Treatment of Chronic and Latent Infections Combined with Nutritional Supplementation Positively Affects Quality of Life of ASD Children: Series of 30 Cases

Alibek K1,2*, Farmer S1,2, Tskhay A2, Moldakozhayev A2, and Isakov T3

1FLAASK, LLC, 30500 Aurora Road, Suite 120, Solon, 44139, OH, USA
2Locus Fermentation Solutions, LLC, 30500 Aurora Road Suite 180, Solon, 44139, OH, USA
3New Family Physicians Associates, Inc, Lyndhurst, OH, USA

*Corresponding Author: Alibek K, FLAASK, LLC, 30500 Aurora Road, Suite 120, Solon, 44139, OH, USA, Locus Fermentation Solutions, LLC, 30500 Aurora Road Suite 180, Solon, 44139, OH, USA, E-mail: kalibek@locusfs.com


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Abstract

Based on a number of publications, which demonstrate that children with ASD have chronic and latent infections complicated with inflammation and immune insufficiency, we suggested to treat these complications for improving the quality of life of 30 ASD children from Central Asia and Eastern Europe. The treatment included antiviral therapy combined with nutritional supplementation, which resulted in a tendency of the blood and immune parameters to shift towards normalization and in some positive changes in symptoms and signs related to ASD. There were no significant side effects observed, except for mild side reactions that continued for no longer than 14 days. Neither of the children experiencing the reactions required termination of treatment.

Keywords: Autistic Disorder; Behavior; Immune System; Infection; Inflammation; Nutritional supplements; Treatment, Valacyclovir

Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental pathology with social communication problems, repetitive/restricted behavior, and language impairment [1]. It is a rapidly growing problem and it is estimated that in the USA in 2018, 1 in 59 children [2,3] will be born with ASD, while 50 years ago, the incidence of ASD was almost 40 times lower-only 1 in 2500 [4].

There have been many scientific studies related to ASD in the recent decades, but a commonly accepted etiology for ASD has not been agreed upon. At the same time, based on a wide range of theories on the etiology and pathogenesis of ASD, there have been many treatment approaches proposed. In one of the studies [5], 764 parents of ASD children were interviewed about the types of treatments that had been prescribed for their children. The results revealed that 111 types of treatment had been used. The most common were interventional corrective therapies, including speech therapy, interventional therapy, ABA therapy, etc., and nutritional supplement administrations including vitamin B6, fatty-acids, and magnesium. Medications were used less frequently. Among the most common medications were sleep aid, antipsychotic, antihypertensive and antidepressant drugs. In a 2018 study the effectiveness of the common drugs and therapies was assessed by meta-analysis of the existing clinical studies. The data showed that despite a variety of approaches, there was no therapy which produced significant improvements in treatment cohorts [6]. It was therefore concluded that there is no effective treatment for ASD children.

It is currently known that ASD is a result of atypical brain development, revealing itself in a form of impairments in mental imagery, sensory acquisition, attention, affective behavior, visual organization, and other functions. The areas of brain, which were found to be altered in ASD children are: dorsolateral prefrontal cortex, temporal cortex, cerebellum, and some other parts [7], and the damage may happen either in the prenatal or infantile period of life [8].
The brain alterations are the result of genetic differences responsible for brain development [9-11]. Genetic alterations are common in ASD children. More than 120 genes affecting brain development and immune system functioning were identified as genes participating in ASD [12,13].

These genetic alterations can be a result of oxidative stress [8,14,15], which apart from causing DNA damage, may also inhibit DNA's repair mechanisms [14,15].

Oxidative stress resulting from overproduction of reactive oxygen species is a hallmark of inflammation [14]. Additionally, inflammation is characterized by induction of a wide range of inflammatory and pleiotropic cytokines and chemokines [16], which also contribute to DNA damage. Signs of systemic and neuro-inflammation [17-20] along with signs of immune system alterations [21] were identified in ASD children in many studies.

Inflammation, in turn, can be induced by various factors, but infections are considered the most important ones. The overwhelming number of publications show a wide variety of infectious agents that are suspected to play a role in the initiation and promotion of ASD: herpes simplex virus 1/2 (HSV) [22], Epstein-Barr virus (EBV) [23], Cytomegalovirus (CMV) [24,25], human herpesvirus 6 (HHV 6) [26], rubella virus [27], Chlamydia spp, and Mycoplasma spp [26,28]. Since, the inciting event of ASD was shown to begin in the prenatal period [8], it is very likely that the chain of events starts from a maternal viral/bacterial infection.

