

## Influence of MNRI on the Immune Status of Children with Down Syndrome

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### Abstract

The clinical and immunological characteristics of 49 children with Down syndrome were studied. Thirty-four boys and 15 girls between the ages of zero and six years old were observed.

It was revealed that children in the Study Group with Down syndrome developed a greater number of disorders starting at the earliest stages of pregnancy and delivery, such as fetal malnutrition, congenital heart defects, and pathology of vision, than children in the control group ( $p < 0.05$ ). All of the children in the Study Group had allergic reactions and were frequently ill. There was a noticed decrease in the numbers of subpopulations of T-lymphocytes (CD45/CD3), CD3/CD4, CD3/CD8 and the absolute number of B-cells (CD45/CD19), and at IgG pool, indicating a certain deficiency in cell-mediated and humoral immune responses which provides a base for frequent diseases, including bacterial diseases. Also an increase in the prevalence of pre-activated cells (CD45/CD25) and NK cells (CD16/CD32/CD56), and a clear increase of IgE ( $1489.5 \pm 467.9$  и  $59.67 \pm 11.8$  IU/L in the control group,  $p < 0.05$ ) was noted, which explains the predisposition in children with Down syndrome to IgE-dependent humoral immune responses and allergic reactions. These specific indicators served as evidence that an evaluation of MNRI as a therapeutic program for improved immunity could be very beneficial. Tests done after two weeks of MNRI therapy showed normalizing of a significant number of abnormal indicators of T- and B- lymphocytes, NK-cells, immunoglobulin levels, and pro- and anti-inflammatory cytokines (IL-2, IL-4, IL-6, IL-10, IL-12, IL-17, IFN- $\gamma$ , TNF- $\alpha$ ).

**Keywords:** Down syndrome; Lymphocytes; Cytokines; Cell-mediated and humoral immunity; Masgutova Neurosensorimotor Reflex Integration (MNRI)

### Introduction

Down syndrome is one of the most common genetic chromosomal abnormalities, which occurs in 1 out of 700 to 1000 newborns. The cause of the defect is a change in the pair at the 21<sup>st</sup> chromosome, which was discovered by Lejeune et al. almost a hundred years after the first description of the syndrome [1]. There are three different syndrome variations: trisomy is the existence of three copies of the 21<sup>st</sup> chromosome, comprising 95% of cases. Translocation is an attachment of a part of the 21<sup>st</sup> chromosome to another chromosome; the frequency of this occurrence is 3-4%. Mosaicism, an error in cell division after fertilization, which results with an extra 21<sup>st</sup> chromosome in some cells, occurs in 1-2% of cases. In 90% of cases a child receives an additional 21<sup>st</sup> chromosome from her mother and 10% from the father. A woman of fertile age has a 0.54% risk to have a child with Down syndrome and this risk increases with age (up to 4.2% woman of 45 and older). In all cases of chromosomal disorders the clinical characteristics are typical and were described by Down [2]. The definition of the "syndrome" includes a various combinations of symptoms and features but always includes two imminent characteristics: intellectual disability and muscle hypotonia.

The most common phenotypes are a flat face with the depressed nasal bridge and nape, short and wide neck, underdeveloped ear lobes, epicanthus, slightly opened mouth, noticeably short limbs with underdeveloped and bent digits and a single line that runs across the palm of the hand called a simian crease. Slow physical development is a typical feature of children with Down syndrome of all ages. This can be combined with abnormal development of the cardiovascular system (50%) and other systems along with hearing difficulties, nearsightedness and cataracts, hypothyroidism, scoliosis, and infertility. The main disorders are low intellectual development, slow development of abilities

and skills, and the disharmonious development of other functions [3,4].

In some cases, children aged 2-4 years with Down syndrome can develop atypical autism [5]. Down syndrome is very rarely accompanied by epileptic symptoms. There is a theory that one of the key reasons for this intellectual disorder is the increased gene-dosage of superoxide dismutase, which is located on the 21<sup>st</sup> chromosome [6].

This pathology is a subject of multiple studies by specialists of different fields. Various methods of social rehabilitation directed toward neurosensorimotor and oral-motor functions [7], amino-acid metabolic therapy, and applying neurotrophic factors with neuroprotective and neurodegenerative effects which increase neuroplasticity and stimulate neurogenesis [8].

Thanks to the modern approaches of special education and care recommended by the National Down Syndrome Society, the average life expectancy has increased and, according to some studies, is at approximately 50 years [9].

Many children with Down syndrome often suffer from illnesses, but despite the existing knowledge about the specifics of the immune system of such children [8,10], the impact of different interventions in congenital and adaptive immunity remains insufficiently unexplored.

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**This defines the purpose of this study:** to research the influence of Masgutova Neurosensorimotor Reflex Integration (MNRI) treatment on the immune status of children with Down syndrome.

## Materials and Methods

Observation and research of 49 children (34 boys and 15 girls) with Down syndrome was done at the I.I. Mechnikov Scientific Research Institute of Vaccines and Serums, Moscow and the Svetlana Masgutova Educational Institute for Neuro-Sensory Motor and Reflex Integration, Orlando, FL, USA in 2010-2016 (Table 1). This Study Group was divided into age groups: under one year old – 10 children; 1-2 years old – 11 children; 2-3 years old – 12 children, and 3-6 years old – 16 children. Fifty-six (29 boys and 27 girls) healthy children were observed and examined as the Control Group. The study did not include children with acute inflammatory diseases, as well as with chronic eczema and atopic dermatitis during the exacerbation.

The last step in a Down syndrome diagnosis was cytogenetic analysis (karyotype testing). Ninety-eight percent of observed children had trisomy disorder (47 xy+21) and just one child had mosaicism (47 xy+21 (10) 46 xy (34)).

