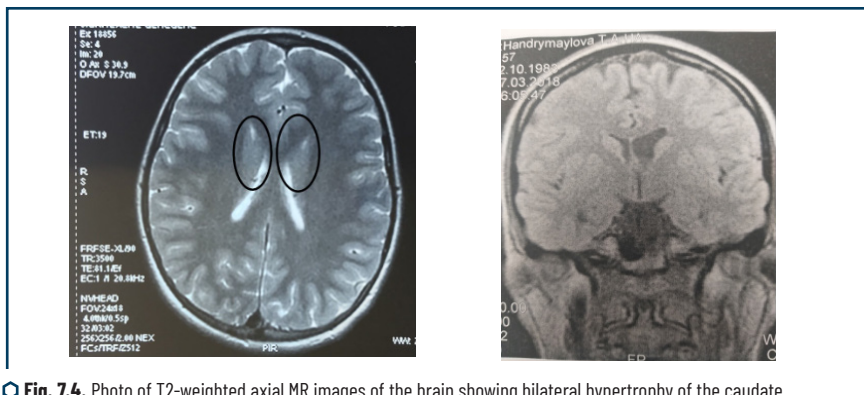


Fig. 7.4. Photo of T2-weighted axial MR images of the brain showing bilateral hypertrophy of the caudate subcortical nuclei with compression of both anterior horns of the lateral ventricles (right; lesions are circled by an oval) and FLAIR coronal images showing unilateral hypertrophy of the caudate nucleus with compression of the ipsilateral anterior horn of the lateral ventricle (left) in children with ASD associated with GDFC (own observations)

The EEG results obtained in this category of patients fit into three patterns of pathological disorders of the bioelectric activity of the cerebral cortex. With the initial MR signs of hypertrophy of the caudate subcortical ganglia, manifestations of local hyperexcitation of bioelectric activity in the projection of the subcortical nodes of the cerebral hemispheres were noted (**Fig. 7.5**). In patients with manifestations of a more pronounced increase in the size of nn. caudati, signs of bilateral lateralized synchronous electrical discharges were recorded (**Fig. 7.6**). And, finally, with MR signs of severe hypertrophy of the caudate subcortical nuclei with complete compression of the anterior horns of the lateral ventricles, diffuse hypersynchronization of bioelectric cortical rhythms took place (**Fig. 7.7**).

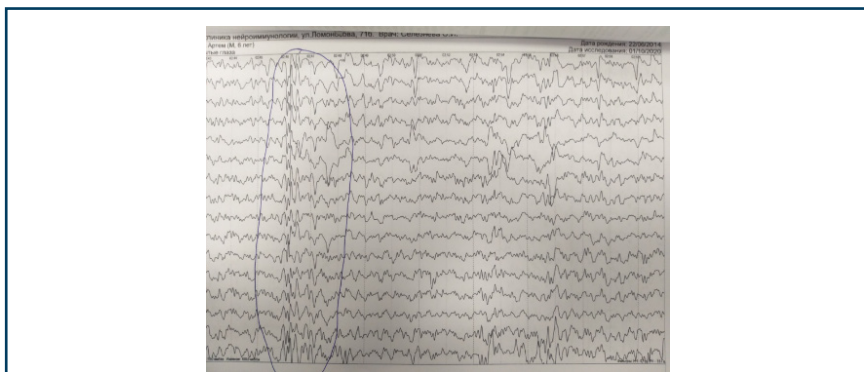


Fig. 7.5. EEG signs of excitation in the subcortical ganglia of the cerebral hemispheres in a child with GDFC associated with ASD, who had MRI signs of subcortical ganglia hypertrophy and positive results of the Cunningham panel (PANDAS; pathological complexes are circled) (own observation)

calcifications (**Fig. 7.8**). The complete neuroradiological phenotype was found in 79 % of cases, while the partial (3–4 signs) was found in 21 % of cases. Only bilateral and exclusively asymmetric lesions occurred. These changes were usually combined with signs of striatal artery vasculopathy, which were detected during neurosonographic examination in the antenatal period and/or during the first year after birth.

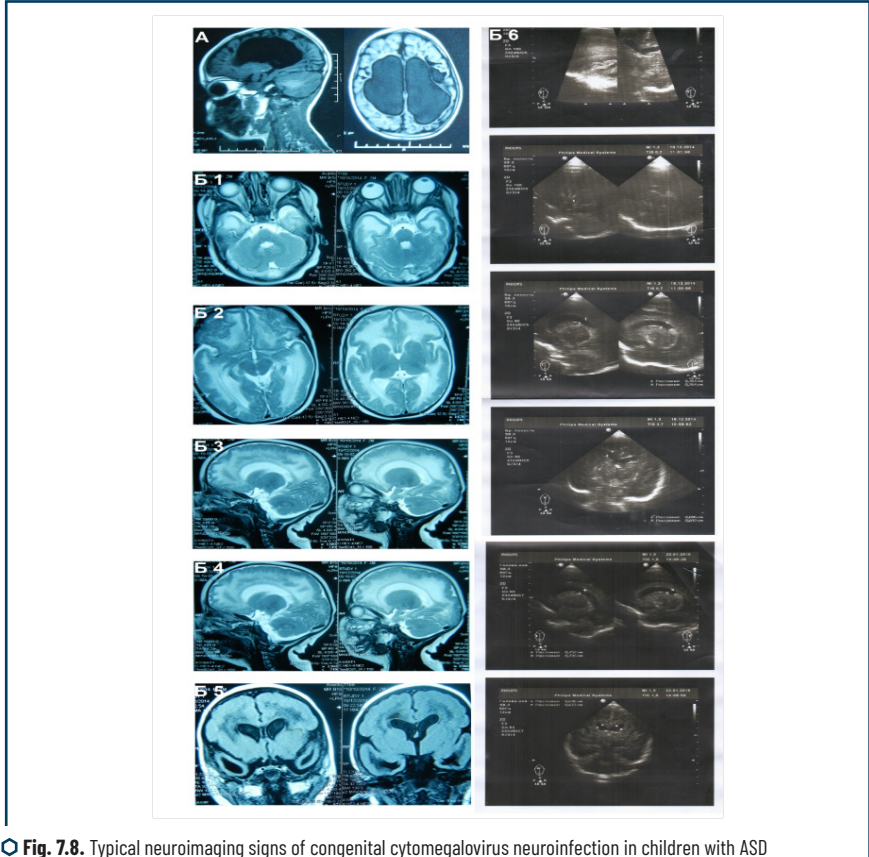


Fig. 7.8. Typical neuroimaging signs of congenital cytomegalovirus neuroinfection in children with ASD associated with GDFC (own observations)

A (FLAIR mode; sagittal (left) and horizontal (right) projections) – severe CNS malformation with pronounced ventriculomegaly and profound hypogenesis of the cerebral hemispheres (porencephaly), probably due to infection with the virus in the early period of intrauterine ontogenesis;

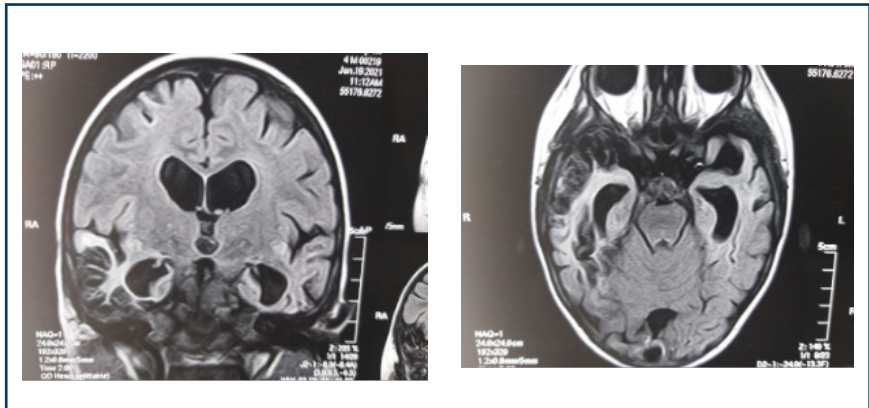
B – complex of classical signs of late antenatal cytomegalovirus infection: symmetrical bilateral cysts in the poles of the temporal lobes (B1, T2-weighted mode, horizontal projections), leukoencephalopathy (B2, T2-weighted mode, horizontal projections); B3, B4, T2-weighted mode, sagittal projections), agenesis of the corpus callosum (B5, FLAIR mode, coronal projections), ventriculomegaly (B2, T2-weighted mode, horizontal projections; B3, B4, T2-weighted mode, sagittal projections) and vasculopathy of the striatal arteries according to EchoEG (B6)

The data obtained on neuroimaging signs of congenital CMV neuroinfection in SG children correspond to the results of a controlled clinical trial by Pinillos-Pisón R. et al. The authors presented the results of an 18-year longitudinal study using CMV DNA detection on filter paper and identified the following manifestations of intrauterine CMV infection: antenatal developmental delay, microcephaly, sensorineural hearing loss, chorioretinitis, mental retardation, behavioral disorders (especially autism spectrum disorders), ventriculomegaly, intracranial calcifications, encephaloclastic disorders, leukoencephalopathy, cortical dysplasia, temporal lobe and hippocampal malformations, including cysts in the poles of the temporal lobes [25].

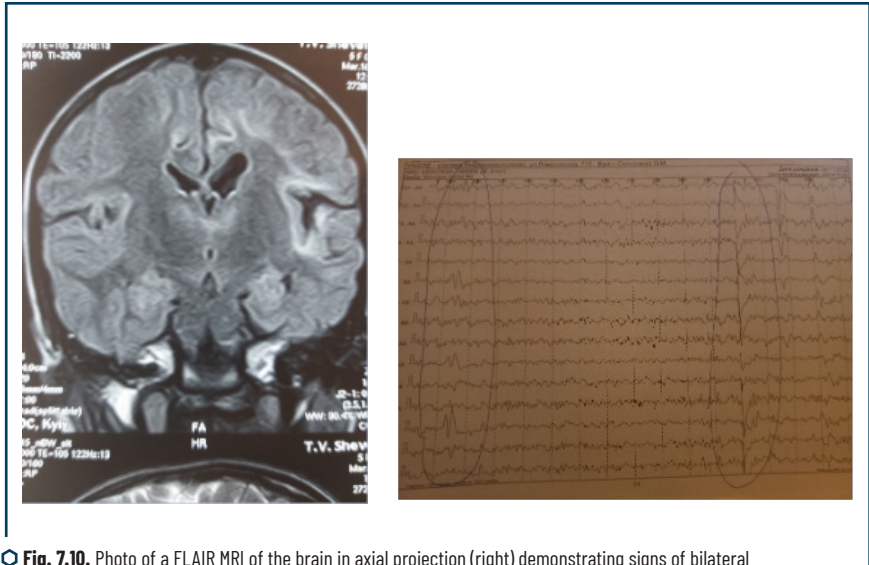
The data on the prevalence of congenital CMV infection obtained in this study correspond to the results of a controlled clinical trial by Sakamoto A. et al. The authors showed that congenital CMV infection with CNS involvement in children with ASD was significantly more common (7.4 %) than in the general population (0.31 %) ($p = 0.004$). CMV was identified by real-time PCR of dried neonatal blood samples and cord blood samples obtained immediately after birth [31].

Among the postnatal encephalitis cases, residual signs of temporal partial hemorrhagic-necrotic encephalitis of HSV-1/2 etiology prevailed (52 % of cases) (**Fig. 9**). Residual phenomena after limbic encephalitis (14 %) (**Fig. 10**), multifocal leukoencephalitis (15 %), stem encephalitis (9 %) and cerebellitis (10 % of cases) were less frequently recorded.

There was a decrease in neuroimaging manifestations of the encephalitis cases and their transformation due to the increase in cystic, atrophic and glial changes in the brain parenchyma in children of the older age group ($p < 0.05$; $Z < Z_{0.05}$), which reflected the natural evolution of postencephalitic foci in the human brain over time.



○ **Fig. 7.9.** Photo of MR images of the brain in the FLAIR mode in coronal (left) and axial (right) projections in a child with ASD associated with GDFC, demonstrating signs of bilateral atrophy and cystic-gliotic transformation in the temporal lobes of the cerebral hemispheres in the areas of bilateral temporal partial necrotic-hemorrhagic encephalitis of HSV1 etiology suffered in the first year after birth (own observation)



◉ **Fig. 7.10.** Photo of a FLAIR MRI of the brain in axial projection (right) demonstrating signs of bilateral autoimmune limbic encephalitis caused by autoantibodies to neuronal potassium channels, with asymmetric hyperintensity of the MR signal and manifestations of atrophy of both hippocampi and insula with vicarious expansion of local CSF pathways and EEG data (left) demonstrating epileptiform activity (abnormal waves circled) in a child with ASD associated with GDHC (own observation)

The prevalence of cases of temporal partial necrotizing hemorrhagic encephalitis of HSV1 etiology among ASD children associated with GDHC is consistent with previously published clinical case reports that have repeatedly described the appearance of the clinical phenotype of ASD in children and adults shortly after transmission of this form of neuroinfection [5, 7, 8].

Among the so-called minor brain developmental anomalies in SG children, neuroradiological manifestations of pineal cyst (52 %), retrocerebellar cyst (27 %), unilateral temporal lobe pole cyst (25 %), Arnold-Chiari malformation of the first degree (27 % of cases), empty sella turcica (16 % of cases) prevailed. Mega cisterna magna (11 %) and Dandy-Walker anomaly (9 % of cases) were less common. In 67 % of cases, several minor brain developmental anomalies occurred in one patient. The obtained data are consistent with the results of a specially designed clinical study by Pavone V. et al. In this study, the authors analyzed MR images of the brain and spinal cord in patients with ASD and psychospeech delay who are carriers of pathogenic polymorphic variants of the folate cycle enzyme gene MTHFR, which showed that small congenital malformations of the brain and spinal cord structures are a typical feature in such children and a characteristic finding during neuroimaging [24].

It is important to clarify the clinical significance of the identified pathological neuroimaging MR signs in children with GDHC associated with ASD. Data on the analysis of associations between certain radiological phenomena and clinical syndromes in SG patients are presented in **Table 7.1**.

● **Table 7.1.** Results of the study of associations (OR; 95 % CI) between pathological radiological phenomena and clinical syndromes in children with GDFC associated with ASD (n = 225)

Sign	Diffuse leukoencephalopathy	Temporal median sclerosis	Hypertrophy of subcortical ganglia	Signs of congenital CMV neuroinfection	Signs of postnatal encephalitis	Minor developmental anomalies
Regressive course of ASD	14,4; 3,0136 – 68,8073*	0,2973; 0,116 – 0,7618	1,6709; 0,5767 – 4,8414	0,8889; 0,3345 – 2,362	1,5235; 0,6101 – 3,8042	0,5365; 0,2122 – 1,3562
Epileptic syndrome	0,5333; 0,1678 – 1,6946	8,7941; 3,0351 – 25,4809*	1,0526; 0,4006 – 2,766	7,5273; 2,7278 – 20,7716*	2,9455; 1,1643 – 7,4515*	0,4068; 0,1614 – 1,0255
Obsessive-compulsive syndrome	0,6327; 0,2095 – 1,9107	1,9685; 0,6853 – 5,6542	13,3333; 4,5013 – 39,4942*	1,4182; 0,5676 – 3,5433	0,5667; 0,2215 – 1,4497	0,4793; 0,192 – 1,1962
Hyperkinesia	0,2605; 0,0678 – 1,0015	1,8158; 0,6296 – 5,2366	10,2222; 3,6044 – 28,9903*	1,5235; 0,6101 – 3,8042	0,6175; 0,2429 – 1,5698	0,5600; 0,226 – 1,3875
Cognitive impairment	0,6636; 0,2589 – 1,7008	18,857; 6,0733 – 58,5495*	0,8889; 0,3345 – 2,362	3,1866; 1,2547 – 8,0929*	7,3981; 2,6492 – 20,6601*	0,7870; 0,3139 – 1,9732
Movement disorders	3,3239; 1,2413 – 8,9007*	0,9502; 0,3316 – 2,7228	0,6545; 0,2536 – 1,689	2,9455; 1,1643 – 7,4515 *	6,4274; 2,3134 – 17,8575*	1,319; 0,5331 – 3,2638

Note. * - $\alpha = 0,05$

As can be seen from the data in **Table 7.1**, certain associations were noted between radiological phenomena and clinical syndromes observed in SG children. In general, the identified associations correspond to modern ideas about the functional purpose of various anatomical structures of the human cerebral hemispheres. Thus, the detection of diffuse leukoencephalopathy increased the chance of clinical symptoms of regressive autism in a child by at least 14 times, and motor disorders - by 3 times. Radiological signs of temporal median sclerosis were associated with epileptic syndrome and cognitive disorders, which corresponds to the theory of MTE-HS, and the idea of the location of the center of short-term memory in the hippocampus [36]. Hypertrophy of the subcortical ganglia increased the chance of developing obsessive-compulsive syndrome by 13 times, and hyperkinetic syndrome - by 10 times, which is consistent with the modern concept of autoimmune subcortical encephalitis [6]. Radiological symptoms of congenital CMV neuroinfection, as well as postnatal encephalitis, were associated with epileptic syndrome, cognitive decline and motor disorders, which is consistent with the results of reports on residual phenomena after neuroinfections [5, 9, 25].

However, the manifestations of minor anomalies of brain development were not associated with the occurrence of any of the studied clinical syndromes. Most likely, these were clinically insignificant manifestations of dysembryogenesis of the nervous system, which is a characteristic feature of GDFC [24].

Another important task is to find connections between radiological phenotypes and the results of special laboratory tests that reflect the implementation of known pathogenetic mechanisms of CNS damage in children with ASD associated with GDFC. Analysis of associations between neuroimaging studies and the results of a specially designed laboratory paraclinical examination in SG children is given in **Table 7.2**.

● **Table 7.2.** Results of the study of associations (OR; 95 % CI) between pathological radiological phenomena and the results of special laboratory tests in children with GDFC associated with ASD (n = 225)

Sign	Diffuse leu-koencephalopathy	Temporal median sclerosis	Hypertrophy of subcortical ganglia	Signs of congenital CMV neuroinfection	Signs of postnatal encephalitis	Minor developmental anomalies
Reactivated HHV-6/HHV-7 infections	5,2662; 2,5064 – 11,0648 *	18,1071; 7,6503 – 42,8568 *	0,7917; 0,3975 – 1,5767	0,8868; 0,4433 – 1,774	1,1485; 0,5744 – 2,2963	0,9787; 0,4919 – 1,9472
Autoantibodies to hippocampal neurons	0,7568; 0,3796 – 1,5088	8,9931; 4,1046 – 19,7036 *	1,1852; 0,5867 – 2,3943	1,0512; 0,525 – 2,1047	0,6686; 0,3352 – 1,3336	1,2536; 0,6276 – 2,5041
Autoantibodies to subcortical ganglia neurons	0,8567; 0,4296 – 1,7085	1,7591; 0,8740 – 3,5407	14,7245; 6,3708 – 34,0318 *	1,9192; 0,9531 – 3,8647	1,3602; 0,6785 – 2,7269	1,200; 0,6005 – 2,398
Signs of autosensitization to myelin	4,4136; 2,1294 – 9,148*	1,4380; 0,7221 – 2,8635	1,5693; 0,7875 – 3,1271	1,1568; 0,5808 – 2,3042	1,040; 0,5246 – 2,0619	0,7653; 0,3849 – 1,5217
Increased serum concentration of tumor necrosis factor alpha	4,6538; 2,2383 – 9,6761*	7,6364; 3,5371 – 16,4866*	7,2100; 3,3498 – 15,4757 *	1,5021; 0,7541 – 2,992	1,6397; 0,8224 – 3,2691	0,9700; 0,4858 – 1,9367
Identification of Streptococcus pyogenes	1,6125; 0,8011 – 3,2457	1,4840; 0,7406 – 2,9737	13,3714; 5,8391 – 30,62*	1,1073; 0,5557 – 2,2065	1,5143; 0,7611 – 3,0128	1,4493; 0,7288 – 2,882

Note. * - $\alpha = 0,05$

As shown in **Table 7.2**, MRI features of diffuse leukoencephalopathy were associated with the detection of reactivated HHV-6 and HHV-7 infections and signs of CNS myelin sensitization. It is known that HHV-6 and HHV-7 can infect oligodendrocytes [3] and cause multifocal demyelinating leukoencephalitis, which resembles autoimmune demyelinating diseases of the nervous system on neuroimaging [26], which may explain the observed association. In addition, the role of HHV-6 and HHV-7 as triggers of autoimmune

reactions in demyelinating diseases of the CNS, which resemble leukoencephalopathy on neuroimaging in children with ASD associated with GDFC, is now well known and studied [29].

MRI features of temporal median sclerosis have been associated with the identification of reactivated HHV-6 and HHV-7 infections and autoantibodies to hippocampal neurons. These findings are consistent with the notion that HHV-6 and HHV-7 are involved in the pathogenesis of MTE-HS, as suggested by a recent meta-analysis and systematic review of randomized controlled trials [36] and the current concept of autoimmune limbic encephalitis in humans [22]. Furthermore, there are reports that HHV-6 and HHV-7 may be triggers for the development of autoimmunity in autoimmune limbic encephalitis [35], and there have been rare cases of sudden onset of ASD symptoms in humans [9, 14] and animals [32] with autoimmune limbic encephalitis.

MRI signs of subcortical ganglion hypertrophy have been associated with serum autoantibodies to basal ganglia neurons and cases of group A beta-hemolytic streptococcus in the oropharynx. These findings are consistent with the current concept of PANDAS as an autoimmune subcortical encephalitis caused by the production of autoantibodies to subcortical ganglion neurons, with *Streptococcus pyogenes* being the typical trigger for autoimmunity [6].

Elevated serum TNF-alpha concentrations have been associated with three radiological phenomena: diffuse leukoencephalopathy, temporal median sclerosis, and subcortical ganglion hypertrophy, which may reflect the well-known inflammatory nature of such CNS lesions [6, 29, 36]. However, an inverse relationship is also possible, since the neurotoxic properties of TNF-alpha with the induction of damage to both neurons and myelin in the CNS have been described and studied [16], and the overproduction of this cytokine may not be secondary, but primary to some of the aforementioned neuroimaging signs, since systemic inflammation, being a consequence of immune dysregulation in GDFC, is considered by some researchers as an independent damaging factor in the formation of encephalopathy in children with ASD [18].

Radiological signs of congenital CMV neuroinfection, postnatal encephalitis, and minor brain anomalies were not associated with any of the laboratory test results that characterize the known mechanisms of CNS damage in ASD associated with GDFC in children. This may be due to the fact that these radiological phenomena do not reflect a current pathological process that is being implemented in real time, but are signs of residual phenomena of cerebral damage that occurred in the past.

The generalization of the results of **Tab. 7.1** and **7.2** allows us to speak about the detection of certain close associations between laboratory signs of known damaging factors of the CNS, radiological signs of nervous system damage and clinical manifestations of cerebral dysfunction in children with ASD associated with GDFC. These associations allow us to distinguish typical laboratory-radiological-clinical complexes, or diagnostic patterns, such as virus-induced temporal median sclerosis, autoimmune limbic encephalitis, autoimmune subcortical encephalitis, autoimmune or virus-induced demyelinating damage to the hemispheres, the consequences of previous neuroinfections and small anomalies of brain development. Encephalopathy in SG children was the result of a combination of these complexes in different ways in different patients. A feature of SG children was the possibility of combining several of these complexes in one patient, which created a large number of heterogeneous combinations and determined the heterogeneity of clinical symptoms of the disease, while in the available scientific literature these complexes were described mostly as isolated phenomena [4, 29, 36].

Conclusions. In children with ASD associated with GDFC, 5 main groups of neuroimaging features are noted, characteristic of leukoencephalopathy, temporal median sclerosis, PANS/PITANDS/PANDAS, congenital CMV neuroinfection and postnatal encephalitis, and minor CNS developmental anomalies.

The identified neuroimaging features are closely associated with the results of special laboratory tests characterizing known immune-dependent mechanisms of CNS damage and with the appearance of corresponding clinical syndromes, consistent with modern concepts of the main infectious or autoimmune lesions of the human nervous system in patients with immunosuppression.

It is possible to distinguish certain laboratory-radiological-clinical complexes in children with ASD associated with GDFC (virus-induced temporal median sclerosis, autoimmune limbic encephalitis, autoimmune subcortical encephalitis, autoimmune or virus-induced demyelinating lesion of the cerebral hemispheres, consequences of previous neuroinfections and small anomalies of brain development), which, combining in different ways in different patients, form a specific encephalopathy with heterogeneous clinical and radiological signs and, most likely, a complex pathogenesis.