In a Danish study that analyzed all children born in Denmark from 1980 to 2005, it was found that admission to a hospital due to maternal viral infection in the first trimester and maternal bacterial infection in the second trimester were associated with the diagnosis of ASD in the offspring [29].

Presumably, based on these studies, it was expected that there were attempts to use antiviral therapy to treat ASD children. We found out that there were attempts to use antiviral therapy for reducing severity of ASD symptoms. For example, on the Treating Autism website [30] there was an anecdotal example of a child treated with Valtrex (Valacyclovir) for a year. The treatment resulted in the disappearance of the child's autism symptoms. Another search led us to a physician’s discussion on the Talk About Curing Autism website [31] concerning the presence of herpes group and other viruses. Based on this, there was a proposal to use antivirals including Valacyclovir, Acyclovir or Famvir combined with nutritional supplementation.

Additionally, we found that in the National Neurological Journal published in Ukraine there were two publications that suggested the use of antiviral medications such as Valacyclovir, Famyclovir, Interferon-alfa and human non-specific immunoglobulin [32,33]. The authors showed that children with ASD have a special form of primary immunodeficiency with a deficiency of natural killers and natural killer T-lymphocytes which leads to a selective decrease in resistance to viral agents and a tendency to generate autoimmune and allergic complications. Prior to treatment, the sample group was tested for the presence of infections and for immune system status. Although the complete picture of these findings was not discussed in detail, it was mentioned that the children were infected with different herpesvirus infections (from HHV-1 to HHV-8) and the rubella virus and most of them showed a dysfunction of the immune system and symptoms of encephalitis. In one of the author’s follow-up article [33] it was recommended that in addition to antiviral and immunomodulating therapy, neurorehabilitation treatment should be used. It was stated that the treatment caused pronounced improvement in a half of the patients. Additionally, the author stated (unfortunately, without referring to specific cases), that a 5-year observation of the children who were treated with the proposed therapy showed “full recovery in almost half of the patients”.

Furthermore, in a study by [34] it was indicated that children with an autism spectrum disorder (ASD) diagnosis have ongoing neuroinflammation or chronic encephalitis. It is conservatively estimated that at least 69% of ASD individuals have microglial activation or neuroinflammation. But children with an ASD diagnosis are not usually assessed for a possible diagnosis of encephalitis. According to the authors, if a child with ASD has neuroinflammation, its treatment could lead to improved outcomes and benefit to these children.

Combined, this preliminary evidence led us to the selection of a composition capable of inducing anti-infectious, anti-inflammatory, and immunocorrective effects. Taking into account the age of these patients, we aspired not to over-use prescription drugs and to use appropriate nutritional supplements with proven positive clinical and non-toxic effects. Therefore, the composition included a non-toxic anti-viral pharmaceutical compound and nutritional supplements with the effect tailored at the main elements of the ASD etiopathogenesis discussed above.

**Description of Treatment Composition**

Valacyclovir is an antiviral Acyclovir prodrug. Compared to Acyclovir, it has a higher bioavailability—55% which
is 2-4 times higher than the bioavailability of Acyclovir [35]. Valacyclovir shows the highest stability profile compared to other Acyclovir prodrugs [36].

Valacyclovir, was reported to be effective in the inhibition of herpes viruses: HHV-1 [37], HHV-2, HHV-3/VZV [38,39], and HHV-4/EBV [40,41], HHV-5/CMV [42]. In a clinical study [43], it was shown that Valacyclovir was both effective against Epstein-Barr virus and well tolerated by children. The only side effects, which appeared in a small number of children, were headache in 8%, and gastrointestinal disturbances in 16%. Many additional studies demonstrated that Valacyclovir is well-tolerated by children [44] and rare side reactions included only headache and diarrhea (meta-analysis by [45]) with no serious adverse effects (stated in the annotation to the drug) described in the meta-analysis, analyzing the existed studies on the usage of Valacyclovir for children.

Valacyclovir is recommended for the treatment of viral infections in children by regulatory agencies of Central Asia and Eastern European countries where treatments were performed [46,47].

