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The effectiveness of combined application of transfer factors in natural killers and natural killers T-cells deficiency in children with autism spectrum disorders

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ABSTRACT

The present study aims to evaluate the efficacy and safety of combination immunocorrection with transfer factors in autism spectrum disorders (ASD) children with genetic deficiency of the folate cycle (GDFC) and natural killer (NK) and/or natural killer T-lymphocyte (NKT) cell deficiency. The single-center, retrospective, controlled, non-randomized clinical trial analyzed medical records of 225 ASD children with GDFC aged 2-9 years. SG received the transfer factor classic in dose 2 caps 3 times a day and transfer factor trifactor in dose 1 caps 3 times a day during 3 months. The control group involved 52 ASD children with GDFC who followed the same age and gender distribution pattern but did not receive immunocorrection. The number of NK cells reached the lower limit of reference range in 109 out of 146 patients (75% of cases) with baseline deficiency of these lymphocytes. The combination of transfer factors is an effective and safe strategy for NK- and NKT-cell deficiency correction in children with ASD.

Keywords: immune status, immunocorrection, immunoprophylaxis

INTRODUCTION

The results of research over the past decades have elucidated that immunodeficiency and the associated immune dysregulation are crucial components in the pathogenesis of multisystemic involvement in children with autism spectrum disorders (ASD) [1-3]. Data from five meta-analyses and systematic reviews of randomized controlled clinical trials confirming the association between ASD and genetic deficiency of the folate cycle (GDFC) shed light on key factors of genetic predisposition to the development of severe neuropsychiatric syndromes in children [4-8]. The findings of the latest three systematic reviews of controlled clinical trials characterized the spectrum of immunological disorders typical for children with ASD, among which a deficiency of natural killers (NK), natural killer T-lymphocytes (NKT), and, conversely, abnormally elevated levels of CD3+ T-lymphocytes in peripheral blood are identified as essential components [9-11].

Immune system disorders shed light on the origin of a range of immunodependent clinical manifestations (allergic, autoimmune, immunoinflammatory, etc.) characteristics of children with ASD, which cannot be solely explained by the presence of mental dysfunction [12-14].

Correction of immune disorders in children with ASD appears to be a promising tool not only for restoring immunoresistance to microorganisms but also for preventing the development of numerous immunodependent complications affecting both the central nervous system 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 exhibiting signs of immune dysfunction. A recent report on the effectiveness of combined immunotherapy with propes and inflamaferin in alleviating key innate immune system disorders in children with ASD associated with GDFC, particularly the deficiency of NK and NKT, suggests the treatability of immune dysfunction and serves as an impetus for further clinical investigations [15]. The search for alternative, more cost-effective, and user-friendly immunocorrective agents that are at least equivalent in efficacy to the tested peptide immunotherapy is imperative. Transfer factors based on immune colostrum extract from bovine milk may serve as such agents for immunocorrection. Placebo-controlled clinical trials have demonstrated their efficacy in immunodeficiency disorders typical of children with ASD. Specifically, they normalize the immune status, enhance resistance to respiratory and gastrointestinal infections, reduce manifestations of intestinal dysbiosis, normalize intestinal permeability, exert anti-allergic effects, alleviate autoimmune manifestations, and normalize certain neurological and psychiatric disorders [16-18].

It was described the specific composition of natural bovine colostrum proteome in the first 9 days [19]. It wasfound the presence of cytokines, chemokines, antibodies, component of complement system, growth factors, and other biologically active molecules with immunosubstituting and

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immunomodulating proprieties. Similarly, the study in [20] confirmed that all immunomodulatory proteins and peptides from natural bovine colostrum are preserved on relevant levels in a so-called standardized derivative of bovine collostrums for manufacturing. Thus, equivalent immunomodulatory proteome compositions have been estimated in natural and manufactured collostrums. These components are able to raise levels of NK and NKT cells in blood; cytokines IL-2 and IFN-gamma, for example, use their specific membrane receptors or immunoglobulins, activating Fc-receptors on NK and NKT cells surface.

There is a justified need for the testing of transfer factors for correcting key disorders of cellular immunity in children with ASD associated with GDFC. This could provide practical medicine with a means of preventing the development of immunodependent complications that impact the quality of life, severity of the condition, and resilience of patients with neuropsychiatric disorders.

Research Objective

To investigate the effectiveness and safety of the combined use of classical transfer factor and trifactored transfer factor in children with ASD associated with GDFC, considering their impact on the quantity of CD3+ T-lymphocytes in the blood.

Research Tasks

1. Evaluate the dynamics of NK lymphocyte count in the blood during the application of classical transfer factor and trifactored transfer factor in children with ASD associated with GDFC.
2. Investigate the dynamics of NKT cell count in the blood during the application of classical transfer factor and trifactored transfer factor in children with ASD associated with GDFC.
3. Determine the dynamics of CD3+ T-lymphocyte count in the blood during the application of classical transfer factor and trifactored transfer factor in children with ASD associated with GDFC.
4. Assess the effectiveness and safety of the application of classical transfer factors and trifactored transfer factors for correcting key signs of immune dysregulation in children with ASD associated with GDFC.

MATERIALS AND METHODS

In pursuit of the research objective and the defined tasks, medical records of children with GDFC and ASD were scrutinized within the experimental group (EG) (n = 225, aged 2 to 9 years). The EG consisted of 183 boys and 42 girls who were patients at the Institute of Immunology and Allergology at O. O. Bogomolets National Medical University (NMU) from 2012 to 2018 and the Vivere Clinic specializing in neuroimmunology from 2019 to 2022.