REFERENCES

1. Maltsev D.V . (2017). Rasshyrenyi klynyko-laboratornyi fenotyp u detei s rasstroistvamy spektra avtyzma, assotsyirovannymi s henetycheskym defitsytom fermentov folatnoho tsykla. *Imunolohiia, alerholohiia*, 1-2, 20–38.
2. 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>
3. Campbell, A., Hogestyn, J. M., Folts, C. J., Lopez, B., Pröschel, C., Mock, D., Mayer-Pröschel, M. (2017). Expression of the Human Herpesvirus 6A Latency-Associated Transcript U94A Disrupts Human Oligodendrocyte Progenitor Migration. *Scientific Reports*, 7 (1). <https://doi.org/10.1038/s41598-017-04432-y>
4. Chen, L., Shi, X.-J., Liu, H., Mao, X., Gui, L.-N., Wang, H., Cheng, Y. (2021). Oxidative stress marker aberrations in children with autism spectrum disorder: a systematic review and meta-analysis of 87 studies (N = 9109). *Translational Psychiatry*, 11 (1). <https://doi.org/10.1038/s41398-020-01135-3>
5. DeLong, G. R., Bean, S. C., Brown, F. R. (1981). Acquired Reversible Autistic Syndrome in Acute Encephalopathic Illness in Children. *Archives of Neurology*, 38 (3), 191–194. <https://doi.org/10.1001/archneur.1981.00510030085013>
6. Dop, D., Marcu, I., Padureanu, R., Niculescu, C., Padureanu, V. (2020). Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (Review). *Experimental and Therapeutic Medicine*, 21 (1). <https://doi.org/10.3892/etm.2020.9526>
7. Ghaziuddin, M., Tsai, L. Y., Eilers, L., Ghaziuddin, N. (1992). Brief report: Autism and herpes simplex encephalitis. *Journal of Autism and Developmental Disorders*, 22 (1), 107–113. <https://doi.org/10.1007/bf01046406>

8. Gillberg, I. C. (1991). Autistic Syndrome with Onset at Age 31 Years: Herpes Encephalitis as a Possible Model for Childhood Autism. *Developmental Medicine & Child Neurology*, 33 (10), 920–924. <https://doi.org/10.1111/j.1469-8749.1991.tb14804.x>
9. González Toro, M. C., Jadraque Rodríguez, R., Sempere Pérez, Á., Martínez Pastor, P., Jover Cerdá, J., Gómez Gosálvez, F. A. (2013). Encefalitis antirreceptor de NMDA: dos casos pediátricos. *Revista de Neurología*, 57 (11), 504–508. <https://doi.org/10.33588/rn.5711.2013272>
10. 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>
11. Haghiri R., Mashayekhi F., Bidabadi E., Salehi Z. Analysis of methionine synthase (rs1805087) gene polymorphism in autism patients in Northern Iran // *Acta Neurobiol. Exp. (Wars)*. – 2016. – Vol. 76(4). – P. 318–323. doi: 10.21307/ane-2017-030.
12. Hardan, A. Y., Fung, L. K., Frazier, T., Berquist, S. W., Minshew, N. J., Keshavan, M. S., Stanley, J. A. (2016). A proton spectroscopy study of white matter in children with autism. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 66, 48–53. <https://doi.org/10.1016/j.pnpbp.2015.11.005>
13. Hegarty, J. P., Gengoux, G. W., Berquist, K. L., Millán, M. E., Tamura, S. M., Karve, S. et al. (2019). A pilot investigation of neuroimaging predictors for the benefits from pivotal response treatment for children with autism. *Journal of Psychiatric Research*, 111, 140–144. <https://doi.org/10.1016/j.jpsychires.2019.02.001>
14. Kiani, R., Lawden, M., Eames, P., Critchley, P., Bhaumik, S., Odedra, S., Gumber, R. (2015). Anti-NMDA-receptor encephalitis presenting with catatonia and neuroleptic malignant syndrome in patients with intellectual disability and autism. *BJPsych Bulletin*, 39 (1), 32–35. <https://doi.org/10.1192/pb.bp.112.041954>
15. 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>
16. Lindhout, I. A., Murray, T. E., Richards, C. M., Klegeris, A. (2021). Potential neurotoxic activity of diverse molecules released by microglia. *Neurochemistry International*, 148, 105117. <https://doi.org/10.1016/j.neuint.2021.105117>
17. Marseglia, L. M., Nicotera, A., Salpietro, V., Giaimo, E., Cardile, G., Bonsignore, M. et al. (2015). Hyperhomocysteinemia and MTHFR Polymorphisms as Antenatal Risk Factors of White Matter Abnormalities in Two Cohorts of Late Preterm and Full Term Newborns. *Oxidative Medicine and Cellular Longevity*, 2015, 1–8. <https://doi.org/10.1155/2015/543134>
18. 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>
19. Mead, J., Ashwood, P. (2015). Evidence supporting an altered immune response in ASD. *Immunology Letters*, 163 (1), 49–55. <https://doi.org/10.1016/j.imlet.2014.11.006>
20. 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>

21. 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>
 22. Nepal, G., Shing, Y. K., Yadav, J. K., Rehrig, J. H., Ojha, R., Huang, D. Y., Gajurel, B. P. (2020). Efficacy and safety of rituximab in autoimmune encephalitis: A meta-analysis. *Acta Neurologica Scandinavica*, 142 (5), 449-459. <https://doi.org/10.1111/ane.13291>
 23. Nicolson, G. L., Gan, R., Nicolson, N. L., Haier, J. (2007). Evidence for *Mycoplasma ssp.*, *Chlamydia pneumoniae*, 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>
 24. Pavone, V., Praticò, A. D., Parano, E., Pavone, P., Verrotti, A., Falsaperla, R. (2012). Spine and brain malformations in a patient obligate carrier of MTHFR with autism and mental retardation. *Clinical Neurology and Neurosurgery*, 114 (9), 1280-1282. <https://doi.org/10.1016/j.clineuro.2012.03.008>
 25. 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>
 26. Poppe, M., Brück, W., Hahn, G., Weißbrich, B., Heubner, G., Goebel, H., Todt, H. (2001). Fulminant Course in a Case of Diffuse Myelinoclastic Encephalitis - A Case Report. *Neuropediatrics*, 32 (1), 41-44. <https://doi.org/10.1055/s-2001-12214>
 27. 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>
 28. 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>
 29. Saberi, A., Akhondzadeh, S., Kazemi, S. (2018). Infectious agents and different course of multiple sclerosis: a systematic review. *Acta Neurologica Belgica*, 118 (3), 361-377. <https://doi.org/10.1007/s13760-018-0976-y>
 30. 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>
 31. Sakamoto, A., Moriuchi, H., Matsuzaki, J., Motoyama, K., Moriuchi, M. (2015). Retrospective diagnosis of congenital cytomegalovirus infection in children with autism spectrum disorder but no other major neurologic deficit. *Brain and Development*, 37 (2), 200-205. <https://doi.org/10.1016/j.braindev.2014.03.016>
 32. Scott, O., Richer, L., Forbes, K., Sonnenberg, L., Currie, A., Eliyashevskaya, M., Goez, H. R. (2013). Anti-N-Methyl-D-Aspartate (NMDA) Receptor Encephalitis. *Journal of Child Neurology*, 29 (5), 691-694. <https://doi.org/10.1177/0883073813501875>
-

33. 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>
34. Strunk, T., Gottschalk, S., Goepel, W., Bucszy, P., Schultz, C. (2002). Subacute leukoencephalopathy 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>
35. Venâncio, P., Brito, M. J., Pereira, G., Vieira, J. P. (2014). Anti-N-methyl-D-aspartate Receptor Encephalitis with Positive Serum Antithyroid Antibodies, IgM Antibodies Against *Mycoplasma pneumoniae* and Human Herpesvirus 7 PCR in the CSF. *Pediatric Infectious Disease Journal*, 33 (8), 882–883. <https://doi.org/10.1097/inf.0000000000000408>
36. Wipfler, P., Dunn, N., Beiki, O., Trinkka, E., Fogdell-Hahn, A. (2018). The Viral Hypothesis of Mesial Temporal Lobe Epilepsy – Is Human Herpes Virus-6 the Missing Link? A systematic review and meta-analysis. *Seizure*, 54, 33–40. <https://doi.org/10.1016/j.seizure.2017.11.015>

RESULTS OF VALACYCLOVIR, VALGANCICLOVIR, ARTESUNATE FOR THE TREATMENT OF REACTIVATED EBV-, HHV-6-, HHV-7-INFECTIONS IN CHILDREN WITH AUTISM SPECTRUM DISORDERS ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

INTRODUCTION

Autism spectrum disorders (ASD), which currently affect at least 1% of the modern child population and continue to increase in prevalence, are a global problem that requires urgent attention [1]. However, the FDA has not yet approved any drug to modify the course of ASD in children or to treat mental illness. The results of 5 recent meta-analyses and systematic reviews of randomized controlled trials demonstrate the association of ASD with genetic deficiencies of folic acid cycle enzymes (GDFC) [2, 3, 4, 5, 6], which sheds light on the pathogenetic pathways of the formation of a state of transmethylation disorders [7], persistent oxidative stress [8], immunodeficiency and related immune dysregulation [9], and reactivation of opportunistic infections [10, 11], which are considered important pathways of brain damage in children with ASD.

According to the data of a systematic review by Hughes H.K. et al. on the state of the immune system, children with ASD have impaired cytokine balance, quantitative disorders of immunocompetent cells, signs of persistent neuroglial inflammation in the CNS, defects in the functioning of the adaptive and innate immune systems, pathological deviations in serum concentrations of immunoglobulins of different classes and subclasses, as well as signs of autoimmune reactions to neurons, myelin, and extracerebral autoantigens [9].

Due to immune dysfunction in ASD, resistance to a number of microorganisms is reduced. A number of clinical reports and results of controlled studies have been published on the development of severe infections caused by opportunistic and conditionally pathogenic microbial agents in children with ASD. This phenomenon can be explained by the damage to the immune system induced by both GDFC and other genetic abnormalities associated with ASD. One of the key intracellular opportunistic agents that undergo reactivation in the body of children with ASD is herpesviruses. Currently, the typical development of infections caused by various types of herpes in children with ASD has been repeatedly reported, including reactivation of HSV-1 [10], EBV [11], CMV [12] and HHV-6 [13].

Ghaziuddin M. et al. were among the first to report the possibility of developing a clinical phenotype of autism after an episode of HSV-1 temporal encephalitis in humans [10]. Subsequently, an abnormally high frequency of registered cases of congenital CMV neuroinfection among children with ASD was established compared with mentally healthy peers, and the impact of this pathological phenomenon on the severity of neuropsychiatric disorders and adverse prognosis of the disease in children with ASD was substantiated [12]. Subsequently, thanks to the efforts of Valayi S. et al. [11] and Nicolson G.L. et al. [13], data were accumulated on abnormally frequent cases of reactivation of lymphotropic herpesviruses EBV and HHV-6 in children with ASD, which were detected both by PCR of blood leukocytes and by serological examinations by identifying specific IgM to viruses in serum. At the same time, little attention has been paid to the study of reactivated HHV-7 infection in children with ASD, although this viral agent is widely distributed in the pediatric population.

Reactivated herpesvirus agents may affect children with ASD through various mechanisms, causing virus-induced encephalitis [10] and/or neurodegenerative processes in the limbic region of the temporal lobes of the brain [14], enhancing GDFC-induced methylation disorders [15], systemic inflammation and oxidative stress [16], modulating allergic [17] and autoimmune [18] pathways of brain damage in the formation of the ASD phenotype. However, there are currently no clinical studies to test specific antiviral treatment with acyclic nucleoside analogues (valacyclovir, valganciclovir) and the antimalarial drug artesunate, which, according to recent scientific evidence, is highly effective in herpesvirus infections [19], in reactivated herpesvirus infections observed in children with ASD. It is reasonable to assume that suppression of these reactivated opportunistic agents may improve the clinical status of children with ASD by reducing the negative impact of viral factors on brain damage pathways, thereby improving the clinical outcomes of the disease and expanding the range of social adaptation of children with ASD. Therefore, there is an urgent need to conduct specially designed clinical trials to study the efficacy and safety of antiviral chemotherapeutic agents of different pharmacological groups in reactivated infections caused by herpes viruses of different types in children with ASD to assess the potential positive impact of such therapy on the mental status of children due to neuroprotection associated with the weakening or elimination of virus-induced pathways of brain damage.

The aim of the study: to study the efficacy of valacyclovir, valganciclovir, artesunate in reactivated herpesvirus infections caused by EBV, HHV-6 and HHV-7 in children with ASD associated with GDFC, considering the achieved neuroprotective effect according to laboratory biomarkers of cerebral damage.

Materials and methods of the study. To achieve the goal and fulfill the tasks, the medical records of 225 children aged 2 to 9 years with genetic folate cycle deficiency and autism spectrum disorders (study group, SG) were studied. The SG included 183 boys and 42 girls. These children were patients of the Vivere clinic, specializing in neuroimmunology, from 2019 to 2022. Registration dossier of the Vivere clinic No. 10/2212-M dated 12/22/2018. Further processing of clinical material after obtaining medical data in the clinic was carried out at the Institute of Experimental and Clinical Medicine of the Bogomolets National Medical University in accordance with the cooperation agreement No. 150221 dated 02/15/2021 and based on the relevant conclusion of the NMU bioethical expertise commission according to the data of protocol No. 140 dated 12/21/2020. The clinical diagnosis of ASD for patients in the observation groups was made by experienced child psychiatrists specializing in the problem of psychospeech disorders in children, according to the validated diagnostic criteria of DSM-IV-TR.

Pathogenic polymorphic variants of nucleotide substitutions in the genes of folate cycle enzymes for diagnosing GDFC in patients of the observation groups were identified using the polymerase chain reaction (PCR) method with restriction in the Sinevo laboratory (Ukraine). In this case, nucleotide substitutions MTHFR C677T were detected both in mono-form (68 patients SG; 30 % of cases) and in combination with other pathogenic nucleotide substitutions, in particular - with MTHFR A1298C, MTR A2756G and/or MTRR A66G (157 people SG; 70 % of cases). The genome containing the double pathological nucleotide substitutions MTHFR C677T + MTHFR A1298C was noted in 26 (12.5 %), MTHFR C677T + MTRR A66G - in 19 (8.5 %), and MTHFR C677T + MTR A2756G - in 25 (11 % of cases) of SG children. The genome containing the triple pathological nucleotide substitutions MTHFR C677T + MTRR A66G + MTR A2756G occurred in 23 (10.5 %),

MTHFR C677T + MTHFR A1298C + MTR A2756G - in 22 (9.5 %), and MTHFR C677T + MTHFR A1298C + MTRR A66G - in 21 (9 % of cases) of SG children. And finally, the genome that had all four studied pathogenic nucleotide substitutions, MTHFR C677T + MTHFR A1298C + MTR A2756G + MTRR A66G, was identified in 21 (9 % of cases) SG children. (**Fig. 8.1**).

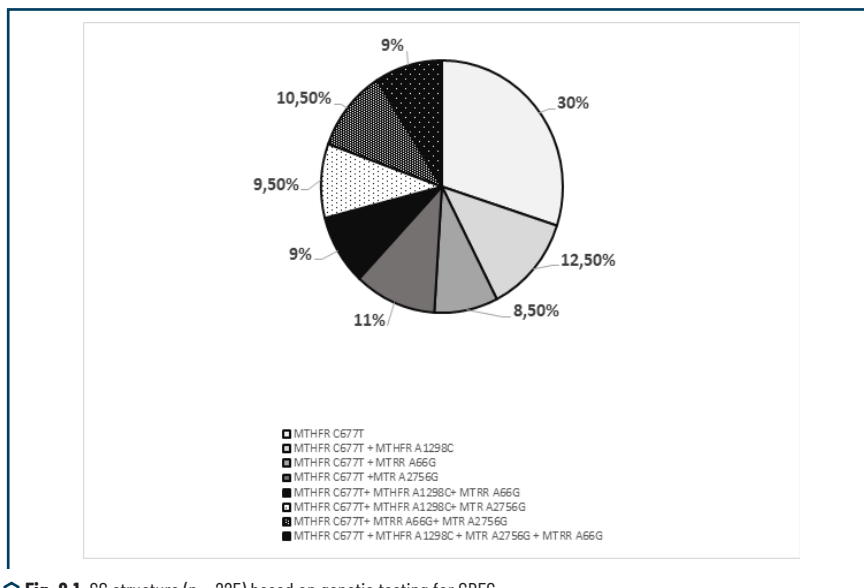


Fig. 8.1. SG structure (n = 225) based on genetic testing for GDFC

The diagnosis of reactivated EBV, HHV-6 and HHV-7 infection was determined by the results of PCR of blood leukocytes (Biocom reagents, Russian Federation) with species-specific primers of these viruses (Institute of Neurosurgery of the National Academy of Medical Sciences of Ukraine, Department of Neurobiochemistry). Reactivated EBV infection was diagnosed in 133 (59 %), HHV-6 infection in 153 patients (68 % of cases), and HHV-7 infection in 178 patients (79 % of cases) in SG. Accordingly, mixed infection, when there was simultaneous reactivation of several herpesviruses of different species, was noted in 135 children (60 % of cases).

NSE and S-100 protein were used as laboratory indicators of cerebral damage. Serum concentrations of NSE (normal - less than 16.3 ng/ml) and S-100 protein (normal - less than 0.105 µg/l) were measured by immunochemical method with electrochemiluminescent detection (ECLIA) using the Cobas 6000 analyzer, Roche Diagnostics (Switzerland) in the Sinevo laboratory (Ukraine). Serum concentrations of these biomarkers among SG patients were elevated, which reflected the presence of encephalopathy with psychiatric symptoms. The mean concentration of NSE was 25.38±3.56 ng/ml, and the mean serum concentration of S-100 protein was 131.94±12.46 µg/l.

Valacyclovir (Valtrex; GlaxoSmithKline, UK) was administered to SG patients at a dose of 1500 to 3000 mg/day (500-1000 mg three times a day) (60 patients, 41 - EBV, 52 - HHV-6, 59 - HHV-7), valganciclovir (Valcyte; Hoffmann-La Roche, Switzerland) - 450 to 900 mg/day (225-450 mg twice a day) (59 patients, 45 - EBV, 49 - HHV-6, 56 - HHV-7), and artesunate (Artesunat; Mekophar Chemical Pharmaceuticals Joint-Stock Company, Vietnam) - 50 to 100 mg/day (25-50 mg twice a day) (59 patients, 47 - EBV, 52 - HHV-6, 63 - HHV-7) depending on age and body weight of the patient daily orally for 3 consecutive months. PCR monitoring of blood leukocytes with species-specific primers for EBV, HHV-6 and HHV-7 was performed monthly during the observation period to assess ongoing viral activity during the course of approved antiviral treatment.

The control group (CG) included medical records of 52 children with GDFC and ASD of similar age (2 to 8 years) and gender distribution (37 boys and 15 girls) to the SG, who had reactivated EBV-, HHV-6-, and HHV-7-infections in proportions similar to those in the SG. The CG patients did not take antiviral medications during the observation period. The CG children also underwent monthly PCR monitoring of blood leukocytes with species-specific primers for EBV, HHV-6, and HHV-7 for 3 consecutive months to assess ongoing viral activity during the natural course of the infection in the patient's body.

Statistical processing of the obtained material was carried out using comparative and structural analyses. The Shapiro- Wilk test was used to study the distribution of the variant in the variation series. To establish the probability of the obtained differences between the values of the studied laboratory indicators in the observation groups, the parametric Student's T-test with an additional measurement of the confidence probability indicator p and the nonparametric Z-test according to Urbach V.Yu. [20] were used. Differences were considered probable in the case of obtaining $p < 0,05$ and $Z < Z_{0,05}$.

The odds ratio (OR) and 95 % confidence interval (95 % CI) were used to study the associations between the results of antiviral treatment and laboratory indicators of cerebral damage. Microsoft Excel (Redmond, WA) was used for statistical calculations.

Sources of funding. This clinical study was implemented as a fragment of research work funded by the State Budget of Ukraine under No. 0121U107940.

Results. All tested antiviral drugs resulted in inhibition of viral reproduction in blood leukocytes according to PCR results both in the case of EBV reactivation and in reactivated HHV-6 and HHV-7 infections, modifying the natural course of herpesvirus infections, as indicated by the results of comparing SG and CG data at all control points during the course of therapy ($p < 0,05$; $Z < Z_{0,05}$) (Figs. 8.1, 8.2, 8.3).

All tested antiviral drugs were also effective in both EBV-, HHV-6- and HHV-7-monoinfections and in the subgroup of patients with mixed infection (EBV + HHV-6, EBV + HHV-7, HHV-6 + HHV-7, EBV + HHV-6 + + HHV-7). No differences in the effectiveness of treatment for each of the studied viruses in mono- and mixed infections were noted, as evidenced by the data comparing the results of SG and CG ($p < 0,05$; $Z < Z_{0,05}$). Most likely, the effectiveness of antiviral treatment was determined primarily by the sensitivity of the virus strain to the antiviral drug used, and not by the total viral load on the human body. Each virus responded to the antiviral treatments used separately, and the formation of a combination with another virus or viruses generally did not change the response pattern to the approved antiviral treatment.

For all approved antiviral drugs, in all the studied reactivated herpesvirus infections, a common profile of therapeutic action was characteristic, which consisted of a progressive increase in the number of responders

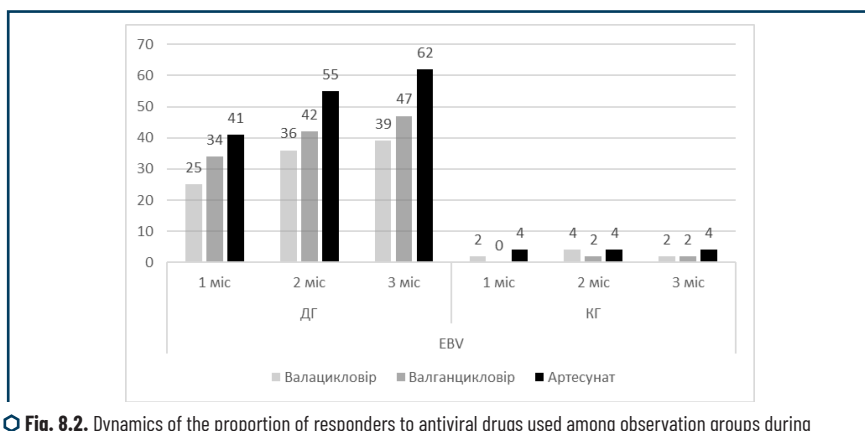
at each subsequent control point, but with a gradual reduction in the magnitude of the increase in the number of cases of negative PCR results of blood leukocytes as the course of therapy continued (Fig. 8.3).

At the same time, the approved antiviral drugs differed in their effectiveness both among themselves in general and in different herpesvirus infections, demonstrating different therapeutic effects in terms of inhibition of herpesvirus reproduction in blood leukocytes as such and significant species-specific differences in treatment efficacy.

Elimination of EBV DNA from blood leukocytes when using valacyclovir was achieved after the first month of treatment in 25 %, the second - in 36 %, and the third - in 39 % of cases. A negative result of PCR of blood leukocytes with EBV species-specific primers when using valganciclovir was obtained in 34 % of cases after the first, in 42 % of cases - after the second, in 47 % - after the third month of therapy. On the other hand, in the subgroup of patients taking artesunate, EBV DNA was eliminated from blood leukocytes after the first month of treatment in 41 %, in the second - in 55 %, and in the third - in 62 % of cases (Fig. 8.2).

Elimination of HHV-6 DNA from blood leukocytes with valacyclovir was achieved after the first month of treatment in 19 %, the second month in 26 %, and the third month in 29 %. Negative PCR results of blood leukocytes with HHV-6 species-specific primers with valganciclovir were obtained in 25 % of cases after the first, 29 % after the second, and 32 % after the third month of therapy. In contrast, in the subgroup of patients receiving artesunate, HHV-6 DNA was eliminated from blood leukocytes after the first month of treatment in 34 %, the second month in 47 %, and the third month in 57 % of cases (Fig. 8.3).

Elimination of HHV-7 DNA from blood leukocytes with valacyclovir was achieved after the first month of treatment in 14 %, the second month in 19 %, and the third month in 24 %. Negative PCR results of blood leukocytes with HHV-7 species-specific primers with valganciclovir were obtained in 22 % of cases after the first, 31 % after the second, and 35 % after the third month of therapy. In contrast, in the subgroup of patients receiving artesunate, HHV-7 DNA was eliminated from blood leukocytes after the first month of treatment in 31 %, the second month in 39 %, and the third month in 44 % of cases (Fig. 8.4).



○ Fig. 8.2. Dynamics of the proportion of responders to antiviral drugs used among observation groups during the course of therapy for reactivated EBV infection

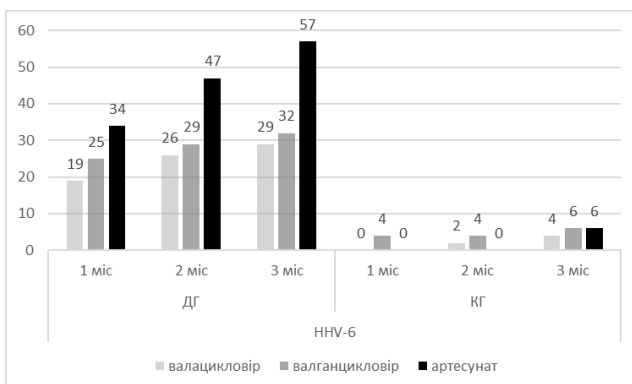


Fig. 8.3. Dynamics of the proportion of responders to antiviral drugs used among observation groups during the course of therapy for reactivated HHV-6 infection

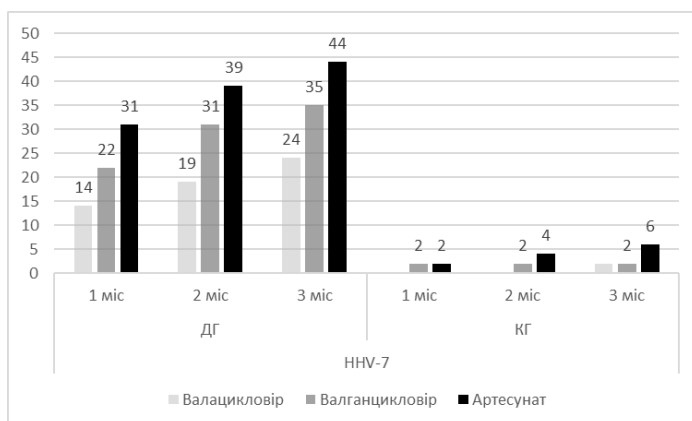


Fig. 8.4. Dynamics of the proportion of responders to antiviral drugs used among observation groups during the course of therapy for reactivated HHV-7 infection

The differences in the effectiveness of the three antiviral drugs used in SG were statistically significant at all control points both in general when comparing the results of treatment of EBV-, HHV-6- and HHV-7-infections, and when comparing the data of subgroups of EBV-, HHV-6- and HHV-7-mono-infections and mixed infections ($p < 0,05$; $Z < Z_{0,05}$).

EBV infection was significantly more sensitive at all control points to all tested antiviral drugs than HHV-7 infection ($p < 0,05$; $Z < Z_{0,05}$), but no significant difference was observed with HHV-6 infection ($p < 0,05$; $Z < Z_{0,05}$).

Although the number of complete responders in chronic reactivated HHV-6 infection for each tested antiviral drug was slightly higher at each control point compared with HHV-7 infection, there was no statistically significant difference in the results obtained ($p < 0,05$; $Z < Z_{0,05}$). The same pattern occurred when comparing the results of treatment of herpesvirus mono-infections and mixed infections with the use of each antiviral drug ($p > 0,05$; $Z > Z_{0,05}$).

The greater efficacy of artesunate compared with acyclic nucleoside analogues can be explained by differences in the mechanism of antiviral action of artemisinin. While valacyclovir and valganciclovir act virostatically only on the virus, inhibiting the process of elongation of viral DNA, artesunate affects both the immediate early synthesis of viral capsid proteins [2] and the sensitivity of human cells to viral invasion, reducing the interaction of viral agent proteins with the human vimentin filament system of the cytoskeleton during viral invasion into susceptible cells [21]. Most likely, this dual antiviral effect of artesunate, which involves an effect on both the virus itself and the host, gives a stronger overall antiviral treatment effect than that of acyclic nucleoside analogues that act only on the virus itself, but not on host cells, in all studied herpesvirus infections at all control points.