L-lysine is an amino acid that is essential for human organism. Usually L-lysine is obtained from the diet: from casein, phosphoprotein which is contained in dairy products [48]. The importance of L-lysine for human organism is in the fact that it participates in such processes as proper growth, muscle protein building and metabolism [48]. L-lysine can inhibit herpes simplex virus by reducing the cyto-pathogenicity of the HHV-1 [49-51]. The study by [49] demonstrated that patients who took L-lysine for 6 months had 2.4 times less cases of HHV-1 infection than patients in placebo-controlled group. In another study by [51] it was shown that recurrence rate of HHV-1 was also lower within those who took L-lysine in comparison with placebo group. It was also reported that L-lysine did not cause any toxic or side effects [49]. Additionally, L-lysine was shown to be effective against such conditions as anxiety, mood disturbances and headaches [48], which are very common for children with ASD.

Elderberry extract in a nutritional supplement extracted from the black elder (Sambucus nigra L.). It has been used for many years in folk medicine because of its effectiveness against viruses, headache, and constipations [52]. It has antiviral (influenza A and B, RSV and parainfluenza), anti-inflammatory, immunomodulatory and antioxidant activity. Several clinical trials showed that elderberry root extract was effective against influenza A and B virus infections [53-55], respiratory syncytial virus (RSV) and parainfluenza [53]. People with symptoms of influenza recovered on average 3 to 4 days faster than control groups in all the mentioned trials. In a placebo-controlled, double blind study with 40 participants with influenza B virus infection, the full recovery of 90% of participants receiving the treatment was within 2 to 3 days, while the control group, which took a placebo showed complete recovery after 6 days [54]. In another study by [56] effectiveness of elderberry against HHV-1 was shown. In a clinical trial with 312 participants it was shown that elderberry extract positively influences both physical and mental health [57]. Furthermore, it was proven that there are no side or adverse effects to taking elderberry extract for all age groups including infants [55]. The effectiveness of the elderberry extract is due cyanidin-3-glucoside and cyanidin-3-sambubioside flavonoids found in the plant [58,59], which modulate the immune system [55]. Flavonoids are also known to have antioxidant and prooxidant activities of low-density lipoprotein [58]. Anthocyanins which are a part of elderberry extract also possess anti-inflammatory properties [55].

Olive leaf extract is also known as a part of folk medicine. Its hypoglycemic, antioxidant, antiviral and antimicrobial effects were reported [60]. The presence of polyphenols and flavonoids in olive leaves provides the spectrum of properties, which were used by humans in medicine [60]. A double-blinded placebo-controlled trial [61] showed that olive leaf extract stimulates downregulation of genes that participate in the inflammatory process (EGR1, COX-2 and ID3). Another study by [62] demonstrated a significant reduction in the production of cytokines participating in the inflammatory processes. These results were supported by other studies [21,63]. Additionally, it was shown that olive leaf extract has antiviral and anti-oxidant activities [64,65]. For example, it decreased the viral infectivity of viral haemorrhagic septicemia virus (VHSV) by 10-30% [65]. Antioxidant activity due to the presence of functional compounds found in olive leaves’ flavonoid was also reported [64]. Also, positive results were obtained by using olive leaf extract for Alzheimer’s disease [66]. Among other plants, olive leaf extract and particularly biophenols contained in it could provide the highest neuroprotection (74%) and strongest inhibitory activity against a number of enzymes participating in the development of Alzheimer’s disease.

Astragalus root extract is extracted from Astragalus membranaceus Moench herb, and as well as plants discussed above, was used for many years in folk medicine because of its ability to stimulate the immune system [67]. It was observed that astragalus root extract intake enhances the production of macrophages and lymphocytes [68,69]. These cells are key actors of the immune system. Similar to olive leaves and elderberry, astragalus contains flavonoids [67].
that as previously stated, have a positive effect on the immune system. Moreover, astragalus extract can stimulate the growth of stem cells [68]. The antiviral, anti-inflammatory and antibacterial activities of the extract were shown by [67] and [70].