An analysis of sensorimotor development of the children with Down Syndrome within their age related differences was done with the use of standardized diagnostic criteria of neurological development [11], their diagnosis of neuropsychological development in the first three years of life compared with children with special needs [12], the *Carolina Curriculum for Infants and Toddlers with Special Needs*, and the Battelle scale [13]. The evaluation of levels of the neuropsychological development of children in actual research was done by rating quality-and-quantity parameters of the child's development, based on performance or completion of tasks corresponding to the child's age.

The rating of neuro-developmental delay in children in the Study Group and Control Groups was based responses and evaluation of ten basic parameters: visual orientation, auditory orientation, sensory development, emotional and social development, speech comprehension, expressive/active speech, gross-motor coordination, and manual abilities based on skills, playing games, and manipulation of objects.

The initial immune status and dynamics of lymphocytes subpopulations, immunoglobulins and cytokines were studied in all 49 children with Down syndrome – in the Study Group after MNRI Neurosensorimotor Reflex Integration, and in the Control Group, where children did not receive the MNRI Program.

The lymphocytes subpopulations were stained using mAb CD45-FITC/CD3-ECD, CD3-ECD/CD4-PE, CD3-ECD /CD8-PE, CD16/CD56-PE, CD3-ECD/CD19-PE, CD45-FITC/CD25-PE, CD45-FITC/CD95-PE and were analyzed by using flow cytometer Cytomix FC 50 (Beckman Coulter, USA) according to the manufacturer's instructions (Biolegend, USA).

Cytokine level in the supernatants of PBMC culture (IL-1, IL-2, IL-4, IL-6, IL-10, IL-12, IL-17, IFN- $\gamma$ , TNF- $\alpha$ ) was determined by using the FlowCytomix Th1/Th2 10 plex kit (Bender MedSystems, Austria) in the flow cytometer Cytomix FC 500 according to the manufacturer's instructions (Beckman Coulter, USA). PBMC ( $1 \times 10^6$  cells) were cultivated 24 hours at 37°C in an air/CO<sub>2</sub> in 1 ml of complete medium composed of RPMI-1640 medium, 0.75 mM glutamine (Sigma, Dorset, UK).

Content of A-, G- and M-class immunoglobulins was determined

by the radial immunodiffusion method in agar gel [14] by using a diagnostic ELISA kit test (Vektor-Best, Novosibirsk, Russia). The IgE test was done by Total IgE ELISA-BEST kit (Vektor-Best, Novosibirsk) following the ELISA method.

## Evaluation of the level of maturity and neurosensorimotor integration of dynamic and postural reflexes

The MNRI program includes a diagnostic and therapeutic assessment procedure [15,16]. The main purpose of the diagnostics was to evaluate the level of maturity and neurosensorimotor integration of the dynamic and postural reflexes. This procedure allows developmental deficiencies in sensorimotor areas and defense mechanisms to be revealed. These deficiencies are considered the result of a delay or poor development of primary sensorimotor patterns – reflexes, or a stressful influence on them. The Assessments of reflexes included such patterns, as: the Asymmetrical Tonic Neck, Hands Supporting, Bauer Crawling, and Leg Cross Flexion-Extension, Spinal Galant and Perez, Moro, Robinson Hand Grasp, and other reflexes. In total, 30 reflexes were tested. The study of the reflex patterns is important and unique for contemporary therapeutic modalities as the assessment of the reflexes gives a much more exact analysis of the developmental deficits in the primary neurosensorimotor area, also in self-regulation and defense mechanisms, which is significant and essential for therapies and corrections. The tests contain five main parameters of evaluation:

- 1) Sensory-motor circuit – a motor or proprioceptive response to a specific stimulus (coordinated work of sensory and motor neurons);
- 2) Direction of a response – movement or posture (coherent work of the alpha and gamma motor neurons for movements);
- 3) Intensity/strength of the response (tone of muscles, ligaments and tendons. The reaction is graded respectively as normal, dysfunctional, pathological, hyper or hypo-active, a-reflexia = absence);
- 4) Response time/latency (normal/hyper/hypo-reaction, a-reflexia = absence);
- 5) Symmetry in response (similar response in circuit, direction, intensity and timing for right and left sides of body).

The parameters were evaluated on a scale of 0 – 20 points. The test results were analyzed using criteria offered for statistical analysis by Professor Anna Kreff [17], where 10 – 11.99 points means that a reflex is at the intermediate stage between dysfunction and functional development. The normal is 16 – 17.99 points.

## The evaluation of anxiety

The C.D. Spielberger and Yu. L. Khanin method was applied to patients to reveal their level of anxiety [18,19]. This test is one of the most often used tools of psychometric evaluation of anxiety level at present. When looking at people who suffer from anxiety, there is reactive anxiety and anxious personality traits. These were examined using a questionnaire with 40 questions to parents of children with Down syndrome.

## The statistical processing of the results

This was accomplished with parametrical and non-parametrical basic statistics with the use of the Mann-Whitney U-test and Wilcoxon test and a standard statistical software package for Windows 7 (StatSoft 7.0), Microsoft Excel and WinMDI software. The differences were considered as significant at  $p < 0.05$ .

## Results

There was an evaluation of the medical histories of 25 children with Down syndrome and 37 children from the Control Group (not all children had early medical history information) (Table 2). It was known that three mothers of the children with Down syndrome had pregnancies with the risk of miscarriage; two women had a history of miscarriage and infants born as stillborn. Only one woman from the Control Group had abnormal pregnancies. Seven children with Down syndrome were prematurely born at 35-36 weeks of gestation, which significantly differs from the Control Group. It was also noted that the children with Down syndrome had neonatal hypoxia-ischemia significantly more often than the control group. Thirteen out of 25 children received care at a Neonatal Intensive Care Unit and two children had received mechanical ventilation.