The registration dossier of the Vivere Clinic is numbered 10/2212-M dated 22.12.2018. Subsequent processing of clinical material, following the acquisition of medical data at the clinic, was conducted at the Research Institute of Experimental and Clinical Medicine at O. O. Bogomolets NMU. To facilitate this, a cooperation agreement (contract no. 150221, 15.02.2021) was established. Additionally, an ethical committee conclusion

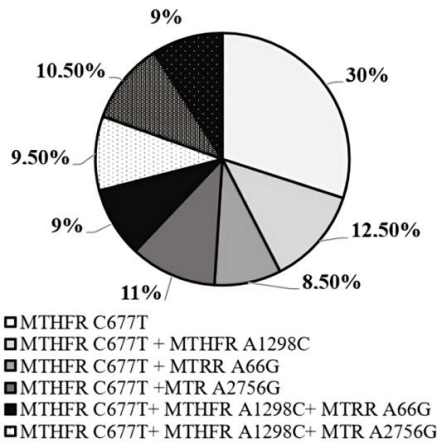

Figure 1. The genetic testing results for GDFC in the structure of the EG (n = 225) (Source: Author's own elaboration)

from NMU, documented in protocol no. 140 (21.12.2020), was obtained. The clinical diagnosis of ASD was ascertained by experienced child psychiatrists with specialization in neurodevelopmental disorders, utilizing validated diagnostic criteria from DSM-IV-TR.

Pathogenic polymorphic nucleotide substitutions in the genes of folate cycle enzymes for the diagnosis of GDFC in the patient observation groups were identified using the polymerase chain reaction (PCR) with restriction enzyme analysis. This molecular analysis was conducted at the neurobiochemistry department of the A. P. Romodanov Institute of Neurosurgery of the National Academy of Medical Sciences of Ukraine from 2012 to 2018 and at the Synevo Laboratory, Ukraine, from 2019 to 2022. According to the manufacturer, the sensitivity of the PCR test used in this study was 92%, and the specificity was 96%. With regard to limitations, PCR analysis can produce both pseudonegative and pseudopositive results in 2-3% of cases.

In this context, nucleotide substitutions in *MTHFR C677T* were identified both in the mono-form (68 patients in the EG; 30% of cases) and in combination with other pathogenic nucleotide substitutions, specifically with *MTHFR A1298C, MTRR A66G,* and/or *MTR A2756G* (157 individuals in the EG; 70% of cases). The genome encompassing dual pathological nucleotide substitutions *MTHFR C677T* and *MTRR A66G* occurred in 19 cases (8.5%), *MTHFR C677T* and *MTHFR A1298C* in 26 cases (12.5%), and *MTR A2756G* and *MTHFR C677T* in 25 children in the EG (11% of cases).

The genome containing triple pathological nucleotide substitutions *MTHFR C677T, MTR A2756G, MTRR A66G* was observed in 23 cases (10.5%), *MTHFR C677T, MTR A2756G, MTHFR A1298C* in 22 cases (9.5%), and *MTHFR C677T, MTRR A66G, MTHFR A1298C* in 21 cases (9%) of children in the EG. A genome containing four pathogenic polymorphic nucleotide substitutions *(MTHFR C677T, MTHFR A1298C, MTRR A66G, MTR A2756G*) was established in 21 cases (9% of cases) among children in the EG (**Figure 1**).

The quantity of CD3+ T-lymphocytes, NK and NKT cells in patients was measured using a laser flow cytometer, Epics XL (USA). In this process, the method of indirect immunofluorescence with monoclonal antibodies to CD markers with single and triple labelling was employed, utilizing reagents from Beckman Coulter (USA). NK cells were defined as a lymphocyte subpopulation with the phenotype CD3-

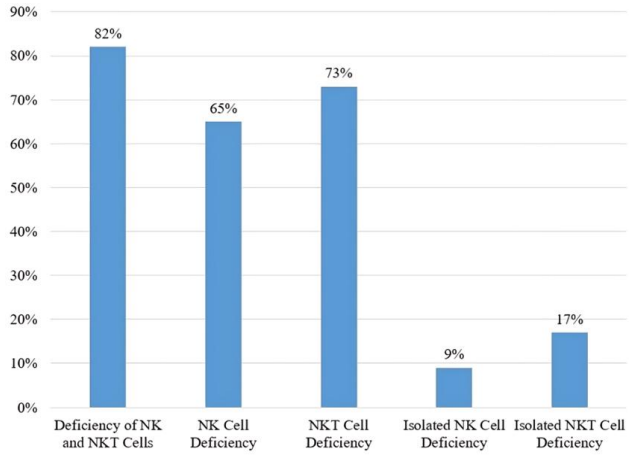

Figure 2. The structure of EG (n = 225) based on deficiencies of NKT and NK cells in peripheral blood (Source: Author's own elaboration)

CD16+CD56+, while NKT cells were defined as a lymphocyte subpopulation with the phenotype CD3+CD16+CD56+. All methodological procedures related to indirect immunofluorescence were performed with accuracy according to the established manufacturing guidelines of Beckman Coulter company (https://www.beckmancoulter.com/products/immunoassay).

The average quantity of CD3+ T-lymphocytes in the peripheral blood of patients in the EG at the beginning of the study was 4.24 ± 0.15 × 10^9/L vs. 4.11 ± 0.18 × 10^9/L in control group (CG). The number of CD3+ T-lymphocytes in the peripheral blood among children in the EG exceeded the upper limit of the norm in 72% of cases, while in the remaining 28% of cases, it was within the normal range. The average number of NK cells in the EG at the beginning of the study was 0.08 ± 0.004 × 10^9/L vs. 0.11 ± 0.005 × 10^9/L in CG, and the average number of NKT lymphocytes was 0.03 ± 0.009 × 10^9/L vs. 0.05 ± 0.004 × 10^9/L in CG. At the beginning of the study, a combined deficiency of NK and NKT cells was present in 82% of patients in the EG, while isolated NK cell deficiency was observed in 65% overall and isolated form in only 9% of cases. In contrast, NKT cell deficiency was observed at 73% overall and isolated form in 17% of cases among patients in the EG (**Figure 2**).