Bacillus coagulans probiotic contains a high concentration of spores ensuring its long-term stability and efficacy in the gut. This bacterium is reported to be non-pathogenic and an efficient producer of L(+) lactic acid [71]. It is capable of surviving in the acidic environment of the stomach, which explains its prolonged activity [72]. A main function of this probiotic is its ability to improve digestive health through activating more efficient digestive absorption [73] and modulation of gut microbiota [74]. It germinates in the small intestine and increases protein and carbohydrates absorption. A placebo-controlled clinical trial with participation of 141 children of age 4-12 showed that Bacillus coagulans probiotic intake significantly improved condition of children with irritable bowel syndrome by relieving such symptoms as bloating, pain in the stomach, discomfort, urgency, etc. [75]. This property of probiotic is especially important for children with ASD since 70% were reported to have various problems of the gastrointestinal tract [76]. It was demonstrated that probiotic intake positively influences children with ASD because it targets two problems at once, microbiota–gut–brain axis, through balancing microbiota, and it inhibits the toxic effect of some compounds on the brain which leads to a decrease in ASD symptoms [77]. Moreover, several studies indicated that bacillus coagulans probiotic intake increases immune response to viral agents. It was found that it increased production of T-cells as a response to viral infections [78]. It was also reported that Bacillus coagulans probiotic has the ability to inhibit 3 viruses of the herpes viruses group: HHV-1, HHV-2 and HHV-3 [71]. The toxicological assessment of Bacillus coagulans probiotic showed that the supplement has no toxic or other side effects [72].

Methods

Participants

All 30 study participants were self-referred by their parents. The study was not prospective and there were no exclusion criteria. The only inclusion criteria were, the request of parents of children with confirmed ASD diagnosis and the parents’ written consent with a guaranteed non-disclosure of identities. The children were from different cities of two post-Soviet countries. The age range of the participants was from 2.5 to 16 years old.

Confirmation of Diagnoses

The study was conducted using written questionnaires and a telemedicine approach. The Skype® program was used for the web-mediated communication and consultations. All participants completed the questionnaire in accordance to DSM-5 via e-mail-based communication assessing the presence of the ASD symptoms and their severity to confirm the ASD diagnoses and prevalence of signs and symptoms. Additionally, the parents answered questions on the physiological and psychological condition of the children (presence of sleep disorders, anxiety, depression, hyperactivity, gastrointestinal tract issues and frequency of respiratory infections), on the history of mother’s pregnancy, parturition, and the initial period of the child’s life and on the types of treatments used prior to enrollment in this study.

It was explained to the parents that the study did not undertake the treatment of autism, but instead the study would be focused on the diagnosis and treatment of chronic/latent infections, reducing the inflammatory state and modulation of the child’s immune system. Initially the parents were asked to have laboratory tests of their ASD diagnosed child’s immune status, presence of antibodies to viral and bacterial infections, and red and white blood cells counts. If the parents agreed, the tests were performed by local clinical diagnostic centers, which the parents were free to select.

Treatment groups and regimens

When the presence of viral/bacterial infection was confirmed by blood test results, the parents received the following recommendations for treatment:

All children were divided into three groups by age and treatment regimens:

Ages up to 3 years: 3 cycles of Valacyclovir treatment (125 mg., twice a day). Each cycle continues for 3 weeks with a 9-day break, along with the continuous intake of 125 mg of each supplement and 0.5 bln CFUs of probiotic per day without breaks.

Ages 3 to 7: 3 cycles of Valacyclovir treatment (250 mg., twice a day). Each cycle continues for 3 weeks with a 9-day break, along with the continuous intake of 250 mg of each supplement and 1 bln CFUs of probiotic per day without breaks.
Ages 7 and older: 3 cycles of Valacyclovir treatment (500 mg., twice a day). Each cycle continues for 3 weeks with a 9-day break, along with the continuous intake of 500 mg of each supplement and 2 bln CFUs of probiotic per day without breaks.

The parents were recommended not to administer any other drugs or supplements apart from cases when there were conditions which required the intake of other medications (for example, appropriate painkillers for headaches or antibiotics in case of bacterial infections, etc.).

Parents submitted the result of tests after either 1 or more (up to 5) monthly cycles of treatment. Discussions on the changes related to ASD were initiated by the parents.

Assessment of the results

The assessments were based on the results of monthly or bi-monthly tests for detection of antibody levels to infections in blood samples, red and white blood cell count tests, and cellular immunity tests, as well as on the parents’ observation of their children in terms of ASD symptoms and ASD-related disorders. The assessment of the treatment results was conducted during videoconferences after a cycle of Valacyclovir (initiated by the parents). Before videoconferences, the parents were asked to prepare a written description of the observed changes in their children. To rule out potential subjectivity, we discouraged the parents from reporting assumptions and requested that they only report obvious changes and compare any changes with previous periods before treatment (if there were any in previous periods).