Individual evaluation of the basic parameters of physical development such as height and body weight revealed essential deviations at birth in the children with Down syndrome, in comparison with the control group, which indicates there was prenatal hypotrophy.

The level of motor and mental skills development in children with Down syndrome depended on accompanying disorders. In the children from the first group, 75.5% had congenital heart defects (37 children) that demanded surgery during their first year of life. By the age of two all the children developed posture problems and flat feet. Chronic allergies such as eczema and atopic dermatitis were displayed in 97% or 48 of these children. By the age of three they developed vision pathologies such as hypermetropia, myopia, or astigmatism (Table 3).

Children with Down syndrome get sick often, with the average being  $5.45 \pm 0.64$  times per year. Children from the control group were sick not more than 2 – 3 times a year (average  $1.8 \pm 0.4$ ,  $p < 0.05$ ). Another difference was also in the way the kids got sick. The group containing children with Down syndrome had ARTI (Acute Respiratory Tract Infection) often complicated by sinusitis, otitis, pneumonia, and development of bronchial obstructive syndrome. All the children needed antibiotic treatment frequently. Only two children from the control group needed antibiotics. This demonstrates the existence of a defect in the cell-mediated and humoral immunity and probable dominance of IgE-dependent allergic reaction.

The children with Down syndrome showed different levels of intellectual disability (Table 4). Because it is impossible to evaluate the degree of mental development in children under two years old, their level wasn't specified.

Neurological problems such as ischemic brain injury during labor and residual changes make it harder to develop physiological reflexes and also cause neurodevelopmental deficits. This leads to the late acquisition of skills and aggravates pre-existing psychomotor delay caused by genetics, particularly in cases of hypotrophy of types 2 and 3, together with muscular hypotonia and joint hypermobility.

The results of three age-related methods and the subsequent tests allowed us to offer a developmental prognosis for the adaptation, communication, and socialization of children with Down syndrome, for practical use in child care facilities (Table 5).

The study of clinical and biochemical blood test indicators of the children with Down syndrome revealed differences from the Control Group (Table 6). Even the average level of leukocytes and lymphocytes was within the normal range but was still lower than in the control group. This may be evidence of a decrease in immune responses. The biochemical indicators were within the age range. There was evidence

Age (months)	Groups of children with Down syndrome			Control Group		
	Number of Children	Boys	Girls	Number of Children	Boys	Girls
Under 12	10	8	2	11	6	5
From 12 to 24	11	7	4	12	6	6
From 24 to 36	12	8	4	15	8	7
Over 36	16	11	5	18	9	9
All children	49	34	15	56	29	27

Table 1: Groups of children by gender and age.

Anamnesis Data	Number of children with this pathology (abs%)	
	Children with Down syndrome (p=25)	Control group (p=37)
Number of the Pregnancy:		
First	6/24	17/45.9
Second	5/20	10/27
Third	7/28	5/13.5
Fourth and more	7/28	5/13.5
Average age of women	31.2 years old	24.9 years old
Pathology while pregnancy	5/20	1/2.7
Born full-term	18/72	36/97.3
Prematurely born	7/28	1/2.7
Born with hypoxia condition	18/72	5/13.5
The Apgar score of 6-7 points	8	5
The Apgar score of 4-6 points	5	0
The Apgar score of 2-4 points	2	0
Central Nervous System Disorders including: intracranial hemorrhages and spasms	9/36	0
3/12		
Weight at birth/grams	2953.7 $\pm$ 179	3276 $\pm$ 95
Height at birth/grams	48.72 $\pm$ 0.60	51.25 $\pm$ 0.55

\*P<0.05 between groups

Table 2: The characteristic of the anamnestic data of the patients with Down syndrome and the children from the control group.

Somatic pathology	Number of children with somatic pathology (abs.%)	
	Children with Down Syndrome (p=49)	Control group (p = 56)
Allergic reactions	48/97	5/8.9
All Hypotrophy	47/95.9	5/8.9
Hypotrophy type 1	33/67.3	4/7.1
Hypotrophy type 2 and 3	14/28.6	1/1.8
Development disorders including:		
Heart defect	37/75.5	1/1.8
Ventricular septal defect (VSD)	21/42.8	1/1.8
Atrial septal defect (ASD)	16/32.6	
Bronchial tubes/lungs defect	2/4	
Intestinal defects	1/2	
Kidney development defect	3/6.1	
Umbilical and inguinal hernias	8/16.3	
Vision pathology	16/32.6	1/1.8
Respiratory disorders:		
Cases per year	97	32
4-5 times a year	9	1
6 times a year and more	5	-

\*P<0.05 between groups

Table 3: Characteristics of accompanying pathology in children with Down Syndrome.

of an increase in alkaline phosphatase levels, which is typical according to some researchers for children with hypotrophy and premature birth who take medication for neurological disorders, possibly due to a liver enzymes disorder [20].

### The specifics of the immunological status in children with Down syndrome and influence of MNRI on effectors of the immune system

The structures of lymphocytes subpopulations, cytokines, and immunoglobulins levels in blood were studied with the goal to evaluate the specifics of the immune status in 49 children with Down syndrome from age one to three years and older than three. The analysis of lymphocytes subpopulations (Table 7) exhibited a steady decrease of

T-lymphocyte, T-cytotoxic counts (CD45/CD3, CD3/CD8), T-helper cells (CD3/CD4), absolute counts of B-lymphocytes (CD45/CD19), with the increase of number of pre-activated lymphocytes (CD45/CD25) and natural killers (CD16/CD32/CD56), that may be a result of a compensatory reaction of the immune system. All the above specifics were registered in the children with Down syndrome at the age from one to three years and older than three should be considered as immunological characteristics of this syndrome.