As the level of lymphocytes differs depending on a child's age, the experiment utilized only lower range of lymphocyte count–1.2 × 10^9/L. A count of lymphocytes below this level has not been recorded since the Chornobyl disaster in Ukraine; therefore, in the current study, this condition was diagnosed as lymphopenia. The criteria for diagnosing a deficiency of NK and NKT cells were based on the manufacturer's data on monoclonal antibodies for laser flow cytometry (Beckman Coulter, USA)–lower than 5% for NK and lower than 3% for NKT.

Thus, the inclusion criteria for EG were ASD, GDFC (MTHFR 677 CT alone or in combination with other polymorphisms), and a deficiency of NK and/or NKT cells in blood. Exclusion criteria were over 9 years, the absence of MTHFR 677 CT, even if other polymorphisms were present. The experiment did not include patients with any psychiatric or somatic conditions comorbid with ASD that required medications in addition to the transfer factor medications.

Patients in the EG, for the purpose of immunocorrection, were administered original standardized immune extracts from bovine colostrum, namely *transfer factor classic 4life (UltraFactor XF®–600 mg)* at a dosage of 2 capsules three times a day during meals, and *transfer factor tri-factor 4life (UltraFactor XF® 600 mg + OvoFactor® + NanoFactor®)* at a dosage of 1 capsule three times a day during meals for a consecutive 3-month period. *UltraFactor XF®* is an ultrafiltrate of immune proteins and peptides from bovine colostrum, *OvoFactor®* is a highly concentrated extract of immune proteins and peptides from chicken egg yolks, and *NanoFactor®* is a concentrated nanofiltrate of bovine colostrum. Measurements of CD3+ T-lymphocytes, NK cells, and NKT cells for patients in the EG were conducted monthly, four times during the course, including before the start of the study and after 1st, the 2nd, and 3rd months of the tested immunotherapy.

The CG consisted of medical records of 52 children with ASD and GDFC, matched in age (2-8 years) and gender distribution (37 boys and 15 girls), where similar deficiencies of NK and NKT cells and the distribution of the polymorphisms of folate cycle genes were observed, resembling the EG. Patients in the CG did not receive transfer factors during the observation period. The children in the CG also underwent a four-time monthly assessment of the quantities of CD3+ T-lymphocytes, NK and NKT cells over a consecutive 3-month period to evaluate the immune status during the natural course of the disease.

The allocation of participants to the study groups was conducted as follows: parents of children with ASD and GDFC were provided with proven immunotherapy using transfer factors in order to compensate for any existing cellular immunodeficiency. The parents made their decisions independently, without any pressure from the researcher. Children whose parents agreed to receive immunotherapy were assigned to the EG group, while those whose parents declined immunotherapy were allocated to the CG group.

Whole exome sequencing is a standard procedure in the laboratory observation of children with ASD in the Vivere Clinic (InVitae Laboratory, USA). The children with confirmed Mendelian genetic diseases were excluded from this study at early first stage and did not participate in this study. This was done to rule out inborn errors of immunity.

Statistical analysis of the obtained data was performed using comparative and structural analyses. The distribution of variants in the variation series was examined using the Shapiro-Wilk test. To assess the likelihood of observed differences between the values of the laboratory parameters investigated in the observation groups, the parametric Student's t-test was applied, with an additional measurement of the probability index (p-value), and the non-parametric sign test Z [21]. Differences were considered significant at $p < 0.05$ and $Z < Z_{0.05}$.

To investigate the correlation between the administration of transfer factors and the dynamics of the studied immune status indicators, the odds ratio (OR) and 95% confidence interval (95% CI) were calculated. Data were processed using the *Microsoft Excel* computer program (Redmond, WA).

RESULTS

Structural analysis results within observation groups indicate that the quantity of NK cells reached the lower limit of the norm in 109 out of 146 patients (75% of cases) with an initial deficit of these lymphocytes. Meanwhile, the average number

Table 1. The structure of EG (n = 225) and CG (n = 52) by relative weight of patients with normal quantity of NK and NKT cells

Parameter	EG	CG
NK	75%	15%*
NKT	77%	21%*

Note. $p < 0.05$ and $Z < Z_{0.05}$

of NK cells in the blood of the study group increased nearly threefold over the 3-month course of transfer factor therapy. In contrast, the quantity of NKT cells normalized in 127 out of 164 patients (77% of cases) with an initial deficit of these cells, and the average number of NKT cells in the blood of the EG increased almost fourfold during the course of immunotherapy compared to the initial level.

The number of NK lymphocytes increased, reaching the lower limit of the norm in 7 out of 36 patients (15%), and the number of NKT cells increased in 9 out of 42 children (21% of cases) in the CG, who initially had a low count of such lymphocytes, which constituted a significant difference from the EG ($p < 0.05$; $Z < Z_{0.05}$) (**Table 1**).

The obtained results of structural analysis within the observation groups indicate that deficits in NK and NKT cells are not only prevalent but also relatively stable laboratory phenomena in children with ASD associated with GDHC, typically not subject to spontaneous resolution during the natural course of the disease. The combined application of transfer factors contributes to the restoration of the previously reduced quantity of NK and NKT lymphocytes in peripheral blood in the majority of children with ASD associated with GDHC, compensating for key disorders of cellular immunity and thereby modifying the immune status of patients.