In any case, there remained a significant number of patients who showed resistance to all approved therapeutic strategies, indicating the need for higher doses of virostatic drugs, longer courses of treatment and/or combination therapy regimens, as well as the feasibility of searching for other potentially effective antiviral drugs.

It is important to determine whether suppression of reactivated herpesvirus infection is beneficial in terms of the dynamics of psychiatric symptoms, and more specifically, whether negative PCR results of blood leukocytes with herpesvirus species-specific primers under the influence of antiviral treatment are associated with some neuroprotective effect in children with ASD associated with GDFC. NSE and S-100 protein are well-known and characterized laboratory indicators of cerebral damage. We studied the associations between negative PCR results of blood leukocytes with EBV, HHV-6, HHV-7 species-specific primers after a course of approved antiviral treatment with normalization of previously elevated serum concentrations of NSE and S-100 protein in SG patients (**Table 8.1**).

● **Table 8.1.** Results of the association study (OR; 95 % CI) between antiviral treatment outcomes and serum concentrations of NSE and S-100 protein

Indicator	EBV	HHV-6	HHV-7
NSE	8.327; 3.858-17.970	4.582; 2.275-9.226	5.370; 2.788-10.342
S-100 protein	5.383; 2.583-11.216	4.138; 2.049-8.355	3.701; 1.942-7.055

As can be seen from **Table 8.1**, in SG there was an association between the phenomenon of negativity of the results of PCR of blood leukocytes at the end of the course of antiviral treatment and the normalization of laboratory indicators of cerebral damage, and the differences in the tightness of the detected associations corresponded to the differences in the sensitivity of herpesviruses of different

species to antiviral treatment – EBV, which was the most sensitive to antiviral drugs, had the closest association with the studied biomarkers, and HHV-7, which was the most resistant to antiviral treatment, had the least tight association. In addition, the normalization of serum NSE protein concentration was more closely associated with the effective results of using antiviral drugs for all studied herpesvirus agents than the serum S-100 protein concentration. The data obtained indicate potential neuroprotective effects of antiviral treatment in children with ASD associated with GDFC, who show reactivated EBV-, HHV-6- and/or HHV-7-infections, which need to be verified in further studies.

The tolerability of the tested antiviral drugs was good. With valacyclovir, a slight increase in serum concentrations of hepatic transaminases was noted in only 7 % of cases, with valganciclovir, a slight increase in serum concentrations of hepatic transaminases occurred in 19 % of cases, neutropenia in 16 % of cases, and with artesunate, mild anemia in 8 % and neutropenia in 11 % of cases. All the identified side effects of the tested drugs were mild and did not cause interruption of the planned therapeutic course.

Discussion. Although there are many studies on the association of ASD with reactivated herpesvirus infections, and the mechanisms of influence of these viral agents on the formation of encephalopathy with induction of mental symptoms have been substantiated, we were unable to find published controlled clinical trials studying the effectiveness of specific antiviral drugs in such cases, which allows us to claim that this trial is the first in the world in this direction. It was possible to demonstrate the different sensitivity of herpesviruses of different species to antiviral drugs of different pharmacological groups and, at the same time, the unequal clinical efficacy of different antiherpetic drugs in reactivated infections caused by herpesviruses of different species, which may be useful for optimizing antiviral treatment in children with ASD associated with GDFC. It has been shown that achieving a negative PCR result with species-specific herpesvirus primers under the influence of antiviral drugs is associated with the normalization of laboratory indicators of cerebral damage, such as NSE and S-100 protein, which indicates a potential neuroprotective effect of antiviral treatment in children with ASD associated with GDFC. The choice of these biomarkers is not accidental, since their informativeness for the assessment of encephalopathy in ASD has been properly established in the results of clinical studies. Zheng Z. et al. published a meta-analysis of randomized clinical trials on the informativeness of serum concentrations of neurotropic calcium-dependent protein S-100 in children with ASD. The meta-analysis analyzed data from 10 clinical trials, which included a total of 822 participants. It was found that the serum concentration of protein S-100 is likely to be higher in ASD than in healthy children, and therefore this laboratory indicator can be used as a biomarker of cerebral damage in such cases [21]. Lv M.N. et al. conducted a controlled clinical trial with the participation of 80 patients, the results of which showed a significantly higher serum concentration of NSE in children with ASD than in mentally healthy individuals of the same age [22].

The identification of signs of immunodeficiency with immune dysregulation in children with ASD creates a scientific basis for understanding the cause of reactivation of opportunistic herpesvirus infections in such cases [9]. Binstock T. was the first to identify selectively reduced resistance of children with ASD to opportunistic and conditionally pathogenic microbial agents, identifying a special subgroup of patients with a predisposition to infections caused by intramonocytic microbes, namely measles virus, CMV, HHV-6 and *Yersinia enterocolitica* [23].

Children with ASD in this subgroup were characterized by signs of hematopoiesis suppression, impaired immune status, abnormally increased permeability of the blood-brain barrier, and demyelination in the white matter of the brain. These clinical and laboratory signs, as it became known today thanks to the results of a clinical study by Marseglia L.M. et al., are very typical of GDFC [24]. Accordingly, Nicolson G.L. et al. in a controlled clinical study, using PCR of blood leukocytes, demonstrated the phenomenon of abnormally frequent detection of *Mycoplasma* and *Chlamydia pneumoniae* DNA, as well as HHV-6 in the blood of children with ASD [13]. Valayi S. et al. in a specially designed controlled clinical study found that specific IgM to EBV in the serum of children with ASD are significantly more common than in the blood of healthy individuals of the same age [11]. Sakamoto A. et al. Another controlled clinical study found that congenital CMV neuroinfection in children with ASD was significantly more common (7.4 %) than in the general population (0.31 %) ($p = 0.004$). In this study, CMV was detected by real-time PCR of dried neonatal blood samples and fetal cord blood [12]. Another controlled clinical study found an association of ASD in children with GDFC with reactivation of EBV, HHV-6, and HHV-7, diagnosed by PCR of white blood cells [19]. However, Sweeten T.L. et al., using PCR of serum but not white blood cells, reported a small number of cases of reactivated herpesvirus infections in children with ASD [25].

It is currently being discussed that herpesvirus agents may be active components in the pathogenesis of encephalopathy in children with ASD associated with GDFC. Herpesviruses are capable of exerting both direct damaging effects on the brain parenchyma, manifesting their neurotropic cytopathic effect, and of realizing damage to the CNS through some indirect immune-mediated mechanisms, for example, modulating systemic and/or intracerebral inflammation or inducing anti-brain autoimmunity.

The direct damaging effect of herpesviruses on the brain of children consists in both the induction of encephalitis and some neurodegenerative processes. A number of cases of acute development of clinical symptoms of autism after an episode of temporal necrotic-hemorrhagic encephalitis of HSV-1 etiology have been published [10, 26]. This is an example of the direct (encephalitic) damaging effect of herpesviruses on the CNS in ASD. It has also been found that HHV-6 is able to carry out transolfactory migration from the upper respiratory tract to the brain [27] and thereby affects the structures of the limbic system of the temporal lobes. At the same time, the virus induces a specific neurodegenerative process called temporal mesial sclerosis [14], the clinical and radiological signs of which, as Monge-Galindo L. et al. have shown, occur in children with ASD [28]. The association of HHV-6 and temporal median sclerosis has recently been confirmed by the results of a meta-analysis and systematic review of randomized controlled clinical trials prepared by Wipfler P. et al. [14]. This may be a second, so-called neurodegenerative, form of direct CNS damage by herpesviruses, which, it is possible, is an important component of the pathogenesis of encephalopathy in children with ASD in GDFC.

If we talk about the potential indirect effects of herpesviruses on the CNS in ASD, two main pathways of cerebral damage should be distinguished. First, herpetic agents can be triggers of autoimmunity to myelin and neurons. Singh V.K. et al. were among the first to establish an association between serological signs of HHV-6 infection and laboratory indicators of anticerebral autoimmunity in children with ASD [29]. Currently, cases of acute development of autism symptoms in children with autoimmune limbic encephalitis with a positive response to recommended immunomodulatory therapy have been described [30, 31], and it has also been established that viruses of the herpes family, including HHV-7, provoke a breakdown of immune

tolerance to autoantigens of hippocampal nerve cells in such cases [18]. On the other hand, herpesvirus agents, especially HHV-6, in the context of immune dysregulation in ASD, can modulate a state of systemic inflammation with a phenomenon of hypercytokinemia, which has a potential neurotoxic effect [16]. The typicality of the phenomenon of systemic hypercytokinemia with an aberrant proinflammatory profile in ASD has now been confirmed by the data of two recent meta-analyses and systematic reviews of randomized controlled clinical trials [32, 33]. It has been shown that HHV-6, which undergoes reactivation in ASD [13], is able to induce a pathological state of hyperactivation of macrophages with the subsequent development of a phenomenon of hypercytokinemia, similar to that occurring in children with ASD [16].

When discussing the inflammatory mechanism of herpesvirus-induced cerebral damage in ASD, it is worth mentioning the involvement of the functional microbiota-gut-brain axis in the pathogenesis of psychiatric illness. According to this concept, by increasing the intensity of inflammation in the intestinal wall, herpesvirus agents can induce further intracerebral inflammation in children with ASD through the abnormal spread of the inflammatory process from the intestinal compartment into the blood and further through the pathologically permeable blood-brain barrier to the brain [34].

Recently, the results of a similar comparative controlled study investigating the efficacy of valacyclovir, valganciclovir, and artesunate in reactivated HHV-6 and HHV-7 infections in adult patients with chronic fatigue syndrome/myalgic encephalomyelitis have been published [19]. It is noteworthy that, despite a similar overall profile of the therapeutic effect of the tested antiviral drugs in various herpesvirus infections in both cases, the efficacy of antiviral therapy in children with ASD is significantly lower than in adults with chronic fatigue syndrome/myalgic encephalomyelitis, despite the use of higher doses of antiviral drugs based on the body weight of patients with ASD. This difference in treatment efficacy may be explained by the reduced intestinal absorption of antiviral drugs associated with a specific immunoinflammatory lesion of the small intestinal wall in children with ASD, described in detail by Torrente F. et al. [35], which indicates the need to study the pharmacokinetics and pharmacodynamics of antiherpetic drugs in ASD. Another explanation may be the potentiating effect of GDFC, which occurred in children with ASD SG, on the reproduction of herpesviruses due to the possible connection between the methylation processes disturbed in GDFC and the reproduction of herpesvirus agents in the human body [15]. It is also known that herpesvirus agents can use human proinflammatory cytokines as a kind of stimulators of their own DNA replication [36], therefore, the state of systemic and neuroglial inflammation with persistent hypercytokinemia, characteristic of children with ASD [37, 38], may be an important factor that also affects the results of the use of antiviral drugs in ASD.

Conclusions. The results obtained indicate that all tested antiviral drugs transform the natural course of chronic reactivated EBV-, HHV-6- and HHV-7-infections in children with ASD associated with GDFC, reducing the DNA content in the blood leukocytes of patients according to PCR data. Artesunate is the most effective of the other studied drugs, with the highest proportion of complete responders at the end of each month of therapy, valganciclovir shows intermediate efficacy, and valacyclovir is the least effective antiviral treatment compared to artesunate and valganciclovir. EBV is more susceptible to all approved antiviral drugs compared to NNV-7. Elimination of herpesvirus agents from blood leukocytes is associated with normalization of laboratory indicators of cerebral damage, indicating a potential neuroprotective effect of antiviral therapy in children with ASD.

Due to the large number of non-responders to various types of herpesvirus infections when using all approved drugs, it is advisable to search for means to enhance the effectiveness of antiviral therapy in ASD. The obtained data may be useful for child psychiatrists, clinical immunologists and infectious disease specialists who are part of multidisciplinary teams for the management of children with ASD, in planning rational antiviral therapy in case of detection of signs of reactivated herpesvirus infections, as well as in planning and implementing further clinical studies in the field of virological aspects of the pathogenesis of ASD.

REFERENCES

1. Indika, N.-L. R., Frye, R. E., Rossignol, D. A., Owens, S. C., Senarathne, U. D., Grabrucker, A. M. et al. (2023). The Rationale for Vitamin, Mineral, and Cofactor Treatment in the Precision Medical Care of Autism Spectrum Disorder. *Journal of Personalized Medicine*, 13 (2), 252. <https://doi.org/10.3390/jpm13020252>
2. 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>
3. 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>
4. 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>
5. 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>
6. 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>
7. Sun, L., Wang, X., Wang, X., Cui, X., Li, G., Wang, L. et al. (2022). Genome-wide DNA methylation profiles of autism spectrum disorder. *Psychiatric Genetics*, 32 (4), 131–145. <https://doi.org/10.1097/ypg.0000000000000314>
8. Liu, X., Lin, J., Zhang, H., Khan, N. U., Zhang, J., Tang, X. et al. (2022). Oxidative Stress in Autism Spectrum Disorder – Current Progress of Mechanisms and Biomarkers. *Frontiers in Psychiatry*, 13. <https://doi.org/10.3389/fpsyt.2022.813304>
9. 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>

10. Ghaziuddin, M., Tsai, L. Y., Eilers, L., Ghaziuddin, N. (1992). Brief report: Autism and herpes simplex encephalitis. *Journal of Autism and Developmental Disorders*, 22 (1), 107-113. <https://doi.org/10.1007/bf01046406>
 11. Valayi, S., Eftekharian, M. M., Taheri, M., Alikhani, M. Y. (2018). Evaluation of antibodies to cytomegalovirus and Epstein-Barr virus in patients with autism spectrum disorder. *Human Antibodies*, 26 (3), 165-169. <https://doi.org/10.3233/hab-180335>
 12. Sakamoto, A., Moriuchi, H., Matsuzaki, J., Motoyama, K., Moriuchi, M. (2015). Retrospective diagnosis of congenital cytomegalovirus infection in children with autism spectrum disorder but no other major neurologic deficit. *Brain and Development*, 37 (2), 200-205. <https://doi.org/10.1016/j.braindev.2014.03.016>
 13. Nicolson, G. L., Gan, R., Nicolson, N. L., Haier, J. (2007). Evidence for *Mycoplasma ssp.*, *Chlamydia pneumoniae*, 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>
 14. Wipfler, P., Dunn, N., Beiki, O., Trinka, E., Fogdell-Hahn, A. (2018). The Viral Hypothesis of Mesial Temporal Lobe Epilepsy - Is Human Herpes Virus-6 the Missing Link? A systematic review and meta-analysis. *Seizure*, 54, 33-40. <https://doi.org/10.1016/j.seizure.2017.11.015>
 15. Engdahl, E., Dunn, N., Niehusmann, P., Wideman, S., Wipfler, P., Becker, A. J. et al. (2017). Human Herpesvirus 6B Induces Hypomethylation on Chromosome 17p13.3, Correlating with Increased Gene Expression and Virus Integration. *Journal of Virology*, 91 (11). <https://doi.org/10.1128/jvi.02105-16>
 16. Lecoine, D., Fabre, M., Habes, D., Mielot, F., Bernard, O., Nordmann, P. (2000). Macrophage activation syndrome in primary human herpes virus-6 infection: a rare condition after liver transplantation in infants. *Gastroentérologie Clinique et Biologique*, 24 (12), 1227-1228.
 17. Hama, N., Abe, R., Gibson, A., Phillips, E. J. (2022). Drug-Induced Hypersensitivity Syndrome (DIHS)/ Drug Reaction With Eosinophilia and Systemic Symptoms (DRESS): Clinical Features and Pathogenesis. *The Journal of Allergy and Clinical Immunology: In Practice*, 10 (5), 1155-1167.e5. <https://doi.org/10.1016/j.jaip.2022.02.004>
 18. Venâncio, P., Brito, M. J., Pereira, G., Vieira, J. P. (2014). Anti-N-methyl-D-aspartate Receptor Encephalitis with Positive Serum Antithyroid Antibodies, IgM Antibodies Against *Mycoplasma pneumoniae* and Human Herpesvirus 7 PCR in the CSF. *Pediatric Infectious Disease Journal*, 33 (8), 882-883. <https://doi.org/10.1097/inf.0000000000000408>
 19. Maltsev, D. (2022). A comparative study of valaciclovir, valganciclovir, and artesunate efficacy in reactivated HHV-6 and HHV-7 infections associated with chronic fatigue syndrome/myalgic encephalomyelitis. *Microbiology and Immunology*, 66 (4), 193-199. <https://doi.org/10.1111/1348-0421.12966>
 20. Urbakh, V. Iu. (1975). *Statisticheskii analiz v biologicheskikh i meditsinskikh issledovaniyakh*. Moscow: Medicina, 295.
 21. Zheng, Z., Zheng, P., Zou, X. (2020). Peripheral Blood S100B Levels in Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. *Journal of Autism and Developmental Disorders*, 51 (8), 2569-2577. <https://doi.org/10.1007/s10803-020-04710-1>
 22. Lv, M., Zhang, H., Shu, Y., Chen, S., Hu, Y., Zhou, M. (2016). The neonatal levels of TSB, NSE and CK-BB in autism spectrum disorder from Southern China. *Translational Neuroscience*, 7 (1), 6-11. <https://doi.org/10.1515/tnsci-2016-0002>
-

23. Binstock, T. (2001). Intra-monocyte pathogens delineate autism subgroups. *Medical Hypotheses*, 56 (4), 523-531. <https://doi.org/10.1054/mehy.2000.1247>
 24. Marseglia, L. M., Nicotera, A., Salpietro, V., Giaimo, E., Cardile, G., Bonsignore, M. et al. (2015). Hyperhomocysteinemia and MTHFR Polymorphisms as Antenatal Risk Factors of White Matter Abnormalities in Two Cohorts of Late Preterm and Full Term Newborns. *Oxidative Medicine and Cellular Longevity*, 2015, 1-8. <https://doi.org/10.1155/2015/543134>
 25. Sweeten, T. L., Croen, L. A., Windham, G. C., Odell, J. D., Stubbs, E. G., Torres, A. R. (2018). Brief Report: Low Rates of Herpesvirus Detection in Blood of Individuals with Autism Spectrum Disorder and Controls. *Journal of Autism and Developmental Disorders*, 49 (1), 410-414. <https://doi.org/10.1007/s10803-018-3691-x>
 26. Gillberg, I. C. (1991). Autistic Syndrome with Onset at Age 31 Years: Herpes Encephalitis as a Possible Model for Childhood Autism. *Developmental Medicine & Child Neurology*, 33 (10), 920-924. <https://doi.org/10.1111/j.1469-8749.1991.tb14804.x>
 27. Harberts, E., Yao, K., Wohler, J. E., Maric, D., Ohayon, J., Henkin, R., Jacobson, S. (2011). Human herpesvirus-6 entry into the central nervous system through the olfactory pathway. *Proceedings of the National Academy of Sciences*, 108 (33), 13734-13739. <https://doi.org/10.1073/pnas.1105143108>
 28. 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>
 29. 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>
 30. González Toro, M. C., Jadraque Rodríguez, R., Sempere Pérez, Á., Martínez Pastor, P., Jover Cerdá, J., Gómez Gosálvez, F. A. (2013). Encefalitis antirreceptor de NMDA: dos casos pediátricos. *Revista de Neurología*, 57 (11), 504-508. <https://doi.org/10.33588/rn.5711.2013272>
 31. Kiani, R., Lawden, M., Eames, P., Critchley, P., Bhaumik, S., Odedra, S., Gumber, R. (2015). Anti-NMDA-receptor encephalitis presenting with catatonia and neuroleptic malignant syndrome in patients with intellectual disability and autism. *BJPsych Bulletin*, 39 (1), 32-35. <https://doi.org/10.1192/pb.bp.112.041954>
 32. 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>
 33. Saghazadeh, A., Ataenia, B., Keynejad, K., Abdolalazadeh, 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>
 34. Saurman, V., Margolis, K. G., Luna, R. A. (2020). Autism Spectrum Disorder as a Brain-Gut-Microbiome Axis Disorder. *Digestive Diseases and Sciences*, 65 (3), 818-828. <https://doi.org/10.1007/s10620-020-06133-5>
-

35. 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>
36. Secchiero, P., Mirandola, P., Zella, D., Celeghini, C., Gonelli, A., Vitale, M. et al. (2001). Human herpesvirus 7 induces the functional up-regulation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) coupled to TRAIL-R1 down-modulation in CD4 + T cells. *Blood*, 98 (8), 2474–2481. <https://doi.org/10.1182/blood.v98.8.2474>
37. Hughes, H. K., Moreno, R. J., Ashwood, P. (2023). Innate immune dysfunction and neuroinflammation in autism spectrum disorder (ASD). *Brain, Behavior, and Immunity*, 108, 245–254. <https://doi.org/10.1016/j.bbi.2022.12.001>
38. Lampiasi, N., Bonaventura, R., Deidda, I., Zito, F., Russo, R. (2023). Inflammation and the Potential Implication of Macrophage-Microglia Polarization in Human ASD: An Overview. *International Journal of Molecular Sciences*, 24 (3), 2703. <https://doi.org/10.3390/ijms24032703>

EFFICACY OF COMBINED IMMUNOTHERAPY WITH PROPES AND INFLAMAFERTIN FOR SELECTIVE NK AND NKT CELL DEFICIENCY IN CHILDREN WITH AUTISM SPECTRUM DISORDERS ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

JUSTIFICATION

Recent meta-analyses of randomized controlled clinical trials indicate an association between autism spectrum disorders and genetic deficiency of the folate cycle in children [14, 15]. It has been established that genetic deficiency of the folate cycle affects the immune status of children with autism spectrum disorders, forming a kind of immunodeficiency, the basis of which is a decrease in the number and functional activity of natural killer (NK) cells and natural killer T lymphocytes (NKT) [10]. Immunosuppression caused by genetic deficiency of the folate cycle mediates the development of a number of immune-dependent complications that determine the formation of inflammatory encephalopathy in children with autism spectrum disorders, in particular, reactivated opportunistic infections [3, 13], autoimmune reactions against neurons and myelin [4, 5] and systemic inflammation with the phenomenon of hypercytokinemia [12, 16]. Compensation of immunodeficiency induced by genetic deficiency of folate cycle seems to be an attractive prospect for preventing or at least reducing the manifestations of related immune-dependent complications that influence the severity of CNS lesions in children with autistic disorders. However, such therapeutic approaches remain undeveloped and therefore inaccessible to patients. Results of previous small clinical studies indicate the potential benefit of combined immunotherapy with Propes and Inflamafertin to compensate for the deficiency of NK and NKT cells in folate cycle deficiency [1, 2], but these encouraging data need to be verified in larger controlled clinical trials with greater validity of the obtained results. Propes is a biological agent containing alpha and beta defensins, which has a pronounced immunoactivating and lymphoproliferative effect. At the same time, Inflamafertin, which includes alarmins and adrenomedullin, on the contrary, has an anti-inflammatory effect mediated by interleukin 10, which is important in preventing autoimmune complications during drug-induced immune activation. As the accumulated experience of using another highly active immunomodulatory agent – recombinant interleukin 2 – indicates, therapeutic immune activation can cause an undesirable increase in the risk of developing autoimmune complications [7], therefore, the combination of the immunoactivating drug Propes with an anti-inflammatory tolerogenic immunotropic agent seems to be the key to achieving a safe immunomodulatory therapeutic effect.

The aim of the research: to study the effectiveness of combined immunotherapy with Propes and Inflamafertin for NK- and NKT-cell deficiency in children with autism spectrum disorders associated with genetic deficiency of the folate cycle.

Materials and methods. This single-center prospective controlled non-randomized clinical study included 225 children aged 2 to 9 years suffering from autism spectrum disorders associated with genetic deficiency of the folate cycle. These patients constituted the study group (SG). The diagnosis of autism spectrum disorders was made by psychiatrists of regional hospitals or specialized departments 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). The basis for including the patient in this trial was the presence of written parental consent for the child's participation in the study (Protocol No. 128 dated December 23, 2019 of the Bioethics Commission of the Bogomolets National Medical University).

To verify the genetic deficiency of the folate cycle, the nucleotide substitutions in the folate cycle genes were determined: MTHFR 677 C > T, MTHFR 1298 A > C, MTRR 66 A > G and MTR 2756 A > G in various combinations in the homozygous and heterozygous state by restriction PCR. Such children were diagnosed with persistent hyperhomocysteinemia – serum homocysteine concentration above 5.2 $\mu\text{mol/l}$, which is a biomarker of folate cycle deficiency. The number of NK and NKT cells in the blood was measured using laser flow cytometry (Epics XI cytometer, USA) using the indirect immunofluorescence method using monoclonal antibodies to CD lymphocyte markers (triple label; Beckman Coulter reagents, USA). NK cells were understood as a subpopulation of lymphocytes with the CD3-CD16 + CD56 + phenotype, and NKT cells were understood as a subpopulation of lymphocytes with the CD3 + CD16 + CD56 + phenotype. Immune status studies were performed monthly for 5 consecutive months both during the 3-month course of immunotherapy and for the next 2 months after the completion of immunotherapeutic interventions.

SG children (n = 225) received approved combination immunotherapy due to NK and/or NKT cell deficiency. Propes was administered at a dose of 2 ml i/m every other day at night for 3 consecutive months (45 injections). Accordingly, Inflamafertin was administered at a dose of 2 ml IM every other day at night for 3 consecutive months, alternating with Propes (45 injections).

The control group (CG) consisted of 51 children of similar age and gender distribution, suffering from autism spectrum disorders associated with genetic deficiency of the folate cycle, but did not receive immunotherapeutic interventions to compensate for NK and NKT cell deficiency. These children underwent only currently recommended educational and developmental programs in specialized centers for patients with special needs.

For statistical analysis of the obtained information, structural and comparative analysis methods were used. In order to establish the reliability of the differences in the results, the Student's T-test was used with the calculation of the confidence probability coefficient p (parametric criterion) and the number of signs Z according to Urbach (non-parametric criterion). To study the relationship between the appointment of immunotherapy and the dynamics of the studied indicators of immune status, Pearson's chi-square (χ^2) was calculated with the definition of the Yates correction. To determine the strength of the detected relationships, the ϕ criterion, Pearson's correlation coefficient (C) and its normalized value (C') were additionally calculated. To verify the obtained data, the calculation of the odds ratio (OR) and 95 % confidence interval (95 % CI) were used. The information was processed using the Microsoft Excel computer program.

The study was carried out as a fragment of scientific work commissioned by the Ministry of Health of Ukraine, grant No. 0118U001218.