The Neurosensorimotor Reflex Integration therapy (Table 8) led to the increase of absolute counts of T-lymphocytes (CD45/CD3) in group 7 (over three years old)  $1.69 \pm 0.03$  to  $2.45 \pm 0.31$  (by 1.5 times) and group 5 (children with Down syndrome, after MNRI)  $1.29 \pm 0.16$  to  $3.22 \pm 0.23$  (by 2.5 times). There was also an increase of T-helper levels (CD3/CD4) compared with levels before the MNRI therapy in group 5,  $0.65 \pm 0.2$  to  $1.31 \pm 0.2$  (by 2 times). The same indicator was increased in group 6 (under one year, after MNRI),  $0.64 \pm 0.18$  to  $1.42 \pm 0.3$  (by 2.2 times), in the group 7 with  $0.49 \pm 0.09$  to  $0.8 \pm 0.1$  (by 1.63 times).

Age (months)	Number of Children	Level of intellectual disability			
		Mild	Moderate	Severe	Profound
Under 12	9	-	1	2	6
From 12 to 24	9	-	1	-	8
From 24 to 36	11	1	2	3	5
Over 36	20	1	10	3	6
All Children	49	2	14	8	25

Table 4: Level of mental development in the children with Down syndrome.

Age	Motor development (coordination)		Speech development (communication)		Self-help skills and adaptation		Socialization (private and public spheres)	
	Healthy (months)	Down syndrome	Healthy (months)	Down syndrome	Healthy (months)	Down syndrome	Healthy (months)	Down syndrome
Under one year	Turns head on sounds - 0-3, Holds head - 2-4 Brings hands together, brings objects to mouth, grasps- 4-5 Turns over - 4-5 Sits - 6-7 Crawls - 10 Stands - 11 Pincer grasp by one year old	Turns over - 8 Sits- 10 Crawls on belly - 12	Babbles - 4 Understands speech -5-6 Vocalizes - 6 Understands words: 'cannot' and 'can' Speaks first words - 8-12	Babbles - 8 Understands a few words - 12 Can do pat-a-cake at a request - 12	Holds a bottle- 6 - 11 Eats solid food with hands- 7 - 12	8-18	Realizes presence of people - 0-6 Smiles back, asks to be picked up, happy to be fed - 3-6 Expresses emotions, responds to name- 6-11	6-12
From 1 to 2 years old	Walks - 13 Carries objects, bends down, squats, throws a ball, walks up steps	Crawls - 15 Stands - 20 Walks - 24 Poor balance, Doesn't squat	Vocabulary up to 250 words, Sentences 2-3 words, Understands words: 'act' and 'quality'	Gestures and first 10 words, connects words and gestures - 24 Can show an object on a picture	Eats with a spoon by self - 12-18 Drinks from a cup - 12-18 Understands purpose of a potty - 15-24	12 - 30 18 - 36	Expresses sympathy, brings a book, shows a picture, expresses possession, mirror self-recognition	Recognizes close people, will feed and rock a doll at a request, stacks blocks by age 2
From 2 to 3 years old	Develops fine motor skills, pincer grasp, walks along a straight line, balances on one foot, walks down steps	Moves their shoulder and forearm instead of her wrist	Vocabulary - 1000 words, 4 words sentences, grammar skills	Vocabulary - 30 words, simple phrases, difficulties with grammar combination of words and gestures	Asks for the potty - 15-24 Understands purpose of objects, pours water from the tap, opens a door	Asks for the potty - 18-36	Welcomes adults, plays with other children, shares, says I and me	Understands words "give me" and "here", knows name, mirrors self-recognition - by age 3 doesn't recognize colors and shapes, doesn't know verbs, doesn't understand questions
From 3 to 6 years old	Draws, molds, constructs, uses scissors, jumps with two feet, walks up and down stairs alternating feet	Retains the palmar grasp, can't perform a series of consecutive tasks	Conceptual level of thinking, motivation, ability to generalize and planning	Reduced perception, retains low volume of information, poor short-term memory, knows only what was taught	Puts toys away, washes her face, puts their clothes on and off, serves them self a portion of food, uses knife, scissors, careful and avoids known danger	Can help to put toys away, can partially undress herself, imitate adults, incapable to make plans, doesn't understand danger	Reacts to a social contact, no separation anxiety, shows enthusiasm, comforts, helps, describes feelings, know their first and last name, gender, and age, chooses friends	By age 4 knows their last name, simulates play, listens to stories, doesn't understand left or right, top or bottom. By age 5 plays with other children, shares, takes offense, hits back, says "I" and "me".

Table 5: Summary of adaptation, communication, and socialization development in children with Down Syndrome.