Comparative analysis data suggest no differences in the achieved relative weight of complete responders to the tested combination of transfer factors regarding the correction of NK and NKT cell deficits among patients in the EG at the end of the observation period ($p > 0.05$; $Z > Z_{0.05}$). This implies that the combined application of transfer factors is equally effective for deficits in NK and NKT lymphocytes in children with ASD associated with GDHC, regardless of the initial low quantities of these cells in peripheral blood. Furthermore, comparative analysis data did not reveal a significant difference in the relative weight of responders to immunocorrection interventions using transfer factors between isolated deficits in NK and NKT cells and combined disorders when a patient exhibits a simultaneous reduction in the quantity of both NK and NKT lymphocytes ($p > 0.05$; $Z > Z_{0.05}$). This may indicate that NK and NKT cells respond to transfer factors separately, in an independent manner, and patients with isolated and combined disorders are equally responsive to the applied immunocorrection interventions.

The data from the dynamic observation of the investigated laboratory indicators of immune status in the EG and CG indicate a gradual increase in the quantity of NK and NKT cells in patients with ASD in each control point, reaching the maximum level at the end of the immunocorrection course, with no significant changes observed in the average quantities of NK and NKT lymphocytes in the peripheral blood of individuals in the CG throughout the observation period. The average quantity of NK cells in the peripheral blood increased threefold over the observation period in the EG, and NKT cells increased by more than fourfold (**Figure 3** and **Figure 4**). A significant difference was noted between the initial average quantities of NK and NKT cells in the EG at the beginning of the

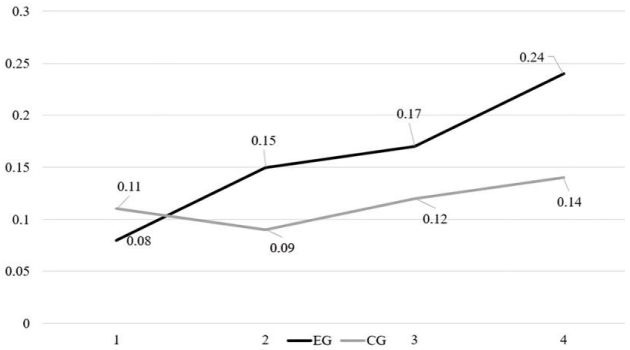

Figure 3. Dynamics of the average quantity of NK cells in the blood of patients in EG (n = 225) and CG (n = 52) (Source: Author's own elaboration)

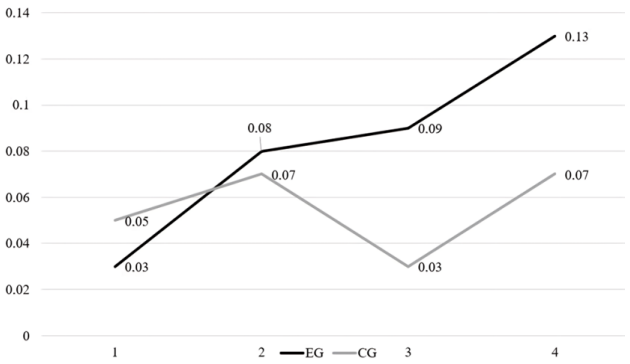

Figure 4. Dynamics of the average quantity of NKT cells in the blood of patients in EG (n = 225) and CG (n = 52) (Source: Author's own elaboration)

research and the end of the 3rd month of immunocorrection ($p < 0.05$; $Z < Z_{0.05}$). A similar significant difference in the average quantities of NK and NKT lymphocytes was found among patients in EG and the CG in the 3rd month of observation ($p < 0.05$; $Z < Z_{0.05}$). However, no significant difference in the average quantities of NK and NKT cells in the CG was established at all control points throughout the entire observation period ($p > 0.05$; $Z > Z_{0.05}$). This may indicate that the application of transfer factors not only normalizes the previously reduced quantity of NK and NKT lymphocytes in the peripheral blood of children with ASD associated with GDHC but also ensures a progressive increase in the number of these cells over time throughout entire course of immunocorrection. This time-dependent response to the application of transfer factors in deficits of NK and NKT cells in children with ASD associated with GDHC allows for justified expectations of the success of immunocorrection interventions by prolonging the duration of their application without increasing the dosage of immunobiological agents when the lower limit of the norm for the investigated laboratory indicator was not achieved after the previous course of immunocorrection.

When assessing the dynamics of the CD3+ T-lymphocyte count in the observation groups, no significant difference was observed in the average quantities of these cells in peripheral blood in patients both in the EG and the CG at all control points throughout the study period ($p > 0.05$; $Z > Z_{0.05}$). Similarly, no significant difference was noted in the average quantities of CD3+ T-lymphocytes in peripheral blood between the EG and CG at all control points throughout the observation period ($p > 0.05$; $Z > Z_{0.05}$) (**Figure 5**). This indicates that the application of

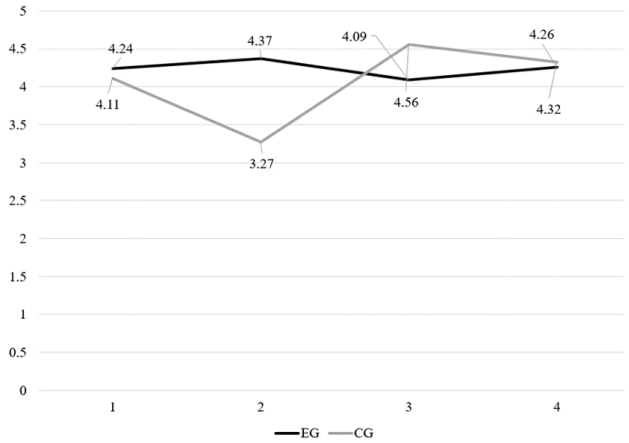

Figure 5. Change in the average quantity of CD3+ T-cells in the blood of patients in EG (n = 225) and CG (n = 52) (Source: Author's own elaboration)