Results and their discussion. Compensation of the immunodeficiency induced by GDFC seems to be an attractive prospect for preventing or at least reducing the manifestations of related immune-dependent complications that affect the severity of CNS damage in children with ASD. However, such therapeutic approaches remain undeveloped at present, and therefore are not available to patients. Earlier, Tucker A. N. et al. in an experimental study showed a positive effect of therapy with a thymus extract preparation in specific disorders of bone marrow hematopoiesis and immunosuppression associated with folate deficiency,

which drew attention to the potential therapeutic properties of peptide immunomodulatory agents in GDFC. The results of previous small clinical studies indicate the potential benefit of combined immunotherapy with Propes and Inflamafertin to compensate for the deficiency of NK and NKT cells in folate cycle deficiency [1, 2], but these encouraging data need to be verified in larger controlled clinical trials with greater validity of the results obtained. Propes is a biological agent containing alpha and beta defensins, which has a pronounced immunoactivating and lymphoproliferative effect. At the same time, Inflamafertin, which includes alarmins and adrenomedullin, on the contrary, has an anti-inflammatory effect mediated by interleukin 10, which is important in preventing autoimmune complications in drug-induced immune activation. As the accumulated experience of using another highly active immunomodulatory agent – recombinant interleukin 2 – indicates, therapeutic immune activation can cause an undesirable increase in the risk of developing autoimmune complications [7], therefore, the combination of the immunoactivating drug Propes with an anti-inflammatory tolerogenic immunotropic agent seems to be the key to achieving a safe immunomodulatory therapeutic effect.

The results of the structural analysis in the observation groups indicate that the number of NK cells reached the lower limit of normal in 39 of 53 patients (74 % of cases) with a baseline deficiency of these lymphocytes, and the average number of NK cells in the blood in SG increased almost twice during the 3-month course of immunotherapy, but returned to almost the baseline level within 2 months after the withdrawal of immunotherapeutic agents. In contrast, the number of NKT cells normalized in 78 of 87 patients (89 % of cases) with a baseline deficiency of these cells, and the average number of NKT cells in the blood in SG increased during the course of immunotherapy by at least half and continued to increase steadily during the 2 months after the withdrawal of the approved immunotropic drugs, increasing almost twice at the end of the observation period.

A significant difference in the mean numbers of NKT cells in the blood in the observation groups occurred in the period from 2 to 5 months of the study ($p < 0,05$; $Z < Z_{0,05}$), but not after the first month of treatment, persisting for at least the next 2 months after the withdrawal of the tested immunotropic agents (**Fig. 9.1**). The data of comparative and variance analyses indicate a significant difference in the average numbers of NK cells in the blood in SG and CG during the period of 1-3 months of immunotherapy ($p < 0,05$; $Z < Z_{0,05}$), but not after the withdrawal of immunotherapeutic drugs (**Fig. 9.2**).

The obtained data indicate the ability of the applied combined immunotherapy to increase the number of NK and NKT cells in the blood in children with autism spectrum disorders associated with genetic deficiency of the folate cycle, normalizing their immune status. However, the response patterns of different lymphocyte subpopulations to the tested immunotherapeutic agents differ from each other. Thus, NK cells respond to immunotherapy faster and more intensively, but the achieved effect is short-lived and lasts only against the background of the applied immunotherapy, while the number of NKT cells in the blood increases more slowly with a 1-month delay, but a prolonged positive effect is achieved, since the gradual increase in the number of these lymphocytes in the blood persists even during the first 2 months after the withdrawal of immunotropic drugs.

To test the association between combined immunotherapy and normalization of the number of NK and NKT cells in the blood, we conducted a Pearson chi-square (χ^2), chi-square with Yates' correction, and chi-square with likelihood correction. These data would allow us to determine whether the immunotherapeutic interventions used were the cause of the changes in the immune status of SG patients. It was assumed that

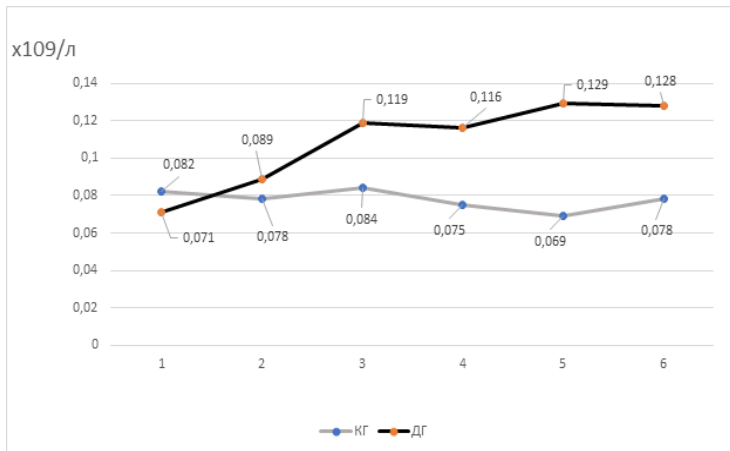


Fig. 9.1. Dynamics of the number of NKT cells in the blood in the observation groups during the clinical study

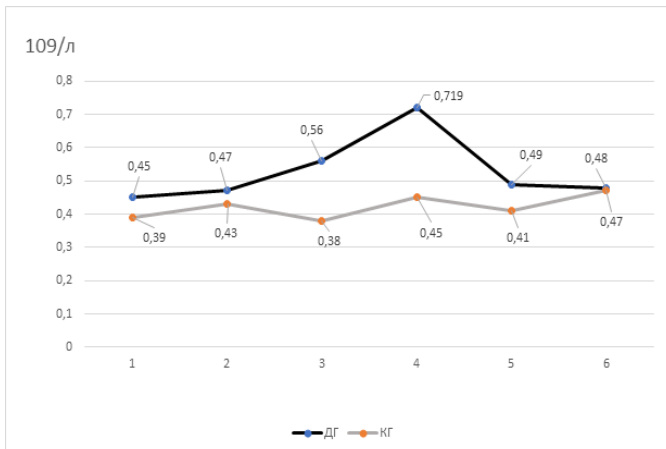


Fig. 9.2. Dynamics of the number of NK cells in the blood in the observation groups during the clinical study

the number of NKT cells was restored in 78 of 87 SG patients with an initial deficiency of these lymphocytes and only in 5 of 32 CG patients with a deficiency of these cells before the start of the study, and the number of NK cells reached normal in 39 of 53 patients with a corresponding deficiency in SG and only in 3 of 18 people with an initial deficiency in CG. The results obtained are presented in **Table 9.1**.

● **Table 9.1.** Evaluation of Pearson's chi-square (χ^2) and other indicators of association between immunotherapy administration and normalization of immune status indicators in SG patients ($n = 225$)

Indicator	NK cells		NKT cells	
	value	probability	value	probability
Pearson's chi-square	18,016	< 0,001	60,65	< 0,001
chi-square with Yates' correction	15,737	< 0,001	57,307	< 0,001
chi-square with likelihood correction	18,613	< 0,001	60,282	< 0,001

The results obtained (**Table 9.1**) indicate a connection between the implementation of immunotherapy and the achievement of normalization of impaired immune status indicators - the number of NK and NKT cells in the blood - in children with autism spectrum disorders associated with genetic deficiency of the folate cycle. This indicates that the prescribed immunotropic drugs were the most likely cause of the positive changes in the immune status of SG patients.

To study the strength of the relationship between the implementation of approved immunotherapeutic interventions and the normalization of the studied indicators of the immune status, the values of the φ coefficient, the Pearson correlation coefficient and its normalized value were calculated. This would allow us to assess how effectively Propes and Inflamafertin act on the impaired immune link in children with autism spectrum disorders associated with GDFC. The results obtained are contained in **Table 9.2**.

● **Table 9.2.** Evaluation of the φ criterion and other indicators of the strength of the relationship between immunotherapy and normalization of immune status indicators in SG patients ($n = 225$)

Indicator	NK cells		NKT cells	
	value	bond strength	value	bond strength
criterion φ	0,504	relatively strong	0,715	strong
Pearson's correlation coefficient (C)	0,450	relatively strong	0,581	relatively strong
normalized value of Pearson's correlation coefficient (C')	0,636	strong	0,822	very strong

As can be seen from the data in **Table 9.2**, there was a predominantly strong or relatively strong relationship between immunotherapy and the achieved changes in immune status, which indicates the high effectiveness of the tested immunotherapeutic agents in SG. NKT cells were somewhat more sensitive to combined immunotherapy than NK lymphocytes, although for both lymphocyte subpopulations convincing data were obtained on a strong relationship between immunotherapy and normalization of their number in the blood.

To verify the data on a strong relationship between the used immunotherapy and normalization of the number of NK and NKT cells in SG patients, the odds ratio (OR), standard error of the odds ratio (S) and 95 %

confidence interval (95 % CI) were calculated. This would avoid errors in assessing the conjugation between the studied processes at the previous stages of statistical analysis. The results obtained are presented in **Table 9.3**.

● **Table 9.3.** Estimation of the odds ratio (OR) and other indicators of the association between immunotherapy and normalization of immune status indicators in SG patients (n = 225)

Indicator	NK cells	NKT cells
odds ratio (OR)	13,929	46,800
standard error of the odds ratio (S)	0,705	0,601
95 % confidence interval (95 % CI)	3,498-55,468	14,415-151,937

As can be seen from the data in **Table 9.3**, the calculation of OR and 95 % CI confirms the previously obtained results on the close relationship between the implementation of the approved immunotherapy and the normalization of the studied indicators of immune status in SG patients. The fact was demonstrated again, identified at the previous stage of statistical analysis of the data, regarding the higher sensitivity of NKT cells compared to NK lymphocytes to combined immunotherapy with Propes and Inflamafertin in SG.

These data indicate the proper immunomodulatory effect of the approved immunotherapeutic strategy in a specific form of immunodeficiency observed in children with autism spectrum disorders associated with genetic deficiency of the folate cycle. Immunodeficiency in children with autism spectrum disorders is most likely responsible for the development of a number of immune-dependent complications that affect both the severity of mental disorders and the level of health in general. In particular, these include abnormally high microbial load on the body [3, 13], persistent immunoinflammatory enterocolitis [6], a tendency to generate allergic manifestations [17], systemic inflammation with hypercytokinemia [12, 16], and autoimmunity against neurons and myelin [4, 5]. Normalization of the impaired immune status is a key to preventing the development of a number of immune-dependent complications in children with autism spectrum disorders, which will contribute to improving their clinical condition and can significantly improve the response to neuroprotective therapeutic strategies [8, 9, 11].

Normalization of the impaired immune status is a key to preventing the development of a number of immune-dependent complications in children with autism spectrum disorders, which will contribute to improving their clinical condition and can significantly improve the response to neuroprotective therapeutic strategies [8, 9, 11].

Conclusions. The results obtained in this controlled non-randomized clinical trial indicate that combined immunotherapy with Propes and Inflamafertin is an effective strategy for the treatment of immunodeficiency caused by genetic deficiency of the folate cycle in children with autism spectrum disorders. These biological immunotropic drugs are able to normalize previously reduced numbers of NK and NKT cells in the blood of this category of patients within a 3-month course of immunotherapy with a more frequent, stronger and more persistent effect on NKT cells compared to NK lymphocytes.

REFERENCES

1. Maltsev, D. V. (2016). Efektyvnost ymmunoterapy pry defytsyte estestvennykh kyllerov y/yly estestvennykh kyllernykh T-lymfotsytov u liudei. Epidemiolohiia, imunopatohenez, diahnozyka, likuvannia khlamidiozu i TORCH-infektsii. Imunolohiia ta alerholohiia. Dodatok, 1, 6-7.
2. Maltsev, D. V. (2017). Efektyvnist kombinovanoi imunoterapii Propesom ta Inflamafertynom pry defytsyti pryrodnykh kirtsiv ta pryrodnykh kilernykh T-limfotsytiv, asotsioovanykh z henetychnym defytsytom folatnoho tsyклу. Novi dosiahnennia v imunolohii ta alerholohii. Imunolohiia ta alerholohiia, 1-2, 48.
3. Binstock, T. (2001). Intra-monocyte pathogens delineate autism subgroups. *Medical Hypotheses*, 56 (4), 523-531. <https://doi.org/10.1054/mehy.2000.1247>
4. 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>
5. Frye, R. E., Sequeira, J. M., Quadros, E. V., James, S. J., Rossignol, D. A. (2012). Cerebral folate receptor autoantibodies in autism spectrum disorder. *Molecular Psychiatry*, 18 (3), 369-381. <https://doi.org/10.1038/mp.2011.175>
6. Furlano, R. I., Anthony, A., Day, R., Brown, A., McGarvey, L., Thomson, M. A. et al. (2001). Colonic CD8 and $\gamma\delta$ T-cell infiltration with epithelial damage in children with autism. *The Journal of Pediatrics*, 138 (3), 366-372. <https://doi.org/10.1067/mpd.2001.111323>
7. He, J., Zhang, R., Shao, M., Zhao, X., Miao, M., Chen, J., Liu, J. et al. (2020). Efficacy and safety of low-dose IL-2 in the treatment of systemic lupus erythematosus: a randomised, double-blind, placebo-controlled trial. *Annals of the Rheumatic Diseases*, 79 (1), 141-149. <https://doi.org/10.1136/annrhumdis-2019-215396>
8. 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>
9. 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 pharmaceutical technology*. - 2019. - Vol. 11(05). - P. 597-609.
10. 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>
11. Maltsev D.V. High-dose intravenous immunoglobulin therapy efficiency in children with autism spectrum disorders associated with genetic deficiency of folate cycle enzymes // *Psychiatry, Psychotherapy and Clinical Psychology*. - 2016. - Vol. 2. - P. 63-80.
12. Maltsev, D., Yevtushenko, S. (2016). High-Dose Intravenous Immunoglobulin Therapy Efficiency in Children with Autism Spectrum Disorders Associated with Genetic Deficiency of Folate Cycle Enzymes. *International Neurological Journal*, (2.80), 35-48. <https://doi.org/10.22141/2224-0713.2.80.2016.74004>

13. 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>
14. Nicolson, G. L., Gan, R., Nicolson, N. L., Haier, J. (2007). Evidence for *Mycoplasma ssp.*, *Chlamydia pneumoniae*, 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>
15. 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>
16. 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>
17. 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.jpsy-chires.2019.05.019>
18. Xu, G., Snetselaar, L. G., Jing, J., Liu, B., Strathearn, L., Bao, W. (2018). Association of Food Allergy and Other Allergic Conditions With Autism Spectrum Disorder in Children. *JAMA Network Open*, 1 (2), e180279. <https://doi.org/10.1001/jamanetworkopen.2018.0279>

EFFICACY OF COMBINED USE OF TRANSFER FACTORS BASED ON COLOSTRUM IMMUNE EXTRACT IN NK AND NKT CELL DEFICIENCY IN CHILDREN WITH AUTISM SPECTRUM DISORDERS ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

JUSTIFICATION

Recent decades of research have shown that immunodeficiency and related immune dysregulation are important components in the pathogenesis of multisystemic damage in children with autism spectrum disorders (ASD). Data from 5 meta-analyses and systematic reviews of randomized controlled trials supporting the association of ASD and genetic deficiency of the folate cycle (GDFC) shed light on key factors in the genetic predisposition to the development of severe neuropsychiatric syndromes in children [1, 2, 3, 4, 5]. The results of the last 3 systematic reviews of controlled clinical trials have characterized the spectrum of immunological disorders characteristic of children with ASD, one of the main components of which is the deficiency of natural killer (NK) cells, natural killer T lymphocytes (NKT) and, conversely, an abnormally increased number of CD3 + T lymphocytes in the peripheral blood [6, 7, 8].

Immune system disorders shed light on the origin of a number of immune-dependent clinical manifestations (allergic, autoimmune, immunoinflammatory, etc.) characteristic of children with ASD, which cannot be explained solely by the existing mental dysfunction [9, 10, 11].

Correction of immune disorders in children with ASD appears to be a promising tool not only for restoring immune resistance to microorganisms, but also for preventing the development of numerous immune-dependent complications that affect both the CNS and other organs and systems of the child's body.

Currently, there is a lack of clinical studies on the testing of immunocorrective interventions in children with ASD with signs of immune dysfunction. A recent report on the effectiveness of combined immunotherapy with Propes and Inflamaferin in eliminating key disorders of innate immunity in children with ASD associated with GDFC, namely, NK and NKT cell deficiency, indicates the potential curability of this immune dysfunction and may serve as an incentive for initiating further research in this direction [12]. It is necessary to find alternative, cheaper and more convenient immunocorrective agents, but at least equivalently effective to the approved peptide immunotherapy. Such immunocorrective agents may be transfer factors based on the immune extract of bovine colostrum, which in placebo-controlled clinical studies have so far demonstrated the ability to eliminate or at least attenuate many immune-dependent disorders characteristic of children with ASD, namely, to improve immune status, increase resistance to respiratory and intestinal infections, reduce manifestations of intestinal dysbiosis, normalize intestinal wall permeability, have antiallergic effects and attenuate manifestations of autoimmunity, as well as normalize some neurological and psychiatric disorders [13, 14, 15]. There is a justified need to test transfer factors to correct key cellular immune disorders in children with ASD associated with GDFC, which would provide practical medicine with an effective means of preventing immune-dependent complications that affect the severity of the condition, quality of life, and endurance of patients with neuropsychiatric disorders.

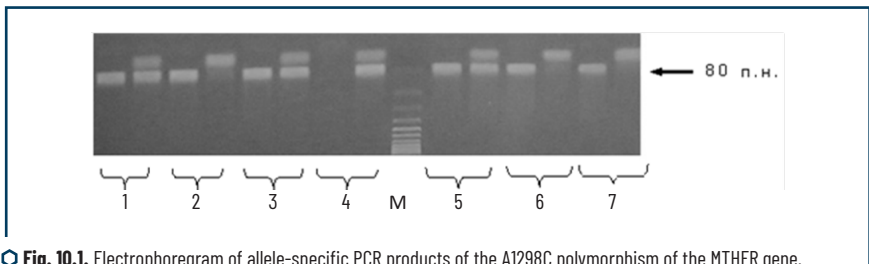
The aim of the research: to study the efficacy and safety of the combined use of classical transfer factor and trifactor transfer factor in NK and NKT cell deficiency in children with ASD associated with GDFC, taking into account the effect on the number of CD3 + T lymphocytes in the blood.

Research objectives:

1. To assess the dynamics of the number of NK cells in peripheral blood using classical transfer factor and trifactor transfer factor in children with ASD associated with GDFC.
2. To investigate the dynamics of the number of NKT cells in the blood using classical transfer factor and trifactor transfer factor in children with ASD associated with GDFC.
3. To establish the dynamics of the number of CD3 + T lymphocytes in the blood using classical transfer factor and trifactor transfer factor in children with ASD associated with GDFC.
4. To assess the effectiveness and safety of the use of classical transfer factor and trifactor transfer factor for the correction of key signs of immune dysregulation in children with ASD associated with GDFC.

Materials and methods. To achieve the goal and fulfill the objectives, the medical records of 225 children aged 2 to 9 years with GDFC and ASD (study group, SG) were studied. The SG included 183 boys and 42 girls. These children were patients of the Institute of Immunology and Allergology of the Bogomolets National Medical University (NMU) of the Ministry of Health of Ukraine (from 2012 to 2018) and the Vivere clinic, specializing in neuroimmunology, for the period from 2019 to 2022. Registration dossier of the Vivere clinic No. 10/2212-M dated 12/22/2018. Further processing of clinical material after receiving medical data in the clinic was carried out at the Institute of Experimental and Clinical Medicine of the Bogomolets National Medical University (NMU) in accordance with the cooperation agreement No. 150221 dated 02/15/2021 and based on the relevant conclusion of the NMU bioethical expertise commission according to the data of protocol No. 140 dated 12/21/2020. The clinical diagnosis of ASD for patients in the observation groups was made by experienced child psychiatrists specializing in the problem of psychospeech disorders in children, according to the validated diagnostic criteria of DSM-IV-TR.

Pathogenic polymorphic variants of nucleotide substitutions in the genes of folate cycle enzymes for diagnosing GDFC in patients of the observation groups were identified using the polymerase chain reaction (PCR) method with restriction in the Department of Neurobiochemistry of the Romodanov Institute of Neurosurgery of the National Academy of Medical Sciences of Ukraine (from 2012 to 2018) (**Figs. 10.1 and 10.2**) and the Sinevo laboratory, Ukraine (from 2019 to 2022).



○ **Fig. 10.1.** Electrophoregram of allele-specific PCR products of the A1298C polymorphism of the MTHFR gene. Pair of lanes 2,6,7 - genotype AA; pair of lanes 1,3,5 - genotype AC; pair of lanes 4 - genotype CC, M - marker 100 bp

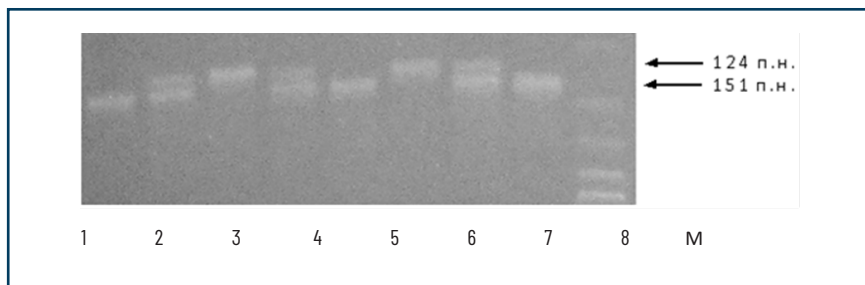


Fig. 10.2. Electropherogram of restriction analysis products of the A66G polymorphism of the MTRR gene. Lanes 3,6 – genotype AA; lanes 2,4,7 – genotype AG; lanes 1,5,8 – genotype GG, M – marker 100 bp

At the same time, MTHFR C677T nucleotide substitutions were detected both in mono-form (68 SG patients; 30 % of cases) and in combination with other pathogenic nucleotide substitutions, in particular with MTHFR A1298C, MTR A2756G and/or MTRR A66G (157 SG patients; 70 % of cases). The genome, which included double pathological nucleotide substitutions MTHFR C677T + MTHFR A1298C, was observed in 26 (12.5 %), MTHFR C677T + MTRR A66G – in 19 (8.5 %), and MTHFR C677T + MTR A2756G – in 25 (11 % of cases) of SG children. The genome containing the triple pathological nucleotide substitutions MTHFR C677T + MTRR A66G + MTR A2756G occurred in 23 (10.5 %), MTHFR C677T + MTHFR A1298C + MTR A2756G – in 22 (9.5 %), and MTHFR C677T + MTHFR A1298C + MTRR A66G – in 21 (9 % of cases) of SG children. Finally, the genome containing all four studied pathogenic nucleotide substitutions, MTHFR C677T + MTHFR A1298C + MTR A2756G + MTRR A66G was identified in 21 (9 % of cases) of SG children (**Fig. 10.3**).

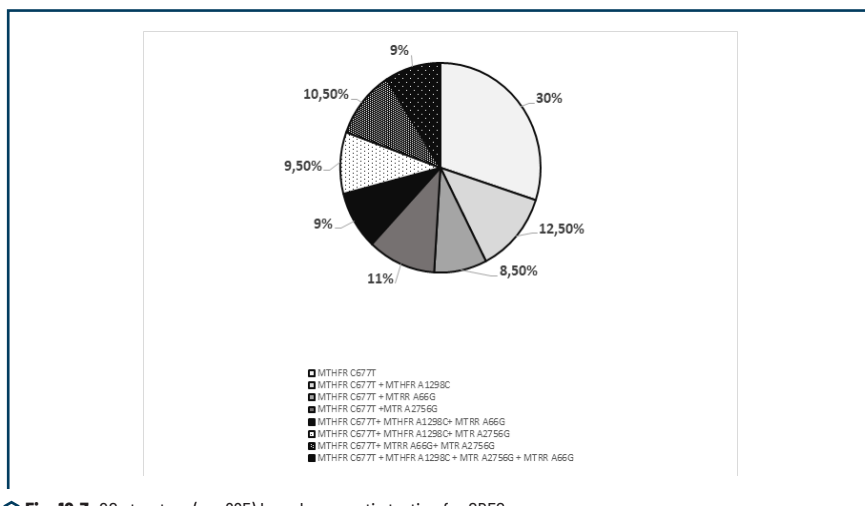


Fig. 10.3. SG structure (n = 225) based on genetic testing for GDGC

10 EFFICACY OF COMBINED USE OF TRANSFER FACTORS BASED ON COLOSTRUM IMMUNE EXTRACT IN NK AND NKT CELL DEFICIENCY IN CHILDREN WITH AUTISM SPECTRUM DISORDERS ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

The number of CD3 + T-lymphocytes, NK- and NKT-cells in the peripheral blood of patients in the observation groups was measured using laser flow cytometry (Epics XI cytometer, USA) using the indirect immunofluorescence method using monoclonal antibodies to CD-markers of lymphocytes (single and triple label; Beckman Coulter reagents, USA). NK-cells were understood as a subpopulation of lymphocytes with the CD3-CD16 + CD56 + phenotype, and NKT-cells were understood as a subpopulation of lymphocytes with the CD3 + CD16 + CD56 + phenotype (**Fig. 10.4**).

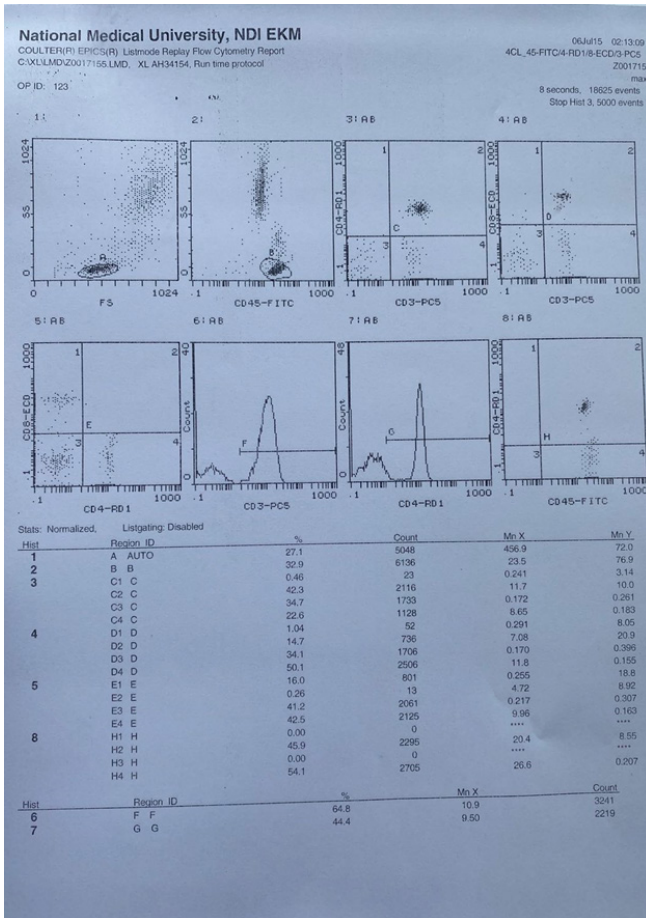


Fig. 10.4. Example of a flow laser cytofluorimetry protocol for studying the subpopulation composition of blood lymphocytes by CD markers on the Epics XI device at the Institute of Experimental and Clinical Medicine (NDI ECM) of the National Medical University

The mean number of CD3 + T lymphocytes in SG patients' peripheral blood at the study's beginning was $4.24 \pm 0.15 \times 10^9/l$. The number of CD3 + T lymphocytes in the peripheral blood of SG children exceeded the upper limit of normal in 72 % of cases, and in the remaining 28 % of cases it was normal. The mean number of NK cells in SG patients' peripheral blood at the study's beginning was $0.08 \pm 0.004 \times 10^9/l$, and the mean number of NKT lymphocytes was $0.03 \pm 0.009 \times 10^9/l$. At the beginning of the study, combined NK and NKT cell deficiency occurred in 82 % of SG patients, while NK cell deficiency in general occurred in 65 %, and in isolated form in only 9 % of cases. In contrast, NKT cell deficiency in general occurred in SG in 73 %, and in isolated form in 17 % of cases (**Fig. 10.5**).