Indicators	Control group (p=56)	Children with Down Syndrome (p=49)	Age groups				With Down Syndrome			
			Under one year (p=11)	From 1 to 2 years (p=12)	From 2 to 3 years (p=15)	Over 3 years (p=18)	Under one year (p=11)	From 1 to 2 years (p=11)	From 2 to 3 years (n=12)	Over 3 years (p=6)
			1	2	3	4	5	6	7	8
Leukocytes, × 10 <sup>9</sup> /mCL	9.14 ± 0.45 <sup>*</sup>	6.02 ± 0.5 <sup>*</sup>	9.6 ± 0.5 <sup>*</sup>	9.16 ± 0.5 <sup>*</sup>	8.8 ± 0.39 <sup>*</sup>	8.6 ± 0.48	7.2 ± 0.37 <sup>*</sup>	5.4 ± 0.33 <sup>*</sup>	5.2 ± 0.29 <sup>*</sup>	5 ± 0.33 <sup>*</sup>
Lymphocytes %	48.7 ± 1.6 <sup>*</sup>	37.9 ± 1.7 <sup>*</sup>	45.2 ± 0.7 <sup>*</sup>	44.8 ± 0.7 <sup>*</sup>	48.1 ± 0.4 <sup>*</sup>	43.1 ± 0.47 <sup>*</sup>	41.1 ± 1.3 <sup>*</sup>	29.8 ± 3.6 <sup>*</sup>	42.2 ± 1.9 <sup>*</sup>	30.2 ± 4.7 <sup>*</sup>
Lymphocytes ABC	3.75 ± 0.2 <sup>*</sup>	1.9 ± 0.15 <sup>*</sup>	3.7 ± 0.12 <sup>*</sup>	3.2 ± 0.12 <sup>*</sup>	4.1 ± 0.2 <sup>*</sup>	3.5 ± 0.14 <sup>*</sup>	1.8 ± 0.13 <sup>*</sup>	1.7 ± 0.14 <sup>*</sup>	2.7 ± 0.11 <sup>*</sup>	1.2 ± 0.06 <sup>*</sup>
Band cells, %	3.4 ± 0.36	2.56 ± 0.14	4.1 ± 0.21	2.8 ± 0.18	3.9 ± 0.21	3 ± 0.3	3 ± 0.26	1.3 ± 0.22	1.1 ± 0.03	1.1 ± 0.041
Segmented cell%	35.9 ± 1.43 <sup>*</sup>	46.3 ± 1.42 <sup>*</sup>	34 ± 1.1 <sup>*</sup>	36 ± 1.6 <sup>*</sup>	38 ± 1.52 <sup>*</sup>	33.9 ± 3.14 <sup>*</sup>	43.3 ± 2.1 <sup>*</sup>	54.1 ± 0.32 <sup>*</sup>	42.8 ± 1.3 <sup>*</sup>	42.2 ± 2.8 <sup>*</sup>
Electrophoresis, %	4.89 ± 0.5	4.53 ± 0.53	4.5 ± 0.5	4.8 ± 0.38	5.2 ± 0.47	5.1 ± 0.5	4.3 ± 0.47	4.9 ± 0.25	4.9 ± 0.4	5.2 ± 0.52
Monocytes, %	4.6 ± 0.44	4.8 ± 0.52	4.2 ± 0.23	4.5 ± 0.4	4.65 ± 0.47	4.39 ± 0.38	5.1 ± 0.51	4.4 ± 0.28	4.8 ± 0.38	4.6 ± 0.56

Table 6: Characteristics of peripheral blood indicators in the children with Down Syndrome.

Indicators	Control group		All children with Down syndrome (p=49) after MNRI			Children with Down syndrome under age one, after MNRI (p=10)		
	Healthy children (p=56)	Healthy children under age one (n=11)	0 day	1 month	3 months	0 day	1 month	3 months
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Leukocytes, ×10 <sup>9</sup> /mCL	9.14 ± 0.45 <sup>*</sup>	9.6 ± 0.5 <sup>#</sup>	6.02 ± 0.5 <sup>*</sup>	9.2 ± .8	8.9 ± 1.1	7.2 ± 0.37 <sup>#</sup>	9.3 ± 0.52	9.9 ± 0.6
Lymphocytes %	48.7 ± 1.6 <sup>*</sup>	45.2 ± 0.7 <sup>#</sup>	37.9 ± 1.7 <sup>*</sup>	47.3 ± 1.8	46.9 ± 2	41.1 ± 1.3 <sup>#</sup>	46.1 ± 1	45.3 ± 0.8
Lymphocytes ABC	3.75 ± 0.2 <sup>*</sup>	3.7 ± 0.12 <sup>#</sup>	1.9 ± 0.15 <sup>*</sup>	3.5 ± 0.5	3.59 ± 0.6	1.8 ± 0.13 <sup>#</sup>	3.4 ± 0.23	3.6 ± 0.29

<sup>\*</sup>p<0.05 reliable difference between groups 1 and 3

<sup>#</sup>p<0.05 reliable difference between groups 2 and 6

Table 7: Characteristics of peripheral blood indicators in children with Down syndrome after MNRI.