Results

Pre-treatment data collection

In our previous study, we described the results of the blood tests related to possible chronic/latent infections, inflammation, and immune system dysregulation. Also, we provided the 57 children’s assessment in accordance to DSM-5 and other disorders, as well as the description of the health problems of the mothers during pregnancy, parturition peculiarities for both mother and a child, and descriptions of slightly noticeable symptoms of the children before they were officially diagnosed with ASD [79]. The 30 children described in this study are a part of these 57 children, with no preselection, but only based on the availability of information from parents and medical and clinical laboratory centers. The initial results of blood tests were taken from the results of these children in the abovementioned study.

Presence of antibodies to chronic infections: The blood tests showed that all 30 children had increased levels of antibodies to certain viral infections and some of them had increased levels of antibodies to bacterial infections. The increased levels were considered only in the cases in which the levels were higher than the established reference levels in their local diagnostic centers. The most prevalent viral indicators were, congenital rubella virus, EBV (HHV-4) and CMV (HHV-5), which were present in the blood samples of 25 and 24 children respectively. HHV-1/2 was detected in 10 children, VZV (HHV-3) was detected in 3 children (not all local centers had capacities for this virus detection). Bacterial infections were detected in the following numbers: H. pylori in 6 children, Mycoplasma spp. in 4 children. Antibodies to Toxoplasma infection were increased in 1 child.

It is important to note that regardless of the type of infection, most children had a poly-infection, ranging from 2 to 5 infections. Out of 30, only 3 children had a single detected infection (but these 3 children were not tested for the presence VZV). The prevalence of each of the infections and the number of poly-infections in the children are presented in the Figure 1.

![Figure 1: The prevalence of infections within children. The left part of the figure represents the number of children in whom the infections were identified. The most common infections were: rubella virus, Epstein-Barr virus, and cytomegalovirus. All children had at least one infection. The chart on the right represents the number of children in relation to the number of infections identified. Most children had 4 identified infections, while the maximum number of infections detected was 5](image-url)
Red and white blood cell counts and cellular immunity assessment: The assessment of alterations was based on the reference ranges accepted in the local diagnostic centers. Red and white blood cell counts showed signs of inflammation, presence of infection (predominantly viral), weakened immune system, and folate deficiency. The indices include:

Red blood cells count:
- Erythrocytosis (increased RBC) in 14 children
- Increased red cell distribution width (RDW) in 12 children
- Red blood cell mean corpuscular volume (MCV) in 8 children (4 increased and 4 decreased),
- Increased erythrocyte sedimentation rate (ESD) in 7 children.

White blood cells count:
- Neutropenia (decreased NEU) in 13 children
- Lymphocytosis (increased LYM) in 11 children.

Alterations in cellular immunity tests: The alterations were assessed in relation to the reference ranges accepted in the local diagnostic centers are shown below.

Out of the 30 children there were:
- Decrease in T-lymphocyte counts in 6 children,
- Decrease in T-helper counts in 8 children,
- Increase in T-suppressor counts in 9 children,
- Decrease in B-lymphocyte counts in 6 children,
- Increase in NK cell counts in 6 children,
- Reduced immune regulatory index (IRI) in 17 children.
- Other minor changes are shown in the Figure 2.

The summary of alterations in relation to reference ranges in red and white blood cell counts and in the cellular immunity is presented in Figure 2.

NOTE: There were other changes such as thrombocytosis and abnormal hemoglobin level, but these changes were in less than 10% of the children. Moreover, since the tests were conducted in different laboratories in accordance with the preferences of the parents and availability of tests in their cities, not all the same parameters were tested. The abovementioned parameters were tested in all 30 children. The other additional parameters were not considered here because they are either not a common feature for ASD children or they were not tested for in all the children. However, when the number of changes in every child was counted, all changes were taken into consideration.

Collection of ASD anamneses: complications in pregnancy, parturition, and in children’s early life: Out of 30 cases, the parents of 25 children answered questions regarding pregnancy, newborn delivery, and initial period of the child’s life, while 5 parents did not provide this information.

NOTE: As with the red and white blood cell counts, there were parameters tested only in some children, or there were parameters that changed only in a small minority of the children. So, only the parameters that are the most common, and that were tested in all the children are included. However, when the number of changes in every child was counted, all changes were taken into consideration.
Pregnancy period: There were 21 mothers who experienced various complications during the pregnancy period. Some of the mothers had more than one complication. There were 13 cases of respiratory infections, 5 cases of increased risk of miscarriage, 2 cases of partial placenta detachment, and 2 cases of vaginal bleeding.