transfer factors does not affect the quantity of CD3+ T-cells in the peripheral blood of children with ASD associated with GDHC. These patients exhibit a state of immune dysregulation associated with a range of immune-related complications, and the abnormally elevated quantity of CD3+ T-lymphocytes in peripheral blood is a significant component of the mentioned immune dysregulation. During the testing of transfer factors, it was crucial to assess not only the effectiveness of correcting NK and NKT cell deficits but also the safety in terms of impact on immune regulatory disorders. Transfer factors do not increase the quantity of CD3+ T-cells in the blood, indicating the absence of a negative impact on the existing immune dysregulation in children with ASD, one manifestation of which is the high quantity of cells in the mentioned lymphocyte population in the blood.

To verify the obtained data regarding the association of applied immunotherapeutic interventions and the normalization of NK and NKT cell quantities in the EG at the end of the observation period, the OR, standard error of the OR (SE), and a 95% CI were calculated. The "outcome" referred to the normalization of the quantity of the investigated lymphocyte subpopulations, while the "risk factor" was the application of transfer factors. This approach aimed to avoid errors in assessing the correlation between the studied processes in the earlier stages of statistical analysis. The results obtained are presented in **Table 2**. As evident from the data in **Table 2**, the calculation of the OR and 95% CI confirms the previously obtained results regarding the close association between the application of transfer factors and the normalization of the investigated indicators of immune status in the EG patients. This reiterates the fact, identified at the earlier stage of statistical data analysis, of the nearly equal high sensitivity of NKT and NK lymphocytes to combined immunocorrection interventions using transfer factors. Specifically, the application of transfer factors in the EG was associated with an almost 12-fold increase in the odds of normalizing previously reduced quantities of NKT and NK cells in peripheral blood.

Thus, a stable immunomodulatory effect from transfer factor therapy was observed throughout the entire treatment course. However, the effect after the completion of the observational period was not studied in this research. There were no significant differences in the transfer immunomodulatory effect based on age, gender, or severity of ASD at the time of inclusion to this study. The tolerability of

Table 2. Results of OR, SE, and 95% CI measurements in assessing the association of immunocorrection interventions and positive changes of immunological indicators in EG (n = 225)

Indicator	NK	NKT
OR	12.205	12.586
SE	0.462	0.420
95% CI	4.934-30.191	5.526-28.661

transfer factors was good, and no side effects were noted during the observation period. According to the reports provided by parents, the frequency of infections was lower in the EG compared to the CG during the treatment course and the next 3 months. However, these data were not confirmed by further observations since this was outside the scope of the current study.

DISCUSSION

The results of this clinical study broaden the list of potential indications for the use of transfer factors based on immune extract from bovine colostrum in correcting immune disorders in children with ASD associated with GFCD. Transfer factors based on immune extract of bovine colostrum are able to normalize previously decreased numbers of NK and NKT cells in blood, avoiding a significant proliferation of overnumbered CD3+ T-cell lymphocytes and preventing any evident side effects in autistic children who suffer from GFCD. These data can be included in general scientific knowledge about immune dysregulation in ASD children and immunomodulatory properties of bovine colostrum extracts. The next part of the discussion provides a general overview of the current state of scientific research in these two fields. It aims to show the potential role and significance of the data obtained regarding new immunomodulatory effects of bovine colostrum extracts.

Currently, a broad spectrum of immune system dysfunctions in children with ASD has been described and characterized. The findings of this scientific inquiry have been synthesized in the data of three recent systematic reviews of clinical studies. For instance, the systematic review in [9] demonstrated signs of immune dysregulation in ASD, including neuroinflammation, autoantibodies, abnormally enhanced T-cell response, impaired activity of NK, and monocytes. These immune aberrations were associated with the severity of ASD-related psychopathologies, including social interaction impairments, stereotypical behavior, and reduction in communicative skills. Notably, experimental animal models allowed the alleviation of clinical symptoms of ASD after the removal of immune factors implicated in aberrant immune reactions [9]. In line with this, the study in [10] described signs of immune dysregulation in their systematic review on ASD in children, encompassing hyperproduction and suppression of anti-inflammatory cytokines, increased blood-brain barrier permeability, abnormal production of anti-brain autoantibodies, and modification of NK cell functional activity.

According to the systematic review in [11] on the immune system status in children with ASD, an imbalanced cytokine profile, altered quantity and functional activity of immune-competent cells, manifestations of neuroglial inflammation, pathology of adaptive and innate immunity systems, including NK cell activity, changes in concentrations of immunoglobulins

of various classes, and autoimmune reactions to neurons, myelin, and extracerebral autoantigens, are documented.

As children with ASD represent a highly heterogeneous group, the features of immunological disturbances may vary among specific subgroups of patients. Consequently, a specific form of immunodeficiency has been described in children with ASD associated with gastrointestinal dysfunction and key disorders characterized by a deficiency of NK and NKT lymphocytes [17]. The results of several studies demonstrate diverse immunological disturbances, including NK deficiency, gastrointestinal dysfunction and folate deficiency in humans [22, 23]. The mechanisms of immunosuppression and immune dysregulation in such cases are attributed to gene regulatory and epigenetic disorders linked to disruptions in processes of DNA methylation, proteins, lipids, and nucleoproteins [14]. Additionally, they are associated with a state of persistent oxidative stress and critical impairment in the antioxidant system [24], deficiencies in vitamins, trace elements, and essential nutrients due to immune-inflammatory damage to the intestinal barrier [25].