	Control Group (n = 56)	Children with Down Syndrome (n=49)	Age		Children with Down Syndrome after MNRI (n=49)	Age	
			0-12 months	3 y.o. and above		0-12 months after MNRI	3 y and above after MNRI
			Group 3	Group 4		Group 6	Group 7
CD45/CD3 (%)	48.67 ± 3.47	40.15 ± 2.7 <sup>*</sup>	53.53 ± 4.67	42.45 ± 4.52	46.5 ± 4.1	52.8 ± 3.1	45.9 ± 4.5
CD45/CD3 (abs.)	3.45 ± 0.3	1.29 ± 0.16 <sup>*</sup>	2.64 ± 0.65	1.69 ± 0.03 <sup>*</sup>	3.22 ± 0.23 <sup>#</sup>	2.87 ± 0.72	2.45 ± 0.31 <sup>^</sup>
CD3/CD4 (%)	29.58 ± 1.82	27.17 ± 1.98	36.3 ± 3.9 <sup>*</sup>	23.55 ± 3.8	27.4 ± 1.35	34.8 ± 3.1 <sup>*</sup>	22.8 ± 2.92
CD3/CD4 (abs.)	2.16 ± 0.19	0.65 ± 0.2 <sup>*</sup>	0.64 ± 0.18 <sup>*</sup>	0.49 ± 0.09 <sup>*</sup>	1.31 ± 0.2 <sup>#</sup>	1.42 ± 0.3 <sup>@</sup>	0.8 ± 0.1 <sup>^</sup>
CD3/CD8 (%)	20.33 ± 1.38	14.98 ± 1.22 <sup>*</sup>	21.8 ± 2.17	14.85 ± 1.22 <sup>*</sup>	17.3 ± 2.1	22.3 ± 1.45	18.1 ± 0.5 <sup>*</sup>
CD3/CD8 (abs.)	1.18 ± 0.1	0.58 ± 0.11 <sup>*</sup>	0.51 ± 0.14 <sup>*</sup>	0.49 ± 0.08 <sup>*</sup>	0.93 ± 0.3	0.87 ± 0.21	0.95 ± 0.1 <sup>*</sup>
CD4/CD8	1.57 ± 0.1	1.83 ± 0.15 <sup>*</sup>	1.66 ± 0.19	2.33 ± 0.21 <sup>*</sup>	1.72 ± 0.3	1.61 ± 0.32	1.88 ± 0.34
CD16/CD32/CD56 (%)	13.11 ± 1.3	19.3 ± 2.5 <sup>*</sup>	19.5 ± 2.72 <sup>*</sup>	17.7 ± 0.34 <sup>*</sup>	17.2 ± 2.2 <sup>*</sup>	17.5 ± 2.7 <sup>*</sup>	15.3 ± 2.45
CD16/CD32/CD56 (a6c.)	0.9 ± 0.1	0.93 ± 0.17	1.17 ± 0.3 <sup>*</sup>	1.2 ± 0.03 <sup>*</sup>	1 ± 0.4	0.9 ± 0.21	1 ± 0.32
CD45/CD20 (%)	21.57 ± 1.3	18.72 ± 2.23	25.1 ± 2.1 <sup>*</sup>	18.4 ± 2.13	19.3 ± 2.32	23.7 ± 2.5	19.3 ± 2.45
CD45/CD20 (abs.)	1.18 ± 0.09	0.61 ± 0.08 <sup>*</sup>	1.62 ± 0.1 <sup>*</sup>	0.39 ± 0.06 <sup>*</sup>	0.98 ± 0.07	1 ± 0.1 <sup>@</sup>	0.89 ± 0.07 <sup>^</sup>
CD45/CD25 (%)	14.73 ± 1.2	20.8 ± 2.31 <sup>*</sup>	22.2 ± 2.8 <sup>*</sup>	16.34 ± 2.1	17.3 ± 1.23 <sup>*</sup>	18.2 ± 2.1 <sup>*</sup>	18.4 ± 2.2 <sup>*</sup>
CD45/CD25 (abs.)	0.62 ± 0.08 <sup>*</sup>	1.25 ± 0.16 <sup>*</sup>	1.19 ± 0.24 <sup>*</sup>	0.5 ± 0.1	1.1 ± 0.09 <sup>*</sup>	1.07 ± 0.11 <sup>*</sup>	0.62 ± 0.09
CD45/CD95 (%)	21.48 ± 0.95	22.83 ± 2.21	18.4 ± 2.1	24.52 ± 2.98	21.8 ± 2.4	19.5 ± 2.7	22.6 ± 2.6
CD45/CD95 (abs.)	1.28 ± 0.08	1.19 ± 0.15	1.32 ± 0.2	0.59 ± 0.04 <sup>*</sup>	1.16 ± 0.06	1.17 ± 0.09	1.3 ± 0.1 <sup>^</sup>

\* p<0.05 – significant results vs. results in group 1; # p<0.05 – difference between groups 2 and 5; ^ p<0.05 - difference between group 4 and 7.