Parturition (mothers): There were 5 women who had stimulated parturition and 4 had cesarean sections due to delivery complications. There were 2 cases of premature birth of low birthweight newborns and 2 cases of prolonged periods of anhydramnios before birth.

Parturition (children): There were 17 children born with perinatal hypoxia, 3 had newborn jaundice, and 3 were diagnosed with CNS damage.

Initial period of life: There were 19 parents who reported the common patterns of a child who was unusually quiet and characterized by the parents as “causing no inconvenience” but having difficulties getting to sleep. Detailed discussion with the parents revealed that these children had different levels of disengagement, reduced social interest, and noticeable passivity. Four other children, according to their parents were “very problematic”, “hypersensitive”, “irritable”, and “suffered from difficulties getting to sleep”. Only 2 children’s states can be described as “usual”, but the parents could not remember details.

The summary of these patterns is presented in Figure 3.

Collection of information on previously treatment methods: According to the parents of these children, prior to the start of the recommended therapy they used many different methods of treatment and a variety of medications and supplements. The data on the types of these are shown in the Tables 1 and 2, with the number of children who had taken the medications and therapies. According to the parents, no dramatic improvement had been achieved using any of the previous treatments.

Side reactions: During the treatment process special attention was paid to any possible side effects caused by the proposed composition. Parents were requested to inform us of any reactions their children experienced. Throughout the period of observation there were no cases of side effects requiring termination of treatment. The following mild side reactions were observed in children in the first two weeks (number of cases): increased tearfulness - 5, headache - 2, unstable behavior - 2, nocturnal enuresis - 2, diarrhea - 2, difficulty falling asleep - 1, reduced appetite - 1, skin rash - 1. In all cases, these reactions were observed together with an initial noticeable improvement and parents insisted on continuing the treatment.

Figure 3: The complications related to pregnancy, parturition, and problems/peculiarities of the children in the initial period of life. Each column represents a time period and different colors are used to identify the related complications or peculiarities during that period. The numbers inside the columns represent the number of mothers/children having the particular problems or peculiarities.

Treatment results
Table 1: The list of medications and therapies that had been taken and received by ASD children prior to antiviral treatment. The most common medication was Cortexin with 15 children taking it and next was supplements with 12 children taking them. The most common therapies were speech therapy with 16 children receiving it, followed by psychological therapy with 14 children receiving it.

<table>
<thead>
<tr>
<th>Medication</th>
<th># of children who took the medication</th>
<th>Therapy</th>
<th># of children who received therapy</th>
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<td>Speech therapy</td>
<td>16</td>
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<tr>
<td>Supplements</td>
<td>12</td>
<td>Psychological therapy</td>
<td>14</td>
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<tr>
<td>Cogitum</td>
<td>9</td>
<td>ABA therapy</td>
<td>12</td>
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<td>Pantogam</td>
<td>8</td>
<td>Massage</td>
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<td>Reflexotherapy</td>
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<td>Cerebrolysin</td>
<td>5</td>
<td>Exercise therapy</td>
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<td>Encephabol</td>
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<td>Defectologist</td>
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<td>Noofen</td>
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<td>Micropolarization</td>
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<td>Gammunol</td>
<td>4</td>
<td>Acupuncture</td>
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<td>Ceraxon</td>
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<td>Sensory integration therapy</td>
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<td>Depakin</td>
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<td>Phenotropil</td>
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Changes in blood tests: According to the blood tests, which when possible, were performed after each course of treatment, there was a general tendency towards normalization of measured parameters. Analyzing the changes in blood parameters, we were mainly focusing on six parameters of red and white blood cell counts and six parameters of cellular immunity. These are: erythrocyte sedimentation rate (ESD), neutrophils count, lymphocytes count, erythrocytes count, mean corpuscular volume (MCV), red blood cell distribution by width, T-lymphocytes, T-suppressors, T-helpers, B-lymphocytes, NK cells, and immunoregulatory index (IRI) in relation to reference levels accepted in the local diagnostic centers. Selection of these parameters is based on the fact that they show the presence/absence of infection, inflammation, and any immune system aberrant changes. For example, erythrocytosis is a sign of hypoxia [80], which may be caused by oxidative stress [81] and of folate-deficiency anemia [82]. Lymphocytosis may be a sign of chronic herpetic infection [83,84]. Neutropenia usually indicates a viral infection [45], inflammation and a disrupted immune system [85]. Increased or decreased MCV, as well as increased RDW, point to a folate deficiency [86-88] and increased ESD is a sign of infection [89], and inflammation [17].