The mechanism of immune transfer from maternal breast milk to the infant's intestine is one of the fundamental components contributing to the unparalleled advantage of mammals in the struggle for existence in the biological world. The application of transfer factors based on immune extracts from colostrum in clinical practice replicates the physiological mechanism of immune transfer as an integral property of mammals. This property has been evolutionarily maintained through natural selection, convincingly demonstrating its effectiveness in the prolonged evolutionary competition of mammals with representatives of other biological entities [26].

The data obtained from this clinical study indicate the proper immunomodulatory effect of tested immunocorrection strategy using transfer factors in children with ASD associated with global developmental delay (GDD). Immunodeficiency and the related immune dysregulation in children with ASD contribute to immune-dependent complications, impacting the severity of psychiatric disorders and overall child health. In ASD, there is an abnormally high microbial load with a predominance of opportunistic pathogens [27], persistent immune-inflammatory enterocolitis [28], various allergic manifestations [29], a state of systemic inflammation with hypercytokinemia [30], and signs of autoimmunity against neurons, myelin, and some extracerebral autoantigens [31].

The normalization of the compromised immune status could be a key preventive measure against the development of several immune-dependent complications in children with ASD associated with GDD, particularly those characterized by abnormally low numbers of NK and/or NKT cells. The development and implementation of effective immunomodulatory strategies specifically targeting the affected components of the immune system are believed to contribute to the improvement of the clinical condition of children with ASD. The success of transfer factors demonstrated in the results of this clinical study raises hopes for a prompt discovery of answers regarding effective, safe, and convenient immunocorrection interventions for children with ASD exhibiting signs of immunodeficiency and immune dysregulation.

The composition of transfer factors based on the standardized immune extract from bovine colostrum was extensively studied in [20], and a comprehensive characterization of bovine milk in the context of immune transfer was provided in [19]. It has been demonstrated 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 subsequent immune transfer to the infant's intestine during breastfeeding. Currently, immunomodulatory and anti-inflammatory effects of transfer factors based on bovine colostrum immune extract in humans have been demonstrated. In particular, results from double-blind, placebo-controlled randomized clinical trials suggest the potential efficacy of transfer factors in preventing frequent respiratory and gastrointestinal infections in children, preventing sepsis episodes in preterm newborns, and HIV-infected children with an inadequate response to antiretroviral therapy [32-35]. Accordingly, the study in [32] demonstrated in a double-blind, placebo-controlled randomized clinical trial that immune extract from bovine colostrum leads to a reduction in symptoms of ongoing diarrhea caused by enterotoxigenic strains of escherichia coli, prevents the development of dehydration, and reduces the need for infusion therapy. In a double-blind, placebo-controlled randomized clinical trial involving 31 patients, the study in [33] found that oral immunotherapy with immune extract from bovine colostrum significantly reduces the frequency and severity of upper respiratory tract infection episodes in individuals with primary selective IgA deficiency without altering the content of secretory IgA in saliva. In contrast, the study in [34] demonstrated in a double-blind, placebo-controlled randomized clinical trial that immune extract from bovine colostrum reduces the number of sepsis episodes in preterm infants with low birth weight and signs of an immature immune system. They observed an increase in the concentration of secretory immunoglobulin A and lactoferrin in biological fluids, indicating enhanced immune resistance through the optimization of local mucosal immunity. Additionally, they noted a decrease in the levels of transforming growth factor-beta, interleukin-1beta, and interleukin-6 in urine and saliva, suggesting the implementation of an anti-inflammatory effect [34]. The study in [35] in a placebo-controlled randomized clinical trial with 75 participants demonstrated that immune extract from bovine colostrum increases the number of CD4+ T-helper cells in the blood of HIV-infected patients weakly responding to highly active antiretroviral therapy conducted according to international protocols.

Previous controlled clinical studies exploring ways to compensate for the deficiency of NK and NKT cells demonstrate the benefits of immunotherapy with propes and inflamafertin in children with ASD associated with GDD [15] and in adults with chronic fatigue syndrome/myalgic encephalomyelitis with GDD [36]. However, these promising findings need to be verified in larger controlled clinical trials with more significant weight in the obtained results. Propes, a biological agent containing alpha- and beta-defensins, exhibits pronounced immune-activating and lymphoproliferative effects. In contrast, inflamafertin, which includes alarmins and adrenomedullin, exerts anti-inflammatory action mediated by interleukin-10, crucial in preventing autoimmune complications during drug-induced immune activation. Accumulated experience with another highly active immunomodulatory agent, recombinant interleukin-2, indicates that therapeutic immune activation may lead to an undesirable increase in the risk of developing autoimmune complications [37]. Therefore, the combination of the immune-

activating drug propes with the anti-inflammatory tolerogenic immunotropic agent appears to be a guarantee of achieving a safe immunomodulatory therapeutic effect. As the results of this clinical study demonstrate, the combined use of transfer factors is associated with outcomes in correcting NK and NKT cell deficiencies and the tolerability of correction interventions similar to those obtained with propes and inflamafertin. Nevertheless, transfer factors proved to be more cost-effective, making them more accessible to patients. Transfer factors are also technically more convenient, as they do not require prolonged intramuscular injections or special storage conditions. Transfer factors can be used in combination with various other medications for children with ASD, including psychotropic agents. Due to the widespread availability of transfer factors and their global distribution, patients are not required to have a prescription from a doctor in order to use them. Limitations of the current study include its retrospective design, the lack of randomization and the small sample size. Based on the promising initial findings, it is essential to improve the research design and conduct further investigations in this area.