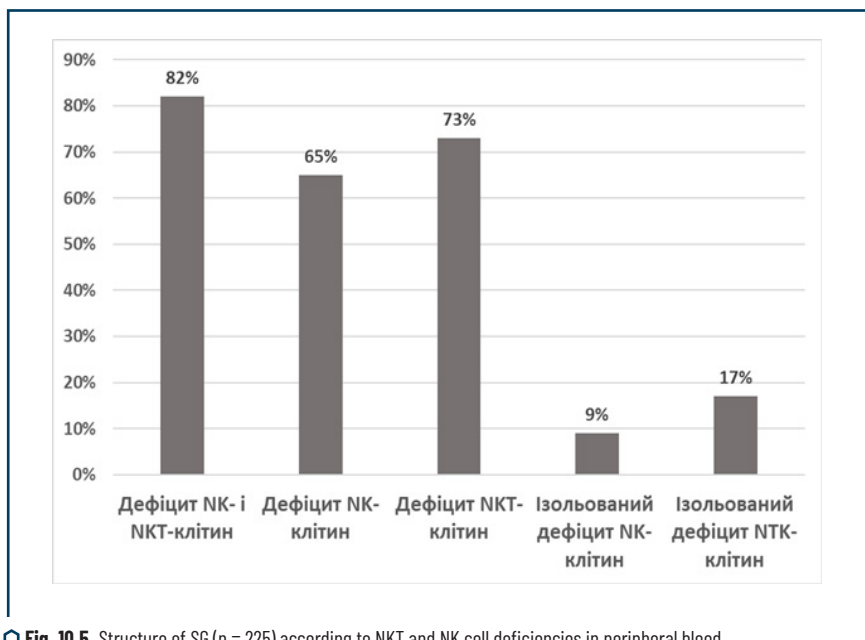


Fig. 10.5. Structure of SG (n = 225) according to NKT and NK cell deficiencies in peripheral blood

For the purpose of immunocorrection, SG patients used the original standardized immune extracts of bovine colostrum Transfer Factor Classic 4Life (UltraFactor XF[®] - 600 mg) 2 capsules 3 times a day during meals and Transfer Factor Tri-Factor 4Life (UltraFactor XF[®] 600 mg + OvoFactor[®] + NanoFactor[®]) 1 capsule 3 times a day during meals for 3 consecutive months. UltraFactor XF[®] is a specific concentrate of ultrafiltrate of immune proteins and other peptides of bovine colostrum, OvoFactor[®] is a specific concentrate of immune proteins and other peptides from chicken egg yolks, and NanoFactor[®] is a proprietary concentrate of nanofiltrate of bovine colostrum. Measurement of the number of CD3 + T-lymphocytes, NK- and NKT-cells in the peripheral blood of SG patients during the observation period was carried out 4 times a month - before the start of the study, after the first, second and third months of transfer factor application.

The same SG was used as in the case of the testing of Propes and Inflamafertin, but in a different time period, after the restoration of the deficiency of NK- and NKT-cells after a certain time (on average – from 6 to 12 months) after the complete cessation of the therapeutic effect of the tested peptide drugs.

The control group (CG) included medical records of 52 children with GDFC and ASD of similar age (2 to 8 years) and gender distribution (37 boys and 15 girls) who similarly had NK- and NKT-cell deficiencies. CG patients did not receive transfer factors during the observation period. CG children also underwent four-time monthly monitoring of the numbers of CD3 + T-lymphocytes, NK- and NKT-cells for 3 consecutive months to assess immune status during the natural course of the disease.

Statistical processing of the obtained material was carried out using comparative and structural analyses. To study the distribution of variants in the variation series, the Shapiro–Wilk test was used. To establish the probability of the obtained differences between the values of the studied laboratory indicators in the observation groups, the parametric Student's T-test with an additional measurement of the confidence probability indicator p and the nonparametric Z-test according to Urbach V.Yu. [16] were used. Differences were considered probable in the case of obtaining $p < 0,05$ and $Z < Z_{0,05}$.

To study the relationship between the appointment of transfer factors and the dynamics of the studied indicators of immune status, the calculation of the odds ratio (OR) and 95 % confidence interval (95 % CI) was used. The information was processed using the Microsoft Excel computer program (Redmond, WA).

Sources of funding. This clinical study was implemented as a fragment of scientific research work with funds from the State Budget of Ukraine under No. 0121U107940.

Research results and their discussion. The results of the structural analysis in the observation groups indicate that the number of NK cells reached the lower limit of normal in 109 of 146 patients (75 % of cases) with a baseline deficiency of these lymphocytes, and the average number of NK cells in the blood in the SG increased almost threefold during the 3-month course of transfer factors. In contrast, the number of NKT cells normalized in 127 of 164 patients (77 % of cases) with a baseline deficiency of these cells, and the average number of NKT cells in the blood in the SG increased almost 4-fold during the course of immunotherapy compared to the baseline level. In contrast, the number of NK cells increased to the lower limit of normal in only 7 of 36 patients (15 %), and the number of NKT cells in only 9 of 42 people (21 %) of the CG with a baseline low number of these lymphocytes in the peripheral blood at the end of the observation period, which was a significant difference from the data in the SG ($p < 0,05$; $Z < Z_{0,05}$). The results of the structural analysis in the observation groups indicate that NK and NKT cell deficiencies are not only common, but also quite persistent laboratory phenomena in children with ASD associated with GDFC, which usually do not undergo spontaneous resolution during the natural course of the disease. The combined use of transfer factors contributes to the restoration of previously reduced numbers of NK and NKT lymphocytes in the peripheral blood in the majority of children with ASD associated with GDFC, compensating for key disorders of cellular immunity and thereby modifying the immune status of patients.

Comparative analysis data indicate no differences in the achieved proportion of complete responders to the tested combination of transfer factors for correction of NK and NKT cell deficiency among SG patients at the end of the observation period ($p > 0,05$; $Z > Z_{0,05}$). This means that the combined use of transfer factors is equally effective in NK and NKT lymphocyte deficiencies in children with ASD associated with GDFC, with initial low numbers of these cells in the peripheral blood.

At the same time, the comparative analysis data did not reveal a significant difference in the proportion of responders to immunocorrective interventions using transfer factors in isolated NK and NKT cell deficiencies and combined disorders, when a single patient has a simultaneous decrease in the number of both NK and NKT lymphocytes ($p > 0,05$; $Z > Z_{0,05}$). This may mean that NK and NKT cells respond to transfer factors separately, in an independent manner, and patients with isolated and combined disorders are equally sensitive to the immunocorrective interventions used.

Data from dynamic observation of the studied laboratory indicators of immune status in SG and CG indicate a gradual increase in the number of NK and NKT cells in the peripheral blood of SG patients at each control point with the achievement of the maximum level at the end of the course of immunocorrection and the absence of significant changes in the average numbers of NK and NKT lymphocytes in the peripheral blood of CG individuals during the observation period. The average number of NK cells in the peripheral blood during the observation period in SG increased 3 times, and NKT cells - more than 4 times (**Figs. 10.6 and 10.7**). There was a significant difference between the initial mean numbers of NK and NKT cells in SG at the beginning of the study and at the end of the 3rd month of immunocorrection ($p > 0,05$; $Z > Z_{0,05}$). A similar significant difference in the mean numbers of NK and NKT lymphocytes was found among SG and CG patients at the 3rd month of observation ($p > 0,05$; $Z > Z_{0,05}$). However, no significant difference was found in the mean numbers of NK and NKT cells in CG at all control points during the entire observation period ($p > 0,05$; $Z > Z_{0,05}$). This may indicate that the use of transfer factors not only normalizes the previously reduced number of NK and NKT lymphocytes in the peripheral blood of children with ASD associated with GDFC, but also provides a progressive increase in the number of these cells over time throughout the course of immunocorrection. We can speak of a time-dependent effect of the use of transfer factors in NK and NKT cell deficiencies in children with ASD associated with GDFC. This progressive manner of response to transfer factors by NK and NKT lymphocytes in SG allows us to consider the hopes of achieving the success of immunocorrection interventions by extending the duration of their use without increasing the dosages of immunobiological agents as justified, when the lower limit of the norm of the studied laboratory indicator after the previous course of immunocorrection was not reached.

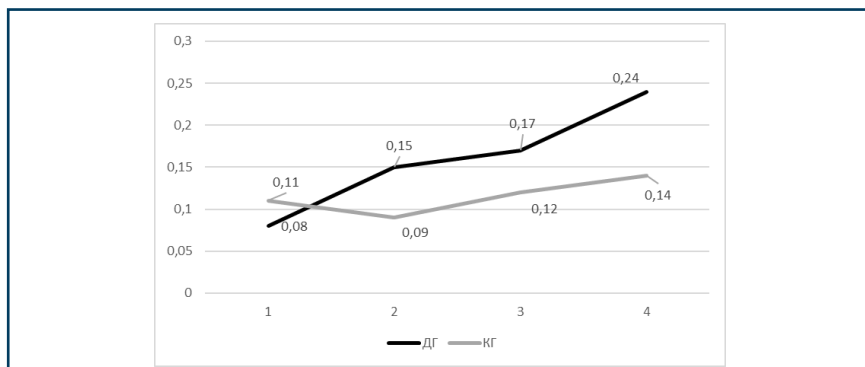


Fig. 10.6. Dynamics of the average number of NK cells in the peripheral blood of patients SG (n = 225) and CG (n = 52) during the observation period

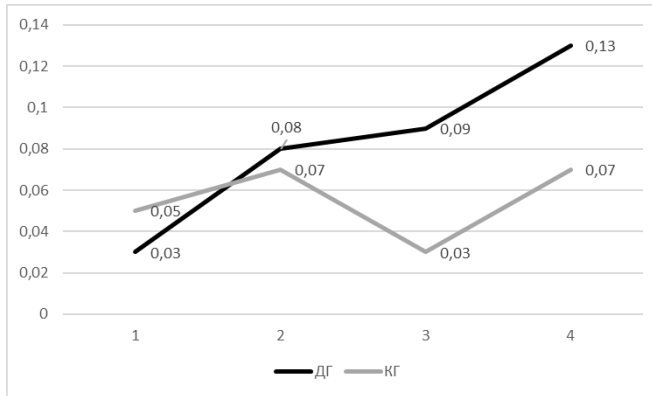
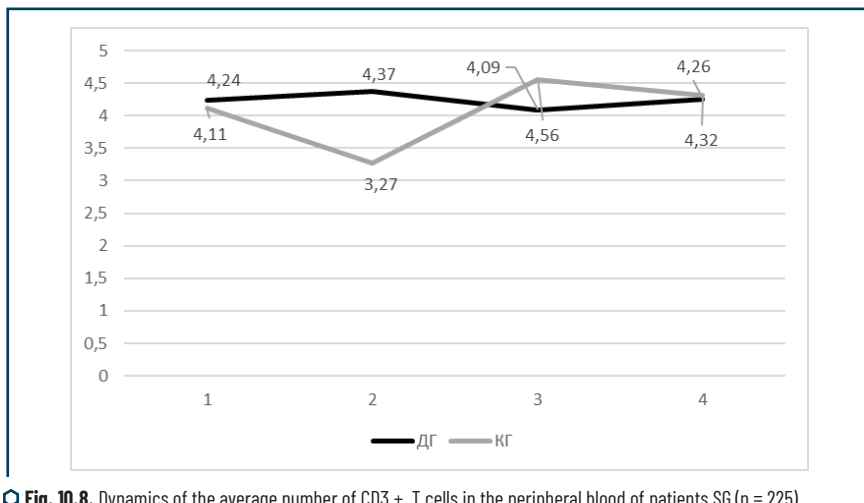


Fig. 10.7. Dynamics of the average number of NKT cells in the peripheral blood of patients SG (n = 225) and CG (n = 52) during the observation period

If we talk about the assessment of the dynamics of the number of CD3 + T lymphocytes in patients of the observation groups, then there was no significant difference in the average numbers of these cells in the peripheral blood of patients in both the SG and the CG at all control points during the study period ($p > 0,05$; $Z > Z_{0,05}$). There was also no significant difference in the average numbers of CD3 + T-lymphocytes in the peripheral blood between the SG and the CG at all control points during the observation period ($p > 0,05$; $Z > Z_{0,05}$) (Fig. 10.8). This indicates that the use of transfer factors does not affect the number of CD3 + T-cells in the peripheral blood in children with ASD associated with GDFC. Such patients are characterized by a state of immune dysregulation, which is associated with a number of immune-dependent complications, and an abnormally increased number of CD3 + T-lymphocytes in the peripheral blood is an important component of the specified immune dysregulation. When testing transfer factors, it was important to check not only the effectiveness of correcting NK and NKT cell deficiencies, but also their safety in terms of their impact on immunoregulatory disorders. Transfer factors do not increase the number of CD3 + T-cells in the blood, which may indicate the absence of a negative effect on the existing immune dysregulation in children with ASD, one of the manifestations of which is a large number of cells of the specified lymphocyte population in the blood.

To verify the available data on the association of the used immunocorrective interventions and the normalization of the number of NK and NKT cells in the peripheral blood of SG patients at the end of the observation period, the odds ratio (OR), standard error of the odds ratio (S) and 95 % confidence interval (95 % CI) were calculated. The "outcome" was understood as the normalization of the number of the studied lymphocyte subpopulations, and the "risk factor" was the use of transfer factors. This would avoid errors in assessing the conjugation between the studied processes at the previous stages of statistical analysis. The results obtained are presented in **Table 10.1**.



● Fig. 10.8. Dynamics of the average number of CD3 + T cells in the peripheral blood of patients SG (n = 225) and CG (n = 52) during the observation period

● Table 10.1. Results of OR, S and 95 % CI measurements in assessing the association of immunocorrective interventions and normalization of immune status indicators in SG patients (n = 225)

Indicator	NK cells	NKT cells
Odds ratio (OR)	12.205	12,586
Standard error of odds ratio (S)	0,462	0.420
95 % confidence interval (95 % CI)	4,934–30,191	5,526–28,661

As can be seen from the data in **Table 10.1**, the calculation of OR and 95 % CI confirms the previously obtained results on the close relationship between the use of transfer factors and the normalization of the studied indicators of immune status in patients with SG. The fact, identified at the previous stage of statistical analysis of the data, of almost equally high sensitivity of NKT and NK lymphocytes to combined immunocorrective interventions using transfer factors, was demonstrated again. Thus, the use of transfer factors in SG was associated with an almost 12-fold increase in the chances of normalization of previously reduced numbers of NKT- and NK-cells in peripheral blood.

Transfer factors were well tolerated. No adverse reactions were noted during their use during the observation period.

Research results and their discussion. A wide range of immune system dysfunctions in children with ASD have been described and characterized. The results of this scientific search are summarized in the data of three recent systematic reviews of clinical studies.

Thus, the data of the systematic review by Mead J. et al. demonstrated signs of immune dysregulation in ASD: neuroinflammation, autoantibodies, abnormally enhanced T-cell response, impaired activity of natural killer cells and monocytes. These immune aberrations were associated with the severity of mental disorders in ASD, in particular, impaired social interaction, stereotyped behavior and reduced communication skills. This review notes that experimental animal models have made it possible to achieve the elimination of clinical symptoms of ASD after removing from the body those immune factors that were involved in aberrant immune reactions [6].

Accordingly, Noriega D.B., Savelkoul H.F. in a systematic review of ASD in children described signs of immune dysregulation, including hyperproduction of pro- and suppression of synthesis of anti-inflammatory cytokines, as well as increased permeability of the blood-brain barrier, abnormal production of anti-brain autoantibodies, and modification of NK functional activity [7]. According to the data of a systematic review by Hughes H.K. et al. on the state of the immune system in children with ASD, there is a disturbed cytokine balance, quantitative disorders of immunocompetent cells, signs of persistent neuroglial inflammation in the CNS, defects in the functioning of the adaptive and innate immune systems, including NK dysfunction, as well as pathological deviations in serum concentrations of immunoglobulins of different classes and subclasses, signs of autoimmune reactions to neurons, myelin and extracerebral autoantigens [8].

Since children with ASD are a rather heterogeneous group, the features of immunological disorders may differ in certain subgroups of patients. Accordingly, a specific form of immunodeficiency has been described in children with ASD associated with GDFC, the key disorders in which there is a deficiency of NK and NKT lymphocytes [17]. The results of a number of studies demonstrate various immunological disorders, including NK deficiency, in GDFC and folic acid deficiency in humans [18]. The mechanism of immunosuppression and immune dysregulation in such cases is seen in gene regulatory and epigenetic disorders associated with impaired DNA, protein, lipid and nucleoprotein methylation processes [11], as well as with a state of persistent oxidative stress and critical weakening of the antioxidant system [19], deficiency of vitamins, trace elements and essential nutrients due to immunoinflammatory damage to the intestinal wall [20].

The mechanism of immune transfer from mother's breast milk to the intestine of infants is one of the main components of the unprecedented advantage of mammals in the struggle for existence in the biological world. The use of transfer factors based on immune extracts of colostrum in the clinic artificially recreates the physiological mechanism of immune transfer as an inherent property of mammals, which was supported by natural selection in the process of evolution of biological organisms, convincingly demonstrating its effectiveness in the long struggle for the existence of mammals with representatives of other classes of biological beings [21].

The data obtained in this clinical study indicate the appropriate immunomodulatory effect of the tested immunocorrective strategy using transfer factors in a specific form of immunodeficiency observed in children with ASD associated with GDFC. Immunodeficiency and the associated immune dysregulation in children with ASD are most likely responsible for the development of a number of immune-dependent complications that affect both the severity of mental disorders and the level of health of the child in general.

As is known, in ASD there is an abnormally high microbial load with a predominance of opportunistic pathogens [22], persistent immunoinflammatory enterocolitis [23], various allergic manifestations [24], a state of systemic inflammation with hypercytokinemia [25] and signs of autoimmunity against neurons, myelin and some extracerebral autoantigens [26]. Normalization of the impaired immune status may be the key to preventing the development of a number of immune-dependent complications in ASD children associated with GDFC, in whom there is a deficiency of NK and/or NKT cells. The development and implementation of effective immunomodulatory strategies with a targeted effect on the affected immune components is believed to contribute to the improvement of the clinical condition of children with ASD and may significantly improve the consequences of the disease. Therefore, the success of transfer factors demonstrated in the results of this clinical study allows us to hope for the earliest possible finding of an answer regarding effective, safe and convenient immunocorrective interventions for children with ASD, who show signs of immunodeficiency and immune dysregulation.

The composition of transfer factors based on a standardized immune extract of bovine colostrum was studied in detail by Sacerdote P. et al. in 2013 [27], and a comprehensive characterization of the proteome of bovine milk in the context of immune transfer was provided by Zhang L. et al. in 2015 [28]. It has been shown that transfer factors contain more than 100 proteins and peptides, including lysozyme, lactoferrin, alpha/beta defensins, cytokines, chemokines, immunoglobulins, soluble lymphocyte receptors, etc., which pass from blood serum to breast milk for the purpose of subsequent immune transfer to the infant's intestine during breastfeeding. Currently, immunomodulatory and anti-inflammatory effects of transfer factors based on an immune extract of bovine colostrum have been demonstrated in humans. In particular, the results of double-blind placebo-controlled randomized clinical trials indicate the potential effectiveness of transfer factors in frequent respiratory and intestinal infections in children, for the prevention of sepsis episodes in premature infants and in HIV-infected children with insufficient response to antiretroviral therapy [29, 30, 31, 32]. In accordance with this, Huppertz H.I. et al. in a double-blind placebo-controlled randomized clinical trial showed that immune bovine colostrum extract leads to a decrease in the symptoms of diarrhea that has already begun, caused by enterotoxigenic strains of *E. coli*, prevents the development of exicosis, and reduces the need for infusion therapy [29]. Patiroğlu T., Kondolot M. in a double-blind placebo-controlled randomized clinical trial involving 31 patients found that oral immunotherapy with immune bovine colostrum extract leads to a sharp decrease in the frequency and severity of upper respiratory tract infections in patients with primary selective IgA deficiency without changing the content of secretory IgA in saliva [30]. On the other hand, Lee J. et al. in a double-blind placebo-controlled randomized clinical trial demonstrated that immune extract of bovine colostrum reduces the number of episodes of sepsis in premature infants with low body weight and signs of immune system immaturity. In parallel, they achieved an increase in the concentration of secretory immunoglobulin class A and lactoferrin in biological media, which indicated an increase in immunoresistance due to optimization of the work of local mucosal immunity. There was also a decrease in the content of transforming growth factor beta, interleukin-1beta and interleukin-6 in urine and saliva, which indicated the implementation of an anti-inflammatory effect [31]. Byakwaga H. et al. in a placebo-controlled randomized clinical trial involving 75 patients, it was shown that an immune extract of bovine colostrum leads to an increase in the

number of CD4+ T-helper cells in the blood of patients with HIV-induced AIDS who poorly respond to highly active antiretroviral therapy administered according to an international protocol [32].

The results of this clinical trial expand the list of potential indications for the use of transfer factors based on immune extract of bovine colostrum for the correction of immune disorders in children with ASD associated with GDFC.

Data from previous controlled clinical trials dedicated to finding ways to compensate for the deficiency of NK and NKT cells indicate the potential benefit of combined immunotherapy with Propes and Inflamafertin in both children with ASD associated with GDFC [12] and adults with chronic fatigue syndrome/myalgic encephalomyelitis in GDFC [33], but these encouraging data need to be verified in larger controlled clinical trials with greater validity of the results obtained. Propes is a biological agent containing alpha- and beta-defensins, which has pronounced immunoactivating and lymphoproliferative effects. At the same time, Inflamafertin, which includes alarmins and adrenomedullin, on the contrary, has an anti-inflammatory effect mediated by interleukin 10, which is important in preventing autoimmune complications during drug-induced immune activation. As the accumulated experience of using another highly active immunomodulatory agent – recombinant interleukin 2 – indicates, therapeutic immune activation can cause an undesirable increase in the risk of developing autoimmune complications [34], therefore, the combination of the immunoactivating drug Propes with an anti-inflammatory tolerogenic immunotropic agent seems to be the key to achieving a safe immunomodulatory therapeutic effect. As the results of this clinical study show, the combined use of transfer factors is associated with results in terms of correction of NK- and NKT-cell deficiencies and tolerability of corrective interventions similar to those obtained with the use of Propes and Inflamafertin, however, transfer factors turned out to be significantly cheaper, and therefore more accessible to patients, and technically more convenient to use, since they do not require long-term intramuscular injections and special storage conditions.

Conclusions. The results obtained in this single-center retrospective controlled non-randomized clinical study indicate that the combined use of transfer factors based on standardized immune extracts of bovine colostrum is an effective strategy for the correction of NK- and NKT-cell deficiencies in children with ASD associated with GDFC, with an appropriate safety profile. These biological immunotropic agents are able to normalize the previously reduced number of NK and NKT cells in the peripheral blood in the specified category of patients already during a 3-month immunocorrection course with the same effect on NKT and NK lymphocytes in a time-dependent manner without increasing the number of CD3+ T cells in the peripheral blood of patients. It seems promising to continue clinical studies in the field of the use of transfer factors in children with ASD, who show signs of immunodeficiency and immune dysregulation.