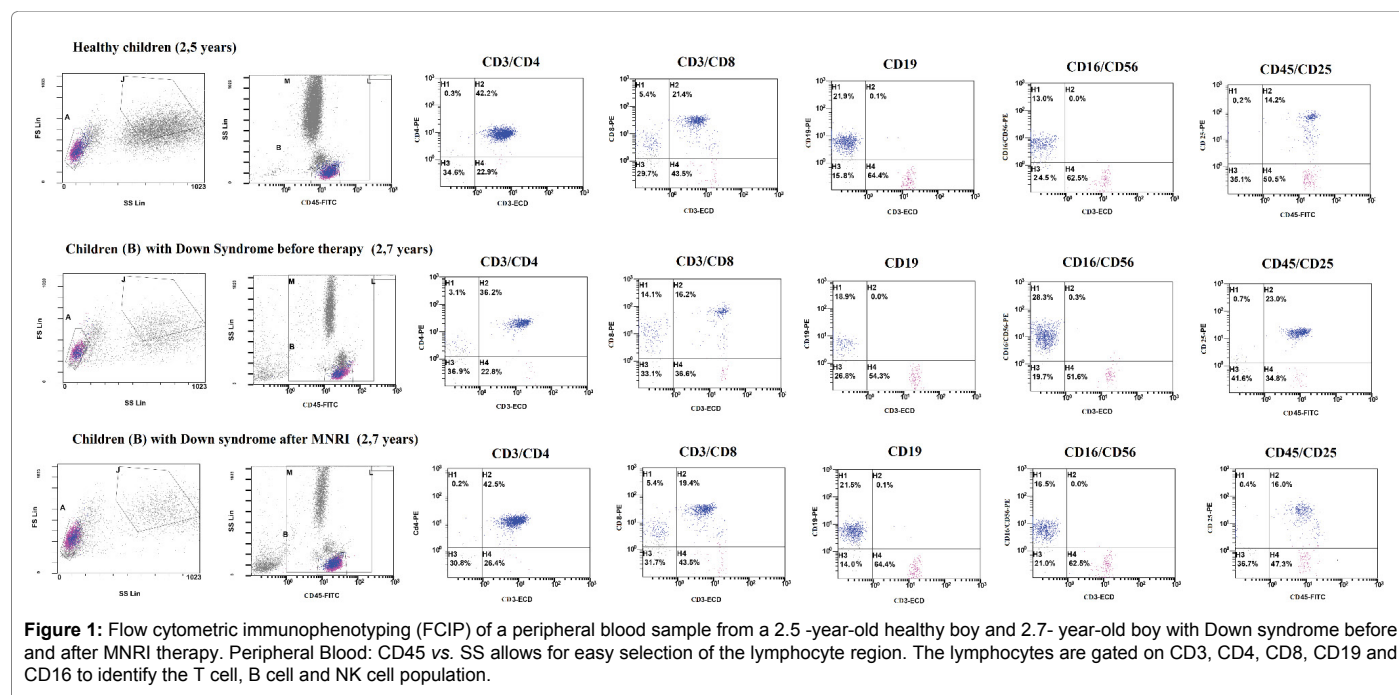
abs. - number of cells in 1 microliter

Table 8: The structure of lymphocytes subpopulations in the children with Down syndrome after MNRI.

An increase of cytotoxic T cell numbers -  $0.49 \pm 0.09$  to  $0.95 \pm 0.1$  (by 1.94 times) was observed in the children over three years old (group 7). There was also a correction in B-lymphocytes (CD45/CD19) -  $0.39 \pm 0.06$  (group 4) to  $0.89 \pm 0.07$  (group 7) (by 2.9 times) and in the number of activated CD45/CD95 blood cells  $0.59 \pm 0.04$  (group 4) to  $1.3 \pm 0.1$  (group 7) (by 2.2 times). Figure 1 presents a cytofluogram of a healthy boy (2.5 y) vs. a boy with Down syndrome (2.7 y) prior and

after the MNRI treatment, and shows also the similar changes observed in their corresponding groups.

Children with Down syndrome that underwent MNRI therapy displayed a correction of absolute cell counts in the cellular immune responses, the content of activated CD45/CD3, CD3/CD4, CD3/CD8 and CD45/CD19 lymphocytes in peripheral blood.



**Figure 1:** Flow cytometric immunophenotyping (FCIP) of a peripheral blood sample from a 2.5-year-old healthy boy and 2.7-year-old boy with Down syndrome before and after MNRI therapy. Peripheral Blood: CD45 vs. SS allows for easy selection of the lymphocyte region. The lymphocytes are gated on CD3, CD4, CD8, CD19 and CD16 to identify the T cell, B cell and NK cell population.

After one year MNRI treatment the number of exacerbations of respiratory disorders have reduced (cases per year) from 97 to 37 (note: the number of children that were sick 4-5 times a year decreased from 9 to 3; those who were sick 6 times a year – the number of them decreased from 5 children to 2). The number of children having allergic reactions have been also reduced from n=48 (97%) to n=21 (42.8%).

The evaluation of humoral immunity was based on immunoglobulins IgM, IgG, IgA, and IgE levels. The children with Down syndrome showed essential decreases in IgG pool, and a tendency to decrease IgM level with their IgA level remaining stable (Table 9). Even when there was no essential immunoglobulin deficiency, the decrease of IgM and IgG levels together with the reduced T- and B-cell numbers predetermined a frequent sickness rate (Table 3) and low level of immune responses (Table 8). The indicators of IgE ( $1489.5 \pm 467.9$  and  $59.67 \pm 11.8$  IU/L,  $p < 0.05$ ) in the Study Group (Table 9) turned out to be significantly high which reflects a predisposition to humoral responses, IgE-dependent, and allergic reactions. According to the existing studies, similar changes such as a decline in cell-mediated immunity in the form of decreasing numbers of T-lymphocyte subpopulation and low IgG and IgM levels in humoral immunity with elevated IgE, are typical for children with organic damage within the central nervous system, which can be caused by chromosomal abnormalities, and for children with allergic disorders [10,21].

The increase of IgM (from 0.75 to 0.97 g/L) and IgG (from 5.59 to 8.3 g/L,  $p < 0.05$ ) levels and the decrease of IgE by almost three times (by 2.9 times, from 1489.5 to 532.5 g/L,  $p < 0.05$ ) were the results of the MNRI therapy. It was observed (Table 9) reduced pro-inflammatory cytokine concentrations IL-2, IL-12, IL-17, IFN- $\gamma$  (respectively by 2.1; 3.34; 4.3; 1.5 times,  $p < 0.05$ ) in the children with Down syndrome in comparison with the control group (healthy children). Also there was an increase in IL-4, IL-6, TNF- $\alpha$  levels (respectively by 3.4; 9.2; 1.56 times,  $p < 0.05$ ).

MNRI (Table 9) caused a noticeable immune-corrective effect on the indicators of the cytokine status in the children with Down

syndrome. There was an increase in the initially low levels of IL-2 (from 21.15 to  $42.2 \pm 4.5$  pg/ml), IL-12 (from 21.7 to 63.2 pg/ml), IL-17 (from 12.7 to 45.2 pg/ml), IFN- $\gamma$  (from 23.7 to 39.7 pg/ml). MNRI also helped to decrease the initially higher levels of the following cytokines: IL-4 (from 78.35 to 31.3 pg/ml), IL-6 (from 218.7 to 51.2 pg/ml), IL-10 (from 323.6 to 111.7 pg/ml), TNF- $\alpha$  (from 115.7 to 69.3 pg/ml).

It is known that the increase in pro-inflammatory cytokine concentration is a part of a chronic inflammatory process, the same way as an increase of anti-inflammatory IL-4 and IL-1 demonstrates poor performance of the immune system in children with Down syndrome who get sick often [21-23]. IL-4 induces switching immunoglobulin synthesis to IgE [24]. The reduced IFN- $\gamma$  synthesis, in comparison to the group containing healthy children, indicate a probable exhaustion of anti-inflammatory resistance in the children with Down syndrome (Table 9) and the immune response is taking the Th-2 way.