CONCLUSION

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 correcting the deficiency of NK and NKT cells in children with ASD associated with GDD, with an appropriate safety profile. These immunotropic agents normalize the reduced counts of NK and NKT lymphocytes during a 3-month course of immunocorrection, exhibiting a similar effect on and NK and NKT in a time-dependent manner without increasing the number of CD3+ T cells in the patients' peripheral blood. The clinical significance of the obtained results may lie in the potential for a new and effective tool for immunocorrection that is able to influence the frequency of infections, the quality of life and developmental progress. Further investigation is needed to explore this potential clinical benefit. Potential applications in clinical practice could lead to significant improvements in the work of clinical immunologists, infectious disease specialists, and child psychiatrists who currently lack efficient and safe agents for treating cellular immunodeficiency in children with ASD. It seems promising to continue clinical research in the field of transfer factor application in children with ASD who exhibit signs of immunodeficiency and immune dysregulation.

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Data sharing statement: Data supporting the findings and conclusions are available upon request from the author.

REFERENCES

1. Sallam DE, Shaker YS, Mostafa GA, El-Hossiny RM, Taha SI, Ahamed MAEH. Evaluation of serum interleukin-17 A and interleukin-22 levels in pediatric patients with autism spectrum disorder: A pilot study. BMC Pediatr. 2024;24(1):18. https://doi.org/10.1186/s12887-023-04484-2 PMid:38183030 PMCid:PMC10768424
2. Ashwood P. Though shall not pass: Blocking lymphocytes from recirculating to the gut as a potential therapeutic approach for autism. Brain Behav Immun. 2023;116:402-3. https://doi.org/10.1016/j.bbi.2023.12.032 PMid:38157947
3. Than UTT, Nguyen LT, Nguyen PH, et al. Inflammatory mediators drive neuroinflammation in autism spectrum disorder and cerebral palsy. Sci Rep. 2023;13(1):22587. https://doi.org/10.1038/s41598-023-49902-8 PMid: 38114596 PMCid:PMC10730823
4. Pu D, Shen Y, Wu J. Association between MTHFR gene polymorphisms and the risk of autism spectrum disorders: A meta-analysis. Autism Res. 2013;6(5):384-92. https://doi.org/10.1002/aur.1300 PMid:23653228
5. Shaik Mohammad N, Sai Shruti P, Bharathi V, et al. Clinical utility of folate pathway genetic polymorphisms in the diagnosis of autism spectrum disorders. Psychiatr Genet. 2016;26(6):281-6. https://doi.org/10.1097/YPG.0000000000000152 PMid:27755291
6. Rai V. Association of methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism with autism: Evidence of genetic susceptibility. Metab Brain Dis. 2016;3(4);727-35. https://doi.org/10.1007/s11011-016-9815-0 PMid:26956130
7. Sadeghiyeh T, Dastgheib SA, Mirzaee-Khoramabadi K, et al. Association of MTHFR 677C>T and 1298A>C polymorphisms with susceptibility to autism: A systematic review and meta-analysis. Asian J Psychiatr. 2019;46:54-61. https://doi.org/10.1016/j.ajp.2019.09.016 PMid:31614268
8. Li Y, Qiu S, Shi J, et al. Association between MTHFR C677T/A1298C and susceptibility to autism spectrum disorders: A meta-analysis. BMC Pediatr. 2020;20(1):449. https://doi.org/10.1186/s12887-020-02330-3 PMid:32972375 PMCid: PMC7517654
9. Mead J, Ashwood P. Evidence supporting an altered immune response in ASD. Immunol Lett. 2015;163(1):49-55. https://doi.org/10.1016/j.imlet.2014.11.006 PMid:25448709
10. Noriega DB, Savelkoul HF. Immune dysregulation in autism spectrum disorder. Eur J Pediatr. 2014;173(1):33-43. https://doi.org/10.1007/s00431-013-2183-4 PMid:24297668
11. Hughes HK, Mills Ko E, Rose D, Ashwood P. Immune dysfunction and autoimmunity as pathological mechanisms in autism spectrum disorders. Front Cell Neurosci. 2018;12:405. https://doi.org/10.3389/fncel.2018.00405 PMid:30483058 PMCid:PMC6242891
12. Alhuzimi TE, Alharbi MH. Perceptions of parents on management of food allergy in children with autism spectrum disorder (ASD) in Saudi Arabia. Children. 2022;10(1):48. https://doi.org/10.3390/children10010048 PMid:36670599 PMCid:PMC9856587
13. Tordjman S, Charrier A, Kazatchkine M, et al. Natural IgG Anti-F (ab')2 autoantibody activity in children with autism. Biomedicines. 2023;11(3):715. https://doi.org/10.3390/biomedicines11030715 PMid:36979694 PMCid:PMC10045297