The comparison of the results over the courses of treatment showed that there was a general tendency towards normalization of these parameters, which means that the treatment resulted in some decrease of infectious and inflammatory states and in immune system status improvement. The trends in the changes of red and white blood cells and in cellular immunity were assessed using a linear regression model. This model allowed us to obtain results in plots that give information on the changes over time and the influence of the covariates [90]. In this particular case the covariates are the number of treatment courses. The Figures 4 and 5 represent these parameters in all 30 children before treatment and the changes observed during the treatment process. The red line in the graphs represents the general trends towards the reference values. Apart from changes in the immune system and decrease of inflammation, it was noticed that there was a decrease in antibodies to infections, which approached reference levels. The most significant changes were noticed in the reduction of CMV and rubella virus.
Changes related to ASD and other health disorders: All the parents reported changes in their children’s ASD and ASD-related signs and symptoms. The most pronounced effect was observed in social communication skills (29 out of 30 children showed improvements), verbal function (23 out of 30 children) and neurological and psychological disorders (20 out of 26 children). Additionally, improvements were reported in restricted and repetitive behavior (12 out of 30 children), gastrointestinal and respiratory tract-related disorders (11 out of 15 children). Nineteen children showed improvements in non-categorized skills, such as, self-dependence, memorization, cognitive function, and motor skills. The number of children having the symptoms in each of these categories as well as the number of children having changes in the symptoms are represented in the Figure 6.

Changes in social communication skills: Almost all, 29 out of 30 children, had changes in social communication. Based on the parents’ reports regarding the changes they observed in their children, we divided these changes in each category into separate skills. In the social communication category these skills include: improved understanding of the addressed speech, more developed communication, improved eye contact, playing with other children, appearance of empathy, appearance of attachment to family members, improved nonverbal communication, appearance of reaction and response to other people, and jokes, and gestures for communication. Many children had more than 1 change. The number of children having changes in each of these skills is indicated in the Figure 7.

Changes in verbal skills: Verbal skill changes were noticed in 23 out of 30. The particular changes included increased vocabulary, usage of full sentences, more conscious and constructive speech, verbal expression of thoughts and emotions, participation in dialogues, repetition of words after parents (relate to those who were nonverbal), clearer pronunciation became. Many children had more than 1 change in the category. Figure 8 represents the number of children having each of these changes.
Figure 6: The number of children having ASD and ASD-related signs and symptoms before initiation of treatment and the improvements in these categories during treatment.

Figure 7: The changes in social communication skills after treatment. The social skills were divided into groups of skills in which the changes were reported after treatment. The graph represents the number of children, in whom these categories of communication skills took place. a) Improved understanding of addressed speech; b) More communicative; c) Improved eye contact; d) Playing with peers; e) Empathy appeared; f) Improved nonverbal communication; g) Attachment to family members; h) Started reacting to other people; i) Began to joke and to understand jokes; j) Started using gestures for communication (nonverbal child).

Figure 8: Changes in verbal skills after treatment. Verbal skills were divided into groups of skills in which the changes were reported after treatment. The graph represents the number of children in whom these categories of communication skills took place. a) Increased vocabulary; b) Full sentences appeared in speech; c) Speech became more conscious and constructive; d) Child stated his/her thoughts and emotions verbally; e) Child started participating in dialogues; f) Child started to repeat some words after parents (those who were nonverbal); g) Pronunciation became clearer.
Changes in restricted and repetitive behavior: Out of 30 children 12 had changes in restricted and repetitive behavior. The 12 children with changes in behavior had one of the changes listed in this category. The changes were: reduced repetitive behavior (including the complete elimination of repetitions), less extensive focusing, and decreased echolalia. The number of children having each of these changes is represented in Figure 9.

Changes in psychological and neurological conditions: Out of 30 children, 26 had some neurological and psychological problems, and 20 of them showed changes in those conditions. The changes included reduced hyperactivity, improved studying process, reduced anxiety, improved attention, improved sleep, and reduced hypersensitivity. The number of children having each of these changes is represented in Figure 10.

Changes related to the immune system and gastrointestinal tract diseases: Out of 30 children 15 initially had problems in these categories. Out of those 15 with problems, 10 showed continuous changes during the process of treatment. The changes included improved appetite and widening of food preferences (in those who had limited preferences), improved resistance to respiratory infections (shorter duration and lesser frequency and milder symptoms), less frequent constipation, and loss of excessive weight. The number of children having each of these changes is represented in Figure 11.

