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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

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**).

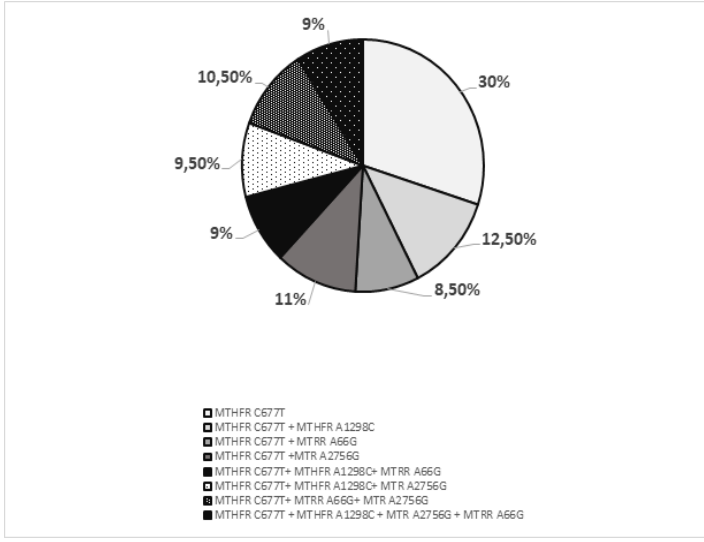

Fig. 8.1. SG structure (n = 225) based on genetic testing for GDFC

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.

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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**).

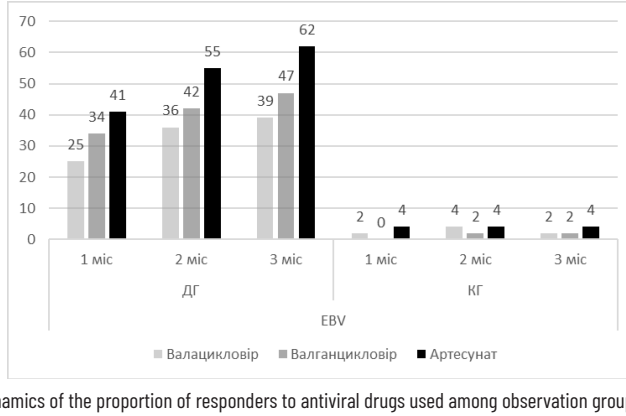

Fig. 8.2. Dynamics of the proportion of responders to antiviral drugs used among observation groups during the course of therapy for reactivated EBV infection

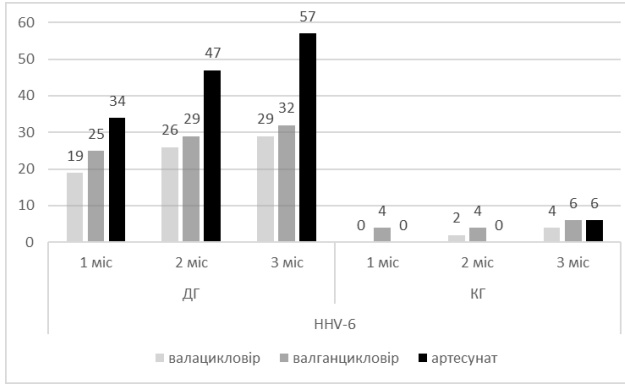

Fig. 8.3. Dynamics of the proportion of responders to antiviral drugs used among observation groups during the course of therapy for reactivated HHV-6 infection

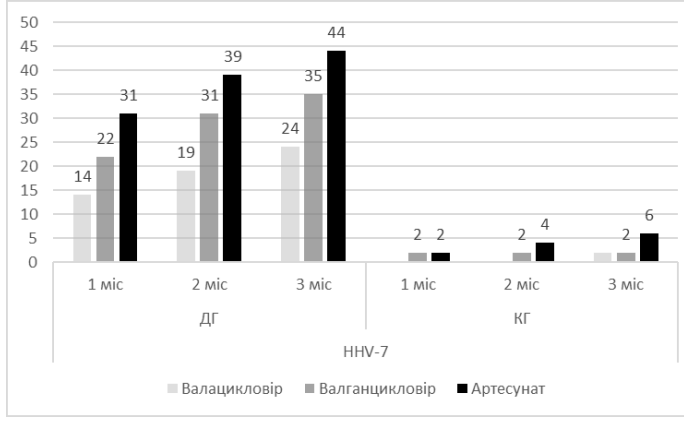

Fig. 8.4. Dynamics of the proportion of responders to antiviral drugs used among observation groups during the course of therapy for reactivated HHV-7 infection

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–monoinfections 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 monoinfections 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.

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