REFERENCES

1. 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>

2. 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>
 3. 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>
 4. 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>
 5. 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>
 6. Mead, J., Ashwood, P. (2015). Evidence supporting an altered immune response in ASD. *Immunology Letters*, 163 (1), 49-55. <https://doi.org/10.1016/j.imlet.2014.11.006>
 7. Noriega, D. B., Savelkoul, H. F. J. (2013). Immune dysregulation in autism spectrum disorder. *European Journal of Pediatrics*, 173 (1), 33-43. <https://doi.org/10.1007/s00431-013-2183-4>
 8. 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>
 9. Alhuzimi, T. E., Alharbi, M. H. (2022). Perceptions of Parents on Management of Food Allergy in Children with Autism Spectrum Disorder (ASD) in Saudi Arabia. *Children*, 10 (1), 48. <https://doi.org/10.3390/children10010048>
 10. Tordjman, S., Charrier, A., Kazatchkine, M., Roubertoux, P., Botbol, M., Bronsard, G., Avrameas, S. (2023). Natural IgG Anti-F (ab')₂ Autoantibody Activity in Children with Autism. *Biomedicines*, 11 (3), 715. <https://doi.org/10.3390/biomedicines11030715>
 11. Alshamrani, A. A., Alshehri, S., Alqarni, S. S., Ahmad, S. F., Alghibiwi, H., Al-Harbi, N. O. et al. (2023). DNA Hypomethylation Is Associated with Increased Inflammation in Peripheral Blood Neutrophils of Children with Autism Spectrum Disorder: Understanding the Role of Ubiquitous Pollutant Di(2-ethylhexyl) Phthalate. *Metabolites*, 13 (3), 458. <https://doi.org/10.3390/metabo13030458>
 12. Maltsev, D., Stefanyszyn, V. (2021). Efficacy of combined immunotherapy with propes and inflamafer-tin in selective deficiency of nk and nkt cells in children with autism spectrum disorders associated with genetic deficiency of the folate cycle. *Current Pediatric Research*, 25 (4), 536-540.
 13. Artym, J., Zimecki, M. (2023). Colostrum and Lactoferrin Protect against Side Effects of Therapy with Antibiotics, Anti-inflammatory Drugs and Steroids, and Psychophysical Stress: A Comprehensive Review. *Biomedicines*, 11 (4), 1015. <https://doi.org/10.3390/biomedicines11041015>
 14. Artym, J., Zimecki, M. (2023). Colostrum Proteins in Protection against Therapy-Induced Injuries in Cancer Chemo- and Radiotherapy: A Comprehensive Review. *Biomedicines*, 11 (1), 114. <https://doi.org/10.3390/biomedicines11010114>
-

15. Bagwe-Parab, S., Yadav, P., Kaur, G., Tuli, H. S., Buttar, H. S. (2020). Therapeutic Applications of Human and Bovine Colostrum in the Treatment of Gastrointestinal Diseases and Distinctive Cancer Types: The Current Evidence. *Frontiers in Pharmacology*, 11. <https://doi.org/10.3389/fphar.2020.01100>
16. Urbakh, V. lu. (1975). *Statisticheskii analiz v biologicheskikh i meditsinskikh issledovaniyakh*. Moscow: Meditsina, 295.
17. 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>
18. Wu, J., You, Q., Lyu, R., Qian, Y., Tao, H., Zhang, F. et al. (2023). Folate metabolism negatively regulates OAS-mediated antiviral innate immunity via ADAR3/endogenous dsRNA pathway. *Metabolism*, 143, 155526. <https://doi.org/10.1016/j.metabol.2023.155526>
19. Liu, X., Lin, J., Zhang, H., Khan, N. U., Zhang, J., Tang, X. et al. (2022). Oxidative Stress in Autism Spectrum Disorder – Current Progress of Mechanisms and Biomarkers. *Frontiers in Psychiatry*, 13. <https://doi.org/10.3389/fpsy.2022.813304>
20. Indika, N.-L. R., Frye, R. E., Rossignol, D. A., Owens, S. C., Senarathne, U. D., Grabrucker, A. M. et al. (2023). The Rationale for Vitamin, Mineral, and Cofactor Treatment in the Precision Medical Care of Autism Spectrum Disorder. *Journal of Personalized Medicine*, 13 (2), 252. <https://doi.org/10.3390/jpm13020252>
21. Lyons, K. E., Ryan, C. A., Dempsey, E. M., Ross, R. P., Stanton, C. (2020). Breast Milk, a Source of Beneficial Microbes and Associated Benefits for Infant Health. *Nutrients*, 12 (4), 1039. <https://doi.org/10.3390/nu12041039>
22. Nicolson, G. L., Gan, R., Nicolson, N. L., Haier, J. (2007). Evidence for *Mycoplasma ssp.*, *Chlamydia pneumoniae*, 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>
23. 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>
24. Li, H., Liu, H., Chen, X., Zhang, J., Tong, G., Sun, Y. (2020). Association of food hypersensitivity in children with the risk of autism spectrum disorder: a meta-analysis. *European Journal of Pediatrics*, 180 (4), 999–1008. <https://doi.org/10.1007/s00431-020-03826-x>
25. Saghazadeh, A., Ataenia, B., Keynejad, K., Abdolalazadeh, 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>
26. Edmiston, E., Ashwood, P., Van de Water, J. (2017). Autoimmunity, Autoantibodies, and Autism Spectrum Disorder. *Biological Psychiatry*, 81 (5), 383–390. <https://doi.org/10.1016/j.biopsych.2016.08.031>
27. Sacerdote, P., Mussano, F., Franchi, S., Panerai, A. E., Bussolati, G., Carossa, S. et al. (2013). Biological components in a standardized derivative of bovine colostrum. *Journal of Dairy Science*, 96 (3), 1745–1754. <https://doi.org/10.3168/jds.2012-5928>

28. Zhang, L., Boeren, S., Hageman, J. A., van Hooijdonk, T., Vervoort, J., Hettinga, K. (2015). Bovine Milk Proteome in the First 9 Days: Protein Interactions in Maturation of the Immune and Digestive System of the Newborn. *PLoS ONE*, 10 (2), e0116710. <https://doi.org/10.1371/journal.pone.0116710>
29. Huppertz, H., Rutkowski, S., Busch, D. H., Eisebit, R., Lissner, R., Karch, H. (1999). Bovine Colostrum Ameliorates Diarrhea in Infection with Diarrheagenic *Escherichia coli*, Shiga Toxin-Producing *E. coli*, and *E. coli* Expressing Intimin and Hemolysin. *Journal of Pediatric Gastroenterology and Nutrition*, 29 (4), 452–456. Portico. <https://doi.org/10.1002/j.1536-4801.1999.tb02450.x>
30. Patiroglu, T., Kondolot, M. (2011). The effect of bovine colostrum on viral upper respiratory tract infections in children with immunoglobulin A deficiency. *The Clinical Respiratory Journal*, 7 (1), 21–26. <https://doi.org/10.1111/j.1752-699x.2011.00268.x>
31. Lee, J., Kim, H.-S., Jung, Y. H., Choi, K. Y., Shin, S. H., Kim, E.-K. et al. (2015). Oropharyngeal Colostrum Administration in Extremely Premature Infants: An RCT. *Pediatrics*, 135 (2), e357–e366. <https://doi.org/10.1542/peds.2014-2004>
32. Byakwaga, H., Kelly, M., Purcell, D. F. J., French, M. A., Amin, J., Lewin, S. R. et al. (2011). Intensification of Antiretroviral Therapy With Raltegravir or Addition of Hyperimmune Bovine Colostrum in HIV-Infected Patients With Suboptimal CD4 + T-Cell Response: A Randomized Controlled Trial. *The Journal of Infectious Diseases*, 204 (10), 1532–1540. <https://doi.org/10.1093/infdis/jir559>
33. Maltsev, D., Stefanyshyn, V. (2022). The efficacy of combined immunotherapy with Propes and Inflamafertin in adult patients with genetic deficiency of the folate cycle and selective deficiency of NK and NKT cells. *Immunology*, 167 (3), 443–450. <https://doi.org/10.1111/imm.13548>
34. Ko, B., Takebe, N., Andrews, O., Makena, M. R., Chen, A. P. (2023). Rethinking Oncologic Treatment Strategies with Interleukin-2. *Cells*, 12 (9), 1316. <https://doi.org/10.3390/cells12091316>

EFFICACY OF INFLIXIMAB IN AUTISM SPECTRUM DISORDERS IN CHILDREN ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

JUSTIFICATION

The notion of systemic inflammation in autism spectrum disorders in children has been established. A recent meta-analysis of randomized controlled trials published in 2019, which included a systematic review of 25 case-control studies, suggests an association between genetic deficiency of the folate cycle and autism spectrum disorders in children [18]. This evidence is consistent with an earlier meta-analysis of randomized controlled trials from 2013, which included data from 8 studies [17].

The encephalopathy that develops in children with genetic deficiency of the folate cycle and manifests as autism spectrum disorders is associated with oxidative stress. The reason for the latter can be seen in the suppression of the immune system with the development of a special form of immunodeficiency, which is based on the deficiency of natural killers, natural killer T lymphocytes and CD8 + cytotoxic T cells [11]. Immunodeficiency mediates all three known mechanisms of brain damage in children with genetic deficiency of the folate cycle, namely the development of opportunistic infections [2, 15], autoimmune reactions against neuronal antigens [3, 6] and manifestations of systemic inflammation, which is based on the phenomenon of hypercytokinemia [13, 20].

Children with autism spectrum disorders have been shown to have overproduction of several proinflammatory cytokines, including tumor necrosis factor alpha (TNF-alpha), interleukin-1beta, and interleukin-6. A recent systematic review and meta-analysis of controlled clinical trials showed increased serum concentrations of the proinflammatory mediators interleukin-1beta ($P < 0.001$), interleukin-6 ($P = 0.03$), interleukin-8 ($P = 0.04$), interferon-gamma ($P = 0.02$), eotaxin ($P = 0.01$), monocyte chemotactic factor 1 ($P < 0.05$), and decreased levels of the anti-inflammatory cytokine transforming growth factor beta 1 ($P < 0.001$) in children with autism spectrum disorders ($n = 743$) compared with healthy controls ($n = 592$) [13].

The results of the latest meta-analysis of randomized controlled clinical trials prepared by Saghazadeh A. et al., which includes 38 studies involving 2487 children, show a significant increase in serum concentrations of TNF-alpha, gamma-interferon, interleukin 1 beta and interleukin 6 in children with autism spectrum disorders compared with healthy individuals [19].

Each of the mechanisms of damage, as well as the causal immunodeficiency, can be the subject of therapeutic interventions. The neurotropic effects of proinflammatory cytokines are well known [19, 20], and persistent hypercytokinemia can lead to impaired cerebral metabolism, neurogenesis, bioelectric activity and disorganization of mental activity in children with genetic deficiency of the folate cycle.

This study aims to investigate the clinical effects of anti-inflammatory therapy aimed at eliminating abnormal hypercytokinemia. It is necessary to determine whether anti-inflammatory therapy will have the desired neuroprotective effect, contributing to the progress of mental development in children with autism spectrum disorders. As is known, TNF-alpha is a master cytokine, the production of which depends on the implementation of the entire pro-inflammatory cytokine cascade in the human body.

Targeted inhibition of TNF-alpha using specific monoclonal antibody preparations is a therapeutic strategy that has proven itself in a number of severe immunoinflammatory diseases, including rheumatoid arthritis, Crohn's disease, autoimmune spondyloarthropathies and psoriatic arthritis [14]. This strategy may also be useful for suppressing persistent systemic inflammation in children with autism spectrum disorders associated with genetic deficiency of the folate cycle.

The aim of the research: to study the clinical efficacy of infliximab in children with autism spectrum disorders associated with genetic deficiency of the folate cycle, in whom elevated serum TNF-alpha concentrations were noted.

Materials and methods. This prospective controlled single-center non-randomized clinical study included 225 children diagnosed with autism spectrum disorders associated with genetic deficiency of the folate cycle. The diagnosis of autism spectrum disorders was made by psychiatrists from regional hospitals or specialized departments according to DSM-IV-TR (Diagnostic and Statistical Manual of mental disorders) and ICD-10 criteria. Children were recruited into the study group (SG) in 2019-2020. These were patients from different regions of Ukraine aged 2 to 9 years, in whom elevated serum TNF-alpha concentrations were observed. As is known, the phenotype of genetic deficiency of the folate cycle includes 5 main syndromes: autism spectrum disorders, intestinal syndrome (persistent enteritis/colitis) [7], PANDAS [4, 9], epileptic syndrome [5] and signs of pyramidal tract damage.

SG children were administered intravenously drip infliximab (a monoclonal antibody preparation to TNF-alpha) at a dose of 3 mg/kg twice a month for 1-3 consecutive months to achieve an anti-inflammatory effect by targeted neutralization of the indicated pro-inflammatory cytokine, according to the cytokine profile.

The control group (CG) consisted of 51 similar children with a similar age and gender distribution, with a corresponding increase in serum TNF-alpha concentration. These patients did not receive infliximab therapy, but underwent only conventional rehabilitation measures, which included work with a speech therapist/specialist, specially trained teachers and psychiatrists. The dynamics of mental symptoms of autism spectrum disorders during the study was assessed using the Aberrant Behavior Checklist (ABC) scale [1].

Folate cycle gene polymorphisms were detected by polymerase chain reaction (PCR) with restriction analysis in three centers: Neurological Research Institute (USA), Kharkiv Specialized Medical Genetic Center and a commercial laboratory in Kyiv. The nucleotide substitutions MTHFR 677 C > T, MTHFR + 1298 A > C, MTRR 66 A > G, and MTR 2756 A > G were detected in various combinations in the homozygous and heterozygous states.

The concentration of the cytokine TNF-alpha in the blood serum was determined by solid-phase enzyme-linked immunosorbent assay (reagents «Vector-Best», RF; norm - 0-8 ng/ml). The average content of this cytokine in the blood serum in SG was 13.4 ± 0.21 ng/ml, and in CG - 12.7 ± 0.24 ng/ml.

All study participants underwent a serial comprehensive immunological examination, which, in addition to a general blood test, included the study of the subpopulation composition of lymphocytes using laser flow cytometry (cytofluorimetry Epics XI, 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 according to the latex test with the determination of the phagocytosis index, phagocytic index, number of active phagocytes and phagocytic capacity of the blood, as well as the activity of the enzymes myeloperoxidase (cytofluorimetry) and NADP-oxidase (NST test). Serum concentrations of immunoglobulins of the main classes (M, G, A) were determined by the results of simple radial immunodiffusion according to Mancini. The concentration of IgE, IgD and IgG subclasses (IgG1, IgG2, IgG3, IgG4) in serum was measured using solid-phase enzyme immunoassay (VectorBEST reagents, RF). SG patients had immunodeficiency associated with genetic deficiency of the folate cycle, which was considered to be the cause of the increased content of TNF-alpha in serum.

In addition, the diagnosis of reactivated viral infection was performed based on the results of quantitative PCR of blood serum with species-specific primers for herpesviruses (herpes simplex viruses 1 and 2 types (HSV-1 and HSV-2), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpes viruses 6, 7 and 8 types (HHV-6, HHV-7, HHV-8)), TTV, measles and KSDS viruses (DNA-Technology reagents, RF). Serological tests were also performed by performing solid-phase enzyme-linked immunosorbent assay to identify specific IgM and IgG in blood serum to *Candida albicans*, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* (Vector-BEST reagents, RF). Western blot was used to identify borreliosis infection.

Intracellular neurotropic pathogens predominated in SG children: viruses with opportunistic properties, especially human lymphotropic herpesviruses (EBV, CMV, HHV-6, HHV-7), which is consistent with the typical deficiency of NK and NKT cells in such patients. The second most frequently detected was reactivated TTV infection, the neurotropic nature of which was recently reported [8]. The third most frequently identified was beta-hemolytic streptococcus group A, involved in the induction of autoimmune complications, including PANDAS. *Candida*, *Borrelia*, *Mycoplasma*, and *Chlamydia* were less frequently detected. The high microbial load was consistent with the data on the presence of immunodeficiency in children with a genetic disorder of the folate cycle.

Serum concentrations of known biomarkers of genetic deficiency of the folate cycle – homocysteine, folic acid, vitamins B12 and B6, creatinine, uric acid – were also assessed using available biochemical methods.

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 and after participation in the study. Typical were signs of leukoencephalopathy of varying severity with a predominant violation of white matter myelination in the parietal lobes of the cerebral hemispheres (**Fig. 11.4**). Also in 57 % of cases there was an additional picture of temporal median sclerosis with the phenomenon of hyperintensity from the hippocampi and insula. Mostly such children suffered from epileptic syndrome of the temporal median epilepsy type or had pathological epileptiform activity on EEG. In 19 % of cases, typical signs of congenital cytomegalovirus neuroinfection were noted in the form of ventriculomegaly, periventricular foci, cysts in the poles of the temporal lobes, hypogenesis of the corpus callosum and zones of delayed myelination of white matter in the parietal lobes of the cerebral hemispheres. These data correspond to the results of an 18-year retrospective study by Pinillos-Pisón R. et al. [16]. Such children usually had symptoms of damage to the pyramidal tracts, and therefore were often diagnosed with cerebral palsy, although autistic mental disorders and other symptoms were also observed.

The criteria for inclusion of the patient in the study were the presence of 2–4 polymorphisms of folate cycle genes, NK and/or NKT cell deficiency, reactivated infection caused by lymphotropic herpesviruses and/or measles virus, signs of leukoencephalopathy/temporal median sclerosis on brain MRI, clinical symptoms of autism spectrum disorders. The criteria for exclusion of the patient from the study were: refusal of the child's parents to participate in the study, the presence of additional 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 make it impossible to continue the approved treatment. The study endpoints were the main clinical manifestations of autism spectrum disorders, serum TNF-alpha concentration, and the dynamics of the main additional clinical manifestations associated with genetic deficiency of the folate cycle, including PANDAS, persistent enteropathy/colitis, temporal median epilepsy, and symptoms of pyramidal tract damage.

Statistical analysis of the obtained information was processed using the Microsoft Excel electronic program using structural and comparative analysis methods. In order to establish the reliability of the differences in the results, the Student's T-test was used with the calculation of the confidence probability coefficient p (parametric criterion) and the number of signs Z according to Urbach (non-parametric criterion). Pearson's chi-square (χ^2) was calculated with the Fisher's exact test and the determination of the Yates correction to study the relationship between the appointment of immunotherapy and the studied indicators' dynamics. The χ^2 criterion, the Pearson correlation coefficient (C) and its normalized value were also calculated to determine the strength of the detected relationships.

This study is part of a grant from the Ministry of Health of Ukraine for the research work "Improvement of diagnostics of human immunodeficiency diseases based on surface plasmon resonance registration and development of modern treatment methods" (state registration number 0113U000709).

Research results and their discussion. This chapter discusses the clinical effects of anti-inflammatory therapy aimed at eliminating abnormal hypercytokinemia. It is necessary to establish whether anti-inflammatory therapy will have the desired neuroprotective effect, contributing to the progress of mental development in children with ASD. As is known, TNF-alpha is a master cytokine, the production of which depends on the implementation of the entire pro-inflammatory cytokine cascade in the human body. Targeted inhibition of TNF-alpha using specific monoclonal antibody preparations is a therapeutic strategy that has proven itself in a number of severe immunoinflammatory diseases, including rheumatoid arthritis, Crohn's disease, autoimmune spondyloarthropathies and psoriatic arthritis [14]. This strategy may also be useful for suppressing persistent systemic inflammation in children with ASD associated with GDFC.

According to the results obtained, infliximab was effective in reducing the clinical symptoms of ASD disorders in 69 of 92 SG children who had elevated serum TNF-alpha concentrations at the beginning of the study (76 % of cases), but the severity of the achieved clinical effect varied in different patients and in relation to different indicators of mental activity (**Table 11.1; Fig. 11.5**). In the CG, during the same observation period, improvement in mental status was noted in only 17 of 47 patients (36 % of cases), which is half as much as in the SG ($\chi^2 < 0,05$; $Z_{0,05}$). These significant differences indicate a certain positive modifying effect of infliximab on the state of the psyche in children with ASD associated with GDFC. Adding infliximab to standard in clinical practice specialized educational programs provides tangible additional benefits,

primarily in stabilizing the emotional state of the child, normalizing everyday activity and increasing the resistance of the psychics to stress factors.

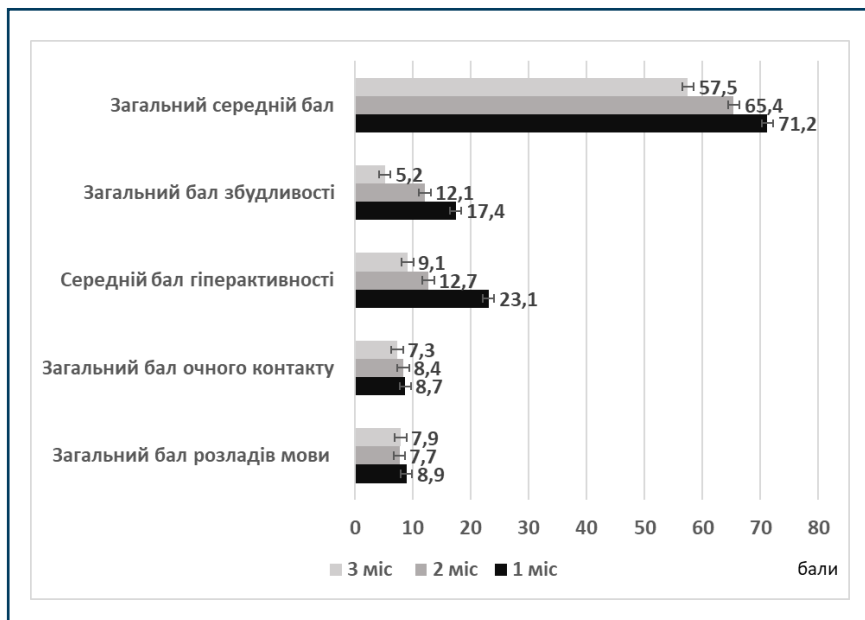
In SG, there was a pronounced positive dynamics in the direction of hyperactivity, hyperexcitability and stereotyped behavior, but no significant effect was noted on the stability of eye contact and the development of expressive-receptive language, while in CG some positive changes were achieved specifically in terms of expressive language and the level of eye contact, which indicates different points of action of infliximab and specialized educational programs (**Table 11.1**). The psychotropic effect obtained with infliximab differs from that of intravenous immunoglobulin, which has also demonstrated clinical efficacy in ASD associated with GDFC [10, 12]. The changes induced by infliximab are more pronounced and develop in a shorter time frame, but they are significantly narrower in terms of the spectrum of positive psychotropic effects compared to high-dose immunoglobulin therapy, which has a total modifying effect on the psyche of such children.

● **Table 11.1.** ABC scale scores in SG (n = 225) and CG patients (n = 51)

Subscales	SG	CG
ABC		
Irritability	5.2 ± 0.6 *	11.5 ± 0.9
Hyperactivity	9.1 ± 0.9 *	19.7 ± 1.4
Inadequate eye contact	7.3 ± 1.8	9.5 ± 1.3
Inappropriate speech	7.9 ± 0.8	8.5 ± 1.4
Symptom checklist		
Drowsiness	8.4 ± 0.8	12.5 ± 1.7
Decreased activity	4.8 ± 0.6	4.9 ± 0.9

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

The average total ASD score in SG children according to the ABC scale decreased slightly, which indicates a moderate positive overall effect of infliximab on the state of the respondents' psyche. However, the achieved positive effect as a result of the immunotherapy was uneven and concerned only individual indicators of the ABC scale. Significant and rapid positive dynamics were achieved in the level of hyperactivity and hyperexcitability. Thus, the average hyperactivity score for a three-month course of immunotherapy decreased by 2.5 times, and the total excitability score – almost three times. At the same time, there was a small and statistically insignificant dynamics in the indicators of language development and the level of eye contact. These data allow us to assume that infliximab acts on the psyche of children with autism spectrum disorders selectively, having mainly a psychostabilizing effect, calming the child by reducing the manifestations of hyperactivity and hyperexcitability. This effect is quite pronounced and develops in a short period of time (**Fig. 11.1**).



○ **Fig. 11.1.** Dynamics of the main indicators of the ABC scale in SG children (n = 225) during a 3-month course of infliximab immunotherapy

If we talk about the structure of psychotropic effects when using infliximab in SG children, then almost complete elimination of manifestations of hyperactivity and hyperexcitability was observed in 21 out of 69 responders to immunotherapy (31 % of cases) among SG patients (**Fig. 11.2**). Such children, in terms of daily motor activity and mental excitability, approached similar levels in healthy children at the end of the immunotherapy course. Thus, every third child among the responders to treatment and every fourth among all SG participants was a complete responder to infliximab immunotherapy in terms of daily motor activity and mental excitability. In another 28 SG children (41 % of cases among responders), the levels of hyperactivity and hyperexcitability during the period of infliximab immunotherapy decreased by at least half compared to the initial ones, which indicates a partial positive response to the treatment. In the remaining 19 patients from the subgroup of responders to immunotherapy among SG participants (28 % of cases), there was a less pronounced decrease in clinical manifestations of hyperactivity and hyperexcitability according to the ABC scale, which allows us to consider them as weak responders to treatment. As noted above, 9 of 38 SG children (24 %) did not respond to infliximab immunotherapy with changes in mental activity and were considered non-responders to the treatment. Their daily motor activity and mental arousal remained at the same level despite immunotherapeutic interventions according to the study protocol.

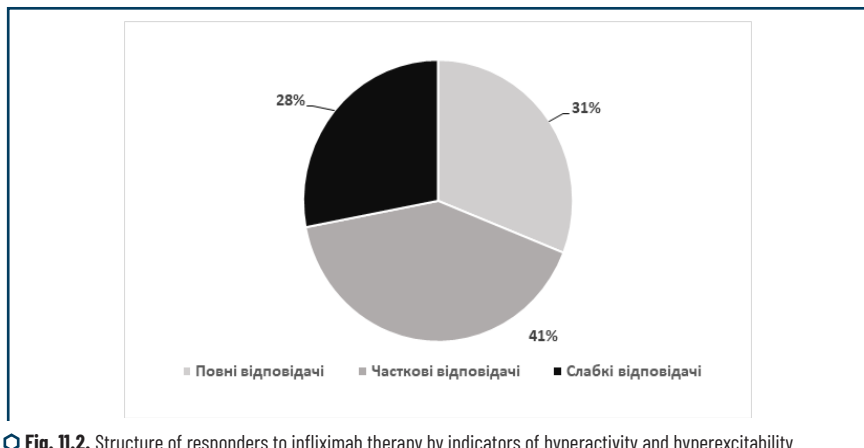


Fig. 11.2. Structure of responders to infliximab therapy by indicators of hyperactivity and hyperexcitability according to the ABC scale among SG patients (n = 225)

The study of the dynamics of serum TNF-alpha concentration in SG and CG children showed normalization of the amount of this pro-inflammatory cytokine in the blood serum of SG children and a significant decrease in this indicator compared to CG, where the average serum TNF-alpha concentration remained elevated at the end of the observation period, as at the beginning of the study (**Fig. 11.3**). These differences can be explained by the direct therapeutic effect of infliximab, whose monoclonal antibodies directly neutralize TNF-alpha molecules by specific recognition and binding [14]. If we talk about the structure of the anti-inflammatory effect of infliximab among SG patients, then the normalization of serum TNF-alpha concentration occurred in the majority of respondents (in 61 of 92 patients; 66 % of cases), and a decrease in this concentration compared to the initial level was observed in almost all children who received infliximab (in 90 of 92 people). At the same time, in the CG, normalization of serum TNF-alpha concentration occurred in only 7 of 47 patients (14 % of cases), and a decrease in the concentration was noted in another 11 respondents (23 % of cases), which was a striking contrast with the SG data ($p < 0,05$; $Z_{0,05}$).

These data suggest that the achieved positive psychotropic effects in SG on hyperactivity and hyperexcitability were associated with the ability of infliximab to targetably reduce serum TNF-alpha concentration, i.e. to have a systemic anti-inflammatory effect. To verify this conclusion, we additionally studied the association between changes in serum TNF-alpha concentration and the dynamics of mental disorders in SG children during the observation period. To study the association between changes in serum TNF-alpha concentration and hyperactivity and hyperexcitability indicators on the ABC scale, we calculated the Pearson chi-square (χ^2) index. We proceeded from the data that there were 69 clinical responders to immunotherapy out of 92 SG patients, but only 61 SG participants had normalization of serum TNF-alpha concentration on the background of infliximab administration. All patients with normalized serum TNF-alpha levels, except for one child, demonstrated a clinical response to immunotherapy according to the ABC scale. The calculation results of conjugation indices are shown in **Tab. 11.2** and **11.3**.

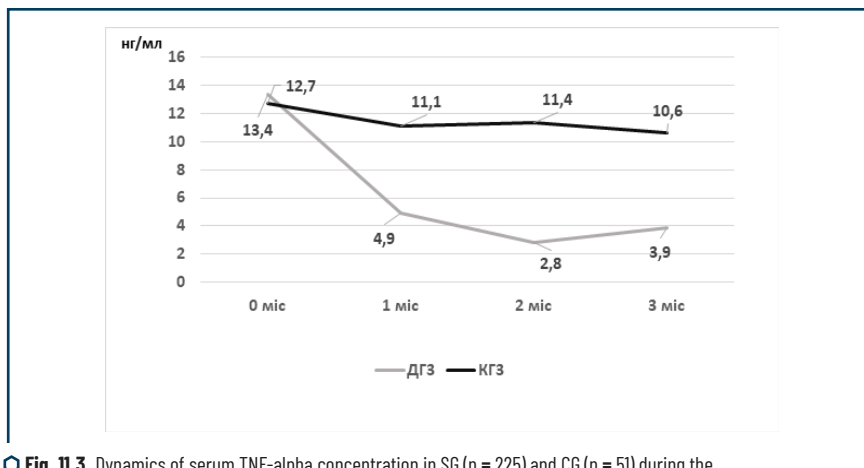


Fig. 11.3. Dynamics of serum TNF-alpha concentration in SG (n = 225) and CG (n = 51) during the observation period

Table 11.2. Results of calculating the Pearson chi-square (χ^2) test in SG (n = 225) when studying the association between serum TNF-alpha concentration and clinical outcome according to the ABC scale

	SG	CG	χ^2	χ^2 at $p = 0,05$	χ^2 at $p = 0,01$	significance	Yates' correction	significance	Fisher's exact test	significance
TNF-alpha/hyperactivity-hyperexcitability	38	22	18,768	3,841	6,635	$p < 0,001$	15,555	$< 0,001$	0,00004	$p < 0,05$

Table 11.3. Calculation of the strength of the relationship between serum TNF-alpha concentration and hyperactivity and hyperexcitability indicators according to the ABC scale in SG children (n = 225)

Criterion name	TNF-alpha / hyperactivity-hyperexcitability	
	value of the criterion	connection strength
criterion φ	0,703	strong
Pearson correlation coefficient (C)	0,575	relatively strong
Normalized value of Pearson coefficient (C)	0,813	very strong

The obtained data prove the existence of a connection between the normalization of serum TNF-alpha concentration and a decrease in the severity of hyperexcitability and hyperactivity in children with ASD SG. This gives grounds to assert that it is the suppression of TNF-alpha and the achievement of a systemic anti-inflammatory

effect in this connection that is the reason for the achieved positive dynamics of mental status indicators in SG children. As is known, TNF-alpha, like other pro-inflammatory cytokines, has certain neurotropic properties, affecting the human psyche [13, 19, 20]. An increase in serum TNF-alpha concentration in children with autism spectrum disorders changes their mental state, potentiating the manifestations of hyperactivity and hyperexcitability, and a targeted effect on TNF-alpha molecules using a drug of specific monoclonal antibodies, leveling the effects of TNF-alpha, contributes to improving the levels of everyday motor activity and mental excitability in children with ASD.

In addition to autism, the effect of infliximab immunotherapy on other manifestations of GDFC, which are also based on an immunoinflammatory reaction that can be attenuated or blocked by a tested immunobiological agent, was evaluated. A decrease in clinical manifestations of such syndromes as intestinal dysfunction, PANS/PITANDS/PANDAS and epileptiform activity of the cerebral cortex was noted, but no positive dynamics were recorded in the movement disorders caused by damage to the pyramidal tracts of the CNS.

Thus, the manifestations of PANS/PITANDS/PANDAS in SG, namely tic hyperkinesia and obsessive-compulsive syndrome, decreased in 77 % of cases among SG patients diagnosed with streptococcal-induced autoimmunity with CNS involvement. There were significant differences from the CG, in which a decrease in PANS/PITANDS/PANDAS symptoms was observed in only 14 % of cases ($p < 0,05$; $Z < Z_{0,05}$). An increase in the number of days with normal bowel movements by at least one third of the initial level in SG children, indicating partial compensation of the intestinal syndrome, occurred in 82 % of cases among patients who had signs of intestinal dysfunction in the form of persistent diarrhea and/or constipation. The differences with the data in the CG were also significant, since similar positive dynamics in intestinal symptoms among these children was observed only in 23 % of cases ($p < 0,05$; $Z < Z_{0,05}$). Reduction or disappearance of epileptiform bioelectric activity of the cerebral cortex according to EEG data in SG was registered in 81 % of cases among patients who had epileptic seizures or, at least, pathological epileptiform activity of the cerebral cortex during EEG. In CG children, positive dynamics of the epileptic syndrome was found only in 27 % of cases, which indicates significant differences with SG data both when calculating the parametric and nonparametric criteria ($p < 0,05$; $Z < Z_{0,05}$) (Table 11.4). However, no significant differences were found in the observation groups regarding the dynamics of clinical manifestations of damage to the pyramidal nerve pathways of the CNS ($p > 0,05$; $Z > Z_{0,05}$).

● **Table 11.4.** Differences in study endpoints between SG (n = 225) and CG patients (n = 51)

End point	SG, %		CG, %		t-test	Number of characters Z
	+	-	+	-		
ASD	76	24	36	64	$p < 0,05$ *	$Z < Z_{0,05}$ *
PANDAS	77	23	14	86	$p < 0,05$ *	$Z < Z_{0,05}$ *
Epileptic syndrome	81	19	27	73	$p < 0,05$ *	$Z < Z_{0,05}$ *
Intestinal syndrome	82	18	23	77	$p < 0,05$ *	$Z < Z_{0,05}$ *
Pyramid disorders	24	76	26	74	$p > 0,05$	$Z > Z_{0,05}$

* - reliable differences

Thus, infliximab helps to reduce not only some manifestations of ASD, but also has a broader, systemic effect, allowing to reduce the manifestations of the main clinical syndromes of the broad GDFC phenotype. Educational programs and corrective exercises, which are still the basis of therapy for children with ASD, do not allow to achieve the indicated positive changes in the work of the brain, intestines and immune system, as evidenced by significant differences with the results in CG.

Conclusions. Infliximab leads to significant improvements in hyperactivity and hyperexcitability, as well as stereotypic behavior in children with autism spectrum disorders associated with genetic deficiency of the folate cycle. Responders to immunotherapy are 76 % of patients with this pathology, which is twice as high as with standard therapy. However, there is no effect of infliximab on such manifestations of autism as the level of eye contact and language development. Psychotropic effects of infliximab immunotherapy are closely related to the normalization of previously elevated serum TNF-alpha concentrations and are probably due to the elimination of the pathological activating effect of this pro-inflammatory cytokine on CNS neurons. In parallel, there is an improvement in other clinical syndromes of genetic deficiency of the folate cycle in children with autism spectrum disorders - intestinal pathology, epileptic syndrome and PANDAS, in the pathogenesis of which, as is known, TNF-alpha and the systemic and intracerebral inflammation induced by this cytokine are involved. However, under the influence of immunotherapy, there is no change in the dynamics of motor deficit in children with symptoms of pyramidal tract damage. Further clinical studies in this direction with a larger number of participants and randomization are necessary to obtain more convincing data.

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. Binstock, T. (2001). Intra-monocyte pathogens delineate autism subgroups. *Medical Hypotheses*, 56 (4), 523-531. <https://doi.org/10.1054/mehy.2000.1247>
3. 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>
4. Esposito, S., Bianchini, S., Baggi, E., Fattizzo, M., Rigante, D. (2014). Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections: an overview. *European Journal of Clinical Microbiology & Infectious Diseases*, 33 (12), 2105-2109. <https://doi.org/10.1007/s10096-014-2185-9>
5. 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>
6. Frye, R. E., Sequeira, J. M., Quadros, E. V., James, S. J., Rossignol, D. A. (2012). Cerebral folate receptor autoantibodies in autism spectrum disorder. *Molecular Psychiatry*, 18 (3), 369-381. <https://doi.org/10.1038/mp.2011.175>
7. Furlano, R. I., Anthony, A., Day, R., Brown, A., McGarvey, L., Thomson, M. A. et al. (2001). Colonic CD8 and $\gamma\delta$ T-cell infiltration with epithelial damage in children with autism. *The Journal of Pediatrics*, 138 (3), 366-372. <https://doi.org/10.1067/mpd.2001.111323>

8. Ikuta, Y., Oba, K., Nai, E., Katori, T., Hashino, M., Inamine, Y. et al. (2019). Aseptic meningitis caused by torque teno virus in an infant: a case report. *Journal of Medical Case Reports*, 13 (1). <https://doi.org/10.1186/s13256-019-2233-2>
9. 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>
10. 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 pharmaceutical technology*. – 2019. – Vol. 11(05). – P. 597-609.
11. 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>
12. Maltsev D.V. High-dose intravenous immunoglobulin therapy efficiency in children with autism spectrum disorders associated with genetic deficiency of folate cycle enzymes // *Psychiatry, Psychotherapy and Clinical Psychology*. – 2016. – Vol. 2. – P. 63-80.
13. 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>
14. Melsheimer, R., Geldhof, A., Apaolaza, I., Schaible, T. (2019). Remicade®(infliximab): 20 years of contributions to science and medicine. *Biologics: Targets and Therapy*, 13, 139-178. <https://doi.org/10.2147/btt.s207246>
15. Nicolson, G. L., Gan, R., Nicolson, N. L., Haier, J. (2007). Evidence for *Mycoplasma ssp.*, *Chlamydia pneumoniae*, 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>
16. 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>
17. 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>
18. 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>
19. Saghazadeh, A., Ataenia, B., Keynejad, K., Abdolalazadeh, 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>
20. Smith, S. E. P., Li, J., Garbett, K., Mirnics, K., Patterson, P. H. (2007). Maternal Immune Activation Alters Fetal Brain Development through Interleukin-6. *The Journal of Neuroscience*, 27 (40), 10695-10702. <https://doi.org/10.1523/jneurosci.2178-07.2007>

EFFICACY OF RITUXIMAB IN AUTISM SPECTRUM DISORDERS ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE WITH SIGNS OF ANTINEURONAL AUTOIMMUNITY

JUSTIFICATION

Advances in genetics, molecular biology, and immunology over the past decades have significantly changed our understanding of the etiology and pathogenesis of autism spectrum disorders (ASD) in children. One of the key advances in this direction is the elucidation of the association of genetic deficiency of the folate cycle (GDFC) with ASD, evidence for which is based on the results of at least 5 meta-analyses of randomized controlled clinical trials and a number of additional controlled trials, the data of which have not yet been properly summarized [14, 21, 27, 28, 29]. It has been established that GDFC leads to pathological biochemical changes in the child's body, which determine the development of encephalopathy with the clinical picture of ASD due to direct (metabolic) and indirect (immune-mediated) mechanisms, and immune-dependent pathways of cerebral damage are currently given a leading role in the pathogenesis of this mental disorder. Among the metabolic disorders induced by GDFC in the child's body, hyperhomocysteinemia, vitamin deficiencies, signs of mitochondrial dysfunction, and impaired nucleotide synthesis and DNA, protein, and lipid methylation processes are distinguished [12, 31, 32]. These pathological biochemical changes lead to the development of persistent oxidative stress, as evidenced by the results of two systematic reviews and meta-analyses of randomized controlled clinical trials on this problem [6, 9]. The result of such disorders are the phenomena of neuro- and immunotoxicity, which underlie the above-mentioned direct and indirect mechanisms of neuronal damage in children with ASD. If we talk about immunotoxicity, it is currently established that in GDFC there is a disturbed development of the child's immune system with the formation of immune dysfunction and dysregulation, which, in turn, cause a phenomenon called a disturbed neuroimmune interface [19, 25]. It is believed that there are at least three main immune-mediated mechanisms of brain damage in GDFC, which can radically affect the development of associated encephalopathy with ASD symptoms. Neurotropic opportunistic and conditionally pathogenic infections [23], autoimmune reactions to neurons, myelin, glial cells of the cerebral hemispheres and cerebral vessels [5, 10], systemic and associated intracerebral persistent aseptic inflammation mediated by existing immune dysregulation [18, 30], constitute the indicated triad of key pathogenetic mechanisms of the development of ASD-forming encephalopathy in GDFC. Suppression or even eradication of these immune-dependent GDFC-induced pathways of CNS damage currently appears to be a promising prospect for effective treatment of ASD in children with GDFC. In particular, it is believed that the suppression of autoimmunity and 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 [17]. At least 10 clinical studies have been conducted to test the immunomodulatory agent intravenous normal human immunoglobulin in ASD, which is believed to improve mental functions

of patients by suppressing intracerebral inflammation and autoimmune reactions against brain autoantigens [1, 3, 4, 7, 8, 12, 13, 16, 20, 24, 26]. Recently, infliximab, a monoclonal antibody against the tumor necrosis factor alpha molecule, has demonstrated efficacy in suppressing hyperactivity and hyperexcitability in children with ASD associated with GDFC in a controlled clinical trial [2].

The prospect of developing new, more effective and safe methods of treating immune-mediated encephalopathy in children with ASD is an important task of modern neuroimmunology. Given that autoimmune reactions to CNS autoantigens in ASD are believed to be mainly mediated by autoantibodies rather than cellular autoimmune reactions, the monoclonal antibody to the CD20 molecule of B lymphocytes, rituximab, which has already undergone a number of successful trials in autoimmune diseases with a similar mechanism of development, seems promising for use in such children. Theoretically, by inducing B-cell depletion, rituximab can significantly suppress or even eliminate the production of autoantibodies to brain autoantigens in children with ASD, having a neuroprotective effect and thereby improving the mental status of patients. A dedicated clinical trial testing rituximab in children with ASD associated with GDFC and evidence of anti-brain humoral autoimmunity is needed.

The aim of the research: to study the effectiveness of rituximab in children with ASD associated with GDFC, who have serological signs of antineuronal autoimmunity, to expand the current arsenal of neuroprotective therapy for immune-mediated encephalopathy in such cases.

Materials and methods. The medical data of 225 children aged 3 to 9 years with GDFC, who had clinical manifestations of ASD, were analyzed. All of them were patients of the specialized neuroimmunological clinic Vivere (registration dossier dated 12/22/2018 No. 10/2212-M). Data for the study and processing of the material were 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 diagnosis of autism spectrum disorders 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 enzyme genes were determined by restriction PCR based on the detection of the MTHFR C677T nucleotide substitution in monoform (27 patients), as well as - in combination with other nucleotide substitutions - MTHFR A1298C, MTRR A66G and/or MTR A2756G.

The results of serological studies of blood serum were evaluated for the detection of specific antineuronal autoantibodies, which are validated as markers of autoimmune limbic encephalitis in children and adults, namely autoantibodies to glutamic acid decarboxylase (GADA), neuronal potassium channels, amphiphysin, NMDA-receptors of neurons, GABA, CV2, Yo, Ro, Hu, AMPAR 1 and 2. Positive results of such laboratory studies were found in 81 patients.

These results were combined with signs of hyperintensity of the MR signal from the structures of the mesolimbic system of the temporal lobes of the cerebral hemispheres in the T2 and FLAIR modes during MR neuroimaging (Fig. 12.1), as well as with the EEG pattern of temporal median epilepsy during neurofunctional studies (Fig. 12.2). Since these individuals had signs of autoimmune limbic encephalitis, with which the existing clinical neuropsychiatric disorders could be associated, they were offered treatment with rituximab according to the latest systematic review on the problem of therapy of the indicated autoimmune lesions of the CNS [22].

Autoantibodies to amphiphysin (3 people, 5 %), NMDA receptors of neurons (3 people, 5 %) and CV2 molecules (2 people, 3 % of cases) were also rarely found (**Fig. 12.3**). Relatives of the other 19 patients with a similar distribution of antineuronal autoantibodies refused such treatment (control group, CG).

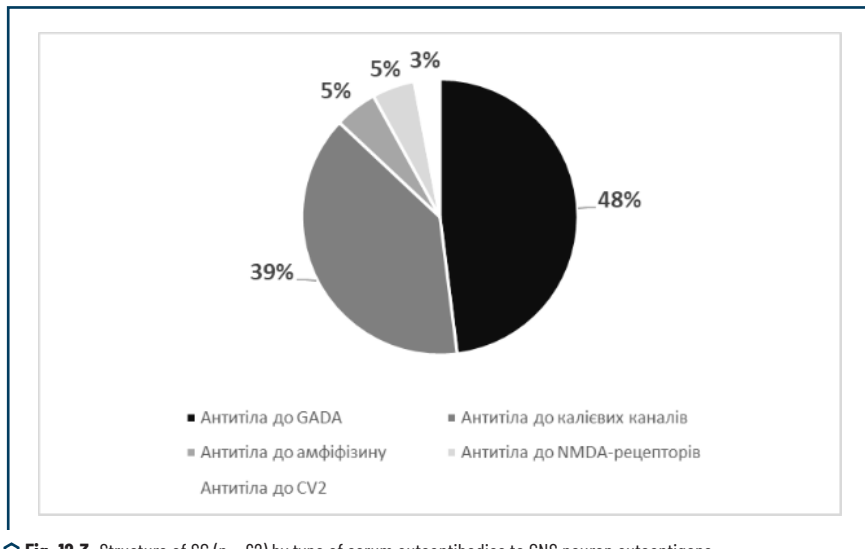


Fig. 12.3. Structure of SG (n = 62) by type of serum autoantibodies to CNS neuron autoantigens

Since serum concentrations of various antineuronal autoantibodies were measured in different units, a special scoring system was used to conduct a generalized data analysis. Exceeding the serum concentration of a particular autoantibody by up to 20 % of the upper limit of reference values was evaluated as 1 point, from 21 to 40 % – 2 points, from 41 to 60 % – 3 points, from 61 to 80 % – 4 points, and more than 81 % – 5 points. Since autoantibodies to GADA and neuronal potassium channels were found in many patients, a separate analysis of the data was performed for these indicators, which could not be carried out for autoantibodies to neuronal NMDA receptors, amphiphysin and CV2 due to the small number of cases of their identification among the examined patients.

Rituximab, a monoclonal antibody preparation to the CD20 molecule of B lymphocytes, was administered intravenously drip at a dose of 375 mg/m² of the child's body surface with a frequency of 1 time per 1 month under the control of the results of determining the serum concentrations of autoantibodies to autoantigens of neurons of the mesolimbic system of the brain until the disappearance of such autoantibodies from the child's blood serum. In total, from 3 to 9 courses of rituximab immunotherapy were performed in SG children.

The dynamics of clinical symptoms of ASD were assessed according to the specialized Aberrant Behavior Checklist (ABS) scale in SG and CG children in order to determine how much the decrease in serum concentrations of antineuronal autoantibodies affects the clinical status of patients.

Statistical processing of the material was carried out by comparative and structural analyses. To determine the probability of differences between the studied indicators in the observation groups, the parametric Student's T-test with the confidence probability indicator p and the non-parametric criterion - the number of signs Z according to Urbach Yu.V. were used. To study the association of the dynamics of serum concentrations of antineuronal autoantibodies and indicators of cerebral damage in children with ASD, the odds ratio (OR) and 95 % confidence interval (95 % CI) were calculated.

Microsoft Excel was used for statistical calculations.

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

Research results and their discussion. Data from the structural analysis of the results of the use of approved rituximab immunotherapy among patients of the observation groups with signs of antineuronal autoimmunity indicate that the normalization of previously elevated serum concentrations of antineuronal autoantibodies in SG children after a 3-month course of rituximab immunotherapy was noted in 37 % of cases, after a 6-month course - in 79 % of cases, and after a 9-month course - in 92 % of cases, while in CG similar indicators corresponded to the levels of 7, 11 and 14 % of cases, which was a significant difference from SG ($p < 0,05$; $Z < Z_{0,05}$) (Fig. 12.4). The average course of rituximab immunotherapy in SG was 4.91 ± 0.65 months.

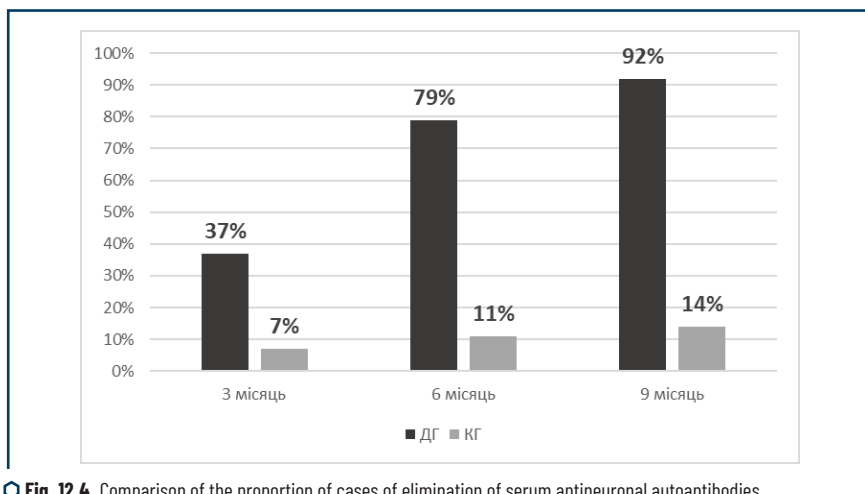


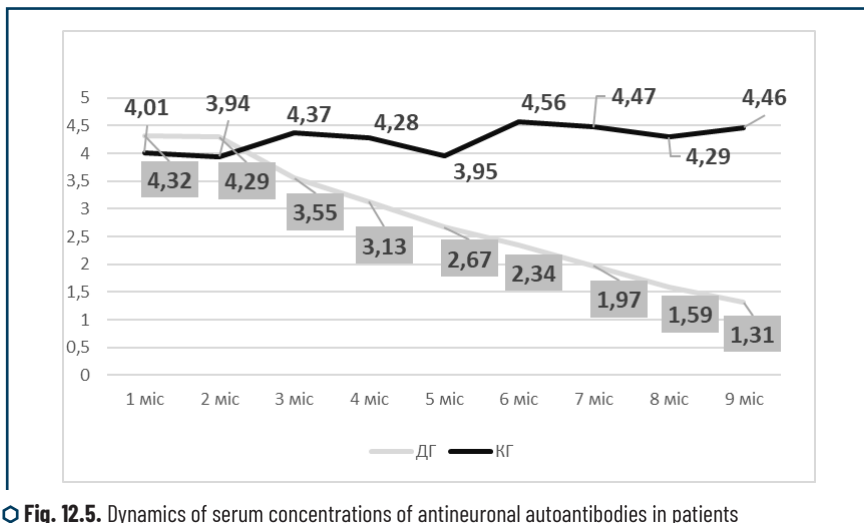
Fig. 12.4. Comparison of the proportion of cases of elimination of serum antineuronal autoantibodies in patients SG ($n = 62$) and CG ($n = 19$) during the course of rituximab immunotherapy

Although there was a small proportion of spontaneous normalization of serum concentrations of antineuronal autoantibodies in CG, immunotherapy with rituximab was associated with a 5-fold increase in the number of cases of obtaining normal serum concentrations of these autoantibodies after 3 months of immunotherapy, an 11-fold increase after 6 months of immunotherapy, and more than 14-fold increase after 9 months of use of the approved monoclonal antibody preparation.

Thus, the use of rituximab was associated with a progressive increase in the cases of negating previously positive results of serum antineuronal autoantibody measurements in SG children as the course of immunotherapy continued. Only 8 % of SG children showed resistance to a 9-month course of rituximab. Additional analysis showed that all of these children had a maximum antineuronal autoimmune response score (5 points) at the time of starting immunotherapy, indicating a high intensity of the autoimmune response. All of these children had decreased serum antineuronal antibody concentrations by the 9th month of immunotherapy by at least 60 %, indicating partial, rather than total, resistance to the immunotherapeutic interventions.

The results of the study of the monthly dynamics of the score of the intensity of the antineuronal autoimmune reaction indicate that throughout the entire course of rituximab, a progressive decrease in the serum concentration of antineuronal autoantibodies was noted in SG children. Thus, the average score of the assessment of the antineuronal autoimmune reaction in SG2 before the start of immunotherapy was 4.32 ± 0.27 points, while after a 9-month course of therapy it was only 1.31 ± 0.14 points, which indicated a decrease in the intensity of the total autoimmune reaction by almost 4 times, although in CG there was no significant dynamics of the score of autoimmunity against CNS neurons (4.01 ± 0.26 and 4.46 ± 0.47 points, respectively), which indicated a significant difference between the results of the observation groups ($p < 0,05$; $Z < Z_{0,05}$) (Fig. 12.5).

There was a delay in the serological response to rituximab immunotherapy for at least 2 months from the start of immunotherapy, which can be explained by the period of complete decay of pre-existing autoantibodies to neurons synthesized by B lymphocytes before the start of immunotherapeutic interventions, which is about 42–46 days.



○ Fig. 12.5. Dynamics of serum concentrations of antineuronal autoantibodies in patients SG (n = 62) and CG (n = 19) during the course of rituximab immunotherapy

These data indicate that rituximab does indeed affect the severity of the autoimmune reaction against CNS neurons in children with ASD associated with GDFC. Moreover, the positive effect of immunotherapy develops rapidly, already during the first 3 months of immunotherapy, consistently increases as the course of immunotherapy continues and leads to the elimination of signs of autoimmunity in almost all cases.

The speed of achieving the endpoint – elimination of antineuronal autoantibodies from the patients' serum – depends on the initial level of their serum concentration, since 89 % of SG patients, in whom the disappearance of serological signs of autoimmunity was noted after the first 3 months of immunotherapy, had a low initial score of the autoimmune reaction at only 1-2 points, while all patients with partial resistance to rituximab, who underwent all 9 months of approved immunotherapeutic interventions without complete elimination of antineuronal autoantibodies from the blood serum, had a high initial score of 5 points ($p < 0,05$; $Z < Z_{0,05}$).

Additionally, a comparative analysis of the effectiveness of rituximab in antineuronal autoimmunity caused by autoantibodies to neuronal potassium channels and GADA was conducted, since this allowed for a significant number of similar cases in SG (Fig. 12.6). Separate analysis of the dynamics of serum concentrations of other autoantibodies to CNS neurons noted in SG children was made impossible by the small number of relevant observations.

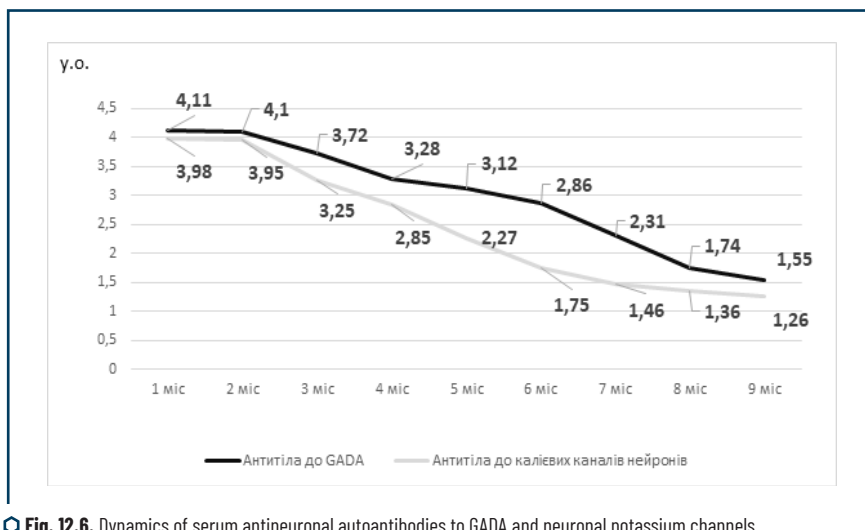


Fig. 12.6. Dynamics of serum antineuronal autoantibodies to GADA and neuronal potassium channels in SG patients ($n = 62$) during a course of rituximab immunotherapy

As shown in Fig. 6, rituximab was more effective in patients with autoantibodies to neuronal potassium channels than in patients with autoantibodies to GADA, although overall the efficacy of immunotherapy was quite high in both cases. These results are consistent with the established notion that rituximab is more effective in antineuronal autoimmunity caused by autoantibodies to surface autoantigens than by autoantibodies

to intracellular neuronal autoantigens, since in the latter case, cellular mechanisms of autoimmunity play a greater role in the pathogenesis of the disease, which are not affected by the monoclonal antibody drug used.

Therefore, when identifying serological signs of antineuronal autoimmunity to GADA in children with ASD associated with GDFC, a longer course of immunotherapy with rituximab should be expected than when detecting autoantibodies to neuronal potassium channels. Perhaps, to equalize the expected timing of immunotherapy in both cases, higher doses of rituximab should be initially used or standard immunotherapy should be combined with glucocorticosteroids specifically in patients with autoantibodies to GADA in the blood serum.

The fundamental question is whether the achieved phenomenon of rituximab-induced elimination of serum antineuronal autoantibodies is associated with the effect of neuroprotection. To this end, we studied the association of negativity of serological test results with normalization of previously elevated serum concentrations of cerebral damage biomarkers neuron-specific enolase (NSE) and S-100 protein (**Table 12.1**), the relevance of which has previously been demonstrated in specially designed controlled clinical trials in children with ASD [14, 32].

● **Table 12.1.** Results of the study of the association between the phenomenon of negativity of serological test results and normalization of serum concentrations of NSE and S-100 protein (OR; 95 % CI) in SG (n = 62)

Indicator	Antibodies to GADA	Antibodies to neuronal potassium channels
NSE	17,875; 4,738- 67,436	41,800; 7,257-240,778
S-100	9,750; 2,707-35,113	18,333; 3,462-97,083

As can be seen from the results of **Table 12.1**, the disappearance of serum autoantibodies to both GADA and neuronal potassium channels was associated with the normalization of previously elevated concentrations of both laboratory biomarkers of cerebral damage studied, which allows us to speak about the neuroprotective effect of immunotherapy with rituximab in children with SG. In the subgroup of patients with autoantibodies to neuronal potassium channels, a more pronounced association of the dynamics of the serological index and cerebral biomarker was noted compared to the subgroup of individuals with autoantibodies to GADA, which is consistent with the results of the analysis of the dynamics of serum concentrations of both types of antineuronal autoantibodies during the course of immunotherapy with rituximab in SG. At the same time, there was a closer association with NSE than the S-100 protein, which can be explained by the tropism of the detected anticerebral autoantibodies in children with SG. Since it was antineuronal autoantibodies that were noted, which primarily affect the gray matter of the brain, it was NSE, which characterizes neuronal damage, that turned out to be more informative, rather than the S-100 protein, the serum concentration of which increases with damage to the white matter of the cerebral hemispheres.

It was also important to investigate the clinical significance of the phenomenon of rituximab-induced disappearance of autoantibodies to CNS neurons in SG children, since there is still ongoing discussion about the role of antineuronal autoimmunity in the pathogenesis of ASD in children.

The data on the dynamics of the mental state score of children on the ABC scale indicate a significant improvement in all studied indicators in children receiving rituximab immunotherapy compared with CG patients. There was a decrease in the severity of clinical manifestations of hyperactivity and hyperexcitability, improvement in eye contact and behavior, progress in speech skills, and an increase in the overall score of the child's mental development. These clinical effects developed and deepened during the course of immunotherapy as serum concentrations of autoantibodies to CNS neurons decreased (**Table 12.2**).

● **Table 12.2.** ABC score in SG (n = 62) and CG (n = 19) patients after completion of rituximab immunotherapy

Nº	Subscales	SG (n = 62)	CG (n = 19)
	ABC		
1	Irritability	6.4±0.8*	14.1±1.5
2	Hyperactivity	10.9±1.4*	22.5±2.1
3	Inadequate eye contact	4.1±0.8*	8.6±1.3
4	Inappropriate speech	1.6±0.5*	7.9±1.5
	Symptom Checklist		
1	Drowsiness	5.7±0.7*	14.2±1.4
2	Decreased activity	1.7±0.4*	5.4±0.5

Note. * - $p < 0.05$; $Z < Z_{0.05}$

These data indicate that autoimmunity to CNS neurons is an important component of the pathogenesis of ASD in children with GDFC, and the elimination of serological manifestations of anti-neuronal autoimmunity with rituximab is associated with a significant improvement in the mental state of children. Therefore, immunotherapy with rituximab modifies the mental state of children with ASD associated with GDFC, uniformly affecting all major clinical signs of mental illness according to the ABC scale.

Conclusions. Rituximab treatment leads to a progressive decrease in serum concentrations of antineuronal autoantibodies in patients with GDFC-associated ASD, with a more pronounced effect in the case of autoantibodies to neuronal potassium channels compared to autoantibodies to GADA, with complete elimination of all types of autoantibodies from the serum of patients after a 9-month course of immunotherapy in at least 92 % of cases. The phenomenon of rituximab-induced elimination of serum antineuronal autoantibodies is associated with a neuroprotective effect, which is confirmed by the normalization of previously elevated concentrations of laboratory biomarkers of cerebral damage NSE and S-100 protein in serum. Most likely, it is the achieved neuroprotective effect that determines the progressive improvement in the main clinical manifestations of ASD in children with GDFC throughout the course of immunotherapy. The obtained data confirm the clinical significance of serum antineuronal autoantibodies in children with ASD associated with GDFC and indicate the effectiveness of rituximab for neuroprotection by suppressing anti-brain autoimmunity and achieving associated improvement in the mental status of the child in such cases.

REFERENCES

1. Maltsev, D., Yevtushenko, S. (2016). High-Dose Intravenous Immunoglobulin Therapy Efficiency in Children with Autism Spectrum Disorders Associated with Genetic Deficiency of Folate Cycle Enzymes. *International Neurological Journal*, (2.80), 35–48. <https://doi.org/10.22141/2224-0713.2.80.2016.74004>
2. Maltcev, D. V., Natrus, L. V. (2020). The Effectiveness of Influximab 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>
3. 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>
4. 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.
5. 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>
6. Chen, L., Shi, X.-J., Liu, H., Mao, X., Gui, L.-N., Wang, H., Cheng, Y. (2021). Oxidative stress marker aberrations in children with autism spectrum disorder: a systematic review and meta-analysis of 87 studies (N = 9109). *Translational Psychiatry*, 11 (1). <https://doi.org/10.1038/s41398-020-01135-3>
7. 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>
8. 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>
9. Frustaci, A., Neri, M., Cesario, A., Adams, J. B., Domenici, E., Dalla Bernardina, B., Bonassi, S. (2012). Oxidative stress-related biomarkers in autism: Systematic review and meta-analyses. *Free Radical Biology and Medicine*, 52 (10), 2128–2141. <https://doi.org/10.1016/j.freeradbiomed.2012.03.011>
10. Frye, R. E., Sequeira, J. M., Quadros, E. V., James, S. J., Rossignol, D. A. (2012). Cerebral folate receptor autoantibodies in autism spectrum disorder. *Molecular Psychiatry*, 18 (3), 369–381. <https://doi.org/10.1038/mp.2011.175>
11. 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>
12. 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>
13. Gupta, S., Samra, D., Agrawal, S. (2010). Adaptive and Innate Immune Responses in Autism: Rationale for Therapeutic Use of Intravenous Immunoglobulin. *Journal of Clinical Immunology*, 30 (S1), 90–96. <https://doi.org/10.1007/s10875-010-9402-9>

14. 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>
15. Lv, M., Zhang, H., Shu, Y., Chen, S., Hu, Y., Zhou, M. (2016). The neonatal levels of TSB, NSE and CK-BB in autism spectrum disorder from Southern China. *Translational Neuroscience*, 7 (1), 6-11. <https://doi.org/10.1515/tnsci-2016-0002>
16. 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 pharmaceutical technology*. – 2019. – Vol. 11(05). – P. 597-609. (глава 9 из 9)
17. 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>
18. 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>
19. Mead, J., Ashwood, P. (2015). Evidence supporting an altered immune response in ASD. *Immunology Letters*, 163 (1), 49-55. <https://doi.org/10.1016/j.imlet.2014.11.006>
20. 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>
21. 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>
22. Nepal, G., Shing, Y. K., Yadav, J. K., Rehrig, J. H., Ojha, R., Huang, D. Y., Gajurel, B. P. (2020). Efficacy and safety of rituximab in autoimmune encephalitis: A meta-analysis. *Acta Neurologica Scandinavica*, 142 (5), 449-459. <https://doi.org/10.1111/ane.13291>
23. Nicolson, G. L., Gan, R., Nicolson, N. L., Haier, J. (2007). Evidence for *Mycoplasma ssp.*, *Chlamydia pneumoniae*, 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>
24. 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>
25. Noriega, D. B., Savelkoul, H. F. J. (2013). Immune dysregulation in autism spectrum disorder. *European Journal of Pediatrics*, 173 (1), 33-43. <https://doi.org/10.1007/s00431-013-2183-4>
26. 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>
27. 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>

28. 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>
29. 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>
30. 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.jpsy-chires.2019.05.019>
31. Wang, Z., Ding, R., Wang, J. (2020). The Association between Vitamin D Status and Autism Spectrum Disorder (ASD): A Systematic Review and Meta-Analysis. *Nutrients*, 13 (1), 86. <https://doi.org/10.3390/nu13010086>
32. 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>
33. Zheng, Z., Zheng, P., Zou, X. (2020). Peripheral Blood S100B Levels in Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. *Journal of Autism and Developmental Disorders*, 51 (8), 2569-2577. <https://doi.org/10.1007/s10803-020-04710-1>

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 research: 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 cytometry (Epics XI cytometry, 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 cytometry) 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.

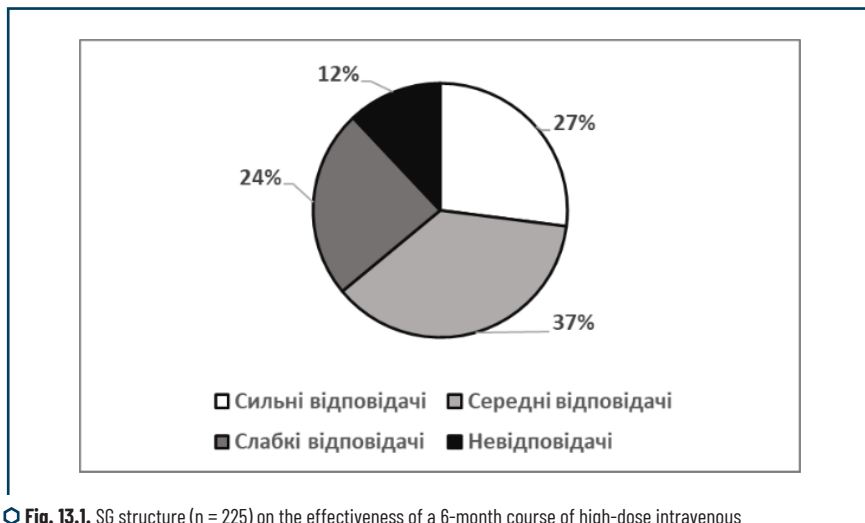
The criteria for inclusion of the patient in this study were the presence of folate cycle gene polymorphisms, NK and/or NKT cell deficiency, reactivated infection caused by lymphotropic herpesviruses, signs of leukoencephalopathy on MRI of the brain, clinical symptoms of ASD according to the ABC scale. 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

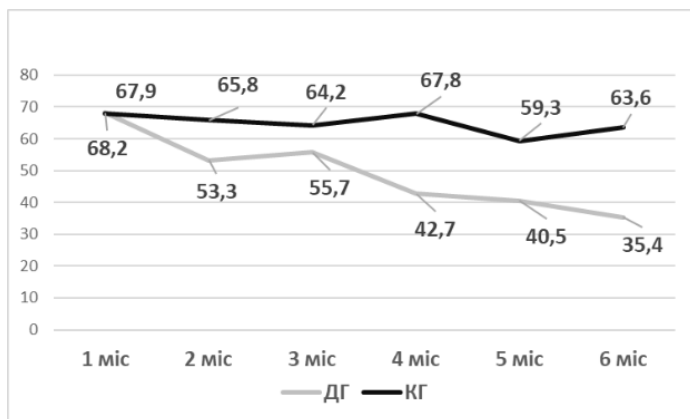
N ^o	Subscales	SG (n = 225)	CG (n = 50)
	ABC		
1	Irritability	7.1±0.6*	14.3±0.8
2	Hyperactivity	12.1±0.7*	23.4±1.2
3	Inadequate eye contact	5.4±0.5*	9.7±0.6
4	Inappropriate speech	2.8±0.3*	7.6±0.7
	Symptom checklist		
1	Drowsiness	6.4±0.6*	13.3±1.0
2	Decreased activity	2.7±0.2*	4.8±0.3

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 ($p < 0,05$; $Z < Z_{0,05}$).

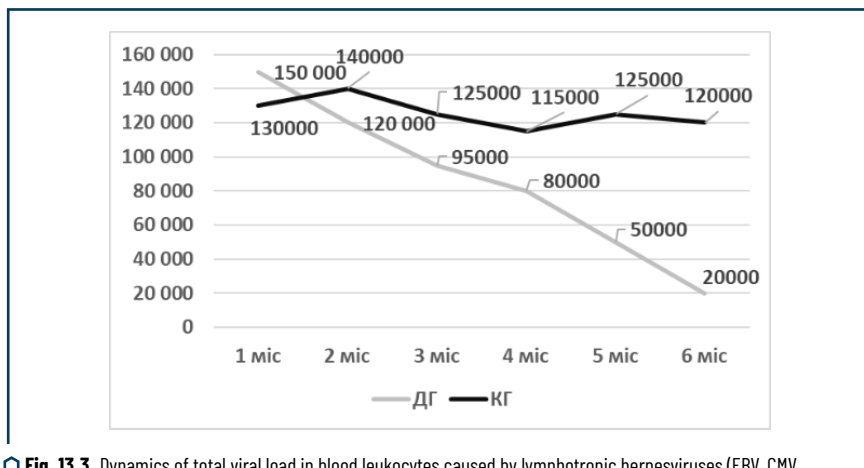


Fig. 13.3. Dynamics of total viral load in blood leukocytes caused by lymphotropic herpesviruses (EBV, CMV, HHV-6, HHV-7), according to PCR data in patients of SG ($n = 225$) and CG ($n = 50$) during the observation period

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 hypogammaglobulinemia 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.

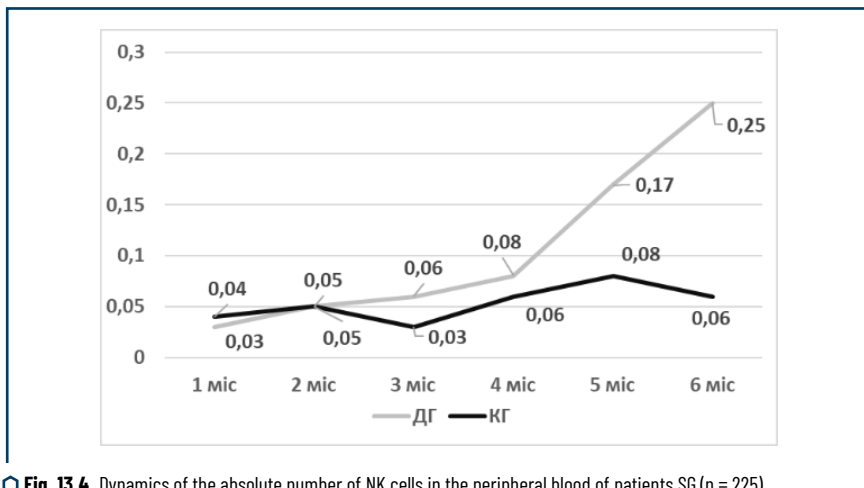
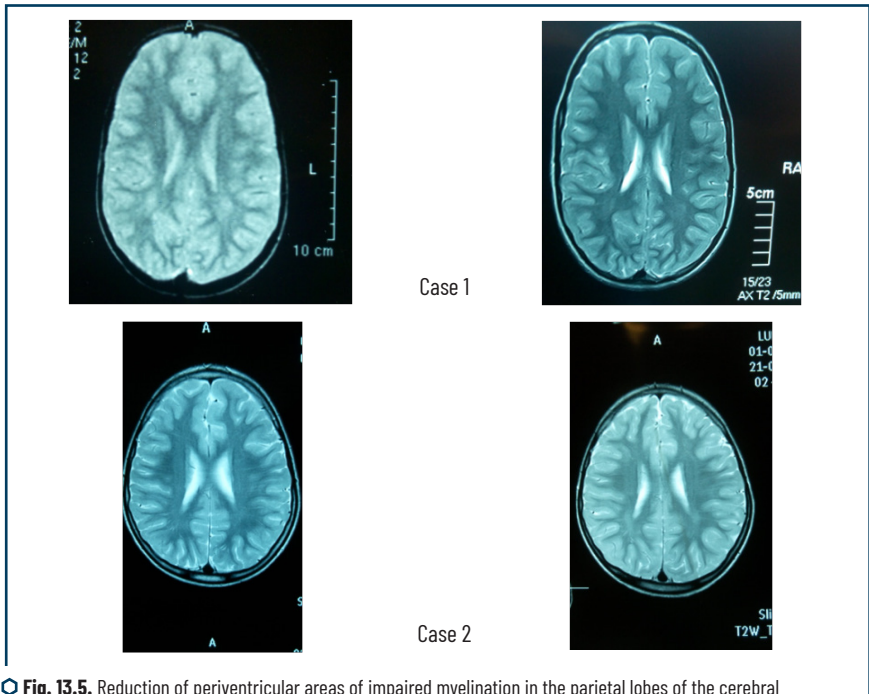


Fig. 13.4. Dynamics of the absolute number of NK cells in the peripheral blood of patients SG (n = 225) and CG (n = 50) during the observation period

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*. Athnem.
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](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* spp., *Chlamydia pneumoniae*, 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](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., Monafa, 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-B Alleles 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., Moulías, 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., Ataenia, 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.jpsy-chires.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 leukoencephalopathy 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>

CONCLUSIONS

GDFC in patients with ASD is associated with specific biochemical changes that are not observed in healthy children, namely hyperhomocysteinemia, deficiency of vitamins B12, B6, folic acid, vitamin D3, hypercreatininemia, increased serum concentrations of lactate dehydrogenase and creatine phosphokinase.

Biochemical abnormalities in children with GDFC who have ASD are associated with specific changes in immune status that are not observed in healthy children, namely deficiency of CD8 + cytotoxic T lymphocytes, natural killers, natural killer T lymphocytes and myeloperoxidase of phagocytes, which are variably combined with manifestations of dysimmunoglobulinemia and are associated with the development of clinical manifestations of immune-dependent syndromes (infectious, allergic, immunoinflammatory, autoimmune and oncological).

Immunodeficiency observed in children with GDFC with ASD is associated with the development of a number of opportunistic and conditionally pathogenic intracellular infections, which are probably observed more often than in healthy children. These infections constitute a specific microbial spectrum, and there is a connection between the deficiency of certain immune factors and the development of certain infections.

Infectious factors that develop in immunodeficiency conditions in children with GDFC with ASD are associated with the development of autoimmune reactions to cerebral and extracerebral autoantigens that are not observed in healthy children, in particular, autoimmunity to the hippocampi, subcortical nuclei and myelin of the CNS (anti-cerebral autoimmunity) and to the nuclei of connective tissue and lumbar striated muscles (autoimmunity to extracerebral autoantigens), and a close relationship is noted between the types of microorganisms and the targets of autoimmunization.

In patients with ASD associated with GDFC, serum concentrations of the pro-inflammatory mediators TM2PK, TNF-alpha, and IL-6 increase, which indicates a state of systemic inflammation and is associated with an increase in serum concentrations of laboratory indicators of cerebral damage.

In children with ASD associated with GDFC, typical laboratory-radiological-clinical complexes are noted (virus-induced temporal median sclerosis, autoimmune parainfectious limbic encephalitis, autoimmune parainfectious subcortical encephalitis, autoimmune or virus-induced demyelinating lesion of the cerebral hemispheres, the consequences of previous neuroinfections and small anomalies of brain development), the manifestations of which, combining variably in different patients, form a specific neuroimaging picture of encephalopathy.

Combined immunotherapy with Propes and Inflamafertin is an effective strategy for treating GDFC-induced immunodeficiency in children with ASD. It normalises previously reduced numbers of NK and NKT cells in the blood with a more frequent, stronger, and more persistent effect on NKT cells than NK lymphocytes.

Rituximab decreases serum concentrations of antineuronal autoantibodies in patients with ASD associated with GDFC, which is associated with normalization of laboratory biomarkers of cerebral damage and progress in children's mental development.

CONCLUSIONS

Infliximab reduces serum TNF-alpha concentrations in children with ASD associated with GDFC. This is associated with improvements in hyperactivity, hyperexcitability, and stereotypic behavior, as well as intestinal pathology, epileptiform activity, hyperkinesia, and obsessive-compulsive syndromes, but not in the quality of eye contact and language development.

High-dose intravenous human normal immunoglobulin exhibits a complex polymodal positive effect on the manifestations of ASD, extrapyramidal disorders, obsessive-compulsive syndrome, epileptiform activity, intestinal lesions, NK cell deficiency, and MRI signs of leukoencephalopathy in children with GDFC.

PRACTICAL RECOMMENDATIONS

Children with ASD associated with GDFC should have serum concentrations of homocysteine, vitamins B12 and B16, folic acid, vitamin D3, creatinine, creatine phosphokinase, and lactate dehydrogenase monitored to assess specific biochemical status and predict immune dysfunction.

Children with ASD associated with GDFC should undergo an immune status assessment to assess the severity of specific immunodeficiency and associated immune dysregulation. This assessment should include determining the number of NK-, NKT-cells, CD3 + , CD3 + CD4 + , CD3 + CD8 + T-lymphocytes, CD3-CD19 + B-cells, phagocyte myeloperoxidase activity, and serum concentrations of immunoglobulins of different classes and subclasses.

In children with ASD associated with GDFC, to assess the infectious syndrome and predict microbe-induced autoimmune complications, it is advisable to diagnose TTV, HHV-7, HHV-6, EBV, CMV, HSV-1/2, *Streptococcus pyogenes*, *Candida albicans*, *Borrelia*, *Yersinia enterocolitica*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Toxoplasma gondii* using appropriate microbiological methods, taking into account the relationships of microorganisms with the identified disorders in the immune status.

For children with ASD associated with GDFC, it is recommended to determine laboratory signs of autoimmunity to brain (hippocampal neurons, subcortical nuclei, myelin) and extracerebral (ANA profile, Myositis profile) autoantigens to assess the autoimmune syndrome, taking into account the relationships between the types of microorganisms and the targets of autoimmunity.

For children with ASD associated with GDFC, it is advisable to monitor serum concentrations of tumour M2pyruvate kinase, tumor necrosis factor-alpha, and interleukin-6 to assess systemic inflammation and related cerebral damage, taking into account the identified differences in the sensitivity and specificity of these indicators.

In children with ASD associated with GDFC, for the diagnosis of specific encephalopathy, neuroimaging signs of temporal median sclerosis, subcortical and limbic encephalitis, leukoencephalopathy and minor anomalies of brain development should be searched for, taking into account the identified clinical, laboratory and radiological correlates.

Children with ASD associated with GDFC, to compensate for the deficiency of blood NK and NKT cells, it is recommended to prescribe a course of combined immunotherapy with Propes and Inflamafertin in an alternating regimen of 2 ml i.m. every other day, alternating with each other, under the control of the blood levels of NK and NKT cells in the blood.

In children with ASD associated with GDFC, to achieve neuroprotection by suppressing anti-brain immunity, it is recommended to prescribe a course of immunotherapy with rituximab in the form of monthly infusions at a dose of 375 mg/m² of the child's body surface area per month under the control of serum concentrations of antineuronal autoantibodies and psychometric scales.

In children with ASD associated with GDFC, to achieve neuroprotection by suppressing systemic inflammation, it is advisable to conduct a course of immunotherapy with infliximab at a dose of 3 mg/kg/month under the control of serum concentrations of tumour necrosis factor alpha.

In children with ASD associated with GDFC, to achieve neuroprotection by suppressing all three immune-dependent mechanisms of cerebral damage (infectious, immunoinflammatory, autoimmune), it is recommended to conduct immunotherapy with normal human intravenous immunoglobulin at a dose of 2 g/kg/month under the control of indicators of infectious load, anti-brain autoimmunization, systemic inflammation, neuroimaging data, and psychometric scales.

IMMUNODIAGNOSTICS AND IMMUNOTHERAPY OF NEUROPSYCHIATRIC DISORDERS IN CHILDREN

Dmytro Maltsev

Monograph

Technical editor I. Prudius
Desktop publishing T. Serhiienko
Cover photo Copyright © 2025 Canva

TECHNOLOGY CENTER PC®

Published in May 2025

Enlisting the subject of publishing No. 4452 - 10.12.2012

Address: Shatylova dacha str., 4, Kharkiv, Ukraine, 61165