Changes in other skills: There were some changes that could not fall into any of the previous categories, so they were grouped into a separate category called “Other skills”. Changes in this category included: increased self-dependence (independent homework performance, toilet usage, dressing up, etc.), improved cognitive function (increased imagination, logical thinking, etc.), improved memorization, improved physical and motor skills, improved writing skills. The number of children having these changes is represented in the Figure 12.
Discussion
Based on numerous research and clinical publications, our treatment was focused on the conditions typical for ASD children including signs, symptoms and laboratory analytical results, which included: inflammation, chronic/latent infections and immune system aberration.

This treatment resulted in positive changes in blood parameters related to infection, inflammation and immune system function of these 30 children. Most important, the treatment resulted in changes in behavior in all three categories of ASD signs and symptoms (social communication skills, verbal function and restricted repetitive behavior), in two categories of ASD-related symptoms (psychological and neurological problems and immune and GI tract diseased), and in some other categories, such as memorization, cognitive function and motor skills.

In all children receiving treatment, with no exception, responses were observed. The extent of the changes varied depending on the treatment period length, a child’s age, severity of infection and of immune disruption, and probably on some other factors. Nevertheless, all children experienced improvements either in one or several categories assessed. The most pronounced effect was observed in social communication skills (29 out of 30 children showed improvements), verbal function (23 out of 30 children) and neurological and psychological disorders (20 out of 26 children). These are the skills essential for a high-quality life, and the relief of these symptoms allows these children to integrate into society. Additionally, some improvements were observed in restricted and repetitive behavior (12 out of 30 children) and gastrointestinal and respiratory tracts health (11 out of 15 children). Nineteen children showed improvements in non-categorized skills: self-dependence, memorization, cognitive function, and motor skills. The results of the treatment focused on comorbidities relieved some symptoms of ASD and improved the quality of life of the treated children, although none of the administered drug and nutritional supplementations were not directly aimed at the signs and symptoms of the disorder.

Limitations of the study
The study’s limitations include: some differences in equipment and reference parameters for lab analyses by local clinical diagnostic centers. Because of this, the results were given in relation to reference ranges. Another limitation is that the study was not prospective, parents with local physicians started a continued treatment at different times and according to recommendations (in many cases) of their local physicians. As well, due to relatively small number of the cases the results cannot be interpreted as representatives of all ASD cases.

Supplementary Materials
The description of individual cases and their improvements in terms of ASD signs and symptoms can be found in the supplementary materials (Supplementary Table 1).

Conclusions
1) Based on the scientific literature about the role of infections, inflammation and immune aberrations in the initiation and progression of ASD we developed a composition of Valacyclovir and 5 nutritional supplements aimed at treatment of ASD comorbidities.

2) The composition was administered to 30 children aged 2.5 to 16 years for 1-5 months in dosages depending on the child’s age.

3) The treatment resulted in the shift of laboratory blood and immune parameters towards normalization.

4) Along with the normalization of the parameters, all children had positive changes in ASD and ASD-related signs and symptoms, which included social communication skills, verbal skills, restricted and repetitive behavior, psychological and neurological conditions, gastrointestinal tract, and the immune system related conditions, and in some other skills.

5) There were no cases of side effects requiring termination of treatment. The following mild side reactions were observed in children in the first two weeks: increased tearfulness, headache, unstable behavior, nocturnal enuresis, diarrhea, difficulty falling asleep, reduced appetite, and skin rash. In all cases, these reactions were observed together with an initial noticeable improvement and parents insisted on continuing treatment.

6) According to the parents of all 30 children, this treatment results are much more positive than results of 42 types of treatment that had been administered and applied to their children before.

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Compliance with Ethical Standards

The authors have no competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from parents of all individual participants included in the study.

Authors’ Contributions

KA contributed to the conceptualization, conceived of the study, participated in its design and coordination, interpretation of the data, and drafting of the manuscript; SF helped to secure funding for the study, participated in its design, coordination and interpretation of the data, and drafting of the manuscript; AT participated in the coordination of the study, in data interpretation, drafting and revising the manuscript; AM participated in data interpretation, drafting and revising the manuscript; TI participated in the initial design of the study, assisted in data interpretation and revision of the manuscript. All authors read and approved the final manuscript.

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