14. Alshamrani AA, Alshehri S, Alqarni SS, et al. 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. 2023;13(3):458. https://doi.org/10.3390/metabo13030458 PMid:36984898 PMCid:PMC10057726
15. Maltsev D, Stefanyshyn V. Efficacy of combined immunotherapy with propes and inflamafertin in selective deficiency of NK and NKT cells in children with autism spectrum disorders associated with genetic deficiency of the folate cycle. Ro J Neurol. 2021;20(2):211-6. https://doi.org/10.37897/RJN.2021.2.13
16. Artym J, Zimecki M. Colostrum and lactoferrin protect against side effects of therapy with antibiotics, anti-inflammatory drugs and steroids, and psychophysical stress: A comprehensive review. Biomedicines. 2023;11(4):1015. https://doi.org/10.3390/biomedicines11041015 PMid:37189633 PMCid:PMC10136316
17. Artym J, Zimecki M. Colostrum proteins in protection against therapy-induced injuries in cancer chemo- and radiotherapy: A comprehensive review. Biomedicines. 2023;11(1):114. https://doi.org/10.3390/biomedicines11010114 PMid:36672622 PMCid:PMC9856106
18. Bagwe-Parab S, Yadav P, Kaur G, Tuli HS, Buttar HS. Therapeutic applications of human and bovine colostrum in the treatment of gastrointestinal diseases and distinctive cancer types: The current evidence. Front Pharmacol. 2020;11:01100. https://doi.org/10.3389/fphar.2020.01100 PMid:33071773 PMCid:PMC7533576
19. Zhang L, Boeren S, Hageman JA, van Hooijdonk T, Vervoort J, Hettinga K. Bovine milk proteome in the first 9 days: Protein interactions in maturation of the immune and digestive system of the newborn. PLoS One. 2015;10(2):e0116710. https://doi.org/10.1371/journal.pone.0116710 PMid:25693162 PMCid:PMC4333125
20. Sacerdote P, Mussano F, Franchi S, et al. Biological components in a standardized derivative of bovine colostrums. J Dairy Sci. 2013;96(3):1745-54. https://doi.org/10.3168/jds.2012-5928 PMid:23332842
21. Urbakh VY. Statistical analysis in biological and medical research. Moscow: Medicine; 1975.
22. Maltsev DV. Features of folate cycle disorders in children with ASD. Bangladesh J Med Sci. 2020;19(4):737-42. https://doi.org/10.3329/bjms.v19i4.46634
23. Wu J, You Q, Lyu R, et al. Folate metabolism negatively regulates OAS-mediated antiviral innate immunity via ADAR3/endogenous dsRNA pathway. Metabolism. 2023;143:155526. https://doi.org/10.1016/j.metabol.2023.155526 PMid:36822494
24. Liu X, Lin J, Zhang H, et al. Oxidative stress in autism spectrum disorder-current progress of mechanisms and biomarkers. Front Psychiatry. 2022;13:813304. https://doi.org/10.3389/fpsyt.2022.813304 PMid:35299821 PMCid:PMC8921264
25. Indika N-LR, Frye RE, Rossignol DA, et al. The rationale for vitamin, mineral, and cofactor treatment in the precision medical care of autism spectrum disorder. J Pers Med. 2023;13(2):252. https://doi.org/10.3390/jpm13020252 PMid:36836486 PMCid:PMC9964499
26. Lyons KE, Ryan CA, Dempsey EM, Ross RP, Stanton C. Breast milk, a source of beneficial microbes and associated benefits for infant health. Nutrients. 2020;12(4):1039. https://doi.org/10.3390/nu12041039 PMid:32283875 PMCid:PMC7231147
27. Nicolson GL, Gan R, Nicolson NL, Haier J. Evidence for mycoplasma ssp., Chlamydia pneunomiae, and human herpes virus-6 coinfections in the blood of patients with autistic spectrum disorders. J Neurosci Res. 2007;85(5):1143-8. https://doi.org/10.1002/jnr.21203 PMid:17265454
28. Torrente F, Ashwood P, Day R, et al. Small intestinal enteropathy with epithelial IgG and complement deposition in children with regressive autism. Mol Psychiatry. 2002;7:375-82. https://doi.org/10.1038/sj.mp.4001077 PMid:11986981
29. Li H, Liu H, Chen X, Zhang J, Tong G, Sun Y. Association of food hypersensitivity in children with the risk of autism spectrum disorder: A meta-analysis. Eur J Pediatr. 2021;180(4):999-1008. https://doi.org/10.1007/s00431-020-03826-x PMid:33145704
30. Saghazadeh A, Ataeinia B, Keynejad K, Abdolalizadeh A, Hirbod-Mobarakeh A, Rezaei N. A meta-analysis of pro-inflammatory cytokines in autism spectrum disorders: Effects of age, gender, and latitude. J Psychiatr Res. 2019;115:90-102. https://doi.org/10.1016/j.jpsychires.2019.05.019 PMid:31125917
31. Edmiston E, Ashwood P, Van de Water J. Autoimmunity, autoantibodies, and autism spectrum disorder. Biol Psychiatry. 2017;81(5):383-90. https://doi.org/10.1016/j.biopsych.2016.08.031 PMid:28340985 PMCid:PMC5373490
32. Huppertz HI, Rutkowski S, Busch DH, Eisebit R, Lissner R, Karch H. Bovine colostrum ameliorates diarrhea in infection with diarrheagenic escherichia coli, shiga toxin-producing E. coli, and E. coli expressing intimin and hemolysin. J Pediatr Gastroenterol Nutr. 1999;29(4):452-6. https://doi.org/10.1002/j.1536-4801.1999.tb02450.x PMid:10512407
33. Patıroğlu T, Kondolot M. The effect of bovine colostrum on viral upper respiratory tract infections in children with immunoglobulin A deficiency. Clin Respir J. 2013;7(1):21-6. https://doi.org/10.1111/j.1752-699X.2011.00268.x PMid:21801330
34. Lee J, Kim HS, Jung YH, et al. Oropharyngeal colostrum administration in extremely premature infants: An RCT. Pediatrics. 2015;135(2):e357-66. https://doi.org/10.1542/peds.2014-2004 PMid:25624376
35. Byakwaga H, Kelly M, Purcell DF, et al. 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. J Infect Dis. 2011;204(10):532-40. https://doi.org/10.1093/infdis/jir559 PMid:21930607
36. Maltsev D, Stefanyshyn V. 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. 2022;167(3):443-50. https://doi.org/10.1111/imm.13548 PMid:35866691
37. Ko B, Takebe N, Andrews O, Makena MR, Chen AP. Rethinking oncologic treatment strategies with interleukin-2. Cells. 2023;12(9):1316. https://doi.org/10.3390/cells12091316 PMid:37174716 PMCid:PMC10177415