## Discussion

Research data proves that children with Down syndrome need cumulative specialized evaluation of their neuropsychological development as they have special needs. All of the children with Down syndrome are predisposed to viral and bacterial infections, allergic reactions, including bronchial obstructive syndrome [4,25]. This reflects the changes in their immune system as their deficiency of cell-mediated and humoral immune responses is, according to the textbooks, one of the frequent causes of diseases [4,8,25,26].

The increase of the pro- (IL-6, TNF- $\alpha$ ) and anti-inflammatory (IL-4, IL-10) cytokines indicate low level of immune system function, while the reduced IL-2, IL-12, IL-17, IFN- $\gamma$  synthesis reflect poor infection resistance. In this Study Group of children, the average number of diseases in one year was three times higher than in the control group. The respiratory diseases were also more severe and had a larger number of complications in the group with Down syndrome. The predisposition to IgE-dependent humoral immune responses and allergic reactions is comparable to IgE indicators.

Cell counts	Control Group (p=56)	Children with Down syndrome (p=49) before the therapy	Children with Down syndrome (p=49) after MNRI
IgM. g/L	1.21 ± 0.05	0.75 ± 0.21	0.97 ± 0.32
IgG. g/L	9.52 ± 0.45	5.59 ± 0.62 <sup>†</sup>	8.3 ± 0.52 <sup>#</sup>
IgA. g/L	0.85 ± 0.11	0.83 ± 0.12	0.82 ± 0.43
IgE. IU/L	59.67 ± 11.8	1489.5 ± 467.9 <sup>†</sup>	532.5 ± 47.3 <sup>#</sup>
IL-1β. pg/ml	18.23 ± 1.72	21.76 ± 1.87	22.6 ± 2.7
IL-2. pg/ml	44.7 ± 3.3	21.15 ± 1.3 <sup>†</sup>	42.2 ± 4.5 <sup>#</sup>
IL-4. pg/ml	23.0 ± 2.45	78.35 ± 5.34 <sup>†</sup>	31.3 ± 3.2 <sup>#</sup>
IL-6. pg/ml	23.87 ± 3.45 <sup>†</sup>	218.7 ± 34.2 <sup>†</sup>	51.2 ± 3.45 <sup>#</sup>
IL-10. pg/ml	56.7 ± 6.87 <sup>†</sup>	323.6 ± 34.6 <sup>†</sup>	111.7 ± 1.5 <sup>#</sup>
IL-12. pg/ml	72.6 ± 8.7 <sup>†</sup>	21.7 ± 2.57 <sup>†</sup>	63.2 ± 6.7 <sup>#</sup>
IL-17. pg/ml	54.6 ± 4.7 <sup>†</sup>	12.7 ± 1.7 <sup>†</sup>	45.2 ± 7.7 <sup>#</sup>
IFN-γ. pg/ml	35.4 ± 3.74 <sup>†</sup>	23.7 ± 2.5 <sup>†</sup>	39.7 ± 5.5 <sup>#</sup>
TNF-α. pg/ml	73.9 ± 5.9 <sup>†</sup>	115.7 ± 11.7 <sup>†</sup>	69.3 ± 7.3 <sup>#</sup>

<sup>†</sup>p<0.05 - in comparison with the control group; <sup>#</sup> - in comparison with the group before the therapy.

**Table 9:** Level of immunoglobulin and intracellular cytokine in the children with Down syndrome, after MNRI®.

Children with Down syndrome who went under MNRI therapy displayed a correction of absolute cell counts in cellular immune responses, of activated T-cells, helper T-cells, cytotoxic T cells (CD45/CD3, CD3/CD4, CD3/CD8) and B-lymphocytes (CD45/CD19), immunoglobulins (IgM, IgG), and indicators of the cytokine status (IL-6, TNF-α, IL-4, IL-10, IL-2, IL-12, IL-17, IFN-γ) in peripheral blood, also the number of exacerbations of respiratory disorders has been reduced. A statistically significant increase in the number of cells expressing differentiation antigens and natural killer cells (CD16) after MNRI was noted. Natural killer cells are the key effectors of innate immunity; they have an important biological role in the mechanisms of immune surveillance (the targeting of tumor cells), in the destruction of viruses and parasite-infected cells, and in the regulation and differentiation of bone marrow cells (they eliminate rapidly proliferating hemopoietic cells) in people with graft-versus-host reaction [27].

However, the question concerned the ability of the individual cell populations to produce cytokines in children with Down syndrome is a separate topic and a target for the future research; this paper is focused on study on the cytokine production by a common pool of peripheral blood lymphocytes (PBMC). The results of the research demonstrate the fact, that the MNRI therapy regulates the production of pro- and anti-inflammatory cytokines, and the regulatory cytokines IL-12, IFN-γ and IL-12 and thus positively affects the interaction of the immune, endocrine, and nervous systems and ultimately homeostasis. We cannot exclude the direct effect of MNRI on circulation and the lymphatic system, because our results revealed a significant decrease in muscle hypertension, hydropic symptoms, vessel spasms, and tissue inflammation after MNRI therapy. We suggest that adding MNRI to the treatment of children with Down syndrome can correct impaired immune system mechanisms, contribute to the resolution of chronic respiratory disease, and enable a longer remission from recurrent disease. However, additional studies of the effects of MNRI therapy on mechanisms regulating immune, endocrine, and nervous system function in children with Down syndrome are of special scientific interest.

The summary of the above leads to the conclusion that poor immunological function in children with Down syndrome is one of the symptoms of the syndrome and should be considered while treating aggravated diseases. The Neurosensorimotor Reflex Integration therapy should be recommended as a rehabilitation method for this group of children